To the Graduate Council:

I am submitting herewith a dissertation written by Peter S. K. Knappett entitled "Sources and Transport Pathways of Fecal Bacteria and Pathogens to Aquifers in Rural Bangladesh". I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Geology.

Larry D. McKay, Major Professor

Alice C. Layton, Co-Major Professor

We have read this dissertation

and recommend its acceptance:

Ed Perfect

Gregory Baker

Alexander van Geen (courtesy member)

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records)

Sources and Transport Pathways of Fecal Bacteria and Pathogens to Aquifers in Rural Bangladesh

A Dissertation Presented for

the Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Peter S. K. Knappett

August 2010

Copyright © 2010 by Peter S. K. Knappett
All rights reserved

This Dissertation is dedicated to

The Children of Bangladesh!

and

The life of Susan Michelle Byars.

Like the golden lining around dark clouds of sorrow,

she beckons me to look beyond the clouds.

Acknowledgements

I appreciate the support and guidance throughout my PhD of my advisors Larry McKay and Alice Layton. Also to Lex van Geen (Columbia Univ.), PI of the NIH/NSF funded project, for assistance in planning and tireless edits to every experiment and chapter in this dissertation. I would like to thank Ed Perfect, Greg Baker and Randy Gentry for their investment in my progress. I appreciate the continuing encouragement of my former advisors, Monica Emelko and Eric Reardon at the University of Waterloo. I would like to thank Jie Zhuang at UTK for his enthusiasm and help in publishing.

Thanks to Liton for 18 months of professionalism and openness to learn in the field. And thanks to Belal for working past midnight many nights during the first 6 months of the project. Thanks to Palash our field manager who helped us work effectively in the field. Thank you to Professor Kazi Matin Ahmed (Dhaka University), who helped all aspects of our travel and stay in Bangladesh run smoothly. And thank you to Babu, our extremely competent driver. Thank you to Char Para who welcomed me and made me feel part of their village. Thank you to Brian Mailloux (Barnard College) and Mike Emch (UNC Chapel Hill) for their coaching and edits from the first day I landed in Bangladesh. I'm grateful to Martin Stute (Barnard College) for the orientation to Araihazar. Patricia Culligan (Columbia Univ.) and Marc Serre (UNC Chapel Hill), statistics guru, are others I would like to thank for help along the way.

I would like to thank Karrie Radloff (Columbia Univ.) who introduced me to Char Para and continually offered me data and support for field operations and writing. I would also like to thank Veronica Escamilla (UNC Chapel Hill) for mapping everything and everyone at Site K. I would like to thank John Feighery (Columbia Univ.) for running column experiments for Chapter IV. And thank you to Jacob Mey (Lamont-Doherty Earth Observatory) for performing chemical analyses on water samples from Chapter IV and Appendix V. Thanks to Yasu Akita and Andy Ferguson for their collaboration as well. I would also like to thank Dan Williams who instructed me on molecular methods in the lab and whose professionalism and expertise contributed so much to the success of this project. I would like to thank Gary Sayler and John Sanseverino, directors of the UTK Center for Environmental Biotechnology for supplementary funding and use of the lab. Thank you to Peter Robertson and Paul Brooks for their excellent work on grain size analysis and sediment core logging. I appreciate the assistance of Kris Bronstad within the Graduate School for help with formatting this dissertation.

I would like to thank many UTK colleagues and friends who listened to me talk about fecal bacteria and diarrheal disease, drinking water and leaking latrines over countless dinners and drinks, causing them to lose their appetites. Thank you to Sarah for being a wonderful

source of encouragement. I would like to thank my parents David and Ruth Knappett for listening to what I learned and supporting my decision to go further in my education, no matter what happened along the way. Thank you to my Uncle, Gary Kuehl (also a hydrogeologist) for ongoing attentiveness and interest in what I do.

Abstract

During the 1980's millions of households in Bangladesh switched from drinking surface water to private groundwater wells to reduce their exposure to fecal microorganisms. Sadly, this switch to shallow groundwater resulted in the largest example of drinking water poisoning in history, with approximately 100 million people exposed to high concentrations of naturally occurring Arsenic in the groundwater. Spatial distribution of Arsenic in the shallow aquifers tends to be patchy, so the most economical mitigation option has been lateral switching from high Arsenic wells to nearby low Arsenic wells. The recently developed Arsenic flushing conceptual model, which explains the spatial distribution of Arsenic throughout the shallow aquifers in Bangladesh, suggests however, that low Arsenic zones are recharged via coarse-grained, rapid flow pathways and therefore represent a higher risk for waterborne pathogens.

The objectives of this dissertation are to evaluate new methods for sampling and detection of waterborne pathogens, while also identifying sources of fecal contamination and transport pathway(s) to private wells emplaced within the shallow aquifers. It was demonstrated that private wells are broadly contaminated with *E. coli*, with prevalence ranging from 30 to 70%. The fact that *E. coli* was detected more frequently in private wells than sealed monitoring wells ($p<0.05$) suggests that well construction and/or daily pumping contribute to fecal contamination of the private wells. Using DNA-based molecular fecal source tracking, contamination was demonstrated to originate from human fecal waste. Unsanitary latrines, which spill effluent onto the open ground, were demonstrated to cause elevated levels of fecal bacteria in ponds, found in every village. These ponds were demonstrated to have an influence

on concentrations of fecal bacteria to at least distances of 12m into the adjacent aquifer. In a culture where latrines, private wells and ponds are frequently clustered closely together, these findings suggest that improvements in the management of human fecal waste changes in placement and construction of private wells could substantially reduce exposure of people to fecal pathogens. Fecal contamination was found to be pervasive in low Arsenic, unconfined, shallow aquifers, and therefore gains from well switching to avoid Arsenic need to be balanced with the risk of consuming waterborne pathogens.

TABLE OF CONTENTS

Chapter I – Introduction 1
I.1 Overview 1
I.2 A Brief History of Drinking Water in Bangladesh 4
I.3 Origins of Arsenic in Bangladesh Groundwater 8
I.4 Transport of Pathogens to Private Wells in Bangladesh 11
I.5 Conceptual Models of Bacteria and Virus Transport in Granular Porous Media 13
I.6 Inactivation of Bacteria and Viruses in Groundwater 21
I.7 Review of Field Studies on Bacteria and Virus Transport 22
I.8 Broader Team Project Goals 28
References 31
Chapter II - Efficacy of Hollow-Fiber Ultrafiltration for Microbial Sampling in Groundwater 41
II.1 Introduction 44
II.2 Methods 48
Site Description 48
Well Installation 49
Well Sampling and Ultrafiltration 49
EDTA Addition and Iron Detection 53
Bacterial and Viral Detection Methods 55
II.3 Results 58
Marker Concentrations in Well Water and Retentate Water 58
Variation in Marker Concentration During Sampling 62
Correlations of Markers in Retentate Water 69
EDTA and Fe Effect 72
II.4 Discussion 74
II.5 Conclusions 77
References 78
Chapter III - Impact of Sanitation on Fecal Bacteria and Pathogens in Ponds of Bangladesh 83

III.1 Introduction 86
III.2 Methods 89
Site Description 89
Field Methods and Pond Classification 90
Microbiological Assays 91
Statistical Analysis 93
III.3 Results 94
Physical and Chemical Attributes of Ponds 94
Concentrations of indicator bacteria and genetic markers 97
Correlations between Spatial Buffer Population Counts and Pond Contamination 100
Latrines and Pond Contamination 103
Correlations of Water Chemistry with Latrines, Population and Pond Contamination 104
III.4 Discussion 105
Implications for the Spread of Diarrheal Disease 105
Ecology of Fecal Bacteria in the Built Environment 106
References 109
Chapter IV - Transport of Fecal Bacteria From Ponds to Aquifers in Rural Bangladesh 114
IV.1 Introduction 117
IV.2 Materials and Methods 119
Field Site and Hydrogeology 119
Seasonal Monitoring 124
Field-scale Infiltration Experiment 126
Lab-scale Experiments 126
Chemical and Microbial Measurements 128
Microbial Transport Modeling 130
IV.3 Results 131
Hydrogeology 131
Seasonal Monitoring 135

Field-scale Transport Experiments 135
Column Experiments 141
Microbial Transport Modeling 145
Bacterial Persistence Modeling 146
IV.4 Discussion 147
Bacteria Occurrence and Transport 147
References 151
Chapter V - Conclusions and Recommendations for Future Research 156
Appendices 161
Appendix II - Ultrafiltration 162
Appendix III – Pond Microbiology 163
Appendix IV – Transect Experiments 183
Appendix V – Village-Scale Fecal Contamination Monitoring 219

List of Tables

I-1. Comparison of Characteristics of Colloids Relevant to Transport 15

I-2. Bacteria and Virus Transport Experiments in Granular Aquifers 23

I-3. Virus Transport Experiments in Granular Aquifers 26

II-1. Groundwater wells sampled and experimental design 56

II-2. Primers and probes used for each Real-time PCR assays to detect *E. coli* and *Bacteroides* rRNA genes and the Adenovirus hexon gene 58

II-3. Summary of Marker Concentrations in 19 Well Water and Retentate Water Samples (9 without and 10 with EDTA added) 61

II-4. Correlation matrix of marker concentrations in retentate water (RW) 70

III-1. Spearman Rank Order correlations of microbial markers with extracted and measured field parameters 102

IV-1. Physical properties of ponds and adjacent aquifers 121

IV-2. Volumetric and Microbial Dilution Factors of Ponds after Filling 125

IV-3. Time decay rate constants (*k*) measured over 11 days in transect KW-39 after KP-04 was artificially flooded 146

A-II-1. Physical and chemical parameters and microbial concentrations 162

A-III-1. Information recorded on each surveyed pond 163

A-III-2. Real-time PCR assays used to detect *E. coli* 164

A-III-3. Summary of data sets for 6 bacterial and viral markers 165

A-III-4. Chemical and Microbiological Information on 43 ponds within Site K 166

A-III-5. Physical Properties of 43 ponds within Site K 167

A-III-6. Locations and Ages of Sanitary Latrines in Site K as of June 30, 2009 172

A-III-7. Locations and ages of Unsanitary Latrines at Site K as of June 30, 2009 173

A-III-8. Pond KP-01 Monthly Monitoring Data 175

A-III-9. Pond KP-04 Monthly Monitoring Data 176

A-III-10. Pond KP-05 Monthly Monitoring Data 177

A-III-11. Pond KP-06 Monthly Monitoring Data 178

A-III-12. Pond KP-07 Monthly Monitoring Data 179
A-III-13. Pond KP-10 Monthly Monitoring Data 180
A-IV-1. Drilling Logs for Transect KW-36 185
A-IV-2. Drilling Logs for Transect KW-37 186
A-IV-3. Drilling Logs for Transect KW-38 187
A-IV-4. Drilling Logs for Transect KW-39 188
A-IV-5. Drilling Logs for Transect KW-40 189
A-IV-6. Drilling Logs for Transect KW-41 190
A-IV-7. Drilling Logs for Transect KW-42 191
A-IV-8. Drilling Logs for Transect KW-43 192
A-IV-9. Drilling Logs for Transect KW-44 193
A-IV-10. Summary of Cored Intervals for Grain Size Analysis 194
A-IV-11. Sediment Sieving Results for KW-36.1a 195
A-IV-12. Sediment Sieving Results for KW-37.1a 196
A-IV-13. Sediment Sieving Results for KW-39.1a 197
A-IV-14. Sediment Sieving Results for KW-39.1c 198
A-IV-15. Sediment Sieving Results for KW-40.1a 199
A-IV-16. Sediment Sieving Results for KW-41.1a 200
A-IV-17. Sediment Sieving Results for KW-41.1d 201
A-IV-18. Sediment Sieving Results for KW-42.1e 202
A-IV-19. Sediment Sieving Results for KW-43.1a 203
A-IV-20. Sediment Sieving Results for KW-44.1d 204
A-IV-21. Major Cations in Ponds Before and After Artificial Filling 205
A-IV-22. Trace Metals in Ponds Before and After Artificial Filling 206
A-IV-23. Major Cations in Deep Transect and Private Wells Before and After Artificial Pond Filling 207
A-IV-24. Trace Metals in Deep Transect and Private Wells Before and After Artificial Pond Filling 208

A-IV-25. Major Cations in Shallow Transect Wells Before and After Artificial Pond Filling 209
A-IV-26. Trace Metals in Shallow Transect Wells Before and After Artificial Pond Filling 211
A-IV-27. Electrical Conductivity Monthly Monitoring Results from Shallow Transect Monitoring wells 213
A-IV-28. *E. coli* Monthly Monitoring Results from Shallow Transect Monitoring wells at Site K 214
A-IV-29. Water Levels from Transects Surrounding Pond KP-10..... 215
A-IV-30. Water Levels from Transects Surrounding Pond KP-04..... 216
A-IV-31. Water Levels from Transects Surrounding Pond KP-05..... 217
A-IV-32. Elevations of Tops of Casings of Transect Wells and L-piezometers..... 218
A-V-1. Classification of Well Types at Site K 220
A-V-2. Depths of Site K Private and Monitoring Wells 221
A-V-3. Elevations of Tops of Casings of Monitoring Wells..... 222
A-V-4. Measured Hydraulic Conductivities of Site K Monitoring Wells..... 223
A-V-5. Locations, Arsenic concentrations and detected *E. coli* frequency in monthly monitored private wells at Site K..... 228
A-V-6. Locations, Arsenic concentrations and detected *E. coli* frequency in monthly monitored monitoring wells at Site K 229
A-V-7. Concentration of Major Cations and Arsenic in Private Wells 230
A-V-8. Concentration of Major Cations and Arsenic in Monitoring Wells 231
A-V-9. Electrical Conductivity Monthly Monitoring Results from Private Wells at Site K..... 232
A-V-10. Electrical Conductivity Monthly Monitoring Results from Monitoring Wells at Site K 233
A-V-11. *E. coli* Monthly Monitoring Results from Private Wells at Site K 234
A-V-12. *E. coli* Monthly Monitoring Results from Monitoring Wells at Site K..... 235

List of Figures

I-1. Arsenic in Bangladesh 6

I-2. Spatial Distribution of Arsenic in Araihazar 7

I-3. Sources and potential transport pathways of fecal bacteria and viruses to tubewells 12

I-4. Physicochemical and Physical Colloid Removal Processes 17

I-5. Electrophoretic mobility of recombinant (inactivated) NV virus and MS2 as a function of solution pH in 0.1 M NaCl 27

I-6. Relative risks of Arsenic and Pathogens in drinking water 30

II-1. Ultrafiltration Apparatus 54

II-2. Comparisons of cultured marker concentrations from 250 ml unfiltered Well Water (WW) samples with 100 L ultrafiltered Retentate Water (RW) samples 64

II-3. Comparisons of molecular marker concentrations from 250 ml unfiltered Well Water (WW) samples with 100 L ultrafiltered Retentate Water (RW) samples 65

II-4. Paired 100 ml pre-filtration Well Water samples (in triplicate) taken from wells early or late in the day for culturing 67

II-5. Paired 250 ml Well Water samples taken from wells early or late in the day for molecular analysis 68

II-6. Comparison of cultured and molecular *E. coli* assays in both WW and RW samples 71

II-7. Comparison of Retentate Water samples from ultrafiltration runs with EDTA and those without EDTA added 73

III-1. Concentration of cultured *E. coli* in each pond classified by pond type with locations of sanitary and unsanitary latrines 95

III-2. Dissolved oxygen, electrical conductivity, temperature and log-transformed concentrations (MPN or copies/100 ml) of three fecal markers in water, from three types of ponds at Site K 96

III-3. Observed and predicted *E. coli* and *Bacteroides* as a function of *mE. coli* in 43 pond water samples 98

III-4. Significance of Spearman rank correlation coefficient with GIS-based spatial buffer counting of population, total, unsanitary and sanitary latrines against concentration of fecal bacteria and Adenovirus in pond water 101
IV-1. Locations of ponds and transects within Site K 122
IV-2. Geologic cross-sections of Transects KW- 37, 39, 42 and 43 133
IV-3. Seasonal *E. coli* concentration in the closest well to each pond 134
IV-4. Fecal bacteria concentrations in transects KW-39, -42 and -43 with lateral distance from pond KP-04, -15 and -05 respectively 138
IV-5. Response of lateral groundwater velocities determined by Darcy's law in transects to natural precipitation events and artificial pond filling 139
IV-6. Triplicate breakthrough curves of microspheres and Bromide in three different 10 cm columns packed with sand from the base of the pond KP-15 143
IV-7. Six-$\log_{10}$ modeled removal distances over time since pond filling for monitoring wells KW-39 and 42, adjacent to ponds KP-04 and KP-15 respectively 144
A-III-1. Relative amounts of fecal contamination from Human or Bovine sources 168
A-III-2. Log-transformed concentrations (copies/100 ml) of 3 markers at Site K 169
A-III-3. Concentrations of Adenovirus in each pond classified by pond type 171
A-IV-1. Response of Vertical Groundwater velocities in transects 183
A-IV-2. Pre-flooding bacterial concentrations in transects KW-39 and -42 184
A-V-1. Detection Frequencies of *E. coli* 225
A-V-2. Monthly proportion of private (P) and sealed monitoring wells (MS) testing positive for *E. coli* 226
A-V-3. Spatial Distribution of Arsenic and *E. coli* 227

CHAPTER I – INTRODUCTION

I.1 OVERVIEW

Beginning in the middle of the 1980's a major shift in drinking water source from surface water to shallow groundwater was made by millions of households throughout Bangladesh to reduce consumption of waterborne pathogens and the resulting diarrheal disease (Ahmed et al., 2006). Due to high natural concentrations of dissolved Arsenic in aquifers throughout the Ganges-Brahmaputra delta, however, this shift has resulted in the largest case of drinking water poisoning in history (Dhar et al., 1997). An estimated 100 million people in Bangladesh, West Bengal, India and other south Asian countries are now exposed to drinking water laden with dissolved Arsenic concentrations many times greater than the WHO recommended guideline of 10 µg/L (Dhar et al., 1997), resulting in elevated rates of internal cancers and skin diseases (Ahmed et al., 2006). A number of inexpensive Arsenic mitigation options have been proposed, including switching drinking water sources back to filtered surface water (Ahmed et al., 2006). Several of these mitigation options raise the concern that avoiding Arsenic, may in fact incur greater overall losses in healthy years lived through raising exposure to waterborne pathogens (Lokuge et al., 2004). The primary Arsenic mitigation option has been switching from high Arsenic private wells emplaced within shallow aquifers to wells of similar depth with low Arsenic concentrations (Ahmed et al., 2006). The heterogeneous distribution of Arsenic in shallow aquifers was recently explained by a hydrologic flushing model which postulates that low Arsenic aquifers are overlain by coarse sediment that has been depleted of sorbed Arsenic by historical flushing by rapidly infiltrating recharge water (van Geen et al., 2008). Based on this

model there is concern that shifting to low Arsenic wells located in shallow unconfined aquifers, which are typically recharged by rapidly flushed pore water could expose people to drinking water high in fecal pathogens. This shift in drinking water source could potentially increase rates of diarrheal disease in a population still struggling with high childhood morbidity and mortality (Emch, 1999).

This dissertation was a key part of an interdisciplinary research effort involving faculty and students from five institutions (Columbia University, University of Tennessee, University of North Carolina at Chapel Hill, Barnard College and Dhaka University in Bangladesh). The project investigated different aspects of the source, fate and transport of fecal contaminants in aquifers of rural Bangladesh and their relationship to Arsenic and occurrence of diarrheal disease. The objectives of this dissertation are to: 1) evaluate new methods for the concentration and detection of waterborne pathogens; 2) identify sources of fecal contamination to both surface and subsurface waters; and 3) determine the dominant transport pathway(s) of fecal contamination to private wells emplaced within shallow aquifers.

Published or expected peer-reviewed papers based on this dissertation are outlined below, along with information on the status of manuscripts. The PhD candidate, Peter Knappett, is or will be first-author on all of the manuscripts included in this dissertation, although other members of the research team are often included as co-authors, reflecting the interdisciplinary nature of this research.

Paper #1 (Chapter II) - Efficacy of Hollow-Fiber Ultrafiltration for Microbial Sampling in Groundwater

Status: In Press in the journal *Ground Water*

Paper #2 (Chapter III) - Impact of Sanitation on Fecal Bacteria and Pathogens in Ponds of Bangladesh

Status: Submitted for review in the journal *Environmental Science & Technology*

Paper #3 (Chapter IV) - Transport of Fecal Bacteria from Ponds to Aquifers in Rural Bangladesh

Status: In Preparation for the journal *Water Resources Research*

Additional manuscripts authored by Mr. Knappett may be submitted over the next year or two based on data in the Appendices and he will likely be a co-author on several of the manuscripts generated by other members of the research team (which are not described in this dissertation).

I.2 A Brief History of Drinking Water in Bangladesh

Bangladesh is a small country (~147,000 km^2) with a very large population of 150 million people (http://www.bbs.gov.bd/dataindex/pby/bulletin.pdf). It is located on the largest delta system in the world, consisting of 100,000 km^2 of braided streams, river channels and floodplains within the confluence of the Ganges and Brahmaputra rivers, which discharge into the Bay of Bengal (Goodbred et al., 2003). Bangladesh is prone to riverine and storm surge flooding, during the late monsoon season (August to November), which pose an annual challenge for its inhabitants. Further, due to the rapid deposition of new high energy sediment, the pathways of the rivers and streams shift continuously (Goodbred et al., 2003), making development of infrastructure such as roads and villages challenging.

Prior to 1980, most people in Bangladesh drank surface water from rivers and ponds which are heavily contaminated with fecal pollution. Accordingly, diarrheal disease morbidity and mortality was high, especially in children under five years old (Pruss et al., 2002). Beginning in the early eighties, prompted by UNESCO and other non-governmental organizations, approximately 10 million private hand pump wells were installed over the next ten years throughout the country, resulting in improved drinking water quality for practically every household (van Geen et al., 2003). During this time, diarrheal disease mortality significantly decreased. This decrease in mortality was at least partially due to the widespread implementation of oral rehydration therapy (ORT), a simple, but life saving procedure whereby patients are orally administered a saline solution. Diarrheal disease morbidity remains high in

Bangladesh, however (Emch, 1999; Rahman et al., 2007), costing untold losses in productive life years (Lokuge et al., 2004).

In 1997 the first paper on Arsenic in groundwater in Bangladesh was published, showing its widespread occurrence in shallow aquifers (Dhar et al., 1997). Earlier experience with Arsenic in groundwater was obtained by investigators working in the Bengal province in India as early as 1987 (Chakraborty et al., 1987 as cited in Zheng et al., 2005). Unlike pathogens, responsible for acute diarrheal disease, the health effects from consuming Arsenic are not immediately obvious. For example, internal cancer and kertosis (skin lesions) result only after years of chronic exposure (Smith et al., 2000).

The highest proportion of private wells testing positive for Arsenic resides in the southern part of the country, with 50 to 100% of wells testing positive (Fig. I-1). In contrast, the northern part of the country is relatively free of Arsenic. Araihazar upazila lies within the transition area between the low Arsenic northern part of the country and the high Arsenic south (Fig. I-1). Araihazar is the focus of ongoing public health and environmental science efforts by Columbia University to understand factors controlling the spatial distribution of Arsenic in private wells. In 2000, six-thousand private wells were analyzed for Arsenic providing unprecedented spatial resolution within the 25 km^2 area of Araihazar upazila (van Geen et al., 2003) resulting in a spatially heterogeneous picture of Arsenic distribution (Fig. I-2). Potential mechanisms accounting for the observed spatial pattern of Arsenic distribution follows in the next section.

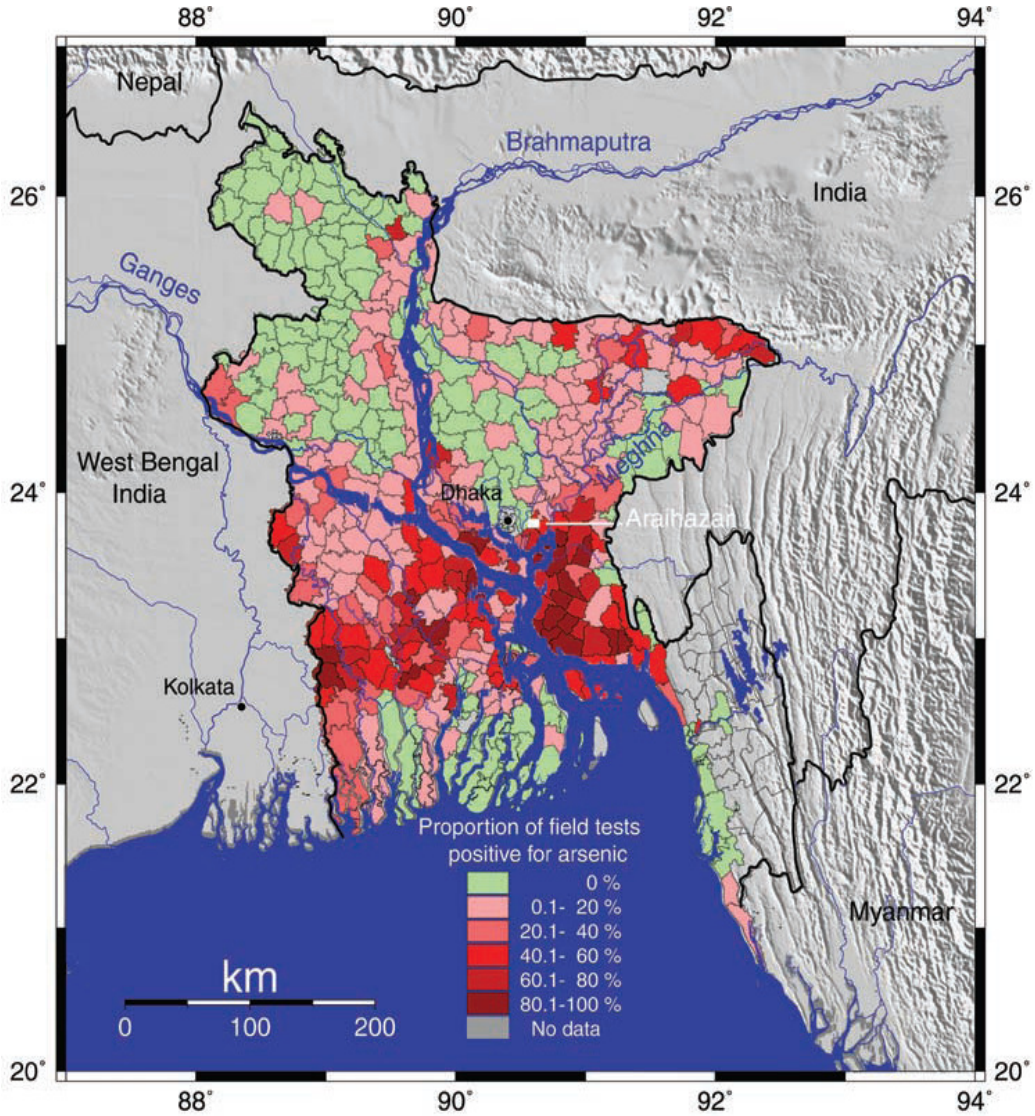


Figure I-1. Arsenic in Bangladesh (DPHE/UNICEF 1997, as cited in van Geen et al., 2003).

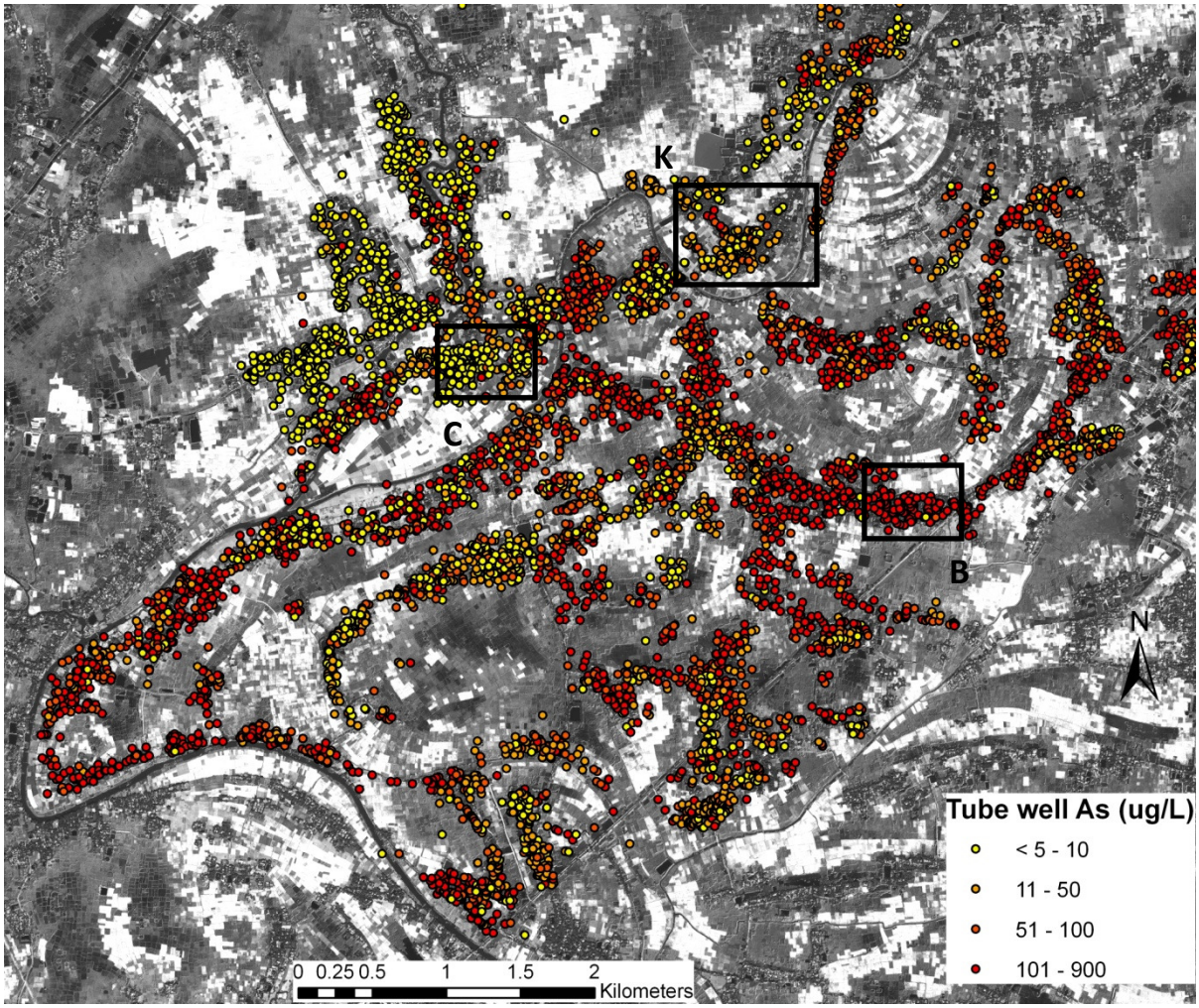


Figure I-2. Spatial Distribution of Arsenic in tube wells in Araihazar upazila (25 km^2), Bangladesh (modified from van Geen et al., 2003). Boxes indicate villages where seasonal *E. coli* sampling has been performed (Leber et al., 2010). Site B is the village Baylakandi (23.780˚N, 90.640˚E), Site C is Satybhadi (23.790˚N, 90.611˚E), Site K is Char Para (23.795˚N, 90.629˚E).

I.3 Origins of Arsenic in Bangladesh Groundwater

Arsenic has two valence states As(III) and As(V) (Cherry et al., 1979 as cited in Appelo and Postma, 1996) with the former being the more toxic form to humans (Amirbahman et al., 2006). In reducing groundwater conditions, such as most shallow aquifers in Bangladesh, arsenite (As(III)) in the form of $H_3AsO_3^0$ predominates at near-neutral pH. In oxidizing conditions, arsenate (As(V)) in the form of the anions $H_2AsO_4^-$ and $HAsO_4^{2-}$ predominates. A mixture of species is usually present in shallow aquifers in Bangladesh (Zheng et al., 2005). It is often thought that Arsenic needs to be in an oxidized (V) form to be sorbed to surfaces and that reduction to arsenite (III) cause its mobilization (Ahmann et al., 1997 as cited in Zheng et al., 2005). Van Geen et al. (2004), however, showed that oxidation of As did not prevent its mobilization in grey (lacking Fe(III)OOH) sediments from shallow aquifers in Bangladesh. Arsenic is thought to reside within sulfide-bearing minerals and its initial liberation and cycling thereafter may be a predominantly a biotic (Islam et al., 2004; Mailloux et al., 2009) or abiotic process (Amirbahman et al., 2006).

Several early hypotheses regarding the liberation of Arsenic from shallow aquifer sediments have had tremendous staying power, in spite of over 10 years of research by hundreds of investigators performing laboratory and field experiments. For example, the earliest papers on the As problem in Bangladesh cite the relatively recent onset of irrigation pumping as a potential cause of the As in the shallow groundwater (Mandal et al., 1996; Dhar et al., 1997; Nickson et al., 1998; Michael and Voss, 2008). Additionally the earliest authors assumed that positively charged Iron Oxyhydroxides (FeOOH) were essential to the storage and

release of As into aquifers and that organic carbon (from recent anthropogenic or detrital sources) was responsible for the simultaneous dissolution of FeOOH's and liberation of As (Nickson et al., 1998; Nickson et al., 2000). These early suggested processes have been retained as central and essential components in the main conceptual model put forward to this day (Harvey et al., 2002; Harvey et al., 2006; Ravenscroft et al., 2005; Polizzotto et al., 2006; Neumann et al., 2010). Recently, Neumann et al. (2010) concluded the primary source of dissolved organic carbon to aquifers comes from ponds.

An alternative, simpler conceptual model proposes that the spatial distribution of Arsenic is controlled by the location of fine layers overlaying shallow (8 to 30 m depth) aquifers where private wells are screened (van Geen et al., 2003). The fine layers increase the pore water residence time of infiltrating water resulting in insufficient historic flushing to deplete the sediment of mobilizable Arsenic (Radloff et al., 2007; van Geen et al., 2008). In contrast coarse sediment overlying unconfined sandy aquifers have rapid flushing (<6 months) resulting depletion of shallow sediments of mobilizable Arsenic (Stute et al., 2007; van Geen et al., 2008). This is the so-called flushing model explaining the spatial distribution of Arsenic in shallow aquifers (van Geen et al., 2003). This conceptual model was modified by Aziz et al. (2008) to include the possibility of lateral flow from unconfined aquifers into confined. The essential retained component of this model was that groundwater which follows rapid infiltration pathways will rapidly deplete the sediment along that flow path of mobilizable Arsenic resulting in low Arsenic groundwater. One of the implications of this model is that the spatial distribution of Arsenic will be stable and concentrations may even decrease due to increased flushing from

infiltrating irrigation water and irrigation pumping (Cheng et al., 2005; van Geen et al., 2008; Aziz et al., 2008).

The flushing model contrasts with a popular view that Arsenic liberation from sediment results from the recent introduction of anthropogenic dissolved organic matter, coupled with increased downward water flux from irrigation pumping (Harvey et al., 2002; Harvey et al., 2006; Neumann et al., 2010). Writing on the effect of DOC and irrigation pumping on As liberation Harvey et al. (2002) state: "The pumping-driven downward velocities imply…travel times to 30 m of 6.8 to 28 years…thus, young carbon could be quickly transported to depth". Further, Ravenscroft et al. (2005) concluded: "Arsenic concentrations in many shallow hand-tube wells are likely to increase over a period of years, and regular monitoring will be essential."

This academic debate has implications for the health of millions of people. Since the vast majority of high Arsenic wells throughout Bangladesh are screened within the depth interval from approximately 8 to 30 m (BGS/DPHE, 2001 as cited in van Geen et al., 2003) one mitigation strategy is to drill deeper wells (>50 m), something that few individual families can afford without government assistance (Ahmed et al., 2006). If Arsenic levels have risen due to increased downward flux of shallow groundwater and dissolved organic carbon due to recent irrigation pumping, then continued irrigation pumping may *increase* Arsenic concentrations in shallow aquifers and continue to draw Arsenic deeper in the future (Michael and Voss, 2008). In contrast, if Arsenic is high in areas of shallow aquifers that are recharged by pathways with long pore water residence times, irrigation pumping will hasten the depletion of these sediments

resulting in a long term *decrease* in Arsenic in shallow aquifers (van Geen et al., 2008). Based on the uncertainty in these models, it is generally agreed that long term monitoring of Arsenic concentrations in private wells of all depths is essential to the protection of public health Bangladesh (Ravenscroft et al., 2005; Ahmed et al., 2006).

I.4 Transport of Pathogens to Private Wells in Bangladesh

Only a few studies have been published evaluating the microbial water quality of tubewells in Bangladesh (Hoque et al., 2006; Leber et al., 2010; van Geen et al., In Preparation). Although recent studies indicate widespread fecal contamination of tubewell water, increasing during the wet season (Leber et al., 2010; van Geen et al., In Preparation), the presence of fecal bacteria, such as Fecal Coliforms or *E. coli*, does not necessarily indicate of the presence of pathogenic bacteria or viruses. The presence of *E. coli* does, however, indicated an elevated risk of both consuming pathogens (Payment, 2009) and acquiring diarrheal disease from contact (Wade et al., 2003).

A number of sources and pathways may be used by fecal-derived bacteria and viruses to reach private drinking water wells in rural Bangladesh (Fig. I-3). Humans and cattle are the main presumed contributors of fecal pollution to the environment although humans are far more abundant than cattle. Human fecal pollution sources are mainly limited to the location of latrines, which may visibly discharge onto the ground surface or consist of concrete rings, which seals the waste from the ground surface. For the purpose of understanding environmental pathways of fecal contamination, the former latrine type is defined here as an “unsanitary” latrine and the latter is defined broadly as a “sanitary” latrine. Fecal waste discharged onto the

ground from unsanitary latrines or livestock will be flushed into ponds or shallow depressions when it rains. Further, many of these unsanitary latrines discharge directly into a pond. Latrines and private hand pump wells, known in Bangladesh as tubewells, are frequently located close together, within 5 m of one another. The reason is due to the necessity of water in hand washing and anal cleansing practices after defecation (Hoque et al., 1995). This clustering of wells, latrines and ponds creates an ideal setting for the transport of fecal contamination through the ground to the wells. It is not known, however, how far fecal bacteria and viruses may be transported in these deltaic deposits. Another possible pathway for fecal bacteria and virus transport is the rapid flow of surface water or shallow groundwater along the annulus of private wells to screen depth, which are installed without seals, sometimes referred to as "short-circuiting" (Fig. I-3).

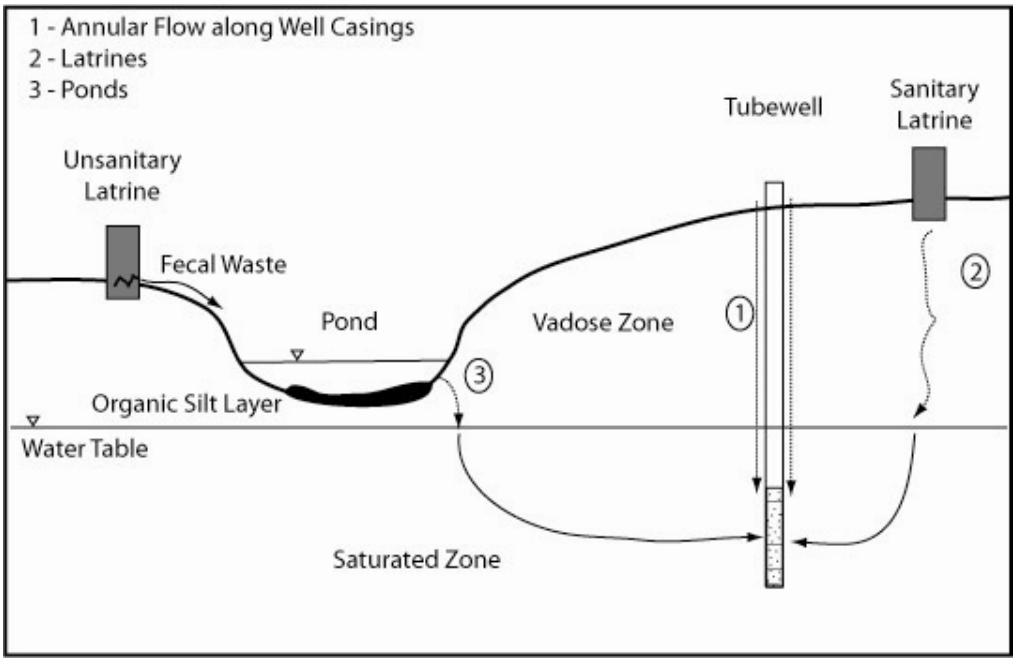


Figure I-3. Sources and potential transport pathways of fecal bacteria and viruses to tubewells.

I.5 Conceptual Models of Bacteria and Virus Transport in Granular Porous Media

A USGS report (USGS, 2006) showed the results of *E. coli* sampling of hundreds of wells across the US from a variety of aquifer types and well depths. Table 6 of the USGS report (2006) shows *E. coli* detection frequencies as a function of aquifer geology. Twenty-six percent of wells in limestone/carbonate aquifers contained *E. coli*, and the concentration ranged up to 1,200 CFU/100 ml (n=253). In contrast only 5% of wells placed in sand and gravel aquifers, and 8% of wells placed in sandstone or shale were positive for *E. coli*, with concentrations that ranged up to 23 and 33 CFU/100 ml, respectively (n=280 and 89 respectively). Another aquifer category named "Semiconsolidated Sand" indicated 12% positive *E. coli* detection with peak concentrations of 12 CFU/100 ml (n=112). Private wells in Bangladesh deltaic sand are frequently much shallower than wells in the United States, with typical depths of 8 to 30 m (van Geen et al., 2003) and therefore may be more vulnerable to fecal contamination (Rudolph et al., 1998; Leber et al., 2010).

A Web of Science search for the words "virus or bacteria", "transport" and "groundwater" turns up 377 papers dating back to 1981. For experiments on bacteria and virus transport through porous media, >90% of that work has been done on granular porous media, sand or gravel (Ryan and Elimelech, 1996; Schijven and Hassanizadeh, 2000). The purposes of that literature are diverse; addressing slow sand filtration, river bank filtration, vadose zone transport, managed aquifer recharge and deep aquifer injection. The likely reason why so much more work has been done on granular media as opposed to other types of geology is that

filtration is recognized to be highly efficient and somewhat predictable over short distances (~1-10 m), whereas the same cannot be said for fractured rock or karst.

The study of bacteria and virus transport in groundwater is a subset of the broader field of colloid transport. A colloid is defined as a particle that is too small to be seen with the naked eye and too large to be considered dissolved. Along with protozoa and other parasites, bacteria and viruses are frequently referred to as bio-colloids in the transport literature. Basic physicochemical and physical transport and removal processes may be generalized for all colloids. Transport of bacteria and viruses differ from inert colloids, however, in a few important ways: 1) bacteria may multiply and both bacteria and viruses may be rendered inactive; 2) they are temperature sensitive; 3) a consortium of bacteria may alter the pore spaces in the subsurface through the formation of biofilms (Cunningham et al., 1991; Baveye et al., 1998); 4) waterborne pathogenic bacteria have metabolisms with some being physiologically favored to persist outside of a host; and 5) their tendency to attach to grain surfaces changes in response to the metabolic state of the bacteria (Maier et al., 2000; Cunningham et al., 2007; Foppen et al., 2007a) or damage of conformational state of the viral protein coat (Grant et al.,1993; Redman et al., 1997).

Table I-1 shows different processes that affect the transport and persistence of bacteria and viruses in aquifers, relative to inert colloids, such as natural clays. Inert colloids encompass a large range of sizes (Grolimund et al. 1996), whereas the sizes of bacteria and viruses are each constrained within an order of magnitude. This is an important point, since colloid size relative to pore size is a master variable in colloid transport, concurrently influencing several transport

and removal processes. There is evidence that even slight changes in colloid or pore size may have dramatic removal responses over short distances (Zhuang et al., 2005; Knappett et al., 2008) although this is not well supported by field-scale experiments (Harvey et al., 1989; Schijven et al., 2000; Foppen et al., 2008).

Each of the eight processes or attributes highlighted in Table I-1 will be discussed in this section showing how the transport of bacteria and viruses are similar and different. As mentioned the most important difference from a transport theory perspective is size, but other processes are operative which make virus and bacteria transport a somewhat separate problem.

The transport of bacteria and viruses through granular media has traditionally been described by Colloid Filtration Theory (or clean bed filtration theory), adapted from the water treatment literature (Yao et al., 1971). Colloid Filtration Theory (CFT) envisions colloid removal as a two stage process whereby the colloids first collide with, and then stick to the grain surface. Each of these processes are described mathematically as probabilities (or efficiencies)

Table I-1. Comparison of Characteristics of Colloids Relevant to Transport

Colloid Type	Physico-chemical Removal	Physical Removal	Macropore Transport	Aggregation	Growth	Decay/Inact-ivation	Biofilm Growth	Size Distribution (µm)
Inert Colloids	yes	yes	yes	yes	no	no	no	0.02 - 10
Bacteria	yes	yes	yes	yes	yes	yes	yes	0.5 - 4
Viruses	yes	depends	depends	yes	no	yes	no	0.02 - 0.2

varying between 0 and 1, with collision and sticking efficiencies described by η and α respectively. Equation 1 shows how these efficiencies relate to the exponential spatial decline of bacteria or virus concentration along a porous media flow path:

Equation 1

$$\ln\left(\frac{C}{C_o}\right) = -\left\{\frac{3(1-\varepsilon)\alpha\eta}{2d_g}\right\}x$$

where C is the concentration of the bacteria or virus at distance x from the injection source with an influent concentration of C_o. Porosity is described by ε and d_g describes the "effective" grain diameter, for which there is little agreement of what this should mean. This is because CFT was derived for spherical, uniform sized beads (collectors) and the flow field encountered by colloids surrounding angular, poorly sorted sand grains bear little resemblance to the idealized scenario with respect to collision opportunities (Saiers and Ryan, 2005). Further, η is calculated semi-theoretically based on the above assumptions (Yao et al., 1971; Rajagopalan and Tien, 1976; Tufenkji and Elimelech, 2004), and therefore in natural media, α is frequently nothing more than a mathematical fitting parameter with questionable physical meaning (Saiers and Ryan, 2005; Knappett et al., 2008). The composite term in parentheses on the right hand side of Equation 1 is termed the filtration efficiency (Rajagopalan and Tien, 1976; Harter et al., 2000) and is a property of the interaction between the colloid and bulk porous medium. Since both the calculated η (Tufenkji and Elimelech, 2004) and filtration efficiency (Equation 1) have reciprocal relationships to collector size, removal of bacteria and viruses is predicted by CFT to increase with subtle decreases in grain size. Experimental results suggest that this relationship

is even more sensitive in natural porous media than suggested by CFT (Knappett et al., 2008; Feighery et al., In Review). In contrast to grain size, moderate increases in flow velocity (50%) are usually required to decrease filtration efficiency (Harter et al., 2000).

Classical CFT only accounts for physicochemical removal (Table I-1); it does not include any term for physical removal. Physical removal entails pore throat straining (shown as "ST" in figure I-4) or wedging between two grain surfaces at a grain-to-grain contact point (Li et al., 2006). Modifications of CFT have been made to include processes such as physical removal (Foppen et al., 2005; 2007a) as well as die off or inactivation (Schijven and Hassanizadeh, 2000).

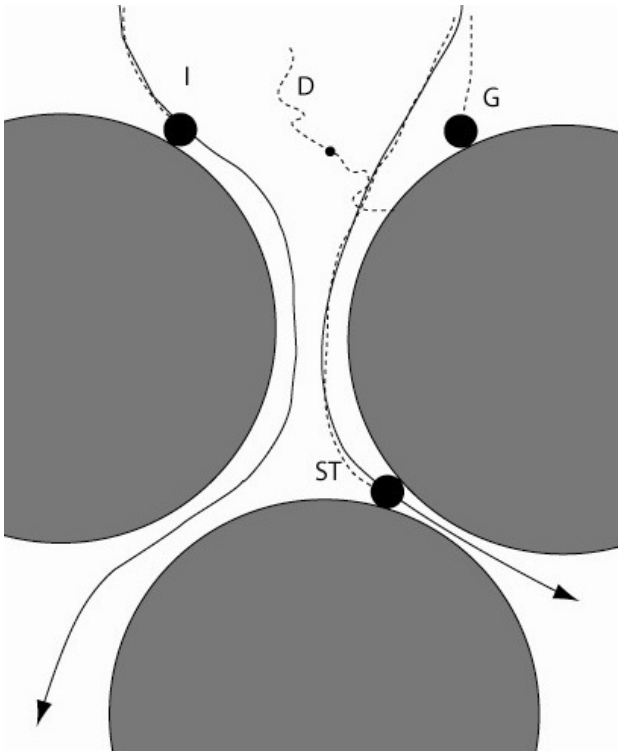


Figure I-4. Physicochemical and physical processes whereby colloids collide with a grain surface in granular porous media (Modified from McGechan and Lewis, 2002). Solid lines with arrows represent streamlines and dotted lines represent transport pathways of colloids to collector surfaces, due to diffusion (D), interception (I), gravitational settling (G) and straining (ST).

Figure I-4 shows a 1985 idealization of colloid removal processes (Vinten and Nye, 1985 as cited in McGechan and Lewis, 2002). This figure provides a basis for discussion of physicochemical and physical removal processes. Colloids may approach a collector surface by diffusion (D), interception (I) or sedimentation (G), where they may attach by a combination of van der Waals attractive force and the interaction of electrostatic force with the diffuse double layer of ions surrounding each charged surface (colloid and grain), the so-called DLVO energy profile (Ryan and Elimelech, 1996). Hydrophobic affinities are also thought to be important in determining the strength and frequency of attachment (Zhuang et al., 2005). These processes, thought to dominate contact of colloids to grains (collectors) in clean bed filtration, were first described quantitatively from basic first principles by Yao et al. (1971) and have been updated several times since (Rajagopolan and Tien, 1978; Tufenkji and Elimelech, 2004).

Removal and transport of bacteria and viruses differ primarily as a function of their size differences. Pore-scale transport of viruses (0.02 – 0.2 µm) to grain surfaces is dominated by diffusion, whereas bacteria (0.5 – 4 µm) are influenced by interception and sedimentation. According to CFT (Yao et al., 1971; Knappett et al., 2008) bacteria lie on a minimum in contact efficiency (η) where interception and sedimentation begin to dominate over diffusion. There is much empirical evidence to support the concept of such a minimum, although quantitatively, CFT is quite poor at predicting filtration in natural angular porous media (Zhuang et al., 2005; Saiers and Ryan, 2005; Johnson et al., 2007; Knappett et al., 2008).

Physical removal mechanisms are pore-throat straining and wedging and are depicted by “ST” in Figure I-5. Bradford et al., (2003) cite McDowell-Boyer et al., (1986) stating: “[Pore-

throat] Straining is the trapping of colloid particles in down-gradient pore throats that are too small to allow particle passage". Wedging was distinguished from this by Cushing and Lawler (1998): "[Wedging] is not a simple factor of straining (particles too large to fit through the small spaces near contact points) but of complex hydrodynamics that funnel particles toward contact points and also make the region near contact points stable regions of collection…" It has been reported that wedging dominates attachment the colloid-grain interaction is repulsive. This is especially true in natural, angular porous media (Li et al., 2006). According to Li et al. (2006) "it is…possible that grain-to-grain contacts serve as the zones in which secondary-minimum associated colloids are retained." If this is true, wedged colloids may be released, since their attachment is at least partially physicochemical. In agreement with this Bradford et al. (2007) showed that physicochemical attachment increases physical removal through pore throat clogging and ripening where the breakthrough concentration of colloids decreases with increased sorption. The opposite of ripening is blocking which tends to dominate under repulsive colloid-colloid interactions. Physical removal has been described mathematically as a function of the ratio of the colloid size to grain size and angularity of the sand grains (Matthess and Pekdeger, 1981; Bradford et al., 2003; Tufenkji et al., 2004; Foppen et al., 2005; 2007a).

Thus far only saturated, *clean bed* filtration has been discussed. Clean bed filtration theory assumes that the likelihood of removal is constant across a homogeneous porous media flow path. In reality, sand filters, river banks, artificial recharge basins, and shallow aquifers become chemically and biophysically altered through the precipitation and dissolution of minerals, such as positively charged iron oxyhydroxides, (Ryan et al., 1999; Flynn et al., 2004),

and the formation of biofilms (Cunningham et al., 1991; Baveye et al., 1998). These processes may change both the affinity that bacteria and viruses have to surfaces as well as the hydrodynamics of the porous media (Cunningham et al., 1991; 2007). Biogeochemical or physical alteration near the contamination source is often invoked to explain observed hyper-exponential decline in bacteria concentrations over the first few meters in field injection experiments (Harvey et al., 1989; Schijven et al., 2000; Dong et al., 2006). Tong et al. (2007) and others (e.g. Bradford et al., 2007), however, have noted the same phenomenon in column experiments run under sterile conditions with inert colloids. In columns this hyper-exponential removal is likely due to: 1) the initial entry of colloids into all pore space sizes at the influent end of the column, after which, 2) transport occurs primarily through preferential pathways in large pore spaces (Harter et al., 2000). Preferential flow paths are almost certainly operative at the field scale as well, albeit at larger (decimeter) scales in addition to the pore scale (Taylor et al., 2004; Dong et al., 2006), and may be the cause of the observed hyper-exponential declines.

The most efficient filtration of bacteria and viruses has been demonstrated experimentally to occur in the partially saturated vadose zone (Wan and Wilson, 1994; Jewett et al., 1999; Chu et al., 2001; Wan and Tokunaga, 2002; Saiers and Lenhart, 2003; DeNovio et al., 2004; McCarthy and McKay, 2004). The reason for this is postulated to be due to film straining (Wan and Wilson, 1994) whereby colloids are pushed up against the grain surface by the receding thickness of the water film during drainage. Once attached these colloids may be remobilized by rapidly infiltrating water following a storm event.

I.6 Inactivation of Bacteria and Viruses in Groundwater

Pathogenic bacteria and viruses are only dangerous to humans if they are infective. Many pathogens are equipped to remain infective for many days outside of a host, often forming cysts or dormant stages where they reduce their size and their metabolic activity drops to zero (Maier et al., 2000). Numerous studies have shown inactivation of both bacteria and viruses to be log-linear with time in batch experiments with groundwater and surface water samples (Thompson and Yates, 1999; Gordon and Toze, 2003; John and Rose, 2005; Bell et al., 2009). Bell et al. (2009) showed increased removal rates of *Bacteroides* in unfiltered vs. filtered stream water samples, attributing this difference to predation. Foppen et al. (2008) demonstrated the same phenomenon with *E. coli* in sterile and unsterile fecally contaminated groundwater. Flowing water and shearing forces along grain surfaces may increase inactivation of attached viruses (Grant et al., 1993; Schijven and Hassanizadeh, 2000). Alternatively, attached viruses may survive longer than free viruses (Grant et al., 1993) increasing transport distances since bacteria and viruses may detach when conditions change. Extreme rainfall flushing events may therefore yield pulses of pathogens higher in concentration than predicted by any removal and die off model that assumes steady-state conditions.

Viruses are extremely sensitive to inactivation at the air-water-solid interface in partially saturated porous media as hydrophobic forces exceed the forces holding the protein coat together (Thompson and Yates, 1999). Schijven and Hassanizadeh (2000) cited an exotic concept called “multiplicity reactivation” whereby aggregates of decaying viruses can actually share “spare parts” and re-assemble whole viruses. Solid evidence of large aggregates of

viruses has very rarely been demonstrated in the water quality literature, however. Aggregates are often invoked to explain puzzling transport results, which is why many column experiments today perform size measurements (using light scattering) on injection water to ensure the colloids are dispersed (Bradford et al., 2007).

I.7 Review of Field Studies on Bacteria and Virus Transport

In most cases conceptual models have evolved to explain what is seen at the column and field scale, such as pore-size exclusion (Taylor et al., 2004). In some instances, however, it seems that conceptual models were created in advance of supporting data, such as Grant et al.'s (1993) classifications of attached/free inactivation scenarios (Schijven and Hassanizadeh, 2000). Equations that describe bacteria and virus transport in porous media have many fitting parameters, and it is difficult to constrain each parameter even under such highly controlled settings as column experiments (e.g. Bradford et al., 2007). Therefore field transport experiments seem to offer little for the fine tuning of equations or introducing new pore-scale concepts such as wedging (Li et al., 2006). Field experiments do, however, represent an integrated real-world measurement of bacteria and virus transport and it is perplexing that there are so few of them in the literature. Phenomena found in column experiments are also observed in the field scale albeit sometimes for different reasons, such as the hyper-exponential deposition profile of colloids away from an injection source (Tong et al., 2007; Bradford et al., 2007; Schijven et al., 2000; Blanford et al., 2005; Dong et al., 2006). In other instances field-scale injection experiments produce results not anticipated from column experiments, such as the extent to which colloids tend to follow the fastest flowing pore spaces

(McKay et al., 1993; 2000; 2002; Auckenthaler et al., 2004; Flynn et al., 2004). For example, although colloids are routinely measured to be up to 2 times faster than conservative chemical tracers through sand columns (e.g. Harter et al., 2000; Knappett et al., 2008) a virus injection experiment in fractured clay demonstrated viral transport approximately 100 times faster than bromide due to matrix diffusion of bromide into the clay and exclusion of the virus (McKay et al., 1993). Six field transport experiments are presented here in groups of three for comparison. Table I-2 describes field transport experiments in granular porous media with bacteria, microspheres and, in some cases, bacteriophages. Table I-3 compares three field virus transport experiments in granular porous media.

Table I-2. Bacteria and Virus Transport Experiments in Granular Aquifers

Study	Aquifer Type	Injection Method	Injection Water	Aquifer Water	Detected (Bio)-colloids	Time Monitored (days)	Number of Monitoring Wells	Distance Monitored (m)
Harvey et al., 1989	Glacial Outwash Sand	Shallow Well Injection, Forced Gradient	clean water	upper clean and lower sewage contaminated	indigineous bacteria, microspheres (0.23, 0.53, 0.6, 0.84, 0.91, 1.35 um)	0.1	2 multi-level	3.2
		Shallow Well Injection, Natural Gradient	clean water	clean groundwater		32	2 multi-level	3.2
Sinton et al., 2000	1 m Silt Loam over Gravel	Surface Infiltration	sewage wastewater	16 m vadose zone occasional sewage wastewater	*E. coli*, somatic coliphage, *Bacillus Subtillus*	4	1	90
Schijven et al., 2000	Dune Sand	Deep Well Injection (300 m)	pre-treated surface water	Anoxic, clean	E. coli, *Clostridium bifermentans*, MS2, PRD-1	93	4	38

A quick comparison of the experimental conditions in Table I-2 reveals a great difference in the distance monitored; a function of the permeability of the aquifer. Sinton et al. (2000) measured breakthroughs 90 m away from a fecal source in a gravel aquifer. In contrast Schijven et al. (2000) measured 5 to 8 $\log_{10}$ decreases in concentration after only 8 m away from the injection well in deltaic sand, after which the concentration leveled off for two of the four bio-colliods and persisted up to 38 m away. All of these studies (Table I-2) used multiple bacteria or viral surrogates, and in each case there were substantial disparities in transport and removal characteristics between bio-colloids. Unlikely groups of bio-colloids were removed at similar rates. Schijven et al. (2000) showed that beyond 8 m the transport of the bacteriophage MS-2 was more similar to Clostridium spores than another bacteriophage (PRD-1), which was attenuated much faster. In general this result would not be predicted by CFT, although CFT provides a mathematical framework to evaluate the concurrent contributions that may have caused this unexpected result, such as a higher sticking efficiency for PRD-1 than MS-2.

Substantial differences in the degree of retardation for three types of similarly-sized microspheres were observed in the natural gradient injection experiment in Harvey et al. (1989) (Table I-2). The order from fastest to slowest was: uncharged latex, polyacrolein and carboxylated latex. The fact that all were retarded indicates that substantial temporary attachment was occurring to counter pore size exclusion effects (Taylor et al., 2004). The difference in breakthrough between the different microspheres may be explained by their different surface charge and hydrophobicity, although this was not done quantitatively in Harvey et al. (1989). All things being equal, CFT predicts substantial differences in breakthrough

times and peak normalized breakthrough (C/C_o) between microspheres of different sizes. Very little difference was shown, however, in the transport or removal of the 0.23, 0.53, 0.91 and 1.35 carboxylated microspheres during the natural gradient experiment (Harvey et al., 1989). Size dependency has been shown in numerous column experiments (Zhuang et al., 2005) so this is perplexing that it wasn't observed at the field scale.

Sinton et al. (2000) reported results that were more consistent with conceptual models of pore size exclusion and CFT. *E. coli* was transported about 2 times faster than rhodamine dye, while MS-2 was transported about 1.2 times faster than the dye. At all points along the 90 m flow path removal was higher with MS-2 than with *E. coli* and this is consistent with CFT since it predicts that ~1 um colloids have the least collisions.

Rather than constrain the problem of microbial transport in groundwater through normalized comparative studies, it seems that the limited number of field transport experiments have multiplied possibilities. Furthermore, there is a lack of published attempts to scale up from columns to field sites (Flynn et al., 2004; Foppen et al., 2008), which would put to the test the underlying assumption of scalability behind hundreds of column experiments.

Viruses tend to be retarded, relative to a conservative tracer, in contrast to bacteria. Although pore-size exclusion may be at work for viruses in some cases (Flynn et al., 2004), in coarse material it is unlikely to make a difference, since all flow paths are much larger than the viruses and they would have access to even the slowest pore spaces.

Table I-3. Virus Transport Experiments in Granular Aquifers

Study	Aquifer Type	Injection Method	Aquifer Water	Detected (Bio)-colloids	Time Monitored (days)	Number of Monitoring Wells	Distance Monitored (m)
Woessner, et al., 2001	Gravel Floodplain	Shallow Injection well	clean groundwater	MS-2, ΦX-174, PRD-1, polivirus type-1	2	17	21.5
Flynn, et al., 2004	Heterogeneous Sand and Gravel	Shallow Injection well	clean groundwater	H40/1	7	9 multi-level	~30
Blanford, et al., 2005	Sandy Glacial Outwash	Shallow Injection well	clean and sewage contaminated	PRD-1	14	50+ sampling ports	13

Woessner et al. (2001) performed in the field what Dowd et al. (1998) performed in a column, making an important contribution by injecting four different virus types at once into a gravel floodplain aquifer (Table I-3). They showed that for MS-2, ΦX-174 and PRD-1 C/C_o was very similar, lying between 10^{-4} and 10^{-5}. Poliovirus was attenuated down to a C/C_o of 10^{-6}. Woessner et al. (2001) were able to correlate the overall removals (mass recovery) and C/C_o values to the isoelectric points of the viruses (pI), inferring that viruses with a lower pI would carry a more negative charge at neutral pH and therefore be more strongly repulsed from a negatively charged grain surface. This was not a valid assumption since Redman et al. (1997) showed that pI and charge at neutral pH doesn't necessarily correlate. Figure I-5 shows the results of measuring electrophoretic mobility (directly related to charge) for two viruses. It shows that the rate of change of surface charge with pH is not uniform between viruses, likely owing to different amino acids in proteins on the viral coat.

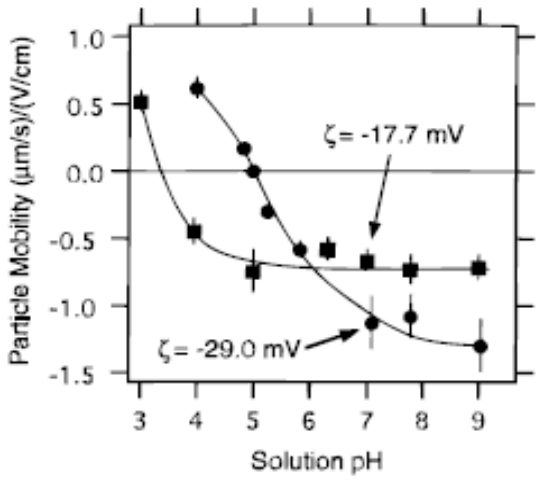


Figure I-5. Electrophoretic mobility of recombinant (inactivated) NV virus (circles) and MS2 (squares) as a function of solution pH in 0.1 M NaCl (Redman et al., 1997)

Flynn et al. (2004) reported a lack of observable variation in mineralogy throughout their site and redox conditions became iron reducing in the lower aquifer. The redox conditions, however, had no effect on the observed inactivation rate of attached viruses. This result is surprising because conceptual models of attached-phase inactivation suggest that the stronger the attachment (strength of attachment is directly related to surface charge) the greater the inactivation (Loveland et al., 1996; Bhattacharjee et al., 2002; Abudalo et al., 2005; Zhuang and Jin, 2008). Blanford et al. (2005) showed that PRD-1 transport was enhanced in the sewage contaminated aquifer over the uncontaminated aquifer. This was explained by blocking of sorption sites (Blanford et al., 2005). Organic matter tends to increase colloid transport, but this may depend on the relative hydrophobicity of the colloid (Zhuang et al., 2005).

Although there are many challenges to be met in the field of colloid transport to better predict transport and persistence, several gaps in the microbial transport and filtration literature have been identified. The first deficiency is the paucity of field transport experiments,

especially ones performed concurrently with column experiments, to examine up-scaling between column experiments and field transport studies (Foppen et al., 2008). A second deficiency is the failure of clean bed CFT to fully describe bacteria and virus transport processes through natural porous media (Saiers and Ryan, 2005; Knappett et al., 2008). A third area with little information available is in the concurrent application of recently developed molecular microbial enumeration methods to help determine whether metabolic state influences transport and filtration (Foppen et al., 2007b). The work presented in this dissertation contributes to improving our understanding of microbial transport processes, as well as addressing problems specific to microbial water quality in Bangladesh.

I.8 Broader Team Project Goals

Several options are available to mitigate the effects of Arsenic, once detected in a well. These include: 1) drilling deeper wells; 2) point-of-use filtering; and 3) lateral switching to a low Arsenic well. The last option is the most economical and low maintenance and hence is the main mitigation practice in rural Bangladesh (Ahmed et al., 2006). Concern arose that avoiding Arsenic, for example by lateral well switching, may lead people to compromise the microbial drinking water quality (Lokuge et al., 2004; Ahmed et al., 2006). One such exposure pathway to waterborne pathogens is based upon the flushing model of Arsenic liberation (Stute et al., 2007; van Geen et al., 2008). This pathway was outlined in the proposal, funded by NIH/FIC Ecology of Infectious Disease Program, “Does Arsenic Mitigation in Bangladesh Raise Exposure to Bacterial and Viral Pathogens?” The global hypothesis of the proposal was that households with low Arsenic wells will have higher rates of diarrheal disease as a result of drawing water

from unconfined aquifers which are vulnerable to fecal pollution and waterborne pathogens (Fig. I-6).

The two upazilas (regions), Matlab and Araihazar, were chosen to test the hypotheses that low Arsenic wells: 1) are correlated to higher household rates of diarrheal disease, and 2) a negative correlation exists between *E. coli* prevalence and Arsenic concentrations in private wells, emplaced within shallow aquifers. Matlab upazila (409 km^2) is much larger than Araihazar upazila (25 km^2) and is the location of a long standing, extensive rural health monitoring program of the International Center for Diarrheal Disease Research, Bangladesh (icddr,b) (http://www.icddrb.org/). Due to the availability of extensive epidemiologic records dating back to 1966, and the availability of Arsenic concentrations on most of the private wells, Matlab is the ideal place to test the hypothesis that low Arsenic wells result in higher rates of diarrheal disease. Further, etiologic agents are frequently identified from the stools of patients in the icddr,b hospital, narrowing down the list of pathogens to look for in water samples.

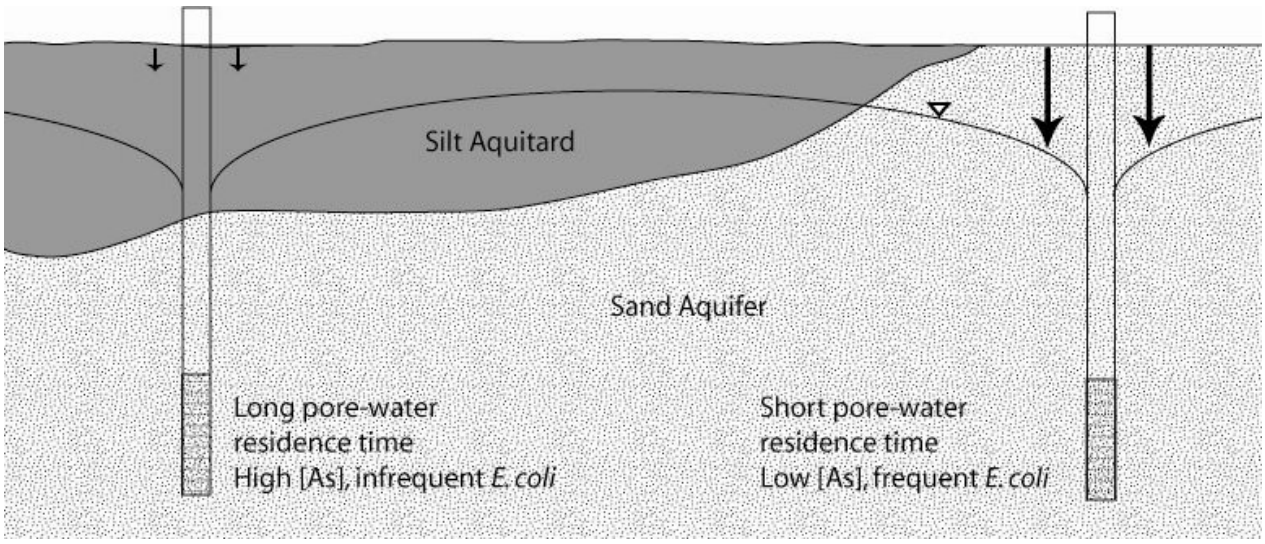


Figure I-6. Two scenarios contrasting in their relative risk of Arsenic and Pathogens.

Araihazar upazila (Fig. I-2) was chosen because pre-existing Arsenic (van Geen et al., 2003; Cheng et al., 2005; Zheng et al., 2005; Radloff et al., 2007; van Geen et al., 2008), hydrologic (Stute et al., 2007) and geologic data (Weinman et al., 2008; Aziz et al., 2008) made it an ideal place to uncover the specific transport mechanisms behind the hypothesized negative correlation between Arsenic and fecal bacteria and viruses in wells. In addition, some *E. coli* sampling had already been performed within two villages of contrasting surficial geology (Fig. I-2) (Leber et al., 2010). In this study Leber et al. (2010) demonstrated that a high Arsenic village underlain by silt (Site B) had a lower proportion of wells testing positive for *E. coli* in both the dry and wet season than a low Arsenic, sandy village (Site C). Another low Arsenic, sandy village (Site K) was chosen as the site for two years of monthly monitoring and experiments to determine the transport pathways of fecal bacteria and pathogens to private wells because of the availability of extensive hydrogeologic and hydrochemical data.

REFERENCES

Abudalo, R.A.; Bogatsu, Y.G.; Ryan, J.N.; Harvey, R.W.; Metge, D.W.; Elimelech, M. Effect of ferric oxyhydroxide grain coatings on the transport of bacteriophage PRD1 and Cryptosporidium parvum oocysts in saturated porous media. *Environmental Science & Technology* **2005** *39*(17), 6412-6419.

Ahmed, M.F.; Ahuja, S.; Alauddin, M.; Hug, S.J.; Lloyd, J.R.; Pfaff, A.; Pichler, T.; Saltikov, C.; Stute, M.; van Geen, A. Epidemiology - Ensuring safe drinking water in Bangladesh. *Science* **2006** *314*(5806), 1687-1688.

Amirbahman, A.; Kent, D.B.; Curtis, G.P.; Davis, J.A. Kinetics of sorption and abiotic oxidation of arsenic(III) by aquifer materials. *Geochimica Et Cosmochimica Acta* **2006** *70*(3), 533-547.

Appelo, C. A. J.; Postma, D. Geochemistry, Groundwater and Pollution; A. A. Balkema: Rotterdam, The Netherlands, 1996.

Appelo, C.A.J.; Van der Weiden, M.J.J.; Tournassat, C.; Charlet, L. Surface complexation of ferrous iron and carbonate on ferrihydrite and the mobilization of arsenic. *Environmental Science & Technology* **2002** *36*(14), 3096-3103.

Auckenthaler, A.; Raso, G.; Huggenberger, P. Particle transport in a karst aquifer: natural and artificial tracer experiments with bacteria, bacteriophages and microspheres. *Water Science and Technology* **2002** *46*(3), 131-138.

Aziz, Z.; van Geen, A.; Stute, M.; Versteeg, R.; Horneman, A.; Zheng, Y.; Goodbred, S.; Steckler, M.; Weinman, B.; Gavrieli, I.; Hoque, M.A.; Shamsudduha, M.; Ahmed, K.M. Impact of local recharge on arsenic concentrations in shallow aquifers inferred from the electromagnetic conductivity of soils in Araihazar, Bangladesh. *Water Resources Research* **2008** *44*(7), W07416.

Baveye, P.; Vandevivere, P.; Hoyle, B.L.; DeLeo, P.C.; de Lozada, D.S. Environmental impact and mechanisms of the biological clogging of saturated soils and aquifer materials. *Critical Reviews in Environmental Science and Technology* **1998** *28*(2), 123-191.

Bell, A.; Layton, A.C.; Mckay, L.; Williams, D.; Gentry, R.; Sayler, G.S. Factors Influencing the Persistence of Fecal *Bacteroides* in Stream Water. *Journal of Environmental Quality* **2009** *38*(3), 1224-1232.

Bhattacharjee, S.; Ryan, J.N.; Elimelech, M. Virus transport in physically and geochemically heterogeneous subsurface porous media. *Journal of Contaminant Hydrology* **2002** *57*(3-4), 161-187.

Blanford, W.J.; Brusseau, M.L.; Yeh, T.C.J.; Gerba, C.P.; Harvey, R. Influence of water chemistry and travel distance on bacteriophage PRD-1 transport in a sandy aquifer. *Water Research* **2005** *39*(11), 2345-2357.

Bradford, S.A.; Simunek, J.; Bettahar, M.; Van Genuchten, M.T.; Yates, S.R. Modeling colloid attachment, straining, and exclusion in saturated porous media. *Environmental Science & Technology* **2003** *37*(10), 2242-2250.

Bradford, S.A.; Torkzaban, S.; Walker, S.L. Coupling of physical and chemical mechanisms of colloid straining in saturated porous media. *Water Research* **2007** *41*(13), 3012-3024.

Cheng, Z.; Van Geen, A.; Seddique, A.A.; Ahmed, K.M. Limited temporal variability of arsenic concentrations in 20 wells monitored for 3 years in Araihazar, Bangladesh. *Environmental Science & Technology* **2005** *39*(13), 4759-4766.

Chu, Y.; Jin, Y.; Flury, M.; Yates, M.V. Mechanisms of virus removal during transport in unsaturated porous media. *Water Resources Research* **2001** *37*(2), 253-263.

Cunningham, A.B.; Characklis, W.G.; Abedeen, F.; Crawford, D. Influence of Biofilm Accumulation on Porous-Media Hydrodynamics. *Environmental Science & Technology* **1991** *25*(7), 1305-1311.

Cunningham, A.B.; Sharp, R.R.; Caccavo, F.; Gerlach, R. Effects of starvation on bacterial transport through porous media. *Advances in Water Resources* **2007** *30*(6-7), 1583-1592.

Cushing, R.S.; Lawler, D.F. Depth filtration: Fundamental investigation through three dimensional trajectory analysis. *Environmental Science & Technology* **1998** *32*(23), 3793-3801.

DeNovio, N.M.; Saiers, J.E.; Ryan, J.N. Colloid movement in unsaturated porous media: Recent advances and future directions. *Vadose Zone Journal* **2004** *3*(2), 338-351.

Dhar, R.K.; Biswas, B.K.; Samanta, G.; Mandal, B.K.; Chakraborti, D.; Roy, S.; Jafar, A.; Islam, A.; Ara, G.; Kabir, S.; Khan, A.W.; Ahmed, S.A.; Hadi, S.A. Groundwater arsenic calamity in Bangladesh. *Current Science* **1997** *73*(1), 48-59.

Dong, H.L.; Scheibe, T.D.; Johnson, W.P.; Monkman, C.M.; Fuller, M.E. Change of collision efficiency with distance in bacterial transport experiments. *Ground Water* **2006** *44*(3), 415-429.

Dowd, S.E.; Pillai, S.D.; Wang, S.Y.; Corapcioglu, M.Y. Delineating the specific influence of virus isoelectric point and size on virus adsorption and transport through sandy soils. *Applied and Environmental Microbiology* **1998** *64*(2), 405-410.

Emch, M. Diarrheal disease risk in Matlab, Bangladesh. *Social Science & Medicine*, **1999**, *49*(4), 519-530.

Feighery, J.; Ferguson, A. S.; Mailloux, B. J.; Ahmed, K. M.; van Geen, A.; Culligan, P. J. Filtration of *E. coli* and Microspheres in Sand Aquifers: Intact versus Re-packed cores. *Ground Water*, In Review.

Flynn, R.; Hunkeler, D.; Guerin, C.; Burn, C.; Rossi, P.; Aragno, M. Geochemical influences on H40/1 bacteriophage inactivation in glaciofluvial sands. *Environmental Geology* **2004** *45*(4), 504-517.

Foppen, J.W.; Van Herwerden, M.; Schijven, J. Measuring and modelling straining of Escherichia coli in saturated porous media. *Journal of Contaminant Hydrology* **2007a** *93*(1-4), 236-254.

Foppen, J.W.; van Herwerden, M.; Schijven, J. Transport of Escherichia coli in saturated porous media: Dual mode deposition and intra-population heterogeneity. *Water Research* **2007b** *41*(8), 1743-1753.

Foppen, J.W.A.; Schijven, J.F. Transport of E-coli in columns of geochemically heterogeneous sediment. *Water Research* **2005** *39*(13), 3082-3088.

Foppen, J.W.A.; van Herwerden, M.; Kebtie, M.; Noman, A.; Schijven, J.F.; Stuyfzand, P.J.; Uhlenbrook, S. Transport of Escherichia coli and solutes during waste water infiltration in an urban alluvial aquifer. *Journal of Contaminant Hydrology* **2008** *95*(1-2), 1-16.

Goodbred, S.L.; Kuehl, S.A.; Steckler, M.S.; Sarker, M.H. Controls on facies distribution and stratigraphic preservation in the Ganges-Brahmaputra delta sequence. *Sedimentary Geology* **2003** *155*(3-4), 301-316.

Gordon, C.; Toze, S. Influence of groundwater characteristics on the survival of enteric viruses. *Journal of Applied Microbiology* **2003** *95*(3), 536-544.

Grant, S.B.; List, E.J.; Lidstrom, M.E. Kinetic-Analysis of Virus Adsorption and Inactivation in Batch Experiments. *Water Resources Research* **1993** *29*(7), 2067-2085.

Grolimund, D.; Borkovec, M.; Barmettler, K.; Sticher, H. Colloid-facilitated transport of strongly sorbing contaminants in natural porous media: A laboratory column study. *Environmental Science & Technology* **1996** *30*(10), 3118-3123.

Harter, T.; Wagner, S.; Atwill, E.R. Colloid transport and filtration of Cryptosporidium parvum in sandy soils and aquifer sediments. *Environmental Science & Technology* **2000** *34*(1), 62-70.

Harvey, C.F.; Ashfaque, K.N.; Yu, W.; Badruzzaman, A.B.M.; Ali, M.A.; Oates, P.M.; Michael, H.A.; Neumann, R.B.; Beckie, R.; Islam, S.; Ahmed, M.F. Groundwater dynamics and arsenic contamination in Bangladesh. *Chemical Geology* **2006** *228*(1-3), 112-136.

Harvey, C.F.; Swartz, C.H.; Badruzzaman, A.B.M.; Keon-Blute, N.; Yu, W.; Ali, M.A.; Jay, J.; Beckie, R.; Niedan, V.; Brabander, D.; Oates, P.M.; Ashfaque, K.N.; Islam, S.; Hemond, H.F.; Ahmed, M.F. Arsenic mobility and groundwater extraction in Bangladesh. *Science* **2002** *298*(5598), 1602-1606.

Harvey, R.W.; George, L.H.; Smith, R.L.; Leblanc, D.R. Transport of Microspheres and Indigenous Bacteria through a Sandy Aquifer - Results of Natural-Gradient and Forced-Gradient Tracer Experiments. *Environmental Science & Technology* **1989** *23*(1), 51-56.

Hoque, B.A.; Hallman, K.; Levy, J.; Bouis, H.; Ali, N.; Khan, F.; Khanam, S.; Kabir, M.; Hossain, S.; Alam, M.S. Rural drinking water at supply and household levels: Quality and management. *International Journal of Hygiene and Environmental Health* **2006** *209*(5), 451-460.

Hoque, B.A.; Mahalanabis, D.; Alam, M.J.; Islam, M.S. Post-Defecation Handwashing in Bangladesh - Practice and Efficiency Perspectives. *Public Health* **1995** *109*(1), 15-24.

Islam, F.S.; Gault, A.G.; Boothman, C.; Polya, D.A.; Charnock, J.M.; Chatterjee, D.; Lloyd, J.R. Role of metal-reducing bacteria in arsenic release from Bengal delta sediments. *Nature* **2004** *430*(6995), 68-71.

Jewett, D.G.; Logan, B.E.; Arnold, R.G.; Bales, R.C. Transport of Pseudomonas fluorescens strain P17 through quartz sand columns as a function of water content. *Journal of Contaminant Hydrology* **1999** *36*(1-2), 73-89.

John, D.E.; Rose, J.B. Review of factors affecting microbial survival in groundwater. *Environmental Science & Technology* **2005** *39*(19), 7345-7356.

Johnson, W.P.; Tong, M.; Li, X. On colloid retention in saturated porous media in the presence of energy barriers: The failure of alpha, and opportunities to predict eta. *Water Resources Research* **2007** *43*(12), W12S13.

Knappett, P.S.K.; Emelko, M.B.; Zhuang, J.; Mckay, L.D. Transport and retention of a bacteriophage and microspheres in saturated, angular porous media: Effects of ionic strength and grain size. *Water Research* **2008** *42*(16), 4368-4378.

Leber, J.; Rahman, M.M.; Ahmed, K.M.; Mailloux, B.J.; Van Geen, A. Contrasting Influence of Geology on *E. coli* and Arsenic in Aquifers of Bangladesh. *Ground Water* **2010**, 10.1111/j.1745-6584.2010.00689.x.

Li, X.Q.; Lin, C.L.; Miller, J.D.; Johnson, W.P. Pore-scale observation of microsphere deposition at grain-to-grain contacts over assemblage-scale porous media domains using X-ray microtomography. *Environmental Science & Technology* **2006** *40*(12), 3762-3768.

Lokuge, K.M.; Smith, W.; Caldwell, B.; Dear, K.; Milton, A. The effect of arsenic mitigation interventions on disease burden in Bangladesh. *Environmental Health Perspectives* **2004** *112*(11), 1172-1177.

Loveland, J.P.; Ryan, J.N.; Amy, G.L.; Harvey, R.W. The reversibility of virus attachment to mineral surfaces. *Colloids and Surfaces a-Physicochemical and Engineering Aspects* **1996** *107*, 205-221.

Maier, R. M.; Pepper, I. L.; Gerba, C. P. *Environmental Microbiology*; Academic Press, Elsevier, USA, 2000.

Mailloux, B.J.; Alexandrova, E.; Keimowitz, A.R.; Wovkulich, K.; Freyer, G.A.; Herron, M.; Stolz, J.F.; Kenna, T.C.; Pichler, T.; Polizzotto, M.L.; Dong, H.L.; Bishop, M.; Knappett, P.S.K. Microbial Mineral Weathering for Nutrient Acquisition Releases Arsenic. *Applied and Environmental Microbiology* **2009** *75*(8), 2558-2565.

Mandal, B.K.; Chowdhury, T.R.; Samanta, G.; Basu, G.K.; Chowdhury, P.P.; Chanda, C.R.; Lodh, D.; Karan, N.K.; Dhar, R.K.; Tamili, D.K.; Das, D.; Saha, K.C.; Chakraborti, D. Arsenic in groundwater in seven districts of West Bengal, India - The biggest arsenic calamity in the world. *Current Science* **1996** *70*(11), 976-986.

Matthess, G.; Pekdeger, A. Concepts of a Survival and Transport Model of Pathogenic Bacteria and Viruses in Groundwater. *Science of the Total Environment* **1981** *21*(Nov), 149-159.

McCarthy, J.F.; McKay, L.D. Colloid transport in the subsurface: Past, present, and future challenges. *Vadose Zone Journal* **2004** *3*(2), 326-337.

McGechan, M.B.; Lewis, D.R. Transport of particulate and colloid-sorbed contaminants through soil, part 1: General principles. *Biosystems Engineering* **2002** *83*(3), 255-273.

Mckay, L.D.; Gillham, R.W.; Cherry, J.A. Field Experiments in a Fractured Clay Till .2. Solute and Colloid Transport. *Water Resources Research* **1993** *29*(12), 3879-3890.

McKay, L.D.; Harton, A.D.; Wilson, G.V. Influence of flow rate on transport of bacteriophage in shale saprolite. *Journal of Environmental Quality* **2002** *31*(4), 1095-1105.

McKay, L.D.; Sanford, W.E.; Strong, J.M. Field-scale migration of colloidal tracers in a fractured shale saprolite. *Ground Water* **2000** *38*(1), 139-147.

Michael, H.A.; Voss, C.I. Evaluation of the sustainability of deep groundwater as an arsenic-safe resource in the Bengal Basin. *Proceedings of the National Academy of Sciences of the United States of America* **2008** *105*(25), 8531-8536.

Microbial Quality of the Nation's Ground-Water Resources, 1993-2004, USGS Scientific Investigations Report 2006-5290; National Water-Quality Assessment Program Principal Aquifers, United States Geological Survey, Reston, VA, USA. 2006.

Neumann, R.B.; Ashfaque, K.N.; Badruzzaman, A.B.M.; Ali, M.A.; Shoemaker, J.K.; Harvey, C.F. Anthropogenic influences on groundwater arsenic concentrations in Bangladesh. *Nature Geoscience* **2010** *3*(1), 46-52.

Nickson, R.; McArthur, J.; Burgess, W.; Ahmed, K.M.; Ravenscroft, P.; Rahman, M. Arsenic poisoning of Bangladesh groundwater. *Nature* **1998** *395*(6700), 338-338.

Nickson, R.T.; McArthur, J.M.; Ravenscroft, P.; Burgess, W.G.; Ahmed, K.M. Mechanism of arsenic release to groundwater, Bangladesh and West Bengal. *Applied Geochemistry* **2000** *15*(4), 403-413.

Payment, P. Utopia or Reality: The Quest for Correlations between Indicators and Pathogens. *Microbial Transport and Survival in Porous Media: First International Conference*, St. Catherines, Ontario, Canada, 2009.

Polizzotto, M.L.; Harvey, C.F.; Li, G.C.; Badruzzman, B.; Ali, A.; Newville, M.; Sutton, S.; Fendorf, S. Solid-phases and desorption processes of arsenic within Bangladesh sediments. *Chemical Geology* **2006** *228*(1-3), 97-111.

Pruss, A.; Kay, D.; Fewtrell, L.; Bartram, J. Estimating the Burden of Disease from Water, Sanitation, and Hygiene at a Global Level. *Environmental Health Perspectives* **2002**, *110*(5), 537-542.

Radloff, K.A.; Cheng, Z.Q.; Rahman, M.W.; Ahmed, K.M.; Mailloux, B.J.; Juhl, A.R.; Schlosser, P.; van Geen, A. Mobilization of arsenic during one-year incubations of grey aquifer sands from Araihazar, Bangladesh. *Environmental Science & Technology* **2007** *41*(10), 3639-3645.

Rahman, M.; Sultana, R.; Ahmed, G.; Nahar, S.; Hassan, Z.M.; Saiada, F.; Podder, G.; Faruque, A.S.G.; Siddique, A.K.; Sack, D.A.; Matthijnssens, J.; Van Ranst, M.; Azim, T. Prevalence of G2P[4] and G12P[6] rotavirus, Bangladesh. *Emerging Infectious Diseases* **2007** *13*(1), 18-24.

Rajagopalan, R.; Tien, C. Trajectory Analysis of Deep-Bed Filtration with Sphere-in-Cell Porous-Media Model. *Aiche Journal* **1976** *22*(3), 523-533.

Ravenscroft, P.; Burgess, W.G.; Ahmed, K.M.; Burren, M.; Perrin, J. Arsenic in groundwater of the Bengal Basin, Bangladesh: Distribution, field relations, and hydrogeological setting. *Hydrogeology Journal* **2005** *13*(5-6), 727-751.

Redman, J.A.; Grant, S.B.; Olson, T.M.; Hardy, M.E.; Estes, M.K. Filtration of recombinant Norwalk virus particles and bacteriophage MS2 in quartz sand: Importance of electrostatic interactions. *Environmental Science & Technology* **1997** *31*(12), 3378-3383.

Rudolph, D. L.; Barry, D. A. J.; Goss, M. J. Contamination in Ontario farmstead domestic wells and its association with agriculture: 2. Results from multilevel monitoring well installations. *Journal of Contaminant Hydrology* **1998**, *32*(3-4), 295-311.

Ryan, J.N.; Elimelech, M. Colloid mobilization and transport in groundwater. *Colloids and Surfaces a-Physicochemical and Engineering Aspects* **1996** *107*, 1-56.

Ryan, J.N.; Elimelech, M.; Ard, R.A.; Harvey, R.W.; Johnson, P.R. Bacteriophage PRD1 and silica colloid transport and recovery in an iron oxide-coated sand aquifer. *Environmental Science & Technology* **1999** *33*(1), 63-73.

Saiers, J.E.; Lenhart, J.J. Colloid mobilization and transport within unsaturated porous media under transient-flow conditions. *Water Resources Research* **2003** *39*(1), DOI 10.1029/2002WR001370.

Saiers, J.E.; Ryan, J.N. Colloid deposition on non-ideal porous media: The influences of collector shape and roughness on the single-collector efficiency. *Geophysical Research Letters* **2005** *32*(21), L21406.

Schijven, J.F.; Hassanizadeh, S.M. Removal of viruses by soil passage: Overview of modeling, processes, and parameters. *Critical Reviews in Environmental Science and Technology* **2000** *30*(1), 49-127.

Schijven, J.F.; Medema, G.; Vogelaar, A.J.; Hassanizadeh, S.M. Removal of microorganisms by deep well injection. *Journal of Contaminant Hydrology* **2000** *44*(3-4), 301-327.

Sinton, L.W.; Noonan, M.J.; Finlay, R.K.; Pang, L.; Close, M.E. Transport and attenuation of bacteria and bacteriophages in an alluvial gravel aquifer. *New Zealand Journal of Marine and Freshwater Research* **2000** *34*(1), 175-186.

Smith, A.H.; Lingas, E.O.; Rahman, M. Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. *Bulletin of the World Health Organization* **2000** *78*(9), 1093-1103.

Stute, M.; Zheng, Y.; Schlosser, P.; Horneman, A.; Dhar, R.K.; Datta, S.; Hoque, M.A.; Seddique, A.A.; Shamsudduha, M.; Ahmed, K.M.; van Geen, A. Hydrological control of As concentrations in Bangladesh groundwater. *Water Resources Research* **2007** *43*(9), W09417.

Taylor, R.; Cronin, A.; Pedley, S.; Barker, J.; Atkinson, T. The implications of groundwater velocity variations on microbial transport and wellhead protection - review of field evidence. *Fems Microbiology Ecology* **2004** *49*(1), 17-26.

Thompson, S.S.; Yates, M.V. Bacteriophage inactivation at the air-water-solid interface in dynamic batch systems. *Applied and Environmental Microbiology* **1999** *65*(3), 1186-1190.

Tong, M.P.; Johnson, W.P. Colloid population heterogeneity drives hyperexponential deviation from classic filtration theory. *Environmental Science & Technology* **2007** *41*(2), 493-499.

Tufenkji, N.; Elimelech, M. Correlation equation for predicting single-collector efficiency in physicochemical filtration in saturated porous media. *Environmental Science & Technology* **2004** *38*(2), 529-536.

Tufenkji, N.; Miller, G.F.; Ryan, J.N.; Harvey, R.W.; Elimelech, M. Transport of Cryptosporidium oocysts in porous media: Role of straining and physicochemical filtration. *Environmental Science & Technology* **2004** *38*(22), 5932-5938.

van Geen, A.; Ahmed, K. M.; Akita, Y.; Alam, Md. J.; Culligan, P. J.; Feighery, J.; Ferguson, A.; Emch, M.; Escamilla, V.; Knappett, P.; Layton, A. C.; Mailloux, B. J.; McKay, L. D.; Mey, J. L.; Serre, M. L.; Streatfield, P. K.; Wu, J.; Yunus; Md. Seasonal contamination of shallow tubewells in Bangladesh with the fecal indicator *E. coli*: Impacts of population density and hydrogeology. In Preparation for *Environmental Science & Technology*.

van Geen, A.; Rose, J.; Thoral, S.; Garnier, J.M.; Zheng, Y.; Bottero, J.Y. Decoupling of As and Fe release to Bangladesh groundwater under reducing conditions. Part II: Evidence from sediment incubations. *Geochimica Et Cosmochimica Acta* **2004** *68*(17), 3475-3486.

van Geen, A.; Zheng, Y.; Versteeg, R.; Stute, M.; Horneman, A.; Dhar, R.; Steckler, M.; Gelman, A.; Small, C.; Ahsan, H.; Graziano, J.H.; Hussain, I.; Ahmed, K.M. Spatial variability of arsenic in 6000 tube wells in a 25 km(2) area of Bangladesh. *Water Resources Research* **2003** *39*(5), DOI 10.1029/2002WR001617.

van Geen, A.; Zheng, Y.; Goodbred, S.; Horneman, A.; Aziz, Z.; Cheng, Z.; Stute, M.; Mailloux, B.; Weinman, B.; Hoque, M.A.; Seddique, A.A.; Hossain, M.S.; Chowdhury, S.H.; Ahmed, K.M. Flushing history as a hydrogeological control on the regional distribution of arsenic in shallow groundwater of the Bengal Basin. *Environmental Science & Technology* **2008** *42*(7), 2283-2288.

Wade, T.J.; Pai, N.; Eisenberg, J.N.S.; Colford, J.M. Do US Environmental Protection Agency water quality guidelines for recreational waters prevent gastrointestinal illness? A systematic review and meta-analysis. *Environmental Health Perspectives* **2003**, *111*(8), 1102-1109.

Wan, J.M.; Tokunaga, T.K. Partitioning of clay colloids at air-water interfaces. *Journal of Colloid and Interface Science* **2002** *247*(1), 54-61.

Wan, J.M.; Wilson, J.L. Colloid Transport in Unsaturated Porous-Media. *Water Resources Research* **1994** *30*(4), 857-864.

Weinman, B., S. L. Goodbred Jr., Y. Zheng, Z. Aziz, M. Steckler, A. van Geen, A. K. Singhvi and Y. C. Nagar. Contributions of floodplain stratigraphy and evolution to the spatial patterns of groundwater arsenic in Araihazar, Bangladesh. *Bulletin of the Geological Society of America* **2008**, *120* (11-12), 1567-1580.

Woessner, W.W.; Ball, P.N.; DeBorde, D.C.; Troy, T.L. Viral transport in a sand and gravel aquifer under field pumping conditions. *Ground Water* **2001** *39*(6), 886-894.

Yao, K.M.; Habibian, M.M.; Omelia, C.R. Water and Waste Water Filtration - Concepts and Applications. *Environmental Science & Technology* **1971** *5*(11), 1105-1112.

Zheng, Y.; van Geen, A.; Stute, M.; Dhar, R.; Mo, Z.; Cheng, Z.; Horneman, A.; Gavrieli, I.; Simpson, H.J.; Versteeg, R.; Steckler, M.; Grazioli-Venier, A.; Goodbred, S.; Shahnewaz, M.; Shamsudduha, M.; Hoque, M.A.; Ahmed, K.M. Geochemical and hydrogeological contrasts between shallow and deeper aquifers in two villages of Araihazar, Bangladesh: Implications for deeper aquifers as drinking water sources. *Geochimica Et Cosmochimica Acta* **2005** *69*(22), 5203-5218.

Zhuang, J.; Jin, Y. Interactions between viruses and goethite during saturated flow: Effects of solution pH, carbonate, and phosphate. *Journal of Contaminant Hydrology* **2008** *98*(1-2), 15-21.

Zhuang, J.; Qi, J.; Jin, Y. Retention and transport of amphiphilic colloids under unsaturated flow conditions: Effect of particle size and surface property. *Environmental Science & Technology* **2005** *39*(20), 7853-7859.

Chapter II - Efficacy of Hollow-Fiber Ultrafiltration for Microbial Sampling in Groundwater

This chapter is adapted from a paper currently in press for a special issue of the journal *Ground Water* on pathogens and fecal indicators.

Knappett, P. S. K.; Layton, A.; McKay, L. D.; Williams, D.; Mailloux, B. J.; Huq, Md. R.; Alam, Md. J.; Ahmed, K. M.; Akita, Y.; Serre, M. L.; Sayler, G. S.; van Geen, A. Efficacy of hollow-fiber ultrafiltration for microbial sampling in groundwater, *Ground Water*, In Press, 2010.

Abstract

The goal of this study was to test hollow-fiber ultrafiltration as a method for concentrating *in situ* bacteria and viruses in groundwater samples. Water samples from nine wells tapping a shallow sandy aquifer in a densely populated village in Bangladesh were reduced in volume approximately 400-fold using ultrafiltration. Culture-based assays for Total Coliforms and *E. coli*, as well as molecular-based assays for *E. coli*, *Bacteroides* and Adenovirus, were used as microbial markers before and after ultrafiltration to evaluate performance. Ultrafiltration increased the concentration of the microbial markers in 99% of cases. However, concentration factors (CF = post-filtration concentration/pre-filtration concentration) for each marker calculated from geometric means ranged from 52 to 1018 compared to the expected value of 400. The efficiency was difficult to quantify because concentrations of some of the markers, especially *E. coli* and Total Coliforms, in the well water collected before ultrafiltration varied by several orders of magnitude during the period of sampling. The potential influence of colloidal iron oxide precipitates in the groundwater was tested by adding EDTA to the pre-filtration water in half of the samples to prevent formation of precipitates. The use of EDTA had no

significant effect on the measurement of culturable or molecular markers across the 0.5-10 mg/L range of dissolved Fe^{2+} concentrations observed in the groundwater, indicating that colloidal iron did not hinder or enhance recovery or detection of the microbial markers. Ultrafiltration appears to be effective for concentrating microorganisms in environmental water samples, but additional research is needed to quantify losses during filtration.

II.1 INTRODUCTION

In the developing world, diarrheal disease remains one of the leading causes of death for children under age five, with estimates ranging from 1 to 5 million deaths per year (Parashar et al., 2003). In the United States, Craun (1988) reported that 49% of the 502 reported cases of waterborne disease outbreaks between the years 1971 and 1985 were attributable to contaminated groundwater. In spite of the importance of water in the transmission of diarrheal disease, most groundwater monitoring programs do not measure pathogens directly. This is partly due to the low concentration and intermittent occurrence of pathogens in aquifers. Instead, fecal indicator bacteria such as cultured *E. coli* (Yates, 2007) are used as surrogates for pathogen contamination, with a value of <1 colony forming units per 100 ml typically considered as the acceptable limit for drinking water (Havelaar et al., 2001). Cultured *E. coli*, however, often correlate weakly with viral and protozoan pathogens (Wilkes et al., 2009), yielding a high percentage of false positives and some false negatives. The weak correlation is due to the intermittent nature of pathogen sources and differences in survival, re-growth and transport in the environment between fecal indicators and various types of pathogens (Schijven et al., 2000; Woessner et al., 2001; Payment, 2009).

Many infectious protozoa, bacteria and viruses may cause disease at levels of only 1-10 viable particles per L, which typically requires that water samples undergo a filtration or concentration procedure to improve the detection limit for the pathogen assays (Rendtorff, 1954; Willshaw et al., 1994; Gale, 2001). In recent years there has been increasing interest in molecular detection methods which can be used for both pathogens and fecal indicators, but

these tests use extremely small samples (a few microliters), which further highlights the need for efficient and reliable methods to concentrate the pathogens prior to measurement.

Over the past several decades a variety of filtration methods have been developed to concentrate viruses and protozoa from large volumes of water (Noble and Fuhrman, 2001; Morales-Morales et al., 2003; Lambertini et al., 2008; Hill et al., 2009). These include the U.S. Environmental Protection Agency's method 1623 for concentrating *Cryptosporidium* and *Giardia* using glass wool (Noble and Fuhrman, 2001) and the Mark D. Sobsey (MDS) charged filters (APHA, 1995) for concentrating viruses. Generally, these filtration methods are time consuming, cumbersome, and yield low recovery efficiencies. An alternative method recently described by Hill et al. (2005) for the filtration of large volumes of water is hollow-fiber ultrafiltration. This is a form of tangential flow filtration where water is cycled through thousands of fibers with sidewalls that are permeable to water, but not to particles greater than approximately 20 nm in diameter. Larger colloids such as viruses and bacteria remain suspended in the retentate water during ultrafiltration (i.e. the water not removed by leakage through the fiber walls). This method can be used to concentrate initial volumes of hundreds of liters of water to a few hundred milliliters in several hours. Most importantly, the microorganisms remain in suspension, rather than attached to the filter material, which eliminates the need for steps to resuspend them prior to measurement with methods such as tissue culture or polymerase chain reaction (PCR).

In laboratory experiments with concentrations of spiked microorganisms ranging from 10^4 to 10^6/ml, Hill et al. (2005; 2007) observed ultrafiltration recovery efficiencies typically

ranging between 50 and 100% using a variety of bacterial and viral markers. Recovery efficiency was calculated by dividing the number of microorganisms enumerated in the retentate water by the known concentration of microorganisms in the initial spiked water sample. Spiked recovery experiments with protozoa, bacteria and viruses on 8 water sources from different regions in the US, with a minimum of two replicates per source, suggested that recovery efficiency is sensitive to a variety of water chemistry parameters including pH, turbidity, conductance, alkalinity, total Fe, total organic carbon, dissolved organic carbon and heterotrophic plate count (Hill et al., 2007). In spite of differences in recovery efficiency in water taken from different regions, no statistically significant correlation between recovery efficiency of the markers with the levels of any single water chemistry parameter was observed (Hill et al., 2007). Since most of the water sources used in previous recovery efficiency studies were tap water, it's uncertain how effective ultrafiltration will prove to be across the broader range of physical and chemical conditions found in wells used for water supply. Furthermore, recovery of pre-existing microorganisms in samples of well water may differ from recovery of spiked microbial markers added to the water sample after collection.

The primary objective of this study was to evaluate the effectiveness of ultrafiltration as a method for concentrating bacteria and viruses from large (typically 100 L) groundwater samples in the field by measuring *in situ* concentrations of fecal indicators before and after a 400-fold reduction in volume. The study was carried out at a field site in Bangladesh because the high levels of fecal contamination common at the site increased the likelihood of the presence of a wide range of fecal microorganisms. The ability of ultrafiltration to increase the

concentration of microorganisms was tested using a suite of *in situ* microbial indicators that included Total Coliforms, *E. coli*, *Bacteroides* and Adenovirus. Measurements of microbial indicator concentrations were carried out with culture-based and DNA molecular-based methods (in this case qPCR). The *E. coli* concentrations were measured with both culture-based and molecular methods to determine whether ultrafiltration effectiveness differs with the type of assay.

A secondary objective was to quantify the effect of high concentrations of dissolved reduced [Fe^{2+}], prevalent in aquifers in Bangladesh, on measurements of bacterial and viral markers in the retentate water. This was done to address the concern that colloidal FeOOH particles formed by the oxidation of iron due to exposure to atmospheric oxygen during sampling might interfere with the filtration and recovery of bacteria and viruses. These particles could clog the filter or form mineral-microbial aggregates, which would reduce the number of colony-forming units in culture-based assays. In addition, the presence of FeOOH particles in the retentate water could interfere with the DNA extraction and PCR amplification in the laboratory. To investigate this potential factor, EDTA was added to one of the paired groundwater samples from each of nine wells spanning a range of natural [Fe^{2+}] before ultrafiltration to prevent formation of FeOOH with the expectation that EDTA would have the greatest effect in wells with high [Fe^{2+}].

The study also provided an opportunity to test the utility of *Bacteroides* as a fecal indicator in groundwater and to compare it to other more commonly used fecal indicators. To the author's knowledge, this study, along with a study by Johnson et al. (this issue), are the first

tests of *Bacteroides* as a quantitative fecal indicator in groundwater. *Bacteroides* sp. have the potential to be useful indicators of fecal contamination in water (Bell et al., 2009; Layton et al., 2006; Lee et al., 2008; Yamara-Iquise et al., 2008) because they are present in the intestines of all warm blooded animals and are one of the dominant (10% by mass) bacterial species in human feces (Matsuki et al., 2002; Bernhard and Field, 2000). In addition, *Bacteroides* are obligate anaerobes and therefore, unlike *E. coli*, unlikely to grow in subsurface environments. However, *Bacteroides* are difficult to enumerate in the laboratory using culture-based tests. This is why *Bacteroides* had not been quantified prior to the development of quantitative PCR assays (Bernhard and Field, 2000; Layton et al., 2006).

II.2 METHODS

Site Description

The field site selected for this study is a sandy floodplain aquifer underlying the village of Char Para, 23.79 N 90.63 E, in Araihazar, Bangladesh, herein referred to as Site K (Radloff et al., 2007; Weinman et al., 2008;). The village is located on sand bar deposits which act as an unconfined aquifer and is tapped by dozens of shallow (10 to 20 m deep) tubewells. The shallow aquifer at this location is low in arsenic, relative to many other wells in the region, possibly because rapid vertical recharge has flushed out the mobilizable arsenic over time (van Geen et al., 2008; Aziz et al., 2008). The village is densely populated, with approximately 1500 people living in an area of 30 hectares. Hundreds of latrines and approximately fifty ponds, many of which receive discharge from latrines, are scattered throughout the village and serve as point sources of fecal pollution to the aquifer. This site is therefore well suited for a study of

microbial sampling methods because of rapid local recharge and abundant sources of fecal contamination.

Well Installation

Two types of wells, 7.6 to 16.8 m deep, were sampled at Site K: i) private tubewells (five) and ii) wells installed for groundwater monitoring (four). For all wells drilling was done by the traditional hand-flapper method, which is essentially a manual mud circulation method that readily penetrates the loose, wet unconsolidated floodplain deposits throughout the Bengal Basin (e.g. Horneman et al., 2004). The monitoring wells were installed to reduce the likelihood of sample contamination due to poor well seals. The annulus of private wells in Bangladesh is typically filled with material removed from the borehole during drilling, whereas the purposely-installed monitoring wells were sealed with cement grout from the top of the sand pack, which itself extends 0.7 m above the 1.5 m screened interval, to the surface. Both types of wells are constructed of 5.1 cm diameter PVC pipes, but private wells are equipped with hand pumps, whereas the monitoring wells were sampled with an electric-powered submersible pump (Typhoon, Groundwater Essentials, LLC).

Well Sampling and Ultrafiltration

All wells were purged for at least three standing wellbore volumes before sampling. One wellbore volume ranged from 11 to 30 L, depending on the well depth and the water level. In monitoring well KW-24, high turbidity was initially observed and ten wellbore volumes were purged until electrical conductivity, temperature and dissolved oxygen concentrations

measured with a multiprobe (556 Multiprobe System, YSI Inc.) stabilized and the water was clear. Steady state values for the nine wells ranged from 25 to 27˚C for temperature, 0.22 – 0.96 µs/cm for electrical conductivity, 6.37 – 7.17 for pH, and 0.2 to 1.1 ppm for dissolved oxygen. Groundwater is typically anoxic in Bangladesh and dissolved oxygen sensors are difficult to calibrate at these very low levels. In the particular setting, however, we cannot rule out that rapid vertical recharge occasionally supplies detectable levels of oxygen to the shallowest aquifer. Monitoring wells were pumped continuously at 7-10 L/min with an electric submersible pump and the excess water pumped, when not filling the 20 L sample reservoirs, flowed into a ditch. In contrast, private wells were pumped intermittently with the existing hand pump at an approximate flow rate of 20-30 L/min while filling the 20 L sample reservoir. Consequently, monitoring wells were sampled at a constant flow rate, as opposed to intermittent flow, and likely with higher daily pumped volumes than private wells, since the submersible pumps ran continuously. The private wells were also utilized for domestic purposes between filling the retentate reservoirs but this additional volume pumped wasn't measured.

The apparatus for performing ultrafiltration (Fig. II-1) was based on a system described by Hill et al. (2005; 2007). Briefly, groundwater was pumped in a closed loop through a hollow fiber single-use ultrafiltration cartridge (Rexeed 25S, Dial Medical Company, Renal Buy) under positive pressure (5-10 kpa) using a portable peristaltic pump (Solinst Model 410, Pine Environmental Services, Inc., Windsor, NJ) and Poly Teflon Lined Tubing (TB30120, Pine

Environmental Services, Inc., Windsor, NJ). The sidewalls of the capillary tubes in the ultrafiltration cartridge have 20 nm pore sizes.

As a sample cycles through the ultrafiltration cartridge, increasing amounts of water, dissolved constituents and colloids <20 nm are lost through the sidewalls as filtrate water. Colloids >20 nm, which include most bacteria and viruses, remain in the retentate water which becomes more concentrated during cycling. To concentrate a 100 L groundwater sample, the retentate reservoir was filled five times with 20 L of well water, then the volume was reduced by ultrafiltration to less than 1 L between each refilling. At the end of the ultrafiltration process, when the retentate reservoir was almost empty, sterile bottled water was used to back flush the tubing and cartridge. The fully saturated volume of the tubing and inner cartridge was calculated to be 187 ml. The final retentate sample represented the first 250 ml of retentate water to exit the back flushed tubing and cartridge, representing approximately 1.3 displaced pore volumes. This method assumes that the microorganisms were in free suspension and not attached to the sidewalls of the capillary fibers in ultrafiltration cartridge. Since the original 100 L groundwater sample was reduced in volume 400 times, the concentration of the markers in the retentate was expected to be 400 times higher than in the unfiltered well water sample. Three 10 ml subsamples of this final retentate were diluted with 90 ml of bottled water to measure cultured *E. coli* and Total Coliform using the Colilert assay (IDEXX Laboratories, Inc.). The remaining retentate (approximately 220 ml) was frozen and transported to the University of Tennessee for molecular DNA analysis. Between sampling of each well, all parts of the ultrafiltration apparatus were soaked in dilute bleach and TWEEN-80 (T164-500, Fischer

Scientific) cleaning solution. The ultrafiltration cartridge was discarded after each use. Powdered Chlorox (5 g) and TWEEN-80 (5 ml) were mixed in 10 L of well water from the next well that was to be sampled. The bleach/TWEEN solution was cycled through the tubing for 5 minutes, followed by rinsing with 10 L of well water containing 5 g of sodium thiosulfate (S446-3, Fisher Scientific) for 2 minutes. A final rinse with 10 L of well water pumped through the tubing was performed over a period of 2 minutes. Sterile techniques were employed throughout. The total time for ultrafiltration of 100 L of groundwater including set up, disinfection and packing up was approximately 3 hours, allowing for sampling of two wells per field day.

Each of the markers were measured directly from samples of unfiltered well water immediately before each ultrafiltration run to obtain background concentrations in the well water. Since two ultrafiltration runs were performed on a well on each field day, unfiltered well water samples were collected twice, once early in the day and once late in the day. For the culture-based assays, triplicate 100 ml Colilert samples for *E. coli* and Total Coliform were collected from each well at the start of each ultrafiltration run to determine marker concentrations in the well during pumping. The exception to this was KW-12.1 which was only sampled at the start of the second ultrafiltration run, in the middle of the 6 hour field day. For the molecular assays a single 250 ml sample was removed from the first of five mixed 20 L reservoirs of well water at the start of each ultrafiltration run. The final retentate was stored in a sterile 250 ml polypropylene bottle. Each ultrafiltration run included one set of triplicate 100 ml samples of unfiltered well water for culture-based analysis, one 250 ml unfiltered well water

sample for molecular analysis and one 250 ml filtered retentate water sample for both culture-based and molecular analysis.

EDTA Addition and Iron Detection

Concentrations of dissolved iron in well water in the form of Fe^{2+} across Site K were measured using a field Iron Test Kit (Model IR-18B, Hach Company) and varied widely from <0.1 to 10 ppm (Table II-1). Initial lab experiments and field observations demonstrated that FeOOH minerals precipitate out of solution within 20 minutes when the reduced, high [Fe^{2+}], 5-10 ppm, groundwater is exposed to atmospheric oxygen. To test for the influence of this on ultrafiltration, 2.5 g of EDTA disodium salt (02793-500, Fisher Scientific) was added to each 20 L reservoir of unfiltered well water immediately after the bucket was filled to prevent the precipitation of FeOOH particulates. EDTA contains 6 metal binding sites for each molecule and therefore, theoretically, all dissolved Fe^{2+} should be bound by a concentration of [EDTA]=0.17x[Fe^{2+}] (Essington, 2004). However, other divalent metal cations in groundwater such as Mn^{2+} and Ca^{2+} may compete for binding sites with Fe^{2+} (Essington, 2004). The concentration of EDTA in each 20 L bucket was 3.36×10^{-4} M, which is twice the maximum concentration of dissolved Fe^{2+} (10 ppm) measured in the samples. Of the two ultrafiltration runs carried out for each well on a given field day, one involved EDTA addition to each 20 L reservoir and the other was run without. The color and clarity difference between the retentate samples of high [Fe^{2+}] water with EDTA added and without was striking, indicating that EDTA effectively prevented precipitation.

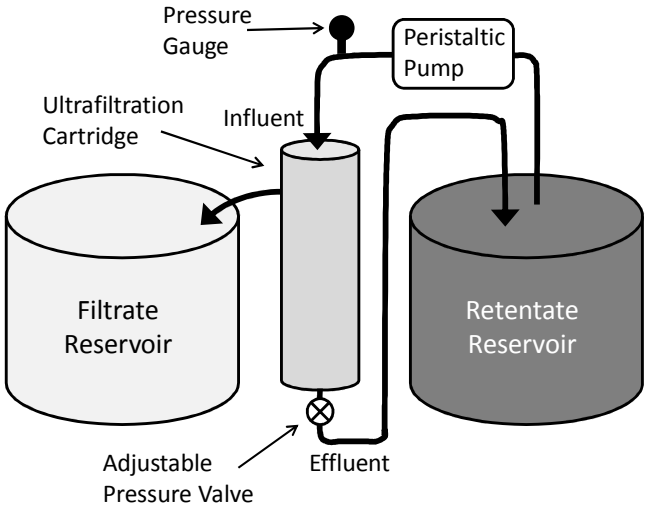


Figure II-1. Ultrafiltration Apparatus. The retentate reservoir represents the 20 L bucket that was filled with groundwater 5 times during each 100 L ultrafiltration run. The pressure valve and gauge were used to control the back pressure which influenced the rate of filtrate water exiting the sidewalls of the capillary tubes in the ultrafiltration cartridge.

The nine wells that were sampled in the field span a limited depth range (7.6 to 16.8 m) but a wide range of Fe^{2+} concentrations (Table II-1). The sequence of sampling at a given well with or without EDTA addition to 100 L of well water was random. The only exception was UTK-7, which was sampled a total of three times (twice with EDTA added) on two different days.

Bacterial and Viral Detection Methods

Culture-based and molecular methods based on analysis of microbial DNA were used to detect fecal indicator bacteria and viruses in all groundwater samples. Samples for *E. coli* and Total Coliform analysis were stored on ice in the field immediately after collection and processed within 8 hours of sampling. Cultured *E. coli* and Total Coliforms were detected using the Colilert™ test kit with the Quanti-tray 2000 (IDEXX Laboratories, Inc.). This is a most probable number method (MPN) that splits a 100 ml water sample into 97 testing wells (49 large, 48 small) and the number of wells positive for each bacterial indicator corresponds to MPN/100 ml according to the solution provided by Hurley and Roscoe (1983). Based on the MPN solution for a given 100 ml water sample, the range of possible concentrations ranges from 1 to >2419 MPN/100 ml. Duplicate or triplicate samples taken directly from the well (WW) or diluted (1:10) from the retentate water were analyzed separately during this study. Because of dilution the detection limit was 10 MPN/100 ml for RW samples, instead of 1 MPN/100 ml for undiluted WW samples. The MPN solution was used to solve the MPN (Hurley and Roscoe, 1983) and associated 95% confidence intervals by combining the numbers of discrete positive wells from all trays of replicate samples. The underlying assumption is that the groundwater from which the 100 ml duplicate or triplicate samples were taken was well mixed, and that the true concentration of bacteria in each 100 ml sample was the same.

Table II-1. Groundwater wells sampled and experimental design.

Well ID	Well Type	Depth (m)	$[Fe^{2+}]$ (ppm)	Times Sampled	
				EDTA Added	No EDTA
KW-12.1	Monitoring	7.6	0.5	1	1
UTK-1	Private	9.1	0.6	1	1
KW-24	Monitoring	11.9	3.5	1	1
UTK-8	Private	16.8	3.8	1	1
UTK-7	Private	7.6	6.2	2	1
UTK-31	Private	12.2	7.6	1	1
KW-30	Monitoring	13.7	8.8	1	1
UTK-30	Private	13.7	9.0	1	1
KW-25	Monitoring	15.5	10.0	1	1
			Total	**10**	**9**

Quantitative PCR (qPCR) was used to measure copies of genes for *E. coli*, *Bacteroides* and Adenovirus in the water samples. To distinguish the cultured *E. coli* values from the molecular *E. coli* values, data collected from qPCR for *E. coli* is denoted as *mE. coli* in this study. For the molecular assays, samples of both unfiltered well water (WW) and retentate water (RW), which is collected after ultrafiltration, were collected in sterile 250 ml polypropylene containers, frozen on dry ice, and brought back to the University of Tennessee for DNA extraction and qPCR analysis. After removal from the -80°C freezer, samples were thawed in cool water for 3-5 hours. Two-hundred and fifty ml of WW samples and 50 ml of the RW samples were vacuum filtered onto autoclaved 0.45 µm cellulose nitrate filters (47 mm, Whatman Filter) for DNA extraction. DNA extraction and purification was performed on ½ or ¼ of each filter using a DNA soil extraction kit following the manufacture's protocols (FastDNA®SPIN for Soil Kit, MP Biomedical). Initial concentrations of gene copies of each

marker microorganism per ng of DNA extracted were obtained by qPCR following previous published methods (Layton et al., 2006), with primers and probes shown on Table II-2.

The basic PCR protocol used for DNA amplification consisted of 50°C for 2 min, followed by 95°C for 10 min and 40 cycles of 95°C for 30 s and 55°C (*E. coli* assay) or 60°C (AllBac and Adenovirus assays) for 45 s. For each sample and assay, the samples were run in triplicate wells and in a fourth well containing the sample and a plasmid DNA spike to determine PCR inhibition. A standard curve containing a positive plasmid DNA target for each assay ranging from 2.5×10^7 copies to 25 copies was run on each plate along with triplicate blanks. Due to the potential for cross-reactivity of the primers with non-target DNA, when the concentration of the target DNA was <1 copy/ng total extracted DNA, the sample was treated as a Non Detect. Since each sample contained a different amount (ng) of total extracted or background DNA the detection limit varied from sample to sample, resulting in more sensitive detection limits for samples with small amounts of background DNA. The pooled average Coefficient of Variation based on triplicate qPCR reaction wells was 30% for all assays.

Table II-2. Primers and probes used for each Real-time PCR assays to detect *E. coli* and *Bacteroides* rRNA genes and the Adenovirus hexon gene.

Assay name (target organism)	Primer/probe name and sequence (5'–3')	Size (bp) of product
EC23S (*E. coli*)[1,2]	EC23Sf 5' GAG CCT GAA TCA GTG TGT GTG 3'	
	EC23Sr 5' ATT TTT GTG TAC GGG GCT GT 3'	78
	EC23Srv1bhq 5' -(FAM)CGC CTT TCC AGA CGC TTC CAC (BHQ-1)- 3'	
AllBac (all *Bacteroides*)[3]	AllBac296f, 5'-GAGAGGAAGGTCCCCCAC-3'	
	AllBac412r, 5'-CGCTACTTGGCTGGTTCAG-3'	106
	AllBac375Bhqr, 5'-(FAM)CCATTGACCAATATTCCTCACTGCTGCCT(BHQ-1)-3'	
Adeno (40/41 hexon gene)[4]	AV40/41-117f 5'- CAGCCTGGGGAACAAGTTCAG 3'	
	AV40/41-258r 5' -CAGCGTAAAGCGCACTTTGTAA 3'	141
	AV40/41-157BHQ 5' -(Fam)ACCCACGATGTAACCACAGACAGGTC (BHQ-1)-3'	

[1] Modified from Smith et al., 1999

[2] Layton et al., 2003

[3] Latyon et al., 2006

[4] Rajal et al., 2007

II.3 Results

Marker Concentrations in Well Water and Retentate Water

The approach followed in this study was to measure the *in situ* concentrations of all markers in unfiltered water collected from the wells after purging approximately 3 well bore volumes or parameter stabilization and then compare these values to measurements from 100 L samples that had been concentrated to a final volume of 250 ml using ultrafiltration (i.e. a 400-fold concentration step). The initial 100 ml samples were referred to as well water (WW)

samples. The post-ultrafiltration samples are referred to as retentate water (RW) samples. From this final retentate, a subset was initially analyzed for cultured Total Coliforms and *E. coli* with the Colilert assay and the rest of each sample was frozen and transported to the University of Tennessee for molecular *E. coli*, *Bacteroides* and Adenovirus assays.

The number of well and retentate water samples that were positive for each marker, as well as the geometric mean and the range of concentrations are listed in Table II-3. The retentate samples contain nine where EDTA was absent and ten where EDTA was added. The number of positive samples (i.e., those containing detectable levels of fecal indicators and molecular markers) ranged from 11 to 18 out of the 19 WW samples (Table II-3) and a large range of marker concentrations was observed in the samples.

All molecular markers, *mE. coli*, *Bacteroides* and Adenovirus, were more abundant than cultured markers in both unconcentrated well water and retentate water samples. In well water, the geometric mean concentration of *mE. coli*, *Bacteroides* and Adenovirus were 5100, 2800 and 5000 copies/100 ml, respectively, and the cultured markers, *E. coli* and Total Coliforms had geometric means of 5 and 37 MPN/100 ml, respectively (Table II-3). In all but 1 out of 83 cases, marker concentrations in the retentate were higher than in the unfiltered well water samples. The addition of EDTA prior to ultrafiltration did not have an obvious impact on the geometric means or ranges of marker concentrations in retentate water samples. In the retentate samples, the highest marker concentrations were observed for *Bacteroides* which had a geometric mean of 3.4×10^6 and 9.1×10^5 copies/100 ml for samples without and with EDTA respectively. The lowest retentate concentrations were observed for the cultured *E. coli* with

geometric means of 76 and 180 MPN/100 ml without and with EDTA respectively. PCR inhibition was detected in only one sample (retentate water for KW-24, +EDTA), as measured by the lack of PCR amplification of the positive control standard in the DNA sample. This PCR inhibition prevented the detection of any of the molecular marker.

The increase in marker concentrations between WW and RW samples is substantial for all markers. This was especially notable in the cases (10 out of 83, or 12%) where a marker was not detected in the WW sample (pre-filtration) but was detected in the RW sample (after ultrafiltration). The 1:1 line in Figures II-2 and II-3, for cultured and molecular markers respectively, indicates the threshold for demonstrating an increase in marker concentration resulting from ultrafiltration. In all but one of the 83 cases for which a marker was detected in the RW sample, the RW vs. WW concentration data point lay above this line (Fig. II-3c). The 1:400 line on each graph represents the expected concentration factor, assuming that a 400x reduction in sample volume results in a 400x increase in marker concentration. For all markers, the RW vs. WW concentration data points straddled the 1:400 line, but with a high degree of scatter. For Total Coliforms (Fig. II-2a) and *Bacteroides* (Fig. II-3b) markers, about equal numbers of data points lay above and below the 1:400 line. In contrast, for the other markers, more data points lay below the 1:400 line then above. Ultrafiltration resulted in substantial increases in concentration of markers in the retentate relative to the well water samples, but the large amount of scatter in the data indicates that the amount of increase is not consistent between wells or between different samples in the same well.

Table II-3. Summary of Marker Concentrations in 19 Well Water and Retentate Water Samples (9 without and 10 with EDTA added).

Marker	Units	Well Water[1,2]		Retentate Water[3] -EDTA		Retentate Water[3] +EDTA	
		# Samples Positive (%)	Geometric Mean (Range)	# Samples Positive (%)	Geometric Mean (Range)	# Samples Positive (%)	Geometric Mean (Range)
E. coli	MPN/100 ml	13 (68)	5.0E+00 (1.0E+00 - 7.5E+01)	6 (67)	7.6E+01 (1.0E+00 - 6.5E+03)	9 (90)	1.8E+02 (1.0E+00 - 7.9E+03)
Total Coliforms	MPN/100 ml	17 (89)	3.7E+01 (1.0E+00 - 1.1E+03)	9 (100)	1.5E+04 (2.2E+02 - 6.6E+05)	9 (90)	2.0E+04 (6.6E+03 - 3.8E+05)
mE. coli	Copies/100 ml	18 (95)	5.1E+03 (6.0E+02 - 1.4E+05)	9 (100)	8.8E+05 (3.7E+04 - 1.5E+07)	9 (90)	5.5E+05 (7.2E+04 - 1.3E+07)
Bacteroides	Copies/100 ml	11 (58)	2.8E+03 (2.9E+02 - 5.9E+04)	9 (100)	3.4E+06 (1.0E+06 - 6.8E+06)	8 (80)	9.1E+05 (2.4E+04 - 1.8E+07)
Adenovirus	Copies/100 ml	15 (79)	5.0E+03 (1.4E+02 - 7.4E+05)	9 (100)	1.6E+05 (1.1E+04 - 2.5E+07)	8 (80)	4.5E+05 (1.4E+04 - 1.1E+07)

[1] A total of 19 well water and 19 retentate water samples were tested

[2] Well water samples for cultured bacteria were sampled directly from the well while samples for molecular markers were taken from the first of five 20 L well-mixed reservoirs

[3] 10 ml subsamples of the 250 ml Rentate sample were diluted for cultured enumeration and 50 ml was extracted for molecular assays

Concentration factor (*CF*) values for each ultrafiltration run were calculated using:

Equation 1

$$CF = \frac{C_{RW}}{C_{WW}}$$

where C_{RW} is the concentration of the marker in the retentate water and C_{WW} is the concentration of the same target in the unfiltered groundwater sample. A line is included in Figures II-2 and II-3 to show the geometric mean concentration factor for each marker based on all the individual ultrafiltration runs. In cases where the marker was detected only in the RW sample, the concentration in the WW sample was set equal to the detection limit for the purpose of calculating the concentration factor. The geometric mean concentration factors, with associated 95% confidence intervals calculated on the log-transformed data were: 105 (26 - 419) for *E. coli*; 794 (252 - 2503) for Total Coliforms; 182 (74 - 446) for *mE. coli*; 1023 (491 - 2130) for *Bacteroides*; and 51 (15 - 179) for Adenovirus.

Variation in Marker Concentration During Sampling

While planning the study, it was assumed that concentrations of bacterial and viral markers collected from the wells would remain relatively constant during the period of sampling and subsequent ultrafiltration for a given retentate water sample. Contrary to expectations, concentrations varied over the course of the day (while pumping for sampling and/or domestic use continued) by as much as three orders of magnitude. Concentrations of the cultured bacteria, *E. coli* and Total Coliforms, decreased in every sample collected later in

the day in those cases where bacteria were initially detected in early in the day (Fig. II-4). Early samples were taken from the well at the beginning of the day, whereas late samples were taken after one complete round of ultrafiltration had been completed from the well, before the second round of ultrafiltration had begun. In the case of well KW-30, four WW samples (rather than the usual two) were collected over a 24 hour period during which 2000 L of water was

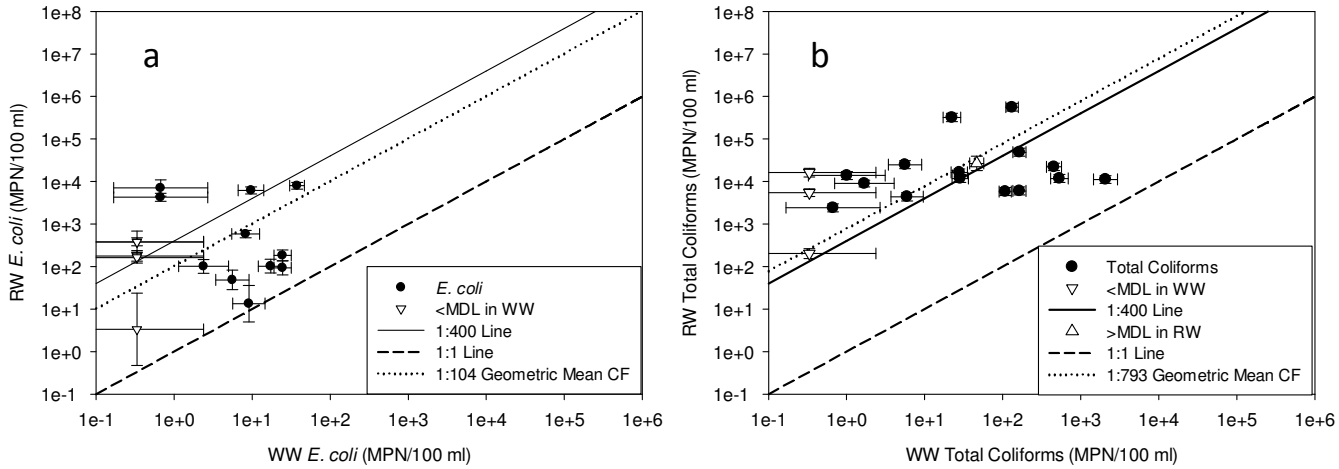


Figure II-2. Comparisons of cultured marker concentrations from 250 ml unfiltered Well Water (WW) samples with 100 L ultrafiltered Retentate Water (RW) samples. Panels a and b represent *E. coli* and Total Coliforms respectively. The 1:1 line is where points would lie if there were no increase in marker concentration during ultrafiltration. The 1:400 line is where points would lie if the 250 ml WW sample was representative of the average concentration within the 100 L WW sample, and if no losses occurred during ultrafiltration. The dotted line represents the geometric mean concentration factor, the ratio of the marker concentration in the RW sample over the WW sample. Inverted triangles indicate non-detects in the WW sample only and are plotted at the detection limit on the x-axis of the graph for each marker. Error bars represent 95% confidence intervals.

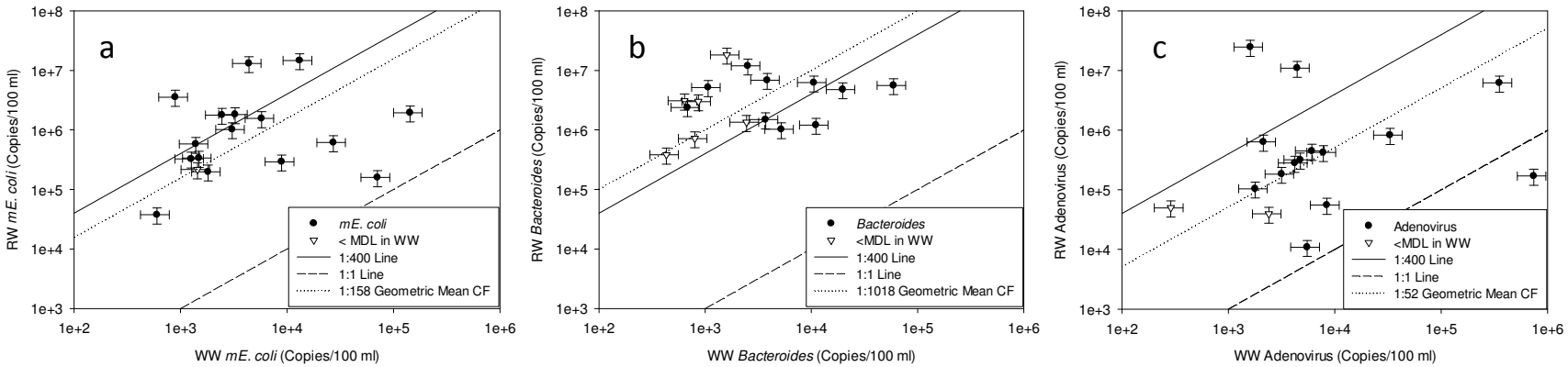


Figure II-3. Comparisons of molecular marker concentrations from 250 ml unfiltered Well Water (WW) samples with 100 L ultrafiltered Retentate Water (RW) samples. Panels a, b and c represent *mE. coli*, *Bacteroides* and Adenovirus. The 1:1 line is where points would lie if there were no increase in marker concentration during ultrafiltration. The 1:400 line is where points would lie if the 250 ml WW sample was representative of the average concentration within the 100 L WW sample, and if no losses occurred during ultrafiltration. The dotted line represents the geometric mean concentration factor, the ratio of the marker concentration in the RW sample over the WW sample. Inverted triangles indicate non-detects in the WW sample only and are plotted at the detection limit on the x-axis of the graph for each marker. Error bars represent 95% confidence intervals.

removed from the well. A consistent log-linear decline in concentration of cultured *E. coli* and Total Coliforms with pumped volume was observed, resulting in decreases of two and three log of *E. coli* and Total Coliforms respectively (data not shown). Between 7 and 12 mm of daily rainfall occurred on six of the ten consecutive days of sampling at Site K during this month in the monsoon season. No systematic relationship was observed between daily precipitation amounts and concentrations of bacteria or viruses in well water during the ten days of sampling.

Molecular marker concentrations in the unfiltered 100 ml well water (WW) samples also showed considerable variability (by up to two orders of magnitude) between paired samples collected at the beginning and the end of the same day, but with approximately equal numbers of cases where concentrations increased or decreased during the day (Fig. II-5). Together, these findings indicate that the concentrations of both cultured and molecular markers were not constant in the unfiltered well water for even relatively short time periods (a few hours to a day) or relatively modest volumes pumped (a few hundred to a few thousand liters).

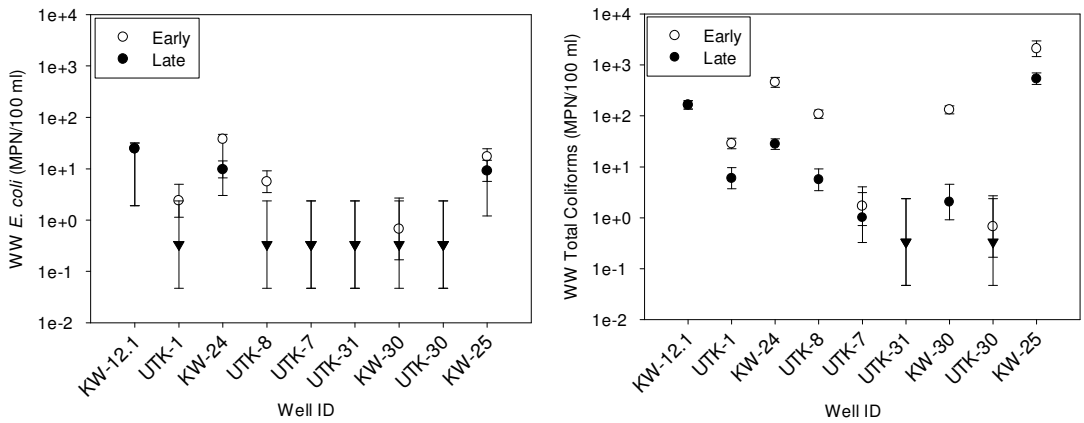


Figure II-4. Paired 100 ml pre-filtration Well Water samples (in triplicate) taken from wells early or late in the day for culturing. Panels a and b represent *E. coli* and Total Coliforms respectively. All wells were purged for at least 3 bore volumes, ranging from 33 to 90 L, before sampling. KW-12.1 only had a single sample taken during the day. Total Coliforms were not detected in UTK-31 at early or late time. *E. coli* was not detected in UTK-7, UTK-31 and UTK-30 at early or late time. Non-detects are indicated by the MDL with inverted triangles. The error bars describe 95% confidence intervals for combined replicates.

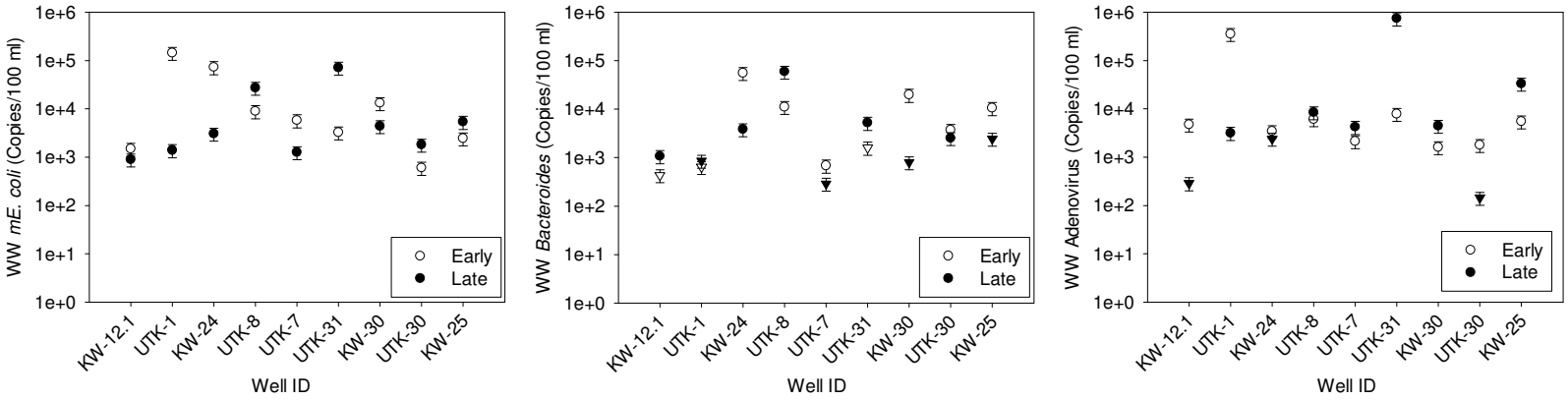


Figure II-5. Paired 250 ml Well Water samples taken from wells early or late in the day for molecular analysis. Panels a, b and c represent *mE. coli*, *Bacteroides* and Adenovirus. Non-detects are indicated by the MDL with inverted triangles. The error bars describe 95% confidence intervals for combined replicates.

Correlations of Markers in Retentate Water

The correlation between the different markers in RW samples was calculated using the Spearmann rank order correlation coefficient (Table II-4). The strongest correlations were observed between *E. coli*, *mE. coli* and Total Coliforms ($p<0.01$). The *E. coli* Colilert assay is a subset of the Total Coliform assay so it would be expected to be correlated. However the strong correlation between the *mE. coli* assay and Total Coliforms, which are based on independent assays suggest the fecal indicator bacteria are the principal source of Coliform bacteria. The other fecal indicator bacteria, *Bacteroides*, did not correlate strongly with either *E. coli* or *mE. coli* in the retentate water, indicating either different die-off (in the environment or during sampling) or transport rates for this bacterium. Correlations were not calculated for the unfiltered well water samples due to the large number of non-detects resulting in comparatively small data sets.

The relative proportion of cultured *E. coli* to *E. coli* genomes, assessed by the molecular assay and the Colilert method, is shown for each sample in Figure II-6. The ratio represents the geometric mean of the number of cultivable *E. coli* to the total number of 23S genes detected. For the RW samples this ratio was 1:6315 (2679 - 14887). Assuming 6 copies of the ribosomal gene in *E. coli* (Klappenbach et al., 2001) the data indicate that the cultivable proportion represents 0.1% of the *E. coli* genomes. The overall proportion of cultivable *E. coli* did not change greatly for unfiltered WW samples and filtered RW samples, indicating that ultrafiltration does not inactivate a large proportion of cultivable *E. coli* cells.

Table II-4. Correlation matrix of marker concentrations in retentate water (RW). Numbers represent the non-parametric Spearmann rank order correlation coefficient (r_s). Numbers in bold indicate statistically significant correlations in paired ranks ($p<0.01$). Paired data set sample sizes vary between 18 and 19, with non-detects included at their respective detection limits.

	E. coli	Total Coliforms	*mE. coli*	*Bacteroides*	Adenovirus
E. coli	1.00				
Total Coliforms	**0.67**	1.00			
mE. coli	**0.68**	**0.80**	1.00		
Bacteroides	0.02	0.30	0.36	1.00	
Adenovirus	0.28	0.39	0.29	-0.37	1.00

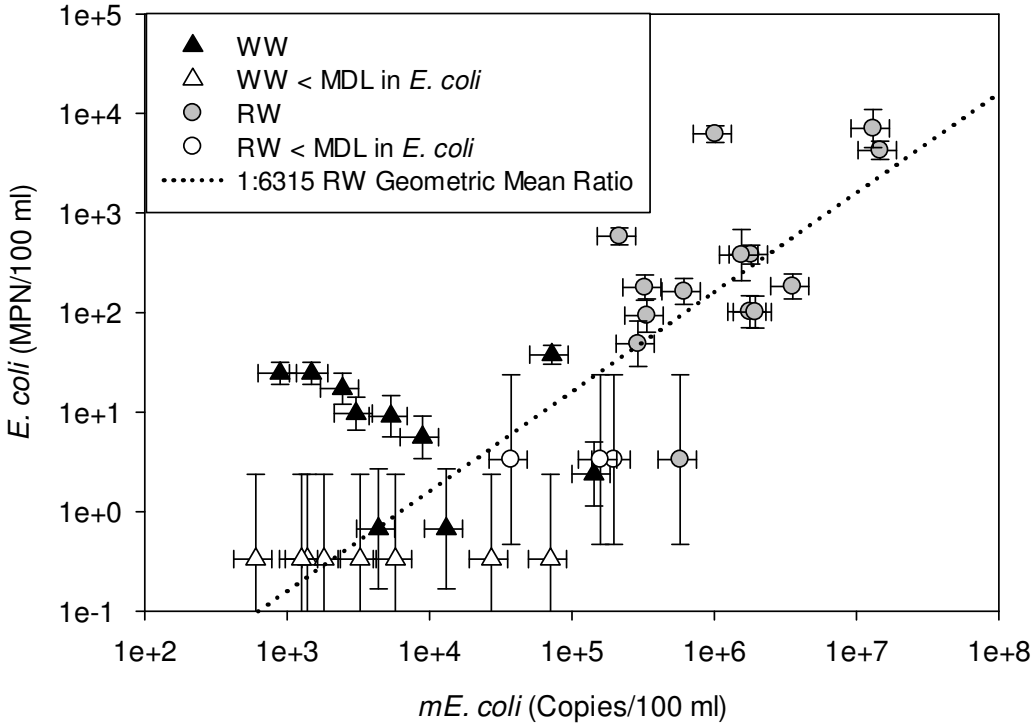


Figure II-6. Comparison of cultured and molecular *E. coli* assays in both WW and RW samples. The geometric mean of the cultivable to molecular *E. coli* ratios in all RW samples is represented by the 1:6315 line, representing approximately 0.1% cultivable *E. coli*. Error bars represent 95% confidence intervals.

EDTA and Fe Effect

The addition of EDTA to well water prior to ultrafiltration did not have any systematic effect on concentrations of the five markers in the retentate (Fig. II-7). There is no evidence that EDTA improved the recovery of any of the five markers, even in a subset of high [Fe^{2+}] waters, as none of markers with EDTA are consistently higher or lower than those without EDTA. One-sided t-tests were performed on the differences between log-transformed concentrations (with and without EDTA added) of each marker pooled from all wells using the statistical software NCSS (version 07.1.14, NCSS, LLC, Kaysville, Utah). The null hypothesis that there was no difference in marker concentration in RW samples with and without EDTA (H_o: $\mu=0$) was not rejected ($p=0.05$) for any of the five markers. The Total Coliform marker data set failed normality tests (skewness and kurtosis) in due to a single outlier (UTK-31, 7.6 ppm Fe^{2+}) where 2 $\log_{10}$ greater RW concentration was observed for the sample with EDTA added (Fig. II-7). Although there was no systematic effect of EDTA or [Fe^{2+}] on molecular marker concentrations in retentate water samples, there was a high degree of variability between subsequent 100 L ultrafiltered samples, which frequently differed by more than an order of magnitude (Fig. II-7). This is consistent with the high degree of variability observed for all the markers in pre-filtration WW samples (Fig. II-4 and II-5). The differences between measured concentrations of markers in RW samples taken from the same well on the same day were apparently random, and could not be explained by any linear combination of parameters measured from the well, such as pumped volume, electrical conductivity, temperature or pH, as assessed by multiple regression ($p=0.05$) using the software NCSS.

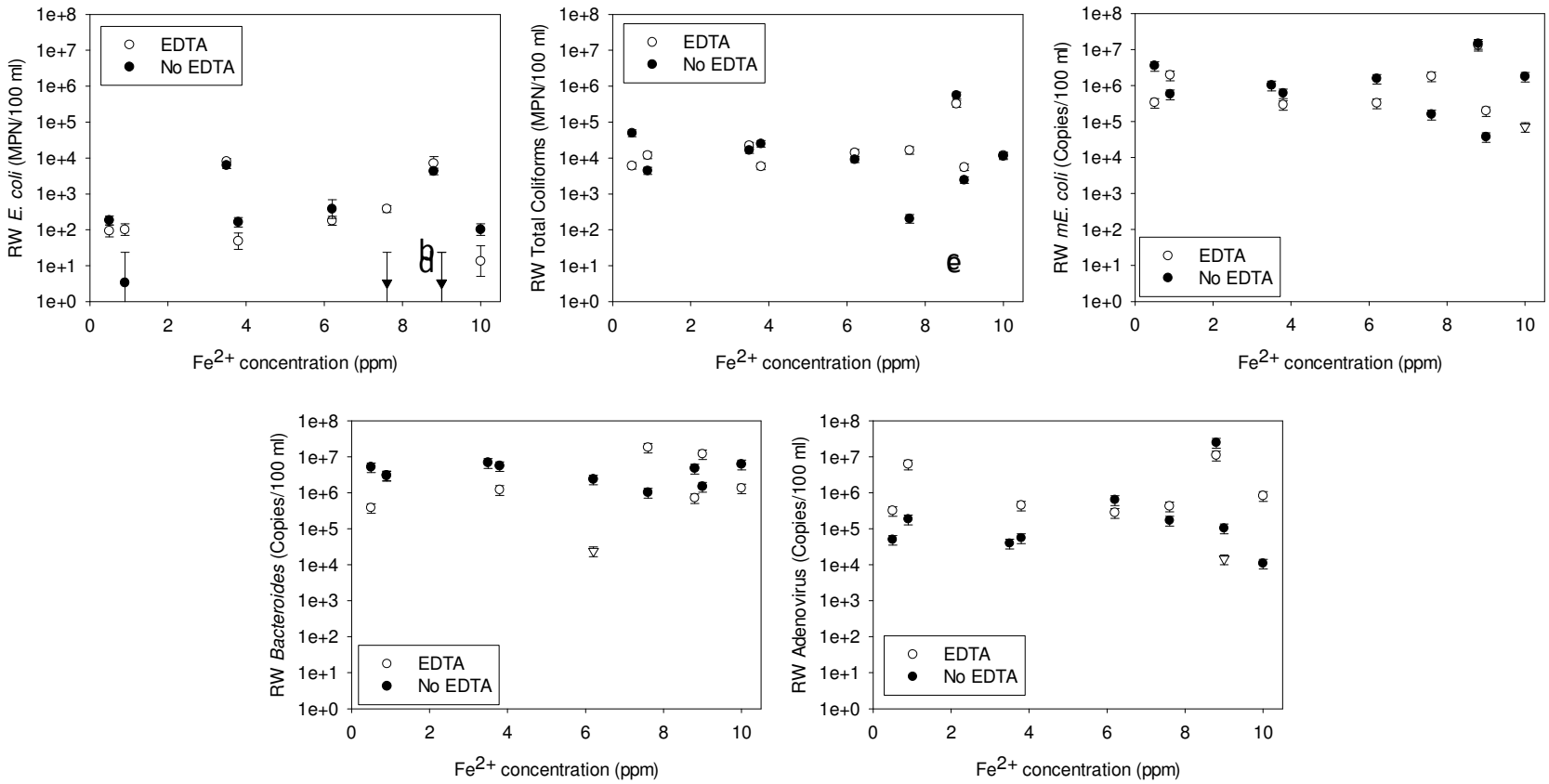


Figure II-7. Comparison of Retentate Water samples from ultrafiltration runs with EDTA and those without EDTA added. Panels a, b, c, d, and e represent *E. coli*, Total Coliforms, *mE. coli*, *Bacteroides* and Adenovirus. No significant difference was found between the two categories across the range of Fe^{2+} concentrations present in the water. Error bars represent 95% confidence intervals.

II.4 Discussion

Ultrafiltration resulted in substantial increases (geometric mean concentration factors of 52 to 1018, relative to an expected value of 400) in concentration of *in situ* bacterial and viral markers from groundwater in 99% of cases where the marker was quantifiable in the retentate water sample (Figures II-2 and II-3). For each marker, measured concentrations in the retentate water (RW) sample tended to be higher for wells which started out with higher concentrations in the pre-filtration well water (WW). There was, however, a substantial range (several orders of magnitude) of concentration factors calculated for each marker for the different ultrafiltration runs. Concentration factors for Total Coliforms and *Bacteroides* tended to be higher than the predicted value of 400 (based on the 400-fold volume reduction and the measured concentration of each marker in the pre-filtration well water). The other three markers (*E. coli*, *mE. coli* and Adenovirus) tended to have concentration factors that were lower than the expected value of 400.

The large variability in calculated concentration factors was at least partly caused by the variability in marker concentrations in the unfiltered well water (WW) samples, as shown in Figures II-4 and II-5. This variability in WW samples could be due to a heterogeneous distribution of microbial markers in the aquifer, but it is perhaps more likely related to conditions in the well. Kwon et al. (2008) found that 36 wellbore volumes were required to reach quasi-steady state in total bacteria cell concentrations and a stable microbial community, however substantial changes in these continued up to 230 wellbore volumes. A possible explanation for the unstable bacteria and virus concentrations in the present study is that

pumping could mobilize microorganisms attached to biofilms in the well, or it could draw in contaminated water through cracks in the well casing. Losses related to the ultrafiltration process (for example: attachment to the filter or die-off during filtration) would also influence concentration factors, but such effects cannot be distinguished from that of marker variability in the pre-filtration water on the basis of the available data.

Several previous ultrafiltration studies (Hill et al., 2005; 2007) have involved carefully-controlled experiments where the sample is spiked with a known concentration of a marker, prior to ultrafiltration to focus on losses due to ultrafiltration. However, it is often not practical to spike samples in the field (especially in Bangladesh) and there would still be uncertainty as to whether ultrafiltration losses of the spiked marker would be similar to losses of *in situ* markers from the sampled aquifer. To separate well water variability from potential ultrafiltration artifacts, a 100 L sample could have been homogenized before ultrafiltration.

The markers Total Coliforms, *E. coli* and *mE. coli* all correlated strongly with one another in the retentate samples (Table II-4). This is expected since *E. coli* is a subset of Total Coliforms. In contrast, *Bacteroides* did not correlate with *E. coli*. Adenovirus, which has been proposed as a possible viral fecal indicator, correlated only weakly with the other fecal indicator bacteria. This could be due to different processes controlling transport through porous media for viruses than bacteria (Schijven et al., 2000; Woessner et al., 2001). *E. coli* represents the cultivable subset of all *E. coli* genomes present in the water sample. Since the *mE. coli* primer targets the 23S rRNA gene on the *E. coli* genome and this sequence is repeated approximately 6 times on each genome (Klappenbach et al., 2001), the results of the qPCR assay will give an approximate

6x larger value than the number *E. coli* genomes present in the water sample. Figure II-6 shows that cultivable *E. coli* typically consisted of 0.1% of the total copies of *E. coli* genomes in retentate water samples, somewhat less than the 1% in previous reports of percent cultivable *E. coli* in low nutrient waters (Garcia-Armisen and Servais, 2004). In the present study substantial changes in the percent cultivable *E. coli* were not observed between WW (n=13) and RW (n=15) samples suggesting that ultrafiltration was not inactivating the bacteria in large numbers.

Although there was considerable variability in concentrations of some markers in paired retentate samples taken from the same well, the addition of EDTA did not explain this variability even in high [Fe^{2+}] wells. It was expected that the negatively charged bacteria and viruses would become attached to the positively charged FeOOH particles, resulting in clumping of bacteria and viruses and perhaps denaturation of the viral protein coat as occurs with viral attachment to metal oxide coated porous media (Abudalo et al., 2005). The lack of a negative correlation between [Fe^{2+}] and cultured bacterial concentration in the retentate in the absence of EDTA suggests that FeOOH particles had no effect on the measured concentration of *E. coli* and Total Coliforms in the retentate samples. This agrees with other studies which found that larger microorganisms such as bacteria and protozoa do not attach as readily as viruses to FeOOH minerals (Abudalo et al. 2005; Dong et al., 2002). The lack of an observable [Fe^{2+}] effect with the molecular markers indicates that FeOOH colloids did not interfere with recovery of markers during the ultrafiltration process, via clumping and denaturing of viral protein coats, nor did it interfere with DNA extraction and qPCR analysis.

II.5 CONCLUSIONS

Groundwater from nine wells was concentrated for fecal microorganisms from a contaminated shallow aquifer in Bangladesh. By measuring concentrations of five *in situ* markers before and after ultrafiltration, it was verified that ultrafiltration resulted in a substantial increase of all the markers in most cases. Measurements on samples collected immediately prior to or during ultrafiltration indicated that both cultured and molecular bacterial and viral concentrations vary greatly with time or pumped volume from both private tubewells and monitoring wells. This suggests that more research is needed to develop better sampling methods for obtaining representative samples of microorganisms from groundwater. The fact that high [Fe^{2+}] in groundwater did not depress the retentate concentrations indicates that FeOOH colloids neither interfered with the persistence of the molecular markers during filtration nor qPCR detection in the laboratory.

REFERENCES

Abudalo, R.A.; Bogatsu, Y.G.; Ryan, J.N.; Harvey, R.W.; Metge, D.W.; Elimelech, M. Effect of ferric oxyhydroxide grain coatings on the transport of bacteriophage PRD1 and Cryptosporidium parvum oocysts in saturated porous media. *Environmental Science & Technology* **2005** *39*(17), 6412-6419.

Aziz, Z.; van Geen, A.; Stute, M.; Versteeg, R.; Horneman, A.; Zheng, Y.; Goodbred, S.; Steckler, M.; Weinman, B.; Gavrieli, I.; Hoque, M.A.; Shamsudduha, M.; Ahmed, K.M. Impact of local recharge on arsenic concentrations in shallow aquifers inferred from the electromagnetic conductivity of soils in Araihazar, Bangladesh. *Water Resources Research* **2008** *44*(7), -.

Bell, A.; Layton, A.C.; Mckay, L.; Williams, D.; Gentry, R.; Sayler, G.S. Factors Influencing the Persistence of Fecal *Bacteroides* in Stream Water. *Journal of Environmental Quality* **2009** *38*(3), 1224-1232.

Bernhard, A.E.; Field, K.G. Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. *Applied and Environmental Microbiology* **2000** *66*(4), 1587-1594.

Craun, G.F. Surface-Water Supplies and Health. *Journal American Water Works Association* **1988** *80*(2), 40-52.

Dong, H.L.; Onstott, T.C.; Deflaun, M.F.; Fuller, M.E.; Scheibe, T.D.; Streger, S.H.; Rothmel, R.K.; Mailloux, B.J. Relative dominance of physical versus chemical effects on the transport of adhesion-deficient bacteria in intact cores from South Oyster, Virginia. *Environmental Science & Technology* **2002** *36*(5), 891-900.

Essington, M. *Soil and Water Chemistry: An Integrated Approach*. CRC Press LLC, 2004.

Gale, P. Developments in microbiological risk assessment for drinking water. *Journal of Applied Microbiology* **2001** *91*(2), 191-205.

Garcia-Armisen, T.; Servais, P. Enumeration of viable E-coli in rivers and wastewaters by fluorescent in situ hybridization. *Journal of Microbiological Methods* **2004** *58*(2), 269-279.

Havelaar, A., U. J. Blumenthal, M. Strauss, D. Kay, J. Bartram. Guidelines: the current position. In *Water Quality: Guidelines, Standards and Health*, ed. L. Fewtrell and J. Bartram, 17-42. IWA Publishing, London, UK, 2001.

Hill, V.R.; Kahler, A.M.; Jothikumar, N.; Johnson, T.B.; Hahn, D.; Cromeans, T.L. Multistate evaluation of an ultrafiltration-based procedure for simultaneous recovery of enteric microbes in 100-liter tap water samples. *Applied and Environmental Microbiology* **2007** *73*(13), 4218-4225.

Hill, V.R.; Polaczyk, A.L.; Hahn, D.; Narayanan, J.; Cromeans, T.L.; Roberts, J.M.; Amburgey, J.E. Development of a rapid method for simultaneous recovery of diverse microbes in drinking water by ultrafiltration with sodium polyphosphate and surfactants. *Applied and Environmental Microbiology* **2005** *71*(11), 6878-6884.

Hill, V.R.; Polaczyk, A.L.; Kahler, A.M.; Cromeans, T.L.; Hahn, D.; Amburgey, J.E. Comparison of Hollow-Fiber Ultrafiltration to the USEPA VIRADEL Technique and USEPA Method 1623. *Journal of Environmental Quality* **2009** *38*(2), 822-825.

Horneman, A.; Van Geen, A.; Kent, D.V.; Mathe, P.E.; Zheng, Y.; Dhar, R.K.; O'Connell, S.; Hoque, M.A.; Aziz, Z.; Shamsudduha, M.; Seddique, A.A.; Ahmed, K.M. Decoupling of As and Fe release to Bangladesh groundwater under reducing conditions. Part 1: Evidence from sediment profiles. *Geochimica Et Cosmochimica Acta* **2004** *68*(17), 3459-3473.

Hurley, M.A.; Roscoe, M.E. Automated Statistical-Analysis of Microbial Enumeration by Dilution Series. *Journal of Applied Bacteriology* **1983** *55*(1), 159-164.

Johnson, T. B., L. D. McKay, A. C. Layton, S. W. Jones, G. C. Johnson, J. L. Cashdollar, D. R. Dahling, L. F. Villegas, G. S. Fout, D. E. Williams and G. Sayler. Viruses and bacteria in karst and fractured rock aquifers in East Tennessee. *Ground Water*, In Review.

Klappenbach, J.A.; Saxman, P.R.; Cole, J.R.; Schmidt, T.M. rrndb: the Ribosomal RNA Operon Copy Number Database. *Nucleic Acids Research* **2001** *29*(1), 181-184.

Kwon, M.J.; Sanford, R.A.; Park, J.; Kirk, M.F.; Bethke, C.M. Microbiological response to well pumping. *Ground Water* **2008** *46*(2), 286-294.

Lambertini, E.; Spencer, S.K.; Bertz, P.D.; Loge, F.J.; Kieke, B.A.; Borchardt, M.A. Concentration of enteroviruses, adenoviruses, and noroviruses from drinking water by use of glass wool filters. *Applied and Environmental Microbiology* **2008** *74*(10), 2990-2996.

Layton, A., D. Williams, G. S. Sayler, L. D. McKay. *Proceedings of the 13th Annual Tennessee Water Resources Symposium*, Tennessee Section of the American Water Resources Association, 2003.

Layton, A.; McKay, L.; Williams, D.; Garrett, V.; Gentry, R.; Sayler, G. Development of *Bacteroides* 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water. *Applied and Environmental Microbiology* **2006** *72*(6), 4214-4224.

Lee, Y.J.; Molina, M.; Domingo, J.W.S.; Willis, J.D.; Cyterski, M.; Endale, D.M.; Shanks, O.C. Temporal Assessment of the Impact of Exposure to Cow Feces in Two Watersheds by Multiple Host-Specific PCR Assays. *Applied and Environmental Microbiology* **2008** *74*(22), 6839-6847.

Matsuki, T.; Watanabe, K.; Fujimoto, J.; Miyamoto, Y.; Takada, T.; Matsumoto, K.; Oyaizu, H.; Tanaka, R. Development of 16S rRNA-gene-targeted group-specific primers for the detection and identification of predominant bacteria in human feces. *Applied and Environmental Microbiology* **2002** *68*(11), 5445-5451.

Morales-Morales, H.A.; Vidal, G.; Olszewski, J.; Rock, C.M.; Dasgupta, D.; Oshima, K.H.; Smith, G.B. Optimization of a reusable hollow-fiber ultrafilter for simultaneous concentration of enteric bacteria, protozoa, and viruses from water. *Applied and Environmental Microbiology* **2003** *69*(7), 4098-4102.

Noble, R.T.; Fuhrman, J.A. Enteroviruses detected by reverse transcriptase polymerase chain reaction from the coastal waters of Santa Monica Bay, California: low correlation to bacterial indicator levels. *Hydrobiologia* **2001** *460*, 175-184.

Parashar, U.D.; Hummelman, E.G.; Bresee, J.S.; Miller, M.A.; Glass, R.I. Global illness and deaths caused by rotavirus disease in children. *Emerging Infectious Diseases* **2003** *9*(5), 565-572.

Payment, P. Utopia or Reality: The Quest for Correlations between Indicators and Pathogens. *Microbial Transport and Survival in Porous Media: First International Conference*, St. Catherines, Ontario, Canada, 2009.

Radloff, K.A.; Cheng, Z.Q.; Rahman, M.W.; Ahmed, K.M.; Mailloux, B.J.; Juhl, A.R.; Schlosser, P.; van Geen, A. Mobilization of arsenic during one-year incubations of grey aquifer sands from Araihazar, Bangladesh. *Environmental Science & Technology* **2007** *41*(10), 3639-3645.

Rajal, V.B.; McSwain, B.S.; Thompson, D.E.; Leutenegger, C.M.; Wuertz, S. Molecular quantitative analysis of human viruses in California stormwater. *Water Research* **2007** *41*(19), 4287-4298.

Rendtorff, R.C. The Experimental Transmission of Human Intestinal Protozoan Parasites .2. Giardia-Lamblia Cysts Given in Capsules. *American Journal of Hygiene* **1954** *59*(2), 209-220.

Schijven, J.F.; Medema, G.; Vogelaar, A.J.; Hassanizadeh, S.M. Removal of microorganisms by deep well injection. *Journal of Contaminant Hydrology* **2000** *44*(3-4), 301-327.

Smith, G.J.; Helf, M.; Nesbet, C.; Betita, H.A.; Meek, J.; Ferre, F. Fast and accurate method for quantitating E-coli host-cell DNA contamination in plasmid DNA preparations. *Biotechniques* **1999** *26*(3), 518-+.

Standard methods for the examination of water and wastewater 19th ed.; American Public Health Association, Washington, DC, 1995.

van Geen, A.; Zheng, Y.; Goodbred, S.; Horneman, A.; Aziz, Z.; Cheng, Z.; Stute, M.; Mailloux, B.; Weinman, B.; Hoque, M.A.; Seddique, A.A.; Hossain, M.S.; Chowdhury, S.H.; Ahmed, K.M. Flushing history as a hydrogeological control on the regional distribution of arsenic in shallow groundwater of the Bengal Basin. *Environmental Science & Technology* **2008** *42*(7), 2283-2288.

Wilkes, G.; Edge, T.; Gannon, V.; Jokinen, C.; Lyautey, E.; Medeiros, D.; Neumann, N.; Ruecker, N.; Topp, E.; Lapen, D.R. Seasonal relationships among indicator bacteria, pathogenic bacteria, Cryptosporidium oocysts, Giardia cysts, and hydrological indices for surface waters within an agricultural landscape. *Water Research* **2009** *43*(8), 2209-2223.

Willshaw, G.A.; Thirlwell, J.; Jones, A.P.; Parry, S.; Salmon, R.L.; Hickey, M. Vero Cytotoxin-Producing Escherichia-Coli O157 in Beefburgers Linked to an Outbreak of Diarrhea, Hemorrhagic Colitis and Hemolytic-Uremic Syndrome in Britain. *Letters in Applied Microbiology* **1994** *19*(5), 304-307.

Woessner, W.W.; Ball, P.N.; DeBorde, D.C.; Troy, T.L. Viral transport in a sand and gravel aquifer under field pumping conditions. *Ground Water* **2001** *39*(6), 886-894.

Yampara-Iquise, H.; Zheng, G.; Jones, J.E.; Carson, C.A. Use of a *Bacteroides* thetaiotaomicron-specific alpha-1-6, mannanase quantitative PCR to detect human faecal pollution in water. *Journal of Applied Microbiology* **2008** *105*(5), 1686-1693.

Yates, M.V. Classical indicators in the 21st century - Far and beyond the coliform. *Water Environment Research* **2007** *79*(3), 279-286.

CHAPTER III - IMPACT OF SANITATION ON FECAL BACTERIA AND PATHOGENS IN PONDS OF BANGLADESH

This chapter is adapted from a paper submitted for review on April 15, 2010 to the journal *Environmental Science & Technology*.

Knappett, P. S. K.; Escamilla, V.; Layton, A.; McKay, L. D.; Emch, M.; Williams, D. E.; Huq, Md. R.; Alam, Md. J.; Farhana, L.; Mailloux, B. J.; Ferguson, A.; Sayler, G. S.; Ahmed, K. M.; van Geen, A. Impact of Sanitation on Fecal Bacteria and Pathogens in Ponds of Bangladesh.

Abstract

The majority of households in Bangladesh obtain their drinking water from tubewells but continue to use surface water for non-drinking purposes including bathing, washing, and oral rinsing. Fecal contamination of pond water could therefore contribute to the spread of diarrheal disease. To assess the impact sanitation, population density, and livestock have on contamination, 43 ponds were analyzed for *E. coli* using culture-based methods and *E. coli*, *Bacteroides* and Adenovirus using quantitative PCR. The highest concentrations of fecal indicator bacteria were found in ponds receiving human waste directly or from a latrine, with the most contaminated pond containing 9.7×10^5 Most Probable Number (MPN) of culturable *E. coli* per 100 mL. All fecal bacteria concentrations in pond water correlated with population surveyed within a distance of 30-70 m ($p<0.01$) and the number of unsanitary latrines (those with visible seepage or open pits) within a pond drainage basin ($p<0.05$). Fecal source-tracking based on *Bacteroides* demonstrated that humans, and not cattle, are the dominant source of fecal pollution in all but 5 of the 43 tested ponds. Unsanitary latrines are a primary cause of

poor pond water quality and may be a factor contributing to still widespread diarrheal disease in rural South Asia.

III.1 Introduction

Despite decades of effort, diarrheal disease continues to kill on the order of 1.5 million children under five every year (UNICEF and WHO, 2009). Research has shown that pathways of diarrheal disease transmission in the developing world are complex and are greatly influenced by sanitation, hygiene and the availability of clean water (Esrey, 1996; Pruss et al., 2002). In rural Bangladesh, for instance, ponds are scattered throughout every village and are used for a variety of purposes including bathing, aquaculture, brushing teeth or, less frequently today, even drinking (Aziz et al., 1990). Many ponds are also surrounded by latrines, however. The multiple uses of ponds and their close proximity to sources of human and livestock feces suggests that they could play a role in transmitting diarrheal disease. By applying both molecular and more traditional culture-based techniques for measuring fecal indicator organisms, this study sheds new light on the influence of sanitation, population density, and livestock on the microbial quality of pond water in a densely populated village of Bangladesh.

Substantial decreases in diarrheal disease morbidity of 25 to 37% (Fewtrell et al., 2005) and improvements in childhood nutritional status (assessed by height to weight ratios) from 4 to 37% (Esrey, 1996) in the developing world have accompanied a gradual switch from open pit latrines to more sanitary disposal methods, such as the use of concrete foundation rings to prevent leakage of human feces onto the open ground. Reductions in diarrheal disease morbidity due to improved sanitation have been reported in epidemiologic studies carried out in rural Bangladesh (Aziz et al., 1990; Hoque et al., 1996; Emch, 1999; Emch et al., 2008), for instance, where approximately 46% of the population today has access to sanitary latrines

(WHO and UNICEF, 2008). However, the transmission of diarrheal disease involves many pathways and sanitation is not the only factor (Esrey, 1996; Pruss et al., 2002). The close proximity of latrines and dwellings suggests that people and domesticated animals might track fecal waste into houses where young children could ingest this waste. However, in Bangladesh and elsewhere, the specific pathways of human exposure to effluent from open pit latrines remain unclear.

In recent decades, the number of ponds excavated in Bangladesh seems to have outpaced population growth (see Supp. Material in Neumann et al., 2010). While many of these ponds are excavated to protect a nearby dwelling from flooding by raising it, they subsequently often fulfill a primary purpose, such as aquaculture, bathing, irrigation or holding latrine effluent, or a combination thereof over the course of the year. Contact with pond water is known to be a major contributor to diarrheal disease in Bangladesh as studies demonstrate that people greatly increase their risk of diarrheal disease when they drink (Emch et al., 2008), bathe in (Emch et al., 2008; Ali et al., 2002) or even live near a pond (Emch et al., 2008). Emch et al. (2008) found that people living in the region of Matlab who bathed in ponds or rivers were approximately 2.5 times more likely to be hospitalized for diarrhea than those who washed with tubewell water. Significant associations between cases of diarrheal disease and the number of unsanitary latrines around a human dwelling have also been identified (Emch et al., 2008; WHO and UNICEF, 2008; Neumann et al., 2010). Several studies have demonstrated a dose-response relationship between fecal bacteria concentrations and diarrheal disease rates in bathers (Pruss, 1998; Wade et al., 2003; Given et al., 2006), with the odds of acquiring

diarrheal disease increasing approximately two fold for every 1 $\log_{10}$ increase in fecal indicator bacteria in ponds (Wade et al., 2003).

The potential sources of fecal contamination around ponds in Bangladesh are numerous, including humans, cattle, goats, dogs, chickens and waterfowl, with humans and cattle representing the largest fecal contributors by volume in densely populated villages. Although cattle are abundant, cattle manure is used as fuel for cooking and therefore collected and traded in villages of Bangladesh. Latrines are ubiquitous in the villages and are often deliberately located close to ponds that effectively become sewage lagoons. The quality of these latrines varies widely in Bangladesh. Some latrines, defined in this study as sanitary, are built out of a concrete rings and with concrete platforms on top. Other latrines are clearly unsanitary, as indicated by cracked rings and effluent spilling or overflowing onto the ground. Unsanitary latrines and simple open pits can also discharge directly into ponds.

This study quantifies the impact of pond use, latrine type, and population density on concentrations of fecal bacteria and viral pathogens in village ponds. Both *E. coli* and *Bacteroides* are used here as fecal indicators. Concentrations of *E. coli* were measured using culture-based methods and quantitative PCR. *Bacteroides* is a known fecal indicator bacteria, and unlike *E. coli* is a dominant species in the intestines of all warm blooded animals, excreted at a rate of 10% by mass in feces (Matsuki et al., 2002). The source of *Bacteroides* can be tracked to humans or livestock by quantitative PCR (Bernhard and Field, 2000; Layton et al., 2006; Lee et al., 2008; Yampara-Iquise et al., 2008). Given that people in Bangladesh live with their livestock, this molecular marker can determine the relative contribution of humans and

livestock to fecal contamination of ponds. Molecular assays were also used to detect the human pathogen Adenovirus (Jiang, 2006). These results have implications for understanding how the built environment influences the transmission of diarrheal disease in densely populated, developing countries.

III.2 METHODS

Site Description

The village of Char Para is located in Araihazar upazila, about 25 km east of Dhaka. On-going public-health and earth-science studies focused on the groundwater arsenic problem were launched in Araihazar in 2000. Char Para, also referred to as Site K (Radloff et al., 2007), is underlain by fine to medium grained deltaic sands, which form a shallow aquifer that is tapped by tubewells (screened from 10 to 20 m) which are the primary drinking water source in the village. The shallow aquifer below Char Para is bounded hydrologically on three sides by a former channel of the Old Brahmaputra River which floods up to the edge of the village during the wet season (van Geen et al., 2003; Weinman et al., 2008). Many ponds in Char Para are empty at the end of the dry season in April when the groundwater table falls below the bottom of the pond. The ponds that do not dry out are often the deepest ponds, or are artificially maintained for fish farming by pumping from the deeper aquifer. Latrine ponds, which receive latrine effluent and runoff of wash water from wells, may also have some standing water year round. At the beginning of the monsoon in late May, the ponds can fill and drain rapidly. Fluctuations in pond water level of up to 1 m were observed within 24 hours in June 2008.

Field Methods and Pond Classification

High accuracy (sub-meter) GPS coordinates were collected for all ponds, latrines and households throughout the village during June 2009 using a Trimble GeoXH receiver and Terrasync 2.4 software (Table A-III-1). Post-processing of the GPS and population data was carried out using Pathfinder Office 3.0. A latrine was classified as sanitary with respect to pond contamination if it was constructed with a concrete platform, a concrete ring without cracks, and no visible sign of effluent discharging onto the ground. A latrine was classified as unsanitary if the ring was cracked or the effluent discharged directly into a pond via a PVC pipe. A survey was conducted to determine the number of people living in each household and the pond owner's name. The number of people and latrines within a given radius of each pond was determined using the buffer and intersect tools in ArcGIS software. The number of people and latrines (sanitary and unsanitary) within a given distance of a pond was calculated between 10 and 50 m at 5-m intervals and between 50 and 100 m at 10-m intervals.

As an alternative method for enumerating potential sources of fecal contamination, latrines within a pond drainage basin that sloped downwards towards the water edge were identified within a distance of ~20 m in June 2008. The rationale is that these latrines could have a greater influence on microbial pond water quality than latrines at a similar distance outside the drainage basin. In cases where a ditch sloped towards a pond, the ditch was included as part of the pond basin. Information collected for each pond drainage basin includes water depth, long and short axes of the pond water surface (using a measuring tape), designated purpose as identified by the owner, number and type of latrines (unsanitary or

sanitary) and the number of cattle residing within the drainage basin (Table A-III-1). In cases where the pond was observed to receive direct latrine effluent, the pond was always classified as a latrine pond. Unless local households identified a specific use such as bathing or aquaculture, ponds that did not receive direct latrine input were categorized as having no specific use. Electrical conductivity, pH, dissolved oxygen and temperature were measured at each pond at the time of microbial sampling in June 2008 using a handheld multiprobe (556 Multiprobe System, YSI Inc.).

Microbiological Assays

Water from 43 ponds was collected in sterile bottles in mid-June 2008, during the early monsoon when surface runoff was common but the ponds were not yet full. Triplicate or duplicate 100 mL pond water samples were collected in sterile containers to measure culturable *E. coli* using the MPN based Colilert™ test kit (IDEXX Laboratories, Inc.). Pond water samples were diluted 1:100 with commercial bottled drinking water before being assayed to avoid exceeding the quantifiable maximum of 2419 bacteria/100 mL of the assay. Taking into account dilution, the method's detection limit was 100 MPN/100 mL. Blanks using bottled water were included every 30 samples.

For the molecular measurements, 200 mL of pond water, or as much as could be filtered before clogging, was filtered through a 0.22 µm nitrocellulose filter (150 mL Vacuum Driven Disposable Filtration System, Stericup, HV Durapore Membrane, Millipore Corp., Bedford, Massachusetts). The filters were removed from the plastic housing, placed in sterile petri plates, frozen and transported on dry ice back to the University of Tennessee. DNA was

extracted and purified from the filters using the FastDNA® SPIN for Soil Kit (MP Biomedicals, LLC, Solon, Ohio) following the manufacturer's protocols.

Quantitative PCR (qPCR) was performed using assays designed for *E. coli* and total *Bacteroides*, three host-associated *Bacteroides* assays (two for human and one for bovine), and the human pathogen Adenovirus (Table A-III-2). The gene targets for the *E. coli* and *Bacteroides* assays were the 23S rRNA gene and the 16S rRNA gene, respectively, with the human and bovine host-associated assays targeting different subgroups within the Bacteroidales order (Bernhard and Field, 2000; Seurinck et al., 2005; Layton et al., 2006). The Adenovirus gene target was the Hexon gene from serotypes 40 and 41 (Rajal et al., 2007) which encodes the major capsid protein for the Adenovirus (Ebner et al., 2005). All qPCR assays were performed in triplicate for each sample with an additional well for each sample containing a known amount of the standard as a spike in order to monitor PCR inhibition as described previously (Layton et al., 2006; Bell et al., 2009). All qPCR reactions were prepared using 12.5 µl PCR mix (QIAGEN, Valencia, CA or Stratagene, LaJolla, CA), 5 pmol of the forward primer and reverse primers, 15 pmol of the probe, 8 µl of sterile water and 2.5 µl of sample or standard. PCR amplification and fluorescent probe detection were performed using the Chromo4 Real-Time PCR Detection system (BioRad, Hercules, CA) and the following amplification protocol: 50˚C for 2 minutes, 95˚C for ten minutes, and 45 cycles of alternating 95 ˚C for 30 seconds and the annealing temperature for 45 seconds (Table A-III-2). The standards used to calibrate the qPCR assays consisted of the target gene cloned into a plasmid for all assays except for the *E. coli* assays which used *E. coli* O157 genomic DNA (Strain EDL 933, ATCC 43895D-5). For plasmid standards,

serial 10-fold dilutions were performed in triplicate from a starting concentration of $1x10^7$ plasmid copies to 10 copies and 2.5 µl of each plasmid dilution were placed in triplicate wells. Similarly the *E. coli* O157 genomic DNA was diluted serially from a starting concentration of $1x10^6$ to 10 genomic equivalents and 2.5 µl of each plasmid dilution was placed in triplicate wells. Data for each sample and assay were calculated as copies/ng of total extracted DNA and then converted to copies/100 mL based on the volume of water filtered. The method detection limit, MDL, was determined to occur when the copies of marker DNA was less than 1 copy per ng of extracted DNA. Since the mass of extracted DNA varied considerably, marker MDL's varied with pond sample. In the rare case where the standard deviation exceeded the mean gene concentration in the 3 wells (C.V. > 100%), the assay for that marker was re-run.

Statistical Analysis

Concentrations of all six markers are compared with one another using a non-parametric correlation matrix which reports the Spearman Rank Order Correlation Coefficient r_s. Each r_s value is accompanied by a p-value, indicating the level of significance of the association. For determining differences in concentrations between groups of ponds based on type, such as latrine vs. fish/bathing, the non-parametric Kruskal-Wallis test was performed using the software NCSS (version 07.1.14, NCSS, LLC, Kaysville, Utah). The significance of associations between the number of people and GIS-based latrine counts (sanitary, unsanitary, total) within 10 to 100 m of a pond and concentrations of markers in pond water was also tested (Spearman Rank Order Coefficient).

III.3 RESULTS

Physical and Chemical Attributes of Ponds

All ponds that could be found within the 0.3 km^2 area of the village were sampled in June 2008. Of the total of 43 sampled, 11 ponds were designated by the owner as fish or bathing ponds and 16 ponds had no designated purpose (Fig. III-1). The remaining 16 ponds were classified as latrine ponds because they were clearly receiving direct effluent from at least one latrine. The surface area of the ponds varied ranged from 20 to 1,200 m^2. The largest ponds were commercial fishing and community bathing ponds found in the northeast section of the village and contain water year round (Fig. III-1). The water level in fish ponds is maintained artificially by pumping from wells. The smallest ponds were latrine ponds, or ponds without a designated purpose, which were located within the village.

Dissolved oxygen concentrations in the latrine ponds (median 0.24 ppm) were lower than in fish/bathing ponds (0.68 ppm) ($p<0.05$), with the fish/bathing ponds having the largest range of dissolved oxygen (0.23 – 1.51 ppm) (Fig. III-2b). The electrical conductivity of water in latrine ponds (median 0.41 mS/cm) was higher than for fish/bathing ponds (0.17 mS/cm). Pond water temperature ranged from 27 to 34 ˚C, with higher temperature ponds located on the edges of the village or in the fields, where there is little to no shade. The smaller and cooler ponds, often latrine ponds, were located in the interior of the village. Median temperature (Fig. III-2c), electrical conductivity (Fig. III-2a), and dissolved oxygen concentrations (Fig. III-2b) for ponds without a defined use were in intermediate median values for fish/bathing and latrine ponds.

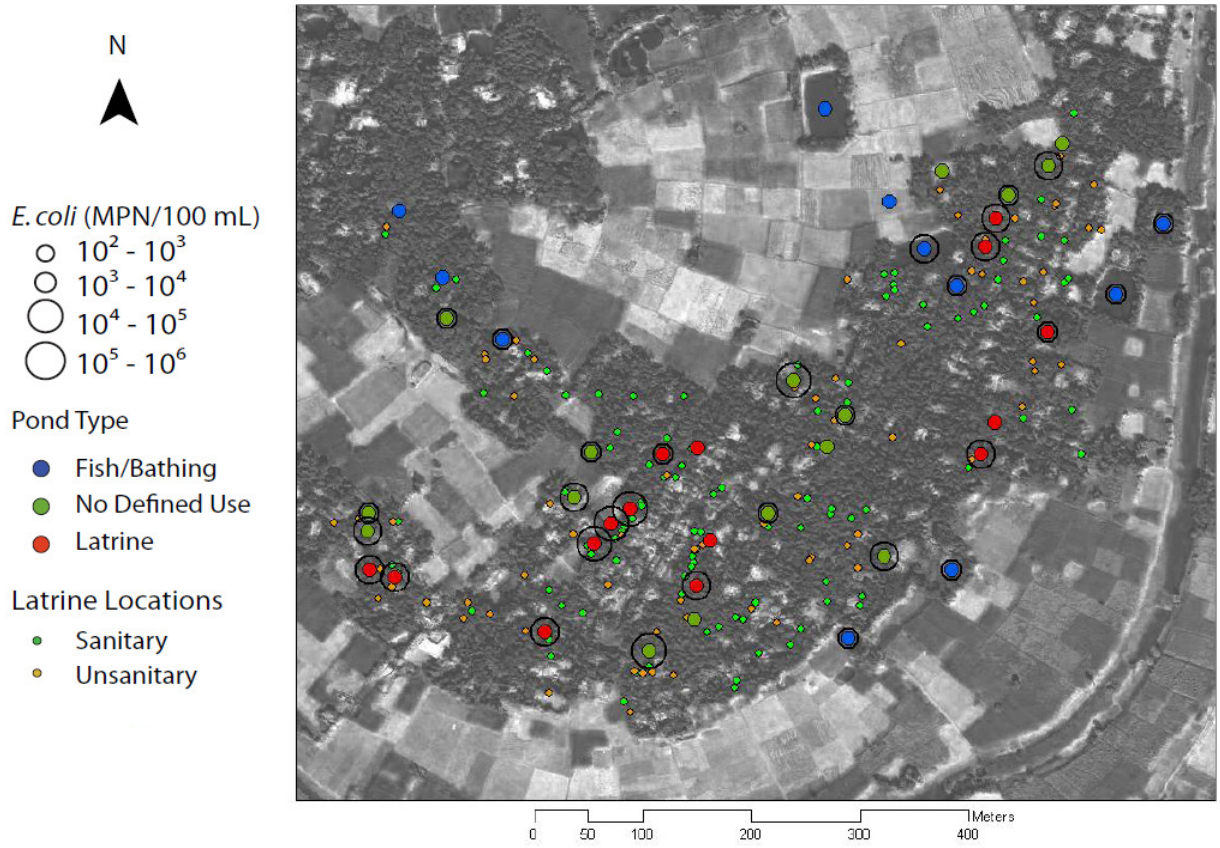


Figure III-1. Concentration of cultured *E. coli* in each pond classified by pond type with locations of sanitary and unsanitary latrines. IKONOS satellite image taken of the entire region of Araihazar at 1 m resolution (van Geen et al., 2003).

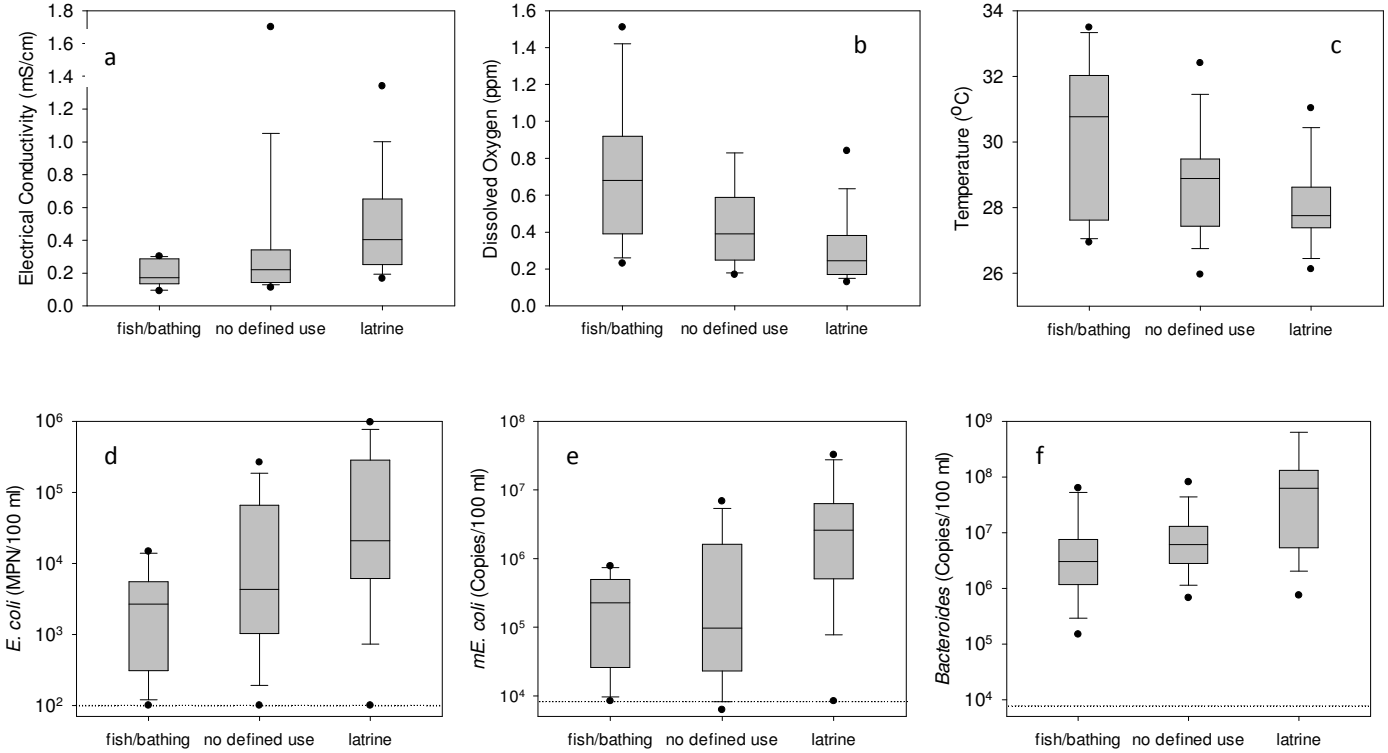


Figure III-2. Dissolved oxygen, electrical conductivity, temperature and log-transformed concentrations (MPN or copies/100 ml) of three fecal markers in water, from three types of ponds at Site K. The center line represents the median, upper and lower bounds of the box are the 75th and 25th percentile and whiskers represent the extent of the data. Outliers are represented by dots. The number of ponds was 43, consisting of 11 fish/bathing, 16 latrine and 16 ponds with no defined use in each category. The geometric mean detection limit for molecular assays was 8,374 copies/100 ml and is indicated by the dotted line.

Concentrations of indicator bacteria and genetic markers

Out of the 43 ponds, cultured *E. coli* were detected in 42, molecular *Bacteroides* were detected in 43, Adenovirus in 41 and *mE. coli* in 39. Human and Bovine *Bacteroides* were detected in a subset of the ponds only (36 and 24, respectively). The molecular markers that were not detectable at quantifiable levels contained <1 copy/ng of extracted DNA from the water sample. Since the amount of DNA extracted from the samples varied from 11 to 413 ng/µl and the volume of pond water filtered ranged from 40 to 295 mL, the analytical detection limit also varied considerably with a geometric mean of 8,374 and a range of 1,400 to 206,500 copies/100 ml respectively (Table A-III-2).

Concentrations of cultured *E. coli* in the 43 ponds ranged from non-detect (<100 MPN/100 mL) to 9.7×10^5 MPN/100 mL, with ponds having the highest *E. coli* concentrations tending to be located in the central part of the village (Fig. III-1). Linear regression was performed on $\log_{10}$-transformed concentrations of culturable *E. coli* and *mE. coli*, resulting in a power law relationship (R^2 = 0.43) (Fig. III-3a). An *E. coli* genome contains 6 copies of the ribosomal operon within the 23S gene (Klappenbach et al., 2001), and in all the ponds, a geometric mean of ~10% of all *E. coli* genomes detected by qPCR were culturable. The relationship between *E. coli* and *mE. coli* (Fig. III-3a) with an exponent of 0.72 (95% CIs, 0.46 – 0.97) indicates that fewer *E. coli* genomes are culturable at higher concentrations. Accordingly the geometric mean ratio of cultured *E. coli* to total genomes for the least contaminated ponds (lower half) was 16%, whereas for ponds with the highest *mE. coli* concentration (upper half) this ratio was only 7%.

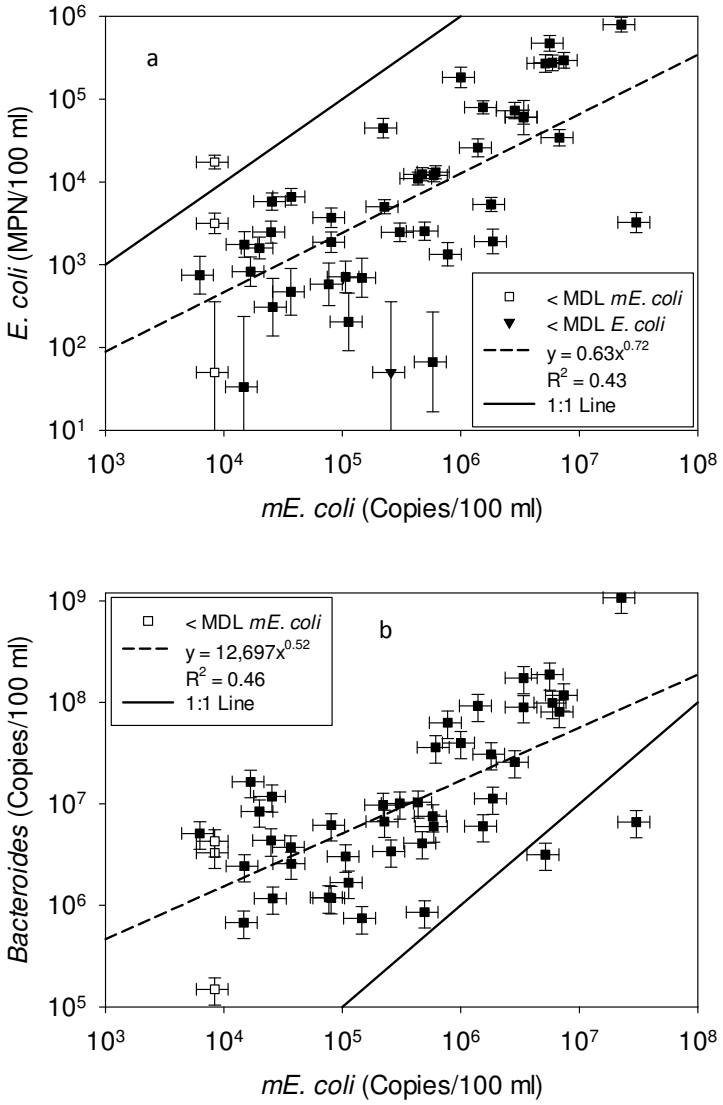


Figure III-3. Observed and predicted *E. coli* and *Bacteroides* as a function of *mE. coli* in 43 pond water samples. One non-detect occurred with culturable *E. coli* and 3 non-detects occurred for *mE. coli*, but not in the same samples. *Bacteroides* was detected in every pond water sample. Error bars represent 95% analytical confidence intervals. Predictive equation is the result of fitting a linear regression model (y = mx + b) to $\log_{10}$-transformed concentrations with R^2. The 1:1 line is shown for comparison (y=x).

Bacteroides is an independent fecal indicator and its concentrations in pond water were correlated to and higher than *mE. coli* concentrations in all but two cases (Fig. III-3b). The degree to which *Bacteroides* markers outnumbered *mE. coli* decreased with increasing *mE. coli* concentration, such that the fitted relationship (R^2 = 0.46), where the exponent was equal to 0.52 (95% CIs, 0.35 – 0.69), approached the 1:1 line (Fig. III-3b). Concentrations of human and bovine *Bacteroides* markers combined were always lower than concentrations obtained with the total *Bacteroides* by approximately one order of magnitude (Table A-III-4). Although total *Bacteroides* were detected in all ponds, neither human nor bovine *Bacteroides* were detected in 5 ponds (Fig. A-III-1). This is not surprising because the total *Bacteroides* assay (AllBac) is more sensitive to fecal pollution than the source-specific *Bacteroides* assays (Layton et al., 2006; Kildare et al., 2007; Okabe and Shimazu, 2007) (Table A-III-4). In 34 out of 38 samples where at least one host-specific marker was detected, the concentration of human *Bacteroides* exceeded that of bovine *Bacteroides* (Fig. A-III-1). Log-transformed concentrations of *E. coli*, *mE. coli*, and *Bacteroides* in pond water are all significantly correlated (Table III-1).

Fish/bathing ponds are significantly less contaminated than latrine ponds according to all three fecal indicator bacteria (Fig. III-2) (non-parametric Kruskal-Wallis test $p<0.05$). The median concentrations of fecal indicators in ponds without a defined use were intermediate of corresponding median concentrations in latrine and fish/bathing ponds. The “no defined use” category includes ponds that were excavated primarily to build up nearby land for a house or for road construction. Adenovirus concentrations were uncorrelated to fecal indicator bacteria (Table III-1) and pond type (Fig. A-III-3).

Correlations between Spatial Buffer Population Counts and Pond Contamination

An estimated total of 1500 people live within Char Para (Site K). Significance of the Spearman rank correlation coefficient between the GIS-based spatial population count and the concentration of the various microbial markers was calculated for a range of counting radii (10-100 m) (Fig. III-4a). The association between population and fecal bacteria concentrations was found to be optimal at a 45 m spatial counting radius with all fecal indicator bacteria showing significant correlations to population ($p<0.05$). A large range in the population within 45 m of each pond was observed from, uninhabited to 126 people (Table A-III-5).

High Spearman correlations were observed between population within 45 m of each pond and all fecal markers with significant r_s values of 0.57, 0.46, and 0.38 for *E. coli*, *mE. coli* and *Bacteroides* respectively (Table III-1). Human *Bacteroides* (HuBac and HF 183) which was detected in 36 out of 43 samples correlated significantly to the population within 45 m of the pond ($r_s = 0.34$) as did Bovine *Bacteroides* ($r_s = 0.36$).

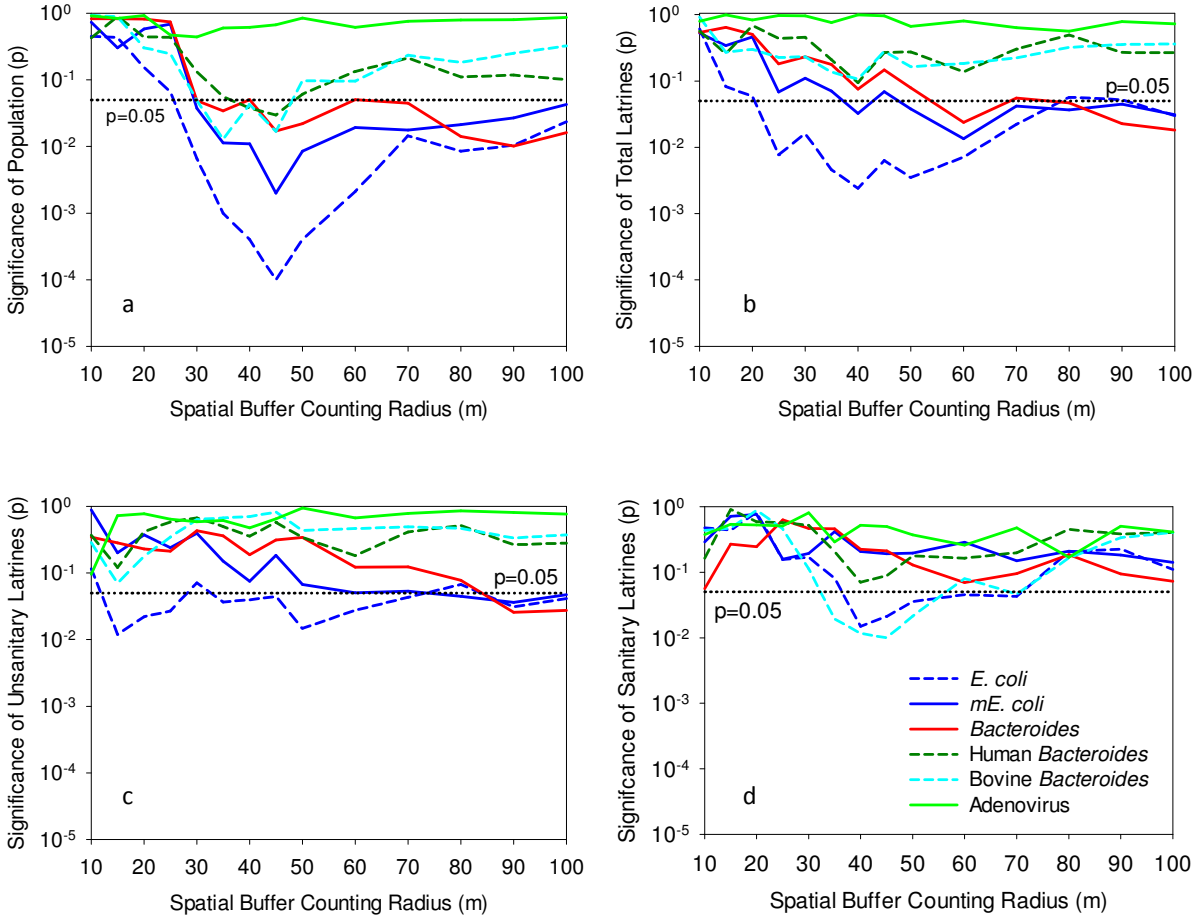


Figure III-4. Significance of Spearman rank correlation coefficient with GIS-based spatial buffer counting of population, total, unsanitary and sanitary latrines against concentration of fecal bacteria and Adenovirus in pond water. Buffer radii were tested at five meter intervals from 10 to 50 m and at ten meter intervals from 50 to 100 m.

Table III-1. Spearman Rank Order correlations of microbial markers with extracted and measured field parameters. Significance in association p<0.05 is indicated by bold. The human population within a 45 m radius of the pond, and latrines within 60 m were determined using a GIS-based spatial counting method. The number of latrines and cattle were also counted on site within the drainage basin of each pond. The size of the data set size was 43. When the target genes in a sample were below the detection limit a concentration of 8,374 copies/100 ml was used.

		GIS				Pond Basin				Measured on Pond Water							
		Population (45 m)	Unsanitary Latrines (60 m)	Sanitary Latrines (60 m)	Total Latrines (60 m)	Unsanitary Latrines	Sanitary Latrines	Total Latrines	Cattle	Temperature (˚C)	Conductivity (mS/cm)	Dissolved Oxygen (mg/L)	*E. coli*	*mE. coli*	*Bacteroides*	Human *Bacteroides*	Bovine *Bacteroides*
GIS	Unsanitary Latrines (60 m)	**0.71**															
	Sanitary Latrines (60 m)	**0.52**	0.27														
	Total Latrines (60 m)	**0.77**	**0.90**	**0.60**													
Pond Basin	Unsanitary Latrines	**0.37**	**0.32**	0.16	**0.35**												
	Sanitary Latrines	0.03	0.08	0.08	0.08	**0.43**											
	Total Latrines	0.28	0.26	0.11	0.27	**0.92**	**0.72**										
	Cattle	0.20	0.10	0.18	0.21	**0.45**	0.17	**0.39**									
Measured on Pond Water	Temperature (˚C)	-0.30	-0.23	-0.25	-0.25	-0.21	-0.06	-0.19	-0.07								
	Conductivity (mS/cm)	**0.38**	0.10	0.20	0.23	**0.43**	0.05	**0.35**	0.27	-0.26							
	Dissolved Oxygen (mg/L)	**-0.36**	**-0.42**	-0.10	**-0.35**	**-0.31**	-0.20	**-0.33**	0.11	**0.69**	-0.29						
	E. coli	**0.57**	**0.33**	**0.31**	**0.40**	**0.46**	0.00	**0.33**	**0.32**	-0.18	**0.47**	-0.14					
	mE. coli	**0.46**	**0.30**	0.16	**0.37**	**0.37**	-0.25	0.18	**0.40**	-0.04	**0.49**	-0.08	**0.69**				
	Bacteroides	**0.38**	0.26	0.28	**0.36**	**0.41**	0.01	**0.31**	**0.41**	-0.09	**0.52**	-0.11	**0.69**	**0.69**			
	Human *Bacteroides*	**0.34**	0.22	0.22	0.24	0.17	-0.04	0.09	0.05	-0.23	**0.41**	-0.04	**0.69**	**0.42**	**0.50**		
	Bovine *Bacteroides*	**0.36**	0.11	0.27	0.21	0.19	-0.05	0.10	0.05	-0.06	**0.41**	-0.04	**0.70**	**0.53**	**0.47**	**0.63**	
	Adenovirus	0.06	0.07	-0.18	-0.04	0.10	-0.20	0.03	0.02	0.11	0.16	0.11	0.09	0.25	0.25	0.22	0.12

Latrines and Pond Contamination

A total of 178 latrines were located during the village-wide GPS survey (Fig. III-1), 79 (42%) of which were sanitary latrines, a similar proportion to that observed elsewhere in Bangladesh (WHO and UNICEF, 2008). Of the 99 unsanitary latrines, 22 were open pit latrines without a concrete ring. To enumerate latrines around each pond, both the GIS-based spatial buffer method was used as wells as counting within the pond drainage basins.

A significant ($p<0.05$) correlation was found between *E. coli* concentrations in a pond and the total number of GIS-based latrines within the range of 20 to 80 m distance from a pond (Fig. III-4b). The total number of GIS-based latrines per pond ranged from 0 to 8 within 20 m and 0 to 34 within 80 m. In the case of GIS-based unsanitary latrines, the correlation between number of latrines and *E. coli* concentration was significant across the distance range of 15 to 80 m from a pond (Fig. III-4c). Correlations between GIS-based unsanitary latrines and concentrations of *mE. coli* and *Bacteroides*, however, were only marginally significant from 80 to 100 m. GIS-based sanitary latrines were correlated to *E. coli* concentrations within 40 to 70 m from a pond (Fig. III-4d). The only other fecal indicator that correlated significantly to sanitary latrines was Bovine *Bacteroides* (35 to 50 m). Adenovirus concentrations were not correlated with counts of any type of GIS-based latrines at any buffer distance (Fig. III-4).

Sixty meters was chosen as the buffer size for optimal correlations between GIS-based latrine counts and fecal bacteria, since this was the only radius that resulted in significant correlations with all three total fecal indicator bacteria, *E. coli*, *mE. coli* and *Bacteroides* (Fig. III-4b). The Spearman correlations are shown in Table III-1 for all bacterial and viral markers. The

correlation between *E. coli* and the total number of GIS-based latrines (r_s = 0.40) within 60 m of a pond was stronger than either unsanitary (0.33) or sanitary latrines (0.31) considered separately. *mE. coli* and *Bacteroides* correlated to GIS-based total latrine counts within 60 m of the pond with r_s equal to 0.37 and 0.36 respectively.

When considering the number of unsanitary latrines within each drainage basin only, which ranged from 0 to 10, the correlations with fecal indicators *E. coli* (r_s = 0.46), *mE. coli* (0.37) and *Bacteroides* (0.41) were stronger than with GIS-based counts of any type of latrine (total, sanitary or unsanitary) within a 60 m radius (Table III-1). No significant correlation was found between human *Bacteroides* in pond water and the number of latrines of any type with either the GIS-based distance or drainage basin counting method (Table III-1). Correlations between all three fecal indicator markers, *E. coli*, *mE. coli* and *Bacteroides*, and the number of cattle within the pond drainage basin were all significant. Bovine *Bacteroides* is not correlated to the number of cattle within the pond drainage area, however (Table III-1).

Correlations of Water Chemistry with Latrines, Population and Pond Contamination

High, positive Spearman correlations were observed between all fecal indicator bacteria and electrical conductivity with *Bacteroides* having the highest r_s of 0.52 (Table III-1). Electrical conductivity was also positively correlated with the GIS-based population count within 45 m of each pond (r_s = 0.38) and the number of unsanitary latrines (r_s = 0.43), but not with the number of sanitary latrines within a pond drainage basin (r_s = 0.05). A high correlation was observed between temperature and dissolved oxygen, and both of these parameters were negatively correlated with population (r_s was -0.30 and -0.36 respectively). Dissolved oxygen was also

negatively correlated to GIS-based unsanitary and total latrines within 60 m (-0.42 and -0.35 respectively) and unsanitary and total latrines within the pond basin (-0.31 and -0.33 respectively).

III.4 Discussion

Implications for the Spread of Diarrheal Disease

In the ponds surveyed in this study, *E. coli* concentrations exceeded the U.S. EPA recreational water quality limit (126 MPN/100 ml) up to 10,000 fold (US EPA, 1986) and were in fact similar in concentration to fecal coliforms detected in raw sewage and wastewater (Sinton et al., 1999). An epidemiological meta-analysis on a world wide data set comparing *E. coli* concentrations to disease determined that for every tenfold increase in *E. coli* the odds of acquiring diarrheal disease from recreational contact approximately double (Wade et al., 2003). The ponds used for bathing or fishing contained 1-2 orders of magnitude less culturable *E. coli* than latrine ponds suggesting that diarrheal disease risk from recreational or bathing exposure to latrine ponds is 2 to 4 times higher than for protected ponds. However, even fishing and bathing ponds had high levels of all fecal indicators *E. coli*, *mE. coli, Bacteroides* and Human *Bacteroides* with median concentrations of $1x10^{3.5}$, $1x10^{5.5}$, $1x10^{6.5}$ and $1x10^{2.5}$ copies/100 mL respectively.

Ecology of Fecal Bacteria in the Built Environment

Bacteroides gene markers were consistently detected in higher numbers than *E. coli* genomes. The fecal indicator bacteria *E. coli*, and molecular markers for *mE. coli* and *Bacteroides* were well correlated with each other indicating that any three of these assays may be used to evaluate the level of fecal contamination in pond water. *E. coli* may re-grow in the environment with recent studies suggesting that *E. coli* is in fact an endogenous soil bacterium (Nautiyal et al., 2010). *Bacteroides* is obligate anaerobe, and the persistence of its DNA is sensitive to both oxygen and temperature (Bell et al., 2009). These important differences between *E. coli* and *Bacteroides* may call for their concurrent use in assessing the level of fecal contamination in a water sample. In every pond where at least one host-specific marker was detected, concentration of human *Bacteroides* concentration exceeded bovine *Bacteroides.* Fecal pollution of pond water throughout the site is therefore overwhelmingly of human origin. The ratio of culturable *E. coli* to *E. coli* genomes was not influenced by pond type and temperature, indicating that neither nutrient availability nor temperature affected the proportion of culturable genomes in the water samples.

Significant positive correlations ($p<0.05$) were observed between all fecal indicator bacteria and electrical conductivity (Table III-1). Conductivity is likely to rise with increased anthropogenic use of the pond catchment area, including salt inputs from human waste (Manahan, 2005) and the use of ash for washing (Hoque et al., 1995; Sengupta et al., 2008) coupled with pond water evaporation and may therefore be a proxy for human usage and contamination of pond water.

All fecal bacteria were highly correlated to the number of people living within 45 m of the pond. Unlike latrines, people are mobile, and in rural Bangladesh, the relatively secluded area around ponds frequently is used for make-shift above-ground latrines consisting of two bricks on the ground. During the surveys, 22 of these "latrines" were recorded as unsanitary latrines when they were found, however, their difficulty to find suggests the presence of more.

The number of unsanitary latrines recorded within each pond drainage basin was reasonably predictive of fecal bacteria concentrations ($p<0.05$), while the numbers of sanitary latrines within drainage basins were uncorrelated with fecal bacteria. This highlights the importance of properly functioning latrines to minimize fecal contamination in bathing ponds of densely populated villages in developing countries like Bangladesh. The number of unsanitary latrines within a pond drainage basin was more predictive of fecal indicator bacteria concentration than GIS-based counts of any type of latrine (total, sanitary, unsanitary) within a 60 m radius from each pond (Table III-1). Unlike the pond basin counting method, where the number unsanitary latrines were most strongly correlated to fecal bacteria concentrations, GIS-based total latrines counted within a 60 m radius were more predictive of fecal bacteria concentrations than either GIS-based unsanitary or sanitary latrine counts alone. The fact that unsanitary latrines counted within pond drainage basins were more predictive is likely the result of topography surrounding each pond that determines the direction latrine effluent and surface runoff will flow. For example, there are many depressions throughout Site K that catch all effluent from latrines that are located within 10 m of a neighboring pond. Therefore the

observations of latrines within each pond's drainage basin are a better estimate of the amount of effluent received by a pond.

These findings underscore the impact that sanitation and population has upon village ponds in Bangladesh, and supports the notion that the built environment profoundly impacts endemic diarrheal disease incidences, especially in developing countries where people are exposed to the aquatic environment. More research is needed to quantify the impact that sanitation practices and pond management have on diarrheal disease in developing countries.

REFERENCES

Ali, M.; Emch, M.; Donnay, J.P.; Yunus, M; Sack, R.B. The spatial epidemiology of cholera in an endemic area of Bangladesh. *Social Science & Medicine* **2002**, *55*(6), 1015-1024.

Aziz, K. M. A.; Hoque, B. A.; Huttly, S. R. A.; Minnatullah, K. M.; Hasan, Z.; Patwary, M. K.; Rahaman, M. M.; Cairncross, S. Water Supply, Sanitation and Hygiene Education: Report of a Health Impact Study in Mirzapur, Bangladesh. United Nations Development Programme – World Bank Water and Sanitation Program. The World Bank, Washington, DC, 1990.

Bell, A.; Layton, A. C.; McKay, L.; Williams, D.; Gentry, R.; Sayler, G. S. Factors influencing the persistence of fecal *Bacteroides* in stream water. *J. Environ. Quality* **2009** *38*, 1224-1232.

Bernhard, A.E.; Field, K.G. Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. *Applied and Environmental Microbiology* **2000**, *66*(4), 1587-1594.

Diarrhoea: Why children are still dying and what can be done; United Nations Children's Fund and World Health Organization, 2009; http://whqlibdoc.who.int/publications/2009/9789241598415eng.pdf

Ebner, K.; Pinsker, W.; Lion, T. Comparative Sequence Analysis of the Hexon Gene in the Entire Spectrum of Human Adenovirus Serotypes: Phylogenetic, Taxonomic, and Clinical Implications. *J. Virology* **2005**, *79*, 12635–12642.

Emch, M. Diarrheal disease risk in Matlab, Bangladesh. *Social Science & Medicine*, **1999**, *49*(4), 519-530.

Emch, M.; Ali, M.; Yunus, M. Risk areas and neighborhood-level risk factors for *Shigella dysenteriae* 1 and *Shigella fexneri*. *Health & Place* **2008**, *14*, 96-115.

Esrey, S. A. Water, Waste, and Well-Being: A Multicountry Study. *Am. J. Epiemiol*. **1996**, *143*(6), 608-623.

Fewtrell, L.; Kaufmann, R. B.; Kay, D.; Enanoria, W.; Haller, L.; Colford, J. M. Jr. Water, sanitation, and hygiene interventions to reduce diarrhea in less developed countries: a systematic review and meta-analysis. *Lancet Infect. Dis.* **2005**, *5*, 42-52.

Given, S.; Pendleton, L. H.; Boehm, A. B. Regional Public Health Cost Estimates of Contaminated Coastal Waters: A Case Study of Gastroenteritis at Southern California Beaches, *Environ. Sci. Technol*. **2006**, *40*(16), 4851-4858.

Hoque, B. A.; Juncker, T.; Sack, R. B.; Ali, M.; Aziz, K. M. A. Sustainability of a water, sanitation and hygiene education project in rural Bangladesh: a 5-year follow-up. *Bulletin of the World Health Organization* **1996**, *74*(4), 431-437.

Hoque, B.A.; Mahalanabis, D.; Alam, M.J.; Islam, M.S. Post-Defecation Handwashing in Bangladesh - Practice and Efficiency Perspectives. *Public Health* **1995,** *109*(1), 15-24.

Jiang, S.C.Human Adenoviruses in water: Occurrence and health implications: A critical review. *Environ. Sci. Technol.* **2006** *40*(23), 7132-7140.

Kildare, B. J.; Leutenegger, C. M.; McSwain, B. S.; Bambic, D. G.; Rajal, V. B.; Wuertz, S. 16S rRNA-based assays for quantitative detection of universal, human-, cow- and dog-specific fecal Bacteroidales: A Bayesian approach. *Water Research* **2007**, *41*(16), 3701-3715.

Klappenbach, J. A.; Saxman, P. R.; Cole, J. R. Schmidt, T. M. rrndb: the Ribosomal RNA Operon Copy Number Database. *Nucleic Acids Research* **2001**, *29*(1), 181-184.

Layton, A.; McKay, L. D.; Williams, D.; Garrett, V.; Gentry, R.; Sayler, G. Development of *Bacteroides* 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water. *Applied & Environmental Microbiology* **2006** *72*(6), 4214-4224.

Lee, Y.J.;Molina, M.; Domingo, J. W. S.; Willis, J. D.; Cyterski, M.; Endale, D. M.; Shanks, O. C. Temporal Assessment of the Impact of Exposure to Cow Feces in Two Watersheds by Multiple Host-Specific PCR Assays. *Applied & Environmental Microbiology* **2008** *74*(22), 6839-6847.

Manahan, S. E. Environmental chemistry 8th Ed., CRC Press LLC: Boca Raton, Florida, 2005.

Matsuki, T.; Watanabe, K.; Fujimoto, J.; Miyamtoto, Y.; Takada, T.; Matsumoto, K.; Oyaizu, H.; Tanaka, R. Development of 16S rRNA-gene-targeted group-specific primers for the detection and identification of predominant bacteria in human feces. *Applied and Environmental Microbiology* **2002**, *70*, 167-173.

Nautiyal, C.S.; Rehman, A.; Chauhan, P.S. Environmental *Escherichia coli* occur as natural plant growth-promoting soil bacterium. *Archives of Microbiology* **2010** *192*(3), 185-193.

Neumann, R.B.; Ashfaque, K.N.; Badruzzaman, A.B.M.; Ali, M.A.; Shoemaker, J.K.; Harvey, C.F. Anthropogenic influences on groundwater arsenic concentrations in Bangladesh. *Nature Geoscience* **2010** *3*(1), 46-52.

Okabe, S.; Shimazu, Y. Persistence of host-specific *Bacteroides*-Prevotella 16S rRNA genetic markers in environmental waters: effects of temperature and salinity. *Applied and Environmental Microbiology* **2007**, *76*(4), 935-944.

Pruss, A. Review of epidemiological studies on health effects from exposure to recreational water. *International J. Epidemiology* **1998,** *27*, 1-9.

Pruss, A.; Kay, D.; Fewtrell, L.; Bartram, J. Estimating the Burden of Disease from Water, Sanitation, and Hygiene at a Global Level. *Environmental Health Perspectives* **2002**, *110*(5), 537-542.

Radloff, K.A.; Cheng, A.; Rahman, M. W.; Ahmed, K. M.; Mailloux, B. J.; Juhl, A. R.; Schlosser, P.; van Geen, A. Mobilization of arsenic during one-year incubations of grey aquifer sands from Araihazar, Bangladesh. *Environ. Sci. Technol*. **2007**, *41,* 3639-3645.

Rajal, V. B.; McSwain, B. S.; Thompson, D. E.; Leutenegger, C. M.; Wuertz, S. Molecular quantitative analysis of human viruses in California storm water. *Water Research* **2007**, *41*(*19*), 4287-4298.

Sengupta, S.; McArthur, J. M.; Sakar, A.; Leng, M. J.; Ravenscroft, P.; Howarth, R. J.; Banerjee, D. M. Do ponds cause arsenic-pollution of groundwater in the Bengal Basin? An answer from West Bengal. *Environ. Sci. Technol*. **2008**, *42*(14), 5156-5164.

Seurinck, S.; Defoirdt, T.; Verstraete, W.; Siciliano, S. Detection and quantification of the human specific HF183 *Bacteroides* 16S rRNA genetic marker with real-time PCR for assessment of human fecal pollution in freshwater. *Environ. Micro.* **2005**, *7,* 249-259.

Sinton, L.W.; Finlay, R.K.; Lynch, P.A. Sunlight inactivation of fecal bacteriophages and bacteria in sewage-polluted seawater. *Applied and Environmental Microbiology* **1999,** *65*(8), 3605-3613.

United States Environmental Protection Agency. Bacteriological Water Quality Criteria for Marine and Fresh Recreational Waters. EPA-440/5-84-002, U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Cincinnati, OH, 1986.

van Geen, A.; Zheng, Y.; Versteeg, R.; Stute, M.; Horneman, A.; Dhar, R.; Steckler, M.; Gelman, A.; Small, C.; Ahsan, H.; Graziano, J. H.; Hussain, I.; Ahmed, K. M. Spatial variability of arsenic in 6000 tube wells in a 25 km^2 area of Bangladesh. *Water Resources Research* **2003**, *39*(5).

Wade, T.J.; Pai, N.; Eisenberg, J.N.S.; Colford, J.M. Do US Environmental Protection Agency water quality guidelines for recreational waters prevent gastrointestinal illness? A systematic review and meta-analysis. *Environmental Health Perspectives* **2003**, *111*(8), 1102-1109.

Weinman, B., S. L. Goodbred Jr., Y. Zheng, Z. Aziz, M. Steckler, A. van Geen, A. K. Singhvi and Y. C. Nagar. Contributions of floodplain stratigraphy and evolution to the spatial patterns of groundwater arsenic in Araihazar, Bangladesh. *Bulletin of the Geological Society of America* **2008**, *120* (11-12), 1567-1580.

World Health Organization and United Nations Children's Fund Joint Monitoring Programme. People's Republic of Bangladesh: improved sanitation coverage estimates (1980-2006), World Health Organization and United Nations Children's Fund, Geneva, Switzerland, 2008. http://documents.wssinfo.org/resources/documents.html

Yampara-Iquise, H.; Zheng, G.; Jones, J.E.; Carson, C.A. Use of a *Bacteroides* thetaiotaomicron-specific alpha-1-6, mannanase quantitative PCR to detect human faecal pollution in water. *Journal of Applied Microbiology* **2008**, *105*(5), 1686-1693.

CHAPTER IV - TRANSPORT OF FECAL BACTERIA FROM PONDS TO AQUIFERS IN RURAL BANGLADESH

This chapter is adapted from a manuscript which is in preparation for submission to a journal for publication.

Abstract

In Bangladesh, numerous ponds within villages represent potential point sources of fecal contamination to drinking water wells, especially during the monsoon when they rapidly fill with runoff water and drain into the ground. Nine transects of monitoring wells radiating away from four ponds were installed in a sandy, unconfined aquifer underlying a village in rural Bangladesh, and sampled monthly for cultured *E. coli* from September 2008 through October 2009. *E. coli* was rarely detected in the aquifer adjacent to the ponds during the dry season. During the early monsoon, however, high concentrations of *E. coli* (>800 MPN/100 ml) and molecular *E. coli* and *Bacteroides* (>100,000 copies/100 ml) were found in the aquifer. In June of 2009, water levels in four ponds were artificially raised by 16 to 63 cm to simulate early monsoon flooding conditions and microbial indicators were monitored in the adjacent transect wells. The distance required for six-$\log_{10}$ (99.9999%) bacteria attenuation, compared to the influent pond water, ranged from 5 to 12 m. This distance was estimated based on the modeled filtration coefficient fitted to fecal indicator bacteria concentrations. Column experiments with 1 µm microspheres were performed to evaluate the assumption of scalability of 12 cm columns to 7 m field transport studies. Similar filtration coefficients were determined from both column and field experiments, indicating that the experiments in the columns provided a good representation of aquifer-scale removal processes. During the early monsoon, the presence of high concentrations of molecular *E. coli* and *Bacteroides* in transects not impacted by

immediately adjacent ponds indicates widespread fecal pollution in the shallow aquifer. Factors determining whether a pond was likely to be a source of groundwater fecal contamination included its geologic setting, depth, and age.

IV.1 Introduction

In rural Bangladesh where there is typically high diarrheal disease morbidity (Emch, 1999), the fecal indicator bacterium *E. coli* is prevalent in rural drinking water wells, with frequency of detection ranging from 30% to 70% and typically peaking in the wet season (Leber et al., 2010). The spatial distribution of *E. coli* in shallow aquifers is erratic and transport pathways from human fecal sources are not well understood (Leber et al., 2010; van Geen et al., In Preparation). Possible pathways for fecal bacteria transport include vertical infiltration and subsequent lateral spreading along highly conductive geologic layers, as well as flow along the annulus of private wells which are constructed without seals. The goal of the present study was to determine whether infiltration from ponds are likely to represent substantial point sources of fecal bacteria to the shallow sandy aquifers in Bangladesh.

Fecal contamination has been reported in many shallow sandy aquifers throughout the world (Rudolph et al., 1998; Schijven et al., 2000; USGS, 2006). This finding is often contrary to expectations based on laboratory-scale column experiments, which routinely demonstrate high removal rates of bacteria and/or micron-sized particles in fine to medium sand, indicating that substantial attenuation should occur within the first meter or two (Zhuang et al., 2004; Foppen et al., 2008; Knappett et al., 2008). Field studies, however, often show that bacterial transport distances are much greater than predicted by laboratory column experiments (Harvey et al., 1989; Schijven et al., 1998; Foppen et al., 2008). One of the main mechanisms thought to be responsible for this over-estimation of filtration efficiency at the column scale is transport along

preferential flow paths operating over larger scales than sampled in the column experiments (Taylor et al., 2004; Foppen et al., 2008).

Fecal contamination in drinking water supplies has typically been assessed by the presence of culturable *E. coli* (Yates, 2007). In recent years the availability of molecular enumeration methods such as quantitative polymerase chain reaction (qPCR) has increased specificity and sensitivity over culture-based and microscopic enumeration methods. With molecular methods it is possible to enumerate all genomes of a target microorganism in a water sample without sensitivity to metabolic states. Further, genomic material contains much information about the identity of a bacterium and even the host organism from which a fecal bacterium was produced (Bernhard and Field, 2000; Scott et al., 2002; Layton et al., 2006; Noble et al., 2006; Kildare et al., 2007). Since qPCR enumerates bacterial genomes and not viable bacteria in a water sample, it may overstate the risk of acquiring diarrheal disease from a drinking water source. Comparing results of culture-based and molecular enumeration methods, however, may shed light on transport and decay processes in an aquifer. Epidemiologic dose-response relationships have not been established for drinking water samples using molecular enumeration methods, as they have been with other enumeration methods such as direct counting (DuPont et al., 1995) and culturing (Gale et al., 2001). It is currently not known how the transport and occurrence of cultured *E. coli* compares with genomes of both *E. coli* and *Bacteroides,* although it has been shown that qPCR detects much higher concentrations of both *E. coli* and *Bacteroides* in groundwater samples than cultured *E. coli* (Knappett et al., In Press).

The primary objective of this study is to determine if groundwater recharge from ponds, which are ubiquitous in rural villages in Bangladesh, are a major source of fecal contamination to shallow sandy aquifers. It is hypothesized that ponds, which typically contain high levels of *E. coli* and other fecal contaminants, rapidly fill with runoff and then drain during the early monsoon due an initially depressed water table. It is further hypothesized that factors such as pond depth and age (which can lead to a build-up of fine-grained sediments in the ponds over time), as well as local variations in sediment grain size, contribute to creating conditions conducive to rapid movement of fecal contamination into the shallow aquifers. Additional objectives are to a) compare the field-scale transport of cultured *E. coli* and molecular *E. coli* and *Bacteroides* through a typical sand aquifer impacted by contaminated pond water; and b) compare field- and laboratory-scale measurements of transport of fecal indicators in this type of aquifer material.

IV.2 Materials and Methods

Field Site and Hydrogeology

Nine transects consisting of five to six monitoring wells (four to five shallow wells and one deep well) were installed radiating away from four ponds receiving latrine effluent (Fig. IV-1). These wells were installed within the village of Char Para, herein referred to as Site K (Radlof et al., 2007). Char Para covers an area of 30 hectares and has a population of approximately 1500 (Knappett et al., In Review). The properties of the ponds and the neighboring wells are described in Table IV-1. The age of each pond was determined by asking the owner. All

transects radiate away from at least one pond. KW-42 was installed as a line of wells between two ponds (KP-15 and KP-05).

A total of forty-seven wells were drilled for the transects near the four ponds (Table IV-1, Fig. IV-1). Drilling was done by the traditional hand-flapper method: a manual mud circulation method that quickly penetrates the loose, wet floodplain deposits throughout the Bengal Basin (e.g. Horneman et al., 2004). The monitoring wells were sealed with cement grout from the top of the sand pack, which itself extends 0.7 m above the 1.5 m screened interval, to the surface. They were constructed of 5.1 cm diameter PVC pipe and sampled with an electric-powered submersible pump (Typhoon, Groundwater Essentials, LLC). The wells were developed by pumping Well depths varied from 5.5 to 7.9 m for the shallow wells and from 8.5 to 10.9 m for the deep wells, which were 3 m deeper than the shallow wells. L-shaped piezometers extending out into the base of each pond were installed to measure pond water elevation. The relative elevation of the top of casing for all wells was measured using a surveyor's level with accuracy of a few millimeters.

Table IV-1. Physical properties of ponds and adjacent aquifers

Pond ID	Pond Basin Depth (m)	Pond Age (yrs)	Pond base material	Transect ID	Shallow Wells			Deep Well
					D_{50} € (mm)	U*	Range K† (m/s)	K (m/s)
KP-10	4	>100	silt	KW-36	0.12	2.4	$1.7 - 5.0 \times 10^{-5}$	3.7×10^{-4}
				KW-37	0.13	2.3	$6.7 - 8.8 \times 10^{-5}$	4.0×10^{-4}
				KW-38	NR‡	NR	$4.5 - 7.6 \times 10^{-5}$	1.9×10^{-4}
KP-04	4.5	20	sand	KW-39	0.30	3.7	$1.1 - 2.7 \times 10^{-4}$	1.5×10^{-4}
				KW-40	0.31	4.2	$4.4 \times 10^{-5} - 2.5 \times 10^{-4}$	1.1×10^{-4}
				KW-41	0.23	3.5	$2.7 - 2.8 \times 10^{-4}$	1.4×10^{-4}
KP-15	4	<1	sand	KW-42	0.33	2.9	$3.1 - 4.5 \times 10^{-4}$	3.3×10^{-4}
KP-05	2	>30	silt	KW-43	0.31	3.1	$3.4 - 4.7 \times 10^{-4}$	2.8×10^{-4}
				KW-44	0.29	4.5	$2.7 - 3.2 \times 10^{-4}$	3.6×10^{-4}

€ Averaged median grain diameter from all 0.3 m cores from shallow wells

* Uniformity Coefficient averaged from all 0.3 m cores

† Range of Hydraulic Conductivities in shallow wells

‡ Not Reported

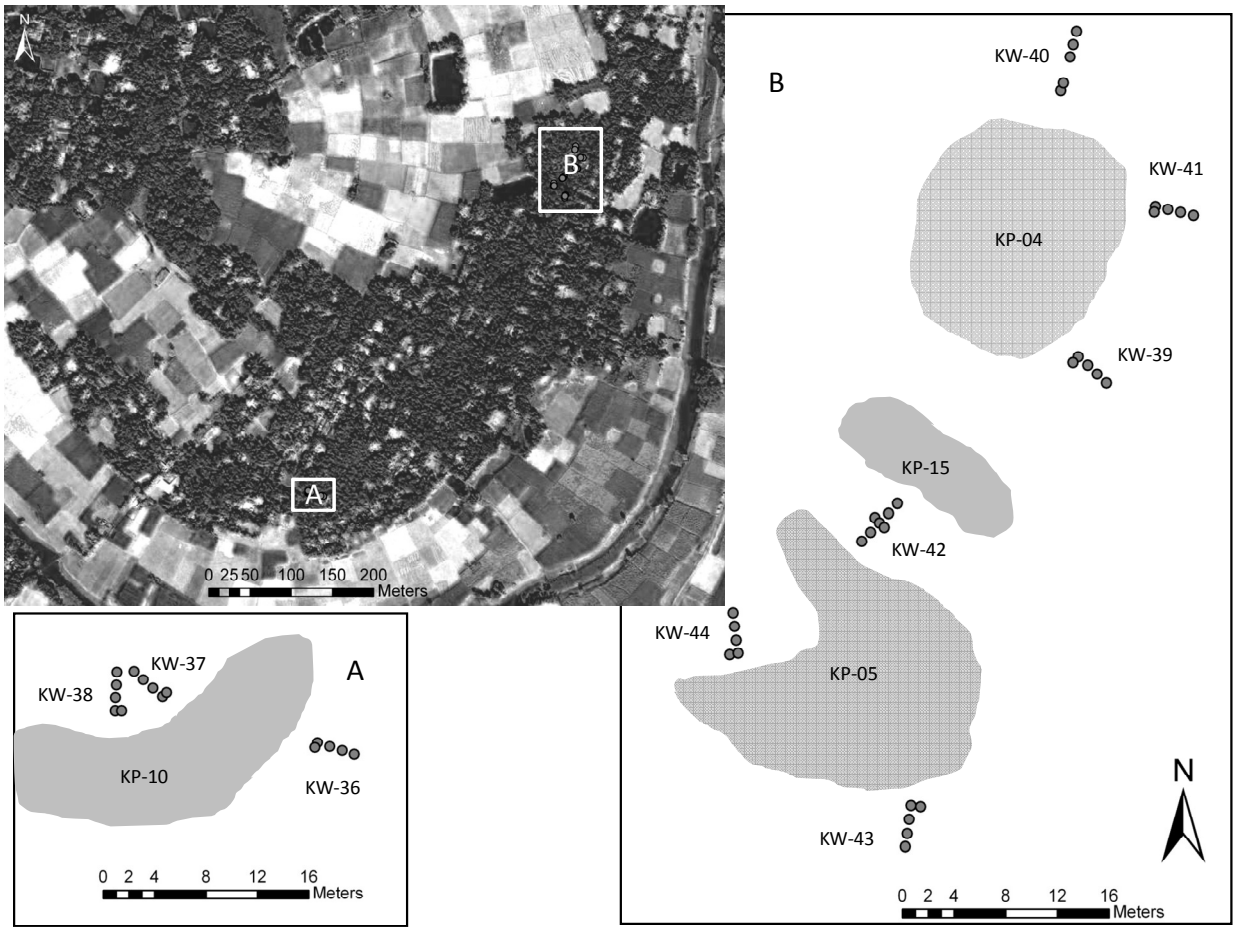


Figure IV-1. Locations of ponds and transects within Site K. Ponds and transect locations (± 0.5 m) are approximate. All shallow wells within a given transect were spaced exactly 1 m apart. Distance of the closest transect well to the pond water edge varied from 1.9 to 5.1 m.

Drill cuttings from each hole were visually logged at 1.5 m intervals. In at least one shallow well per transect, 0.3 m long core samples were collected using manual direct push coring methods with an AMS 424.45 core sampler (AMS, American Falls, Idaho, USA), from 3 m below the surface to the bottom of the borehole. For each 0.3 m core, silt layers were identified at sub-centimeter resolution and dry sieving was performed on the sand component only. Logarithmic interpolation was used to obtain the tenth percentile (d_{10}), median (d_{50}) and sixtieth percentile (d_{60}) grain diameters (Bardet, 1997) and the uniformity coefficient ($U=d_{60}/d_{10}$) was calculated for each core.

Water levels were measured using water level tapes (Dipper-T, Heron Instruments Inc., Burlington, Ontario, Canada) and pressure transducers (Levelogger Model 3001, Solinst Canada Ltd., Georgetown, Ontario, Canda). Manual water level monitoring of all transect wells and ponds was performed at a minimum of once a week from June 11 to July 20, 2009 and once a month through November, 2009 thereafter. Rising head slug tests were performed in triplicate on each well using a pneumatic pressuring device which seals to the top of the well and a pressure transducer. The average Coefficient of Variation for the triplicate measurements made for all wells was 5%. Lateral average linear groundwater velocities were calculated from measured hydraulic gradients and average conductivity of the shallow wells within each transect assuming a porosity of 0.4 (Table IV-1). Vertical velocities were estimated from measured vertical gradients by assuming an anisotropy factor of 10 (K_x/K_z). Anisotropy factors measured on 61 core samples of fluvial and lacustrine sediments in California typically ranged from 2 to 10 (Johnson and Morris, 1962 as cited in Freeze and Cherry, 1979). Precipitation data

was downloaded from the National Climatic Data Center (www7.ncdc.noaa.gov/CDO/cdo) for the time period from June 1 through July 20 from the Dhaka weather station, which is located 25 Km west of Site K. Several missing days were filled in using data recorded by a HOBO Weather Logger (model H21-001, Onset Computer Corporation, Bourne MA) equipped with a rain gauge (model S-RGB-M002, Onset Computer Corporation, Bourne MA) located in the region of Matlab 50 Km south of Site K.

Seasonal Monitoring

Bangladesh experiences a dry season from November through April and a wet season which lasts from May through the end of October, during which the vast majority of annual precipitation occurs. Year-round monthly monitoring for *E. coli* was performed on the closest and furthest shallow wells in eight transects (excluding KW-42) from September 2008 through May 2009. In addition to these eight transects, KW-42 was also monitored monthly from June through the end of October 2009.

The well sampling protocol was as follows. Sixty to one-hundred liters were purged from each well using an electric-powered submersible pump (Typhoon, Groundwater Essentials, LLC), representing approximately three wellbore volumes (Knappett et al., In Press). At the start of sampling a new transect all tubing and pumps were soaked in a cleaning solution consisting of powdered Chlorox (5 g) and TWEEN-80 (5 ml) (T164-500, Fischer Scientific) mixed in 10 L of water from a nearby private well. The cleaning solution was cycled through the tubing for 5 minutes, followed by rinsing with 10 L of private well water containing 5 g of sodium thiosulfate (S446-3, Fisher Scientific) for 2 minutes. Well pumping flow rates varied depending on battery

strength, but were generally between 5 and 10 L/min. Submersible pumps ran continuously while 4 to 8 L of groundwater was filtered through a 0.22 µm nitrocellulose filter (150 ml Vacuum Driven Disposable Filtration System, Stericup, HV Durapore Membrane, Millipore Corp., Bedford, Massachusetts). Filtration times typically varied from 30 to 60 minutes, with the flow rate and filtered volume related to the turbidity of the water.

A six week period of intensive quasi-weekly monitoring (June 11 to July 20) was performed on six transects (KW-36, 37, 39, 41, 42, 43) during the early 2009 monsoon. During the first week of intensive monitoring, sampling was performed under natural gradient flow conditions.

Table IV-2. Volumetric and Microbial Dilution Factors of Ponds after Filling

Pond ID	Filling Date	Pond Level Rise (m)	Initial Volume (m^3)†	Added Volume (m^3)†	Volumetric Dilution Factor	*E. coli* Dilution Factor‡
KP-10	23-Jun	0.16	72	21	0.77	0.01
KP-04	25-Jun	0.16	148	22	0.87	0.82
KP-15	1-Jul	0.63	0	97	NA*	NA
KP-05	27-Jun	0.53	296	32	0.90	0.35

† Estimated from measured pond dimensions
‡ Based on measured E. coli concentrations before and after flooding
* Not Applicable

Field-scale Infiltration Experiment

The second phase of sampling was performed after the ponds were partially filled by introducing groundwater from deep transect wells (~10 m) to simulate a major rainfall event. There is strong evidence for the existence of a fine-grained layer on the base of the ponds, which may inhibit recharge into the aquifer when pond levels are low (Sengupta et al., 2008). During the early monsoon, pond levels had been observed to increase and then decrease by as much as one meter within 24 hours in response to rainfall events. It is possible that during storms water levels can rise above the silt-clogged lower portion of the ponds and then drain rapidly into the adjacent sand aquifer, creating ideal conditions for rapid movement of bacteria into the aquifer. To simulate the condition of monsoon-induced rises in pond level water levels were increased by pumping. These increases in pond levels correspond to estimated additional volumes of water shown in Table IV-2 along with approximate volumetric dilution factors. KP-15 did not have natural standing water during this monitoring period and water from KP-05 was channeled into KP-15 two days after KP-05 had itself been filled.

Lab-scale Experiments

To compare transport at the field scale (~7 m) with the column scale, saturated flow transport experiments were performed in triplicate with sand collected from the base of the pond KP-15 in repacked columns 12 cm long with an inner diameter of 1.9 cm. Glacial Blue microspheres 1 µm in diameter (Bangs Laboratories, Fishers, IN) were chosen as surrogates for microbial contaminants since they were previously shown to be transported very similarly to *E. coli* in similar deltaic sand from Bangladesh (Feighery et al., In Review). The microspheres were

added to the spiked influent solution at a concentration of 10^6 spheres/ml. For the influent solution KCl was added to deionized water to achieve an ionic strength of 3.5 mM, similar to pond water. Bromide was added with the microspheres in the spiked influent solution as KBr (20 mg/L) and the background KCl concentration was reduced to keep ionic strength consistent (+/- 20 µS/cm). Influent and effluent bromide concentrations were monitored using an Orion 9635BNWP ion-selective bromide electrode (Thermo Scientific, Waltham, MA). Bromide was used as a conservative tracer to measure pore flow velocity (v), calculate longitudinal dispersivity (α_x) and to verify consistent packing between columns by fitting the experimental data to a traditional one-dimensional convection-dispersion equation using the software CXTFIT (Toride et al., 1995; Knappett et al., 2008).

After initial upward flow saturation, to ensure air pockets were not present in the sand, 10 pore volumes of a KCl solution was pumped downward followed by 8 pore volumes of spiked influent solution followed by flushing with the KCl solution for 10 pore volumes. A constant flow rate of 1 ml/min was used throughout the experiment. Forty-two samples (3-10 ml) were taken for each trial with a sampling interval of every 10 minutes for the flushing and steady-state breakthrough phases and every 3 minutes during the rising and falling limbs of the breakthrough curve. Enumeration of the microspheres were performed on 0.5 ml aliquots of the samples using a 4-Laser BD LSR-II benchtop flow cytometer (BD Biosciences, San Jose, CA) using an excitation wavelength of 355 nm and a detection wavelength of 450 +/- 25 nm. In all other respects, the experimental method followed was identical to that presented in Feighery

et al. (In Review) for a disturbed, unwashed sand sample, and similar to other published column transport studies (e.g. Zhuang et al., 2004; Knappett et al., 2008).

Chemical and Microbial Measurements

Major cations and trace metals were analyzed from water samples to determine chemical indicators of pond water entering the aquifer. Water samples from five transect (KW-36, 37, 39, 42, and 43) and all four ponds were analyzed, at a minimum, once before and once after artificial pond filling. Twenty mL vials were brought back to Lamont-Doherty Earth Observatory of Columbia University to analyze using ICP-MS. The elements analyzed were the major cations Na, Mg, Si, P, S, K, Ca, Mn, Fe, and the trace metals Ni, As, Mo, Ba, U, Cd, Sb and Pb. Principal components analysis was performed using the software NCSS (version 07.1.14, NCSS, LLC, Kaysville, Utah) on the major cation concentrations to determine whether water chemistry differences existed between shallow and deep transect wells and pond water and whether there were temporal changes.

Two types of detection methods were utilized to measure fecal microorganisms, culture- and molecular-based methods. To measure culturable *E. coli,* the MPN based Colilert™ test kit was used (IDEXX Laboratories, Inc.). Duplicate 100 mL groundwater samples were collected in sterile containers for culturable *E. coli*. For all Colilert assays, lab blanks using bottled water were performed every 30th sample. For pond samples, dilution with bottled water was required with the Colilert assay since the culturable bacteria exceeded the maximum detection limit of 2419 bacteria/100 ml.

To enumerate fecal bacteria genomes, 4 to 8 L of groundwater or approximately 0.2 L of pond water, was filtered through a 0.22 µm nitrocellulose filter (150 ml Vacuum Driven Disposable Filtration System, Stericup, HV Durapore Membrane, Millipore Corp., Bedford, Massachusetts). The filters were removed from the plastic housing, placed in sterile petri plates, frozen and transported on dry ice back to the University of Tennessee. DNA was extracted and purified from the filters using the FastDNA® SPIN for Soil Kit (MP Biomedicals, LLC, Solon, Ohio) following the manufacturer's protocols.

Quantitative PCR was performed to detect *E. coli* and *Bacteroides* using the identical assays and laboratory methods as described in Knappett et al. (In Review). The gene targets for the *E. coli* (herein referred to as *mE. coli*) and *Bacteroides* assays were the 23S rRNA gene and the 16S rRNA gene, respectively (Bernhard and Field, 2000; Layton et al., 2006; Knappett et al., In Press). Data for each sample and assay was calculated as copies/ng of total extracted DNA and then converted to copies/100 ml based on the volume of water filtered. The method detection limit, MDL, was determined to be when the copies of marker DNA was less than 1 copy per ng of extracted DNA. Since the mass of extracted DNA varied between water samples, the marker MDL's varied. However, a geometric mean of 40 copies/100 ml was used as the effective MDL for molecular assays in groundwater. In all pond water samples, gene concentrations exceeded the MDL by several orders of magnitude. The average Coefficient of Variation between the three trials for each sample was 31% for all *mE. coli* and *Bacteroides* assays.

Microbial Transport Modeling

The filtration of bacteria through porous media may be described by the exponential spatial decay equation first proposed by Iwasaki (1937):

Equation 1

$$C(x) = C_{x=0}e^{-\beta x}$$

where *C(x)* is the bacteria concentration at *x* distance from the input source, $C_{x=0}$ is the initial input concentration at the source, and β is the filtration coefficient. In this study the filtration coefficient describes the number of $\log_e$ concentration cycles that are lost per meter of transport through the aquifer. Equation 1 was fit to $\log_e$-standardized concentrations ($C(x)/C_{x=0}$) of each fecal bacteria in groundwater using linear regression to obtain β with 95% CI's.

An exponential temporal decay equation was used to describe bacteria die-off or decay in the aquifer:

Equation 2

$$C(t) = C_{t=0}e^{-kt}$$

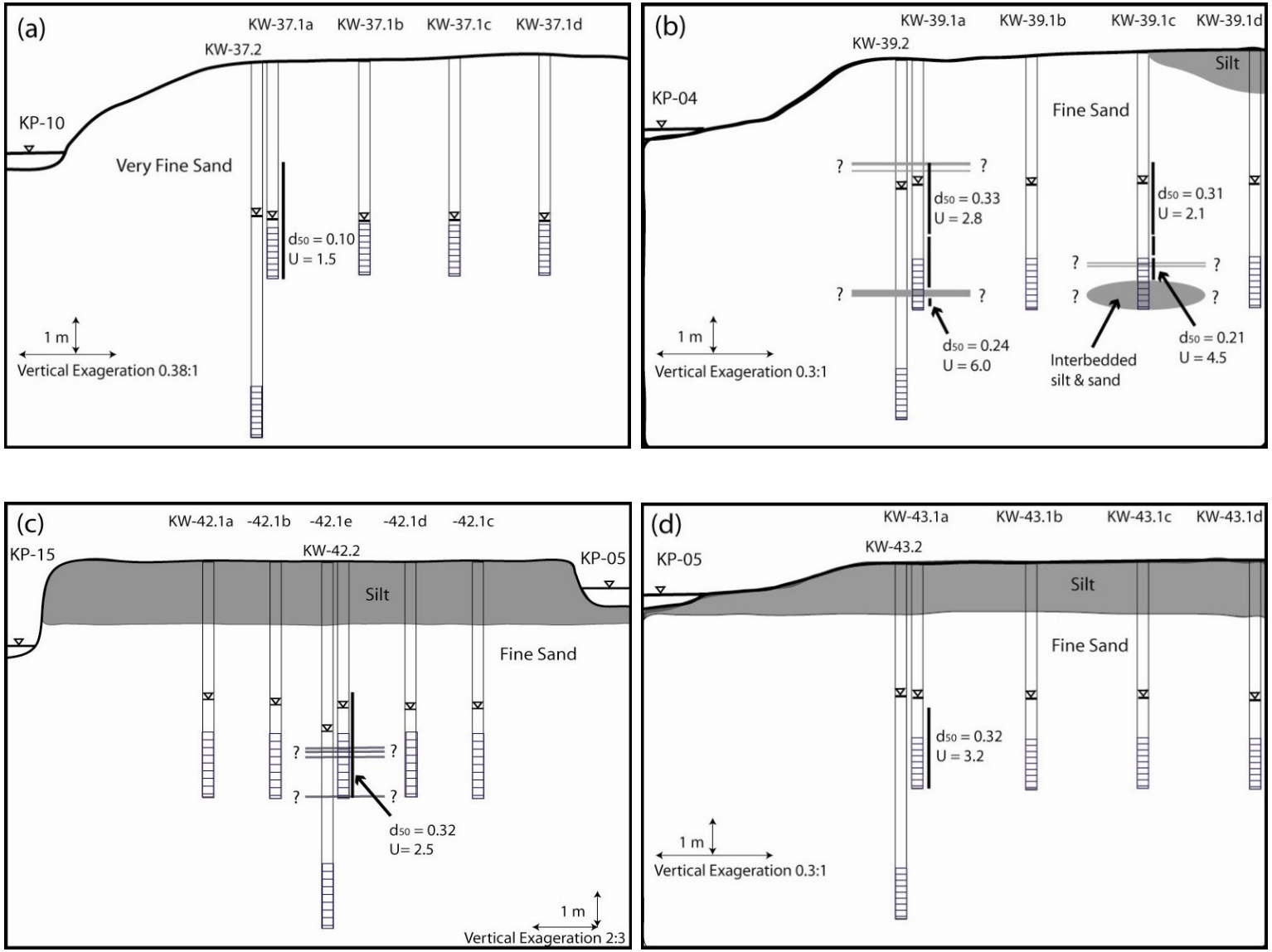
where *C(t)* is bacteria concentration at time *t* from the initial measured concentration $C_{t=0}$. The die-off or decay rate constant is *k*. In the present study *k* represents the number of $\log_e$ cycles of bacterial marker concentrations that are lost per day (Sinton et al., 2002; Bell et al., 2009). Equation 2 was fit to $\log_e$- standardized concentrations ($C(t)/C_{t=0}$) of each fecal bacteria markers in transect KW-39 using linear regression to obtain *k*.

IV.3 Results

Hydrogeology

The locations of the monitoring wells and sediment types for the four transects; KW-37, 39, 42, 43 are displayed in Figure IV-2. Physical properties of the sediments and ponds are shown in Table 1. Transects KW-39, 42 and 43 are located in the northeast corner of Site K (Box B, Fig. IV-1) where the fine sand aquifer is overlain by a 1.5 to 3 m layer of silt (Fig. IV-2b, c, d). With the exception of transect KW-40, where the hydraulic conductivity in the three shallow wells furthest from the pond ranged from 4.4 – 6.2x10^{-5} m/s (Table IV-1), shallow transect wells and deep wells had similar hydraulic conductivities in this area ranging from 1.1 - 4.7x10^{-4} m/s (Table IV-1). Silt layers were encountered in transects KW-39 and 42, as evidenced by a 20 cm thick, laterally continuous layer within the screened interval of the shallow wells in KW-39 (Fig. IV-2b). A fining of the median grain diameter (d_{50}) and an increase in Uniformity Coefficient (U) with depth was observed in each of the cored KW-39 wells (Fig. IV-2b). More silt layers were encountered in the well furthest from the pond (KW-39.1d) than the well closer to the pond (KW-39.1a) indicating a fining to the right (Fig. IV-2b). In the southern part of the village (Box A, Fig. IV-1), the shallow wells were emplaced within a very fine sand aquifer (d_{50} = 0.10 mm, K=2.9 – 8.1x10^{-5} m/s), underlain by a highly conductive aquifer (K=4.0x10^{-4} m/s) (Table IV-1).

Figure IV-2. Geologic cross-sections of Transects KW- 37, 39, 42 and 43. Cored sections of boreholes are indicated by a vertical black bar. Silt is indicated by dark grey shading. The level of the water table and ponds are indicated by the grad symbol.


(a)
KW-37.1a
KW-37.1b
KW-37.1c
KW-37.1d
KW-37.2
KP-10
Very Fine Sand
d50 = 0.10
U = 1.5
1 m
Vertical Exageration 0.38:1
(b)
KW-39.1a
KW-39.1b
KW-39.1c
KW-39.1d
KW-39.2
Silt
KP-04
Fine Sand
d50 = 0.33
U = 2.8
d50 = 0.31
U = 2.1
d50 = 0.24
U = 6.0
Interbedded
silt & sand
d50 = 0.21
U = 4.5
1 m
Vertical Exageration 0.3:1
(c)
KW-42.1a
-42.1b
-42.1e
-42.1d
-42.1c
KP-15
KW-42.2
KP-05
Silt
Fine Sand
d50 = 0.32
U= 2.5
1 m
Vertical Exageration 2:3
(d)
KW-43.1a
KW-43.1b
KW-43.1c
KW-43.1d
KW-43.2
KP-05
Silt
Fine Sand
d50 = 0.32
U = 3.2
1 m
Vertical Exageration 0.3:1

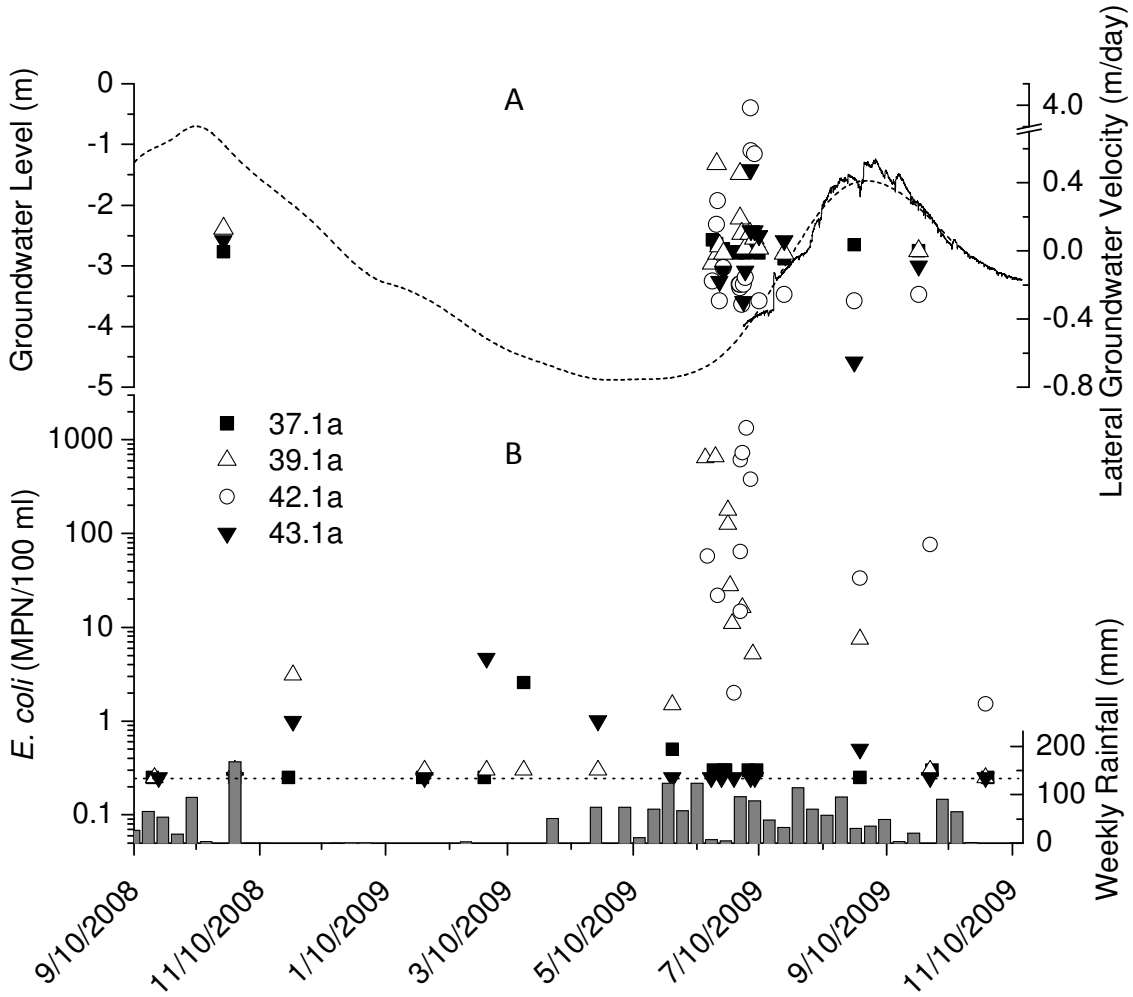


Figure IV-3. Seasonal *E. coli* concentration in the closest well to each pond (dotted horizontal line is the detection limit). Transect KW-42.1a was not monitored until 06/11/09. Weekly precipitation is shown for Matlab (50 Km south of Site K) (Panel B). In Panel A, manual groundwater levels are displayed at Site K (dashed line) from 09/10/08 through 11/11/09 whereas continuous water levels (solid line) were available from 07/10/09 through 11/11/09. Lateral groundwater velocities in each of the four transects are displayed (Panel A) with positive velocity indicating flow away from a pond.

Seasonal Monitoring

Monthly monitoring of the transect wells from September 2008 through May 2009, showed that these wells were largely free of cultured E. coli (Fig. IV-3b). During the 2009 monsoon season, however, substantial concentrations of fecal bacteria were observed in the adjacent aquifer in transects KW-39 and KW-42 prior to artificial pond filling (Fig. A-IV-2). These increases in E. coli were accompanied by the onset of the 2009 monsoon, when the local water table began to rise with an increase in lateral groundwater velocities away from ponds KP-04 and KP-15 (Fig. IV-3a). Water levels were not measured in transect wells between September, 2008 and June, 2009. Later in the 2009 wet season the hydraulic gradients reversed to flow towards the ponds, and E. coli concentrations decreased in the transect wells.

Field-scale Transport Experiments

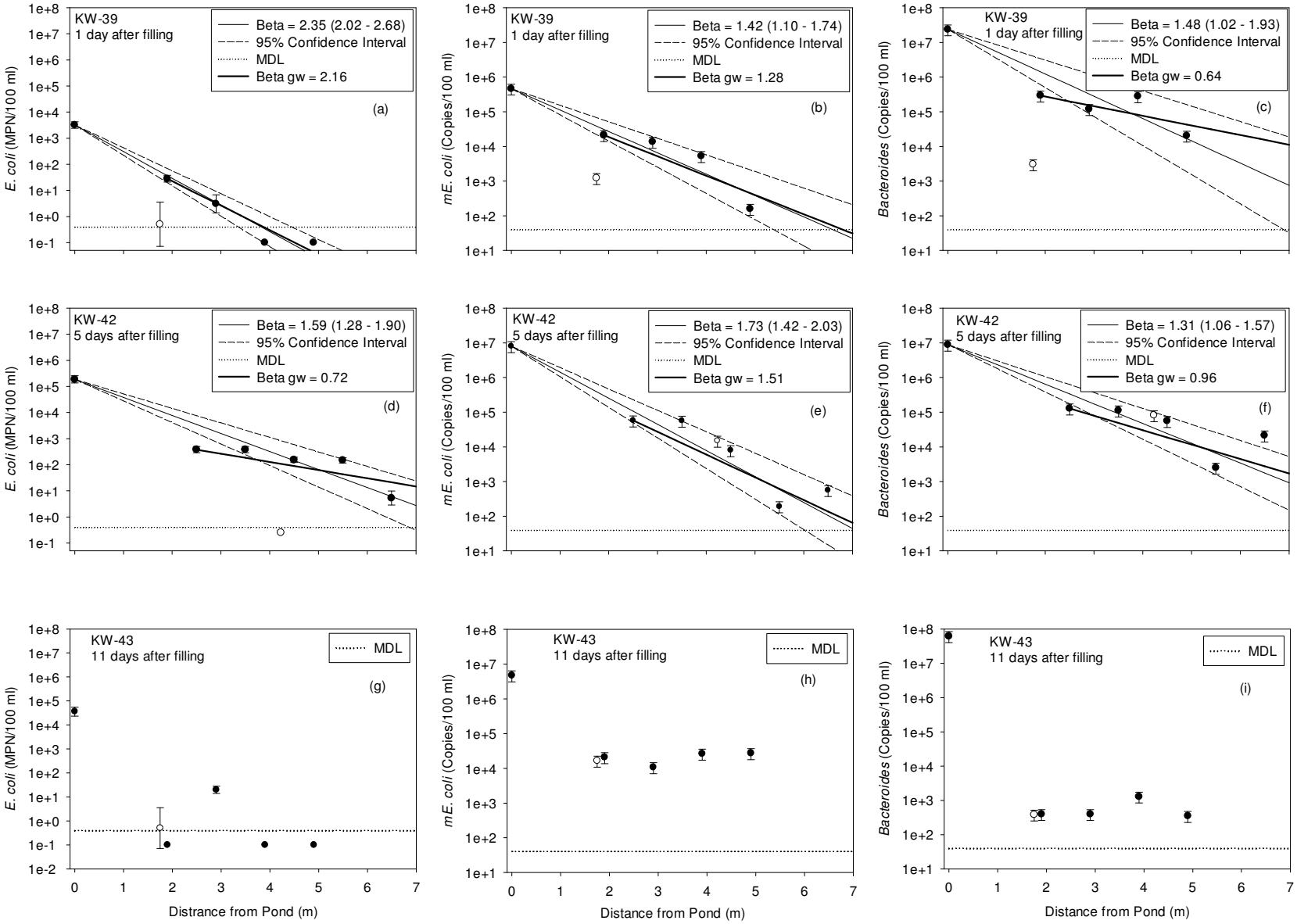
All three latrine ponds in this study (KP-04, KP-05 and KP-10) have high concentrations of cultured *E. coli* throughout the year varying between 10^4 and 10^6 MPN/100 ml (Table A-III-9, A-III-10, A-III-13). *E. coli* concentrations in ponds varied substantially, however, during the experiments when groundwater was pumped into the ponds to raise the water level and increase recharge to the aquifer. Estimated volumetric dilution factors, based on the change in pond water level as a result of pumping for KP-04, KP-05 and KP-10 were 0.87, 0.90 and 0.77, respectively (Table IV-2). Measured dilution factors in *E. coli* concentrations for these same ponds were 0.82, 0.35 and 0.01, respectively (Table IV-2). The measured dilution factor using *E. coli* was very similar to that predicted based on the estimated amount of water added for KP-04. *E. coli* concentrations in KP-05 and KP-10, however, apparently decreased much further

than expected based on estimated volumetric dilution factors (Table IV-2). This is likely due to a lack of vertical mixing in the pond during filling with groundwater resulting in underestimated *E. coli* concentrations. After dilution, pond water *E. coli* concentrations increased within several days after filling with groundwater, following several intense rainfall events.

A decline in concentration of fecal bacteria with distance away from ponds was observed before and after filling during the intense monitoring period (Fig. IV-4). Although filling KP-04 (June 25) produced an increased lateral hydraulic gradient (Fig. IV-5) it did not greatly increase fecal bacteria concentrations in those same transects since they were already contaminated (Fig. A-IV-2). The deep well, KW-39.2, had at least 2-$\log_{10}$ lower concentration of each fecal bacteria marker than the shallow well nearest the pond (KW-39.1a).

The strongest increase in hydraulic gradient and fecal bacteria concentration was observed in transect KW-42 after filling KP-15 with water from KP-05 (Fig. IV-4). Filling KP-15 on July 1 immediately increased lateral flow velocity to 4 m/s (Fig. IV-5). Transect KW-42 is oriented perpendicular to the edges of both KP-05 and KP-15 (Fig. IV-1) and a positive velocity indicates flow away from KP-15. No cultured *E. coli* was detected in the deep well for transect KW-42 either before (Fig. A-IV-2d) or after filling KP-15 (Fig. IV-4d). In contrast high concentrations of both *mE. coli* and *Bacteroides* genes (10^4 and 10^5 copies/100 ml respectively) were found in the KW-42 deep well after filling (Fig. IV-4e, f).

Figure IV-4. Fecal bacteria concentrations in transects KW-39, -42 and -43 with lateral distance from pond KP-04, -15 and -05 respectively. Concentrations in transects KW-39 and -42 increased after filling but were relatively stable thereafter. The results for KW-39 represent one day after filling (June 26) and KW-42 represents five days after filling (July 6). No lateral gradient in fecal bacteria was evident in KW-43. Black filled symbols represent pond and shallow wells, and hollow symbols represent deep wells. The light solid line represents the curve fitted with linear regression using Equation 1. "Beta" indicates the filtration coefficient when pond water is used as the initial concentration ($C_{x=0}$), whereas "Beta gw" indicates the filtration coefficient regressed on the groundwater concentration only (the closest well to the pond becomes $C_{x=0}$).


KW-39
1 day after filling
Beta = 2.35 (2.02 - 2.68)
95% Confidence Interval
MDL
Beta gw = 2.16
(a)
E. coli (MPN/100 ml)
KW-39
1 day after filling
Beta = 1.42 (1.10 - 1.74)
95% Confidence Interval
MDL
Beta gw = 1.28
(b)
mE. coli (Copies/100 ml)
KW-39
1 day after filling
Beta = 1.48 (1.02 - 1.93)
95% Confidence Interval
MDL
Beta gw = 0.64
(c)
Bacteroides (Copies/100 ml)
KW-42
5 days after filling
Beta = 1.59 (1.28 - 1.90)
95% Confidence Interval
MDL
Beta gw = 0.72
(d)
E. coli (MPN/100 ml)
KW-42
5 days after filling
Beta = 1.73 (1.42 - 2.03)
95% Confidence Interval
MDL
Beta gw = 1.51
(e)
mE. coli (Copies/100 ml)
KW-42
5 days after filling
Beta = 1.31 (1.06 - 1.57)
95% Confidence Interval
MDL
Beta gw = 0.96
(f)
Bacteroides (Copies/100 ml)
KW-43
11 days after filling
MDL
(g)
E. coli (MPN/100 ml)
KW-43
11 days after filling
MDL
(h)
mE. coli (Copies/100 ml)
KW-43
11 days after filling
MDL
(i)
Bacteroides (Copies/100 ml)
Distrance from Pond (m)
Distance from Pond (m)
Distance from Pond (m)

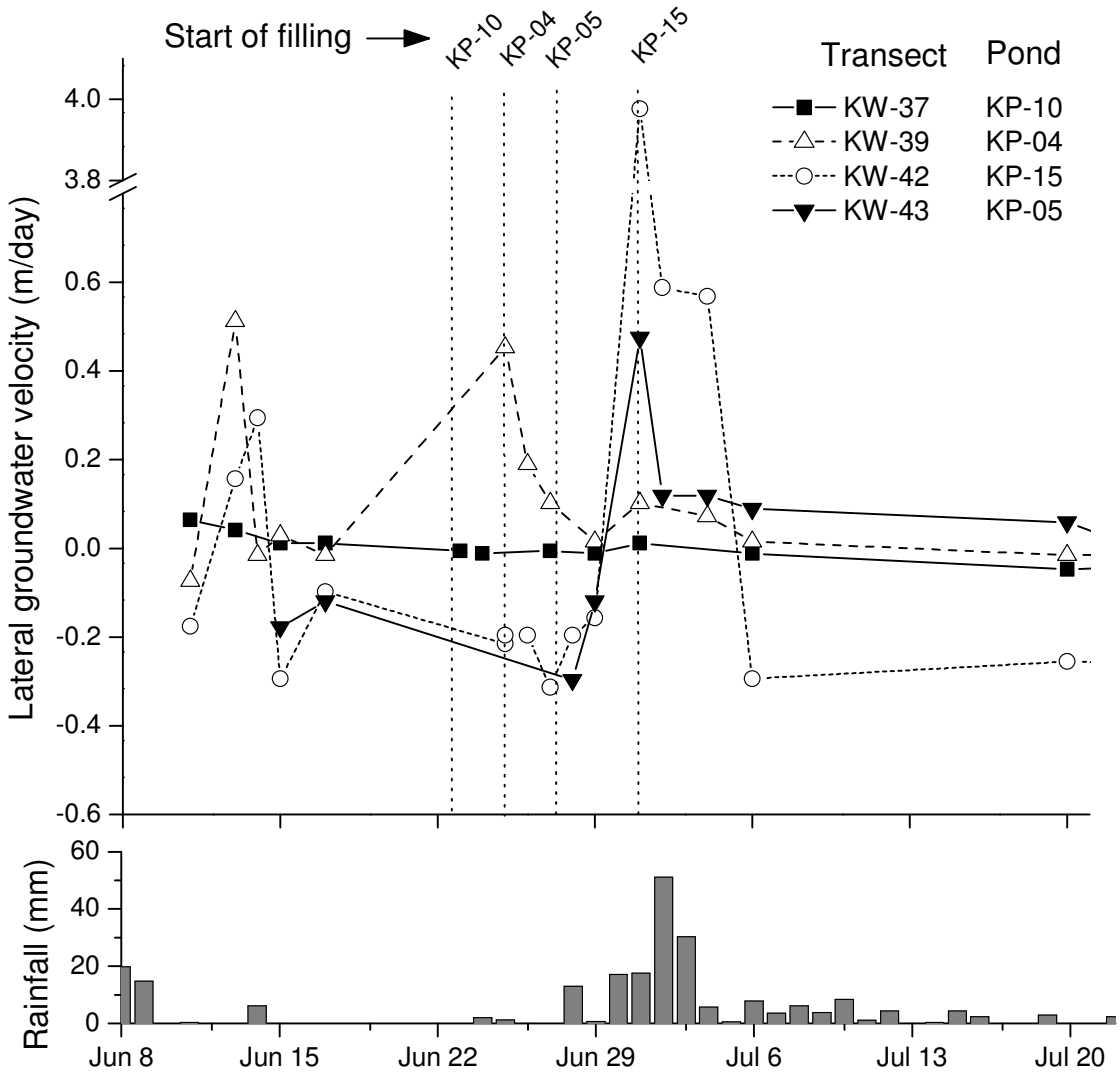


Figure IV-5. Response of lateral groundwater velocities determined by Darcy's law in transects to natural precipitation events and artificial pond filling. Dates of pond filling are indicated with arrows and dashed vertical lines. Precipitation histogram shows daily rainfall for Dhaka 25 Km west of Site K. Lateral average linear groundwater velocities are positive away from each transect's pond indicated in the legend. Transect KW-42 is in between KP-05 and KP-15.

The level of the ponds and the unconfined water table in the four transects several hours after artificial pond filling is shown in Figure IV-2. KP-15 went dry only 24 hours after filling and produced the highest lateral (Fig. IV-5) and vertical (Fig. A-IV-1) gradients of all the monitored transects. An approximately 1 m thick vadose zone was present between the base of the pond and the saturated zone (Fig. IV-2). Groundwater levels rose approximately 30 cm during the period from June 11 to July 20 (Fig. IV-3). The water table rose above the base of each pond during the last week of August 2009 eventually rising to within 1 m of ground surface by the end of September 2009 (Fig. IV-3).

Only two ponds produced increased lateral hydraulic gradients in the adjacent aquifer, with rapid flow occurring in KW-39 and KW-42 immediately after filling (0.5 and 4.0 m/s respectively) (Fig. IV-5). Transects KW-37 and KW-43 did not respond hydraulically to filling of ponds KP-10 and KP-05 respectively (Fig. IV-5), and no hydraulic response was observed in any of the other transects around these ponds. The hydraulic gradient in KW-43 did, however, increase in response to filling KP-15 (Fig. IV-5) approximately 20 m away (Fig. IV-1). Frequent rainfall beginning on June 30th interfered with the assessment of the rate of decline in pond water level after artificial filling.

In transects KW-37 (KP-10) and KW-43 (KP-05) cultured *E. coli* was rarely detected during the intense monitoring period (June 11 to July 20) (Fig. IV-3). Very low concentrations (<100 Copies/100 ml) of *mE. coli* and *Bacteroides* were present in KW-37 (data not shown),

whereas these markers were present well above method detection limits in transect KW-43 (Fig. IV-4). *mE. coli* and *Bacteroides* concentrations were relatively high in KW-43 (>10,000 and ~700 copies/100 ml respectively), however, no lateral concentration gradient was observed (Fig. IV-4h, i). Similar to the neighboring transect KW-42, no decrease in *mE. coli* and *Bacteroides* concentration with depth occurred in KW-43 (Fig. IV-4h, i). This sampling event represents the only time that concentrations of *mE. coli* exceeded *Bacteroides* in this study.

No single cation or trace metal concentration, or linear combination thereof (Principal Components Analysis), indicated pond water recharge into the aquifer. Groundwater was generally high in the cations Ca, Mg, Na, K, Fe and Si with average concentrations of 30, 13, 16, 8, 14 and 23 ppm respectively. Pond water chemistry was more variable between the three ponds KP-10, KP-04 and KP-05 (KP-15 was filled with water from KP-05) than groundwater. Ponds were artificially filled with groundwater from deep transect wells thus changing the pond water chemistry. Before artificial filling, KP-10 and KP-04 were both elevated relative to groundwater in Ca (55 and 42 ppm, respectively) and Mg (19 and 18 ppm, respectively). Further, KP-10 was also elevated relative to groundwater in Na and K (38 and 28 ppm, respectively). All ponds were initially lower in [Fe] (<4 ppm) than groundwater (14 ppm), however pond [Fe] approximately doubled due to groundwater input.

Column Experiments

Triplicate column experiments conducted with 1 µm microspheres using repacked sand taken from the base of KP-15 showed that normalized steady-state breakthrough concentrations (C/C_o) resulted in a measured filtration coefficients (β) ranging from 1.44 to

2.10. These filtration coefficients were calculated using equation 1 by averaging C/C_o across the steady-state portion of the breakthrough curve and setting *x* to 0.12 m. This range of filtration coefficients corresponds to a 6-$\log_{10}$ removal distances of 6.6 to 9.6 m. Fitted velocities (*v*) and longitudinal dispersivities (α_x) to the bromide tracer, using the program CXTFIT (Toride et al., 1995) were estimated to range from 9.5 to 10.3 m/day, and 0.22 to 0.41 m, respectively, indicating consistent packing between replicate columns (Knappett et al., 2008) (Fig. IV-6). Switching influent water from the spiked solution (KCl, KBr and microspheres) to a colloid-free solution (KCl) coincided with an increase in effluent microsphere concentration to approximately two times the influent concentration (Fig. IV-6). This increase was due mostly to the velocity instability or short interruption occurring during the switch of solutions (Zhuang et al., 2007; 2009). The resulting estimated pore water velocities in the columns (~10 m/day) were higher than the measured peak average linear groundwater velocities in transect KW-42 (~4 m/day). The larger velocity observed in the columns than in the field might have resulted in smaller microsphere filtration efficiencies in columns because pore velocity is inversely correlated to filtration efficiency and (Harter et al., 2000; Zhuang et al., 2004).

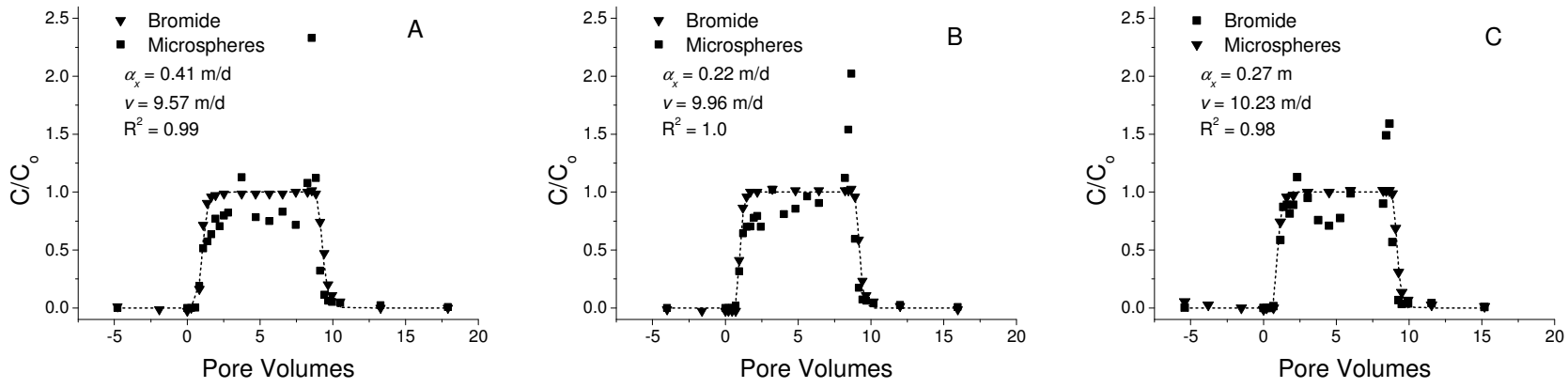


Figure IV-6. Triplicate breakthrough curves of microspheres and Bromide in three different 10 cm columns packed with sand from the base of the pond KP-15. Longitudinal dispersivity (α_x) and velocities were calculated with bromide using the software CXTFIT (Toride et al., 1995).

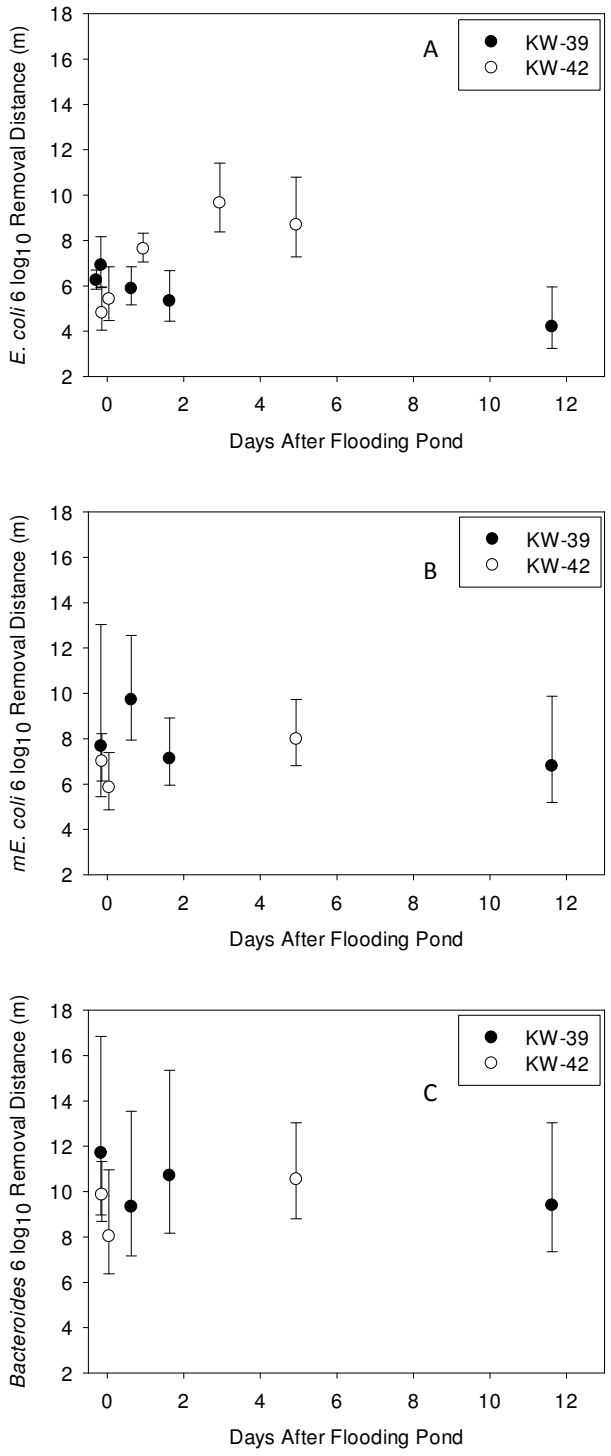


Figure IV-7. Modeled 6-$\log_{10}$ removal distances over time since pond filling for monitoring wells KW-39 and 42, adjacent to ponds KP-04 and KP-15 respectively. Panels A, B and C represent modeled 6-log10 removal distances for *E. coli*, *mE. coli* and *Bacteroides*, respectively.

Microbial Transport Modeling

Modeled filtration coefficients (Equation 1) on aquifer concentrations in KW-39 and -42 of *E. coli*, *mE. coli* and *Bacteroides* (Fig. IV-4) were measured using regression on two different data sets. The first method measured the filtration coefficient along the pathway from the base of the ponds to the aquifer ("Beta"). The second method measured the filtration coefficient along the saturated aquifer flow path only ("Beta gw"). It was found that the filtration coefficient measured along the saturated flow pathway (Beta gw) was always lower than that found along the entire flow path from the pond base through the aquifer (Fig. IV-4). For example, the fitted concentration curve for *E. coli* in along the saturated flow path (Beta gw=0.72) KW-42 shows a more gradual concentration decline than that fitted along the entire flow path (Beta=1.59) (Fig. IV-4d).

Filtration coefficients measured along the entire flow path from pond to aquifer (Beta) were used to calculate expected 6-$\log_{10}$ (99.9999%) removal distances (Fig. IV-7). This is the maximum predicted distance that *E. coli* from a latrine pond (~10^6 MPN/100 ml) will have a measurable impact on the microbial groundwater quality using the Colilert assay with (1 MPN/100 ml detection limit). This estimated distance ranged from 5 to 12 m, with cultured *E. coli* being removed across a shorter distance than *mE. coli* and *Bacteroides*. In transect KW-42 the maximum distance for *E. coli* transport seemed to peak at 10 m after three days of filling. Shorter transport distances were indicated by the data fit to Equation 1 in KW-39 with a maximum 5 m, one day after filling.

Bacterial Persistence Modeling

Bacteria concentrations were measured at three sampling events over 11 days in shallow wells in the transect KW-39 from the day after artificial filling of KP-04 (June 26) through July 6. Decay rate constants (*k*) were derived from linear regression fitted to $\log_e$-transformed normalized concentrations ($C(t)/C_{t=0}$) using Equation 2 (Table IV-3). Decay rate constants were similar for all three markers with k = 0.11, 0.17 (± 0.05) and 0.16 (± 0.03) day^{-1} for *E. coli*, *mE. coli* and *Bacteroides* respectively (Table IV-3). Goodness of fit was high, as indicated by an R^2 of over 0.95 in all but one well/marker (KW-39.1b/*Bacteroides*) where an R^2 of 0.82 was observed. Standard deviations (indicated in parentheses) were based on decay rates derived from the three closest wells to the pond for *mE. coli* and *Bacteroides* whereas only the closest well had high enough initial levels of *E. coli* to measure decay rate. Sampling events on KW-42 were too infrequent, following filling to estimate decay rates.

Table IV-3. Time decay rate constants (*k*) measured at three sampling events over 11 days in transect KW-39 after KP-04 was artificially flooded. Only the well closest to the pond had high enough initial concentrations of *E. coli* to estimate decay rate. Time to <MDL is the predicted number of days required for initial concentrations to fall below the method detection limit. The 6-$\log_{10}$ removal time is the predicted time required for pond water with a concentration of 10^6 MPN /100 ml to be reduced to below the detection limit of the Colilert assay (1 MPN /100 ml).

	k [€]	**Time to < MDL (days)**	**6-$\mathbf{Log_{10}}$ Removal Time (days)**
E. coli	0.11	28	127
mE. coli	0.17 (± 0.05) [†]	34 (± 6)	84 (± 22)
Bacteroides	0.16 (± 0.03)	56 (± 12)	90 (± 19)

[€] *k* is decay constant of $\log_e$ concentration per day

[†] Standard deviation based on decay rates measured in three closest wells to pond

IV.4 Discussion

Bacteria Occurrence and Transport

During the 2009 monsoon, two ponds KP-04 and KP-15 were sources of fecal pollution in the adjacent aquifer under both natural and forced gradient conditions. An explanation of why the other two ponds KP-10 and KP-05 did not contaminate groundwater can be found by examining the geology, age and depth of the pond (Table IV-1, Fig. IV-2). KP-10 is a deep, old pond (>100 yrs), with a well developed layer of organic silt on the bottom and emplaced within a very fine sand aquifer (Fig. IV-2a). This silt layer effectively seals the pond from leaking into the aquifer, even after an extreme simulated rainfall event. These findings agree with Sengupta et al. (2009) who found there was no chemical evidence that pond water mixes with groundwater in West Bengal, India during the dry season. Further, the fine grained aquifer surrounding KP-10 should be an effective filter for microorganisms (d_{50}=0.1 mm) should pond water enter the aquifer (Knappett et al., 2008). Similarly, KP-05 is an old (>30 yrs), shallow pond emplaced within the local 1.5 to 3 m silt layer covering much of the northeast corner of Site K (Fig. IV-2c, d). In contrast KP-04 and KP-15 penetrated the surficial silt in the northeast of Site K, exposing the sandy aquifer below to rapid infiltration (Fig. IV-2b, c). KP-15 was being actively excavated until the day of filling, precluding the possibility of an organic silt layer that would filter out bacteria before they entered the subsurface environment.

According to the filtration coefficients determined from *in situ* measurements along the entire flow path from pond to aquifer (Fig. IV-4), 6-$\log_{10}$ removal of bacteria occurs within 10 m for *E. coli* and *mE. coli* and within 12 m for *Bacteroides* from KW-42 (Fig. IV-7). Longer transport

distances would be predicted based on the filtration coefficients measured along the saturated flow path only, however these are not reported here for simplicity. The shorter 6-$\log_{10}$ removal distance for *E. coli* in KW-39 (5 m) likely results from an increasing amount of silt away from the pond (Fig. IV-2b). This difference in 6-$\log_{10}$ removal distances was not observed between KW-42 and KW-39 for molecular *E. coli* and *Bacteroides*, indicating that total bacterial gene populations may be transported further in the form of shrunken non-culturable bacteria than cultured bacteria particles (Foppen et al., 2007). Further, high concentrations of *mE. coli* (~10,000 copies/100 ml) and *Bacteroides* (~500 copies/100 ml) detected in KW-43 suggest that a substantial proportion of fecal bacteria genes in the aquifer are being transported beyond 12 m from a source.

In contrast to the overestimation of filtration efficiency found in other studies (Schijven et al., 1998; Foppen et al., 2008), *ex situ* filtration coefficients for 1 µm microspheres in 12 cm repacked columns of sand collected from the base of KP-15 were very similar to those measured in the aquifer for fecal bacteria. This suggests that the results of these bench-scale measurements may be predictive of field filtration within the aquifer under KP-15.

During the early monsoon, contaminated pond water must pass vertically through an unsaturated zone before entering the saturated aquifer (Fig. IV-2). Although bacterial filtration in unsaturated porous media is more efficient than in saturated media, as indicated by the larger filtration coefficients measured along the entire flow path from ponds to aquifer than those measured only in the aquifer (Fig. IV-4), bacterial filtration is highly affected by percent saturation (DeNovio et al., 2004). Removal of bacteria in the sediment adjacent to ponds would

potentially be less efficient when the water table rises above the base of the ponds and a direct saturated hydraulic connection between the pond and the aquifer exists. When the groundwater table rose above the base of the pond in late August, however, *E. coli* concentrations later in the wet season (Aug, Sept, Oct, Nov) were far lower than that measured in KW-39 and KW-42 in the early monsoon (Fig. IV-3). This can be explained by: 1) the vertical distance between the base of the pond and the water table during the early monsoon creates ideal conditions for rapid downward flow of contaminated water when it rains, and 2) lateral gradients reverse from the early monsoon, and flow towards the ponds during the later wet season. In temperate climates a thick vadose zone is considered essential protection from fecal pathogens. This study however, shows that monsoonal rainfall events causing rapid flow through the vadose zone increase the threat to microbial quality of shallow aquifers, than when an unsaturated zone is absent under the ponds. As indicated by the peak concentration of microspheres (Fig. IV-6) resulting from the flow interruption or associated change in flow velocity when solution was switched in the column experiments, fluctuation of water table facilitated the remobilization of bacteria retained in the vadose zone between the bottom of pond and the pre-monsoon water table.

Once high concentrations of fecal bacteria enter the aquifer, in this system at 26˚C, they may persist for an estimated 28 (*E. coli*) to 56 days (*Bacteroides*), corresponding to decay rates (*k*) of 0.11 and 0.16 day^{-1} for *E. coli* and *Bacteroides,* respectively (Table IV-2). Foppen et al. (2008) found similar decay rates of cultured *E. coli* in groundwater at 20˚C of 0.15 log_e day^{-1}. Sinton et al. (2002), however, found a higher *E. coli* decay rate of 0.55 day^{-1} in unfiltered surface

water at 14˚C. Bell et al. (2009) found that *Bacteroides* concentration decreased approximately 0.81 day^{-1} in unfiltered (aerobic) surface water at 25 ˚C. Presumably the higher *E. coli* and *Bacteroides* decay rates in surface water were due to processes less active in groundwater, such as exposure to oxygen and predation.

This study implicates ponds as seasonal sources of fecal contamination to shallow aquifers in Bangladesh with transport of *E. coli* from ponds potentially accounting for the broad distribution of *E. coli* observed at Site K (van Geen et al., In Preparation) and in other sandy village sites in Bangladesh (Leber et al., 2010). Typically during the end of the monsoon (Aug-Sept), substantial areas of Site K are inundated by surface water from local precipitation and the nearby river, and this also may result in broad distribution of fecal contamination sources. Since latrines are ubiquitous in rural villages in Bangladesh, improved sanitation would greatly improve the microbial drinking water quality of shallow aquifers (Knappett et al., In Review). In Bangladesh drinking water wells are frequently installed next to latrines and ponds, which serve as a place to hold feces overflowing from leaky (unsanitary) latrines, due to the necessity of obtaining clean water for hygiene. Without detailed geologic information to identify ponds with high bacterial transport potential, every pond represents a potential point source in fecal contamination. This study indicates that installing drinking water wells further away from ponds, ideally at least 12 m away, would greatly decrease the risk of consuming fecal bacteria from the contaminated aquifer.

REFERENCES

Bardet, J. *Experimental Soil Mechanics*, Prentice-Hall, Inc. Upper Saddle River, New Jersey, USA, 1997.

Bell, A.; Layton, A. C.; McKay, L.; Williams, D.; Gentry, R.; Sayler, G. S. Factors influencing the persistence of fecal *Bacteroides* in stream water. *J. Environ. Quality* **2009** *38*(3), 1224-1232.

Bernhard, A.E.; Field, K.G. Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. *Applied and Environmental Microbiology* **2000**, *66*(4), 1587-1594.

Bradford, S. A; Simunek, M. B.; van Genuchten, M. T.; Yates, S. R. Modeling Colloid Attachment, Straining, and Exclusion in Saturated Porous Media. *Environmental Science & Technology* **2003**, *37*(10), 2242-2250.

DeNovio, N. M.; Saiers, J. E.; Ryan, J. N. Colloid Movement in Unsaturated Porous Media: Recent Advances and Future Directions. *Vadose Zone Journal* **2004**, *3*(2), 338-351.

DuPont, H.L., Chapell, C.L., Sterling, C.R., Okhuysen, P.C., Rose, J.B., Jakubowski, W. Infectivity of *Cryptosporidium parvum* in healthy volunteers. *New Engl. J. Med.* **1995**, *332*(13), 855-859.

Emch, M. Diarrheal disease risk in Matlab, Bangladesh. *Social Science & Medicine* **1999**, *49*(4), 519-530.

Esrey, S. A. Water, Waste, and Well-Being: A Multicountry Study. *Am. J. Epiemiol*. **1996**, *143*(6), 608-623.

Feighery, J.; Ferguson, A. S.; Mailloux, B. J.; Ahmed, K. M.; van Geen, A.; Culligan, P. J. Filtration of *E. coli* and Microspheres in Sand Aquifers: Intact versus Re-packed cores. *Ground Water*, In Review.

Foppen, J. W. A.; van Herwerden, M.; Kebtie, M.; Noman, A.; Schijven, J. F.; Stuyfzand, P. J.; Uhlenbrook, S. Transport of Escherichia coli and solutes during waste water infiltration in an urban alluvial aquifer. *Journal of Contaminant Hydrology* **2008**, *95*(1-2), 1-16.

Foppen, J.W.; van Herwerden, M.; Schijven, J. Transport of Escherichia coli in saturated porous media: Dual mode deposition and intra-population heterogeneity. *Water Research* **2007** *41*(8), 1743-1753.

Freeze, R. A.; Cherry, J. A. *Groundwater*; Prentice Hall: Englewood Cliffs, NJ, USA, 1979.

Gale, P. Developments in microbiological risk assessment for drinking water. *J. Appl. Microbiol.* **2001** *91*(2), 191-205.

Harter, T.; Wagner, S.; Atwill, E. R. Colloid Transport and Filtration of Cryptosporidium parvum in Sandy Soils and Aquifer Sediments. *Environmental Science & Technology* **2000**, *34*(1), 62-70.

Harvey, R. W.; George, L. H.; Smith, R. L.; LeBlanc, D. R. Transport of Microspheres and Indigenous Bacteria through a Sandy Aquifer: Results of Natural and Forced-Gradient Tracer Experiments. *Environmental Science & Technology* **1989**, *23*(1), 51-56.

Horneman, A.; van Geen, A.; Kent, D. V.; Mathe, P. E.; Zheng, Y.; Dhar, R. K.; O'Connell, S.; Hoque, M. A.; Aziz, Z.; Shamsudduha, M.; Seddique, A. A.; Ahmed, K. M. Decoupling of As and Fe release to Bangladesh groundwater under reducing conditions. Part I: Evidence from sediment profiles. *Geochimica et Cosmochimica Acta* **2004,** *68*(17), 3459-3473.

Iwasaki, T. Some notes on sand filtration. *Journal of AWWA* **1937**, *29*, 1591-1597.

Kildare, B. J.; Leutenegger, C. M.; McSwain, B. S.; Bambic, D. G.; Rajal, V. B.; Wuertz, S. 16S rRNA-based assays for quantitative detection of universal, human-, cow- and dog-specific fecal Bacteroidales: A Bayesian approach. *Water Research* **2007**, *41*(16), 3701-3715.

Knappett, P. S. K.; Emelko, M. B.; Zhuang, J.; McKay, L. D. Transport and retention of a bacteriophage and microspheres in saturated, angular porous media: Effects of ionic strength and grain size. *Water Research* **2008** *42*(16), 4368-4378.

Knappett, P. S. K.; Layton, A.; McKay, L. D.; Williams, D.; Mailloux, B. J.; Huq, Md. R.; Alam, Md. J.; Ahmed, K. M.; Akita, Y.; Serre, M. L.; Sayler, G. S.; van Geen, A. Efficacy of hollow-fiber ultrafiltration for microbial sampling in groundwater. *Ground Water*, In Press, 2010.

Knappett, P. S. K.; Escamilla, V.; Layton, A.; McKay, L. D.; Emch, M.; Williams, D. E.; Huq, Md. R.; Alam, Md. J.; Farhana, L.; Mailloux, B. J.; Ferguson, A.; Sayler, G. S.; Ahmed, K. M.; van Geen, A. The impact of sanitation on fecal bacteria and pathogens in village ponds in Bangladesh. *Environmental Science & Technology*, In Review.

Layton, A.; McKay, L. D.; Williams, D.; Garrett, V.; Gentry, R.; Sayler, G. Development of *Bacteroides* 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water. *Applied & Environmental Microbiology* **2006** *72*(6), 4214-4224.

Leber, J.; Rahman, M. M.; Ahmed, K. M.; Mailloux, B.; van Geen, A. Contrasting Influence of Geology on *E. coli* and Arsenic in Aquifers of Bangladesh. *Ground Water* **2010**, DOI: 10.1111/j.1745-6584.2010.00689.x.

Li, X; Lin, C.; Miller, J. D.; Johnson, W. P. Role of Grain-to-Grain Contacts on Profiles of Retained Colloids in Porous Media in the Presence of an Energy Barrier to Deposition. *Environmental Science & Technology* **2006**, *40*(12), 3769-3774.

Noble, R. T.; Griffith, J. F.; Blackwood, A. D.; Fuhrman, J. A.; Gregory, J. B.; Hernandez, X.; Liang, X.; Bera, A. A.; Schiff, K. Multitiered approach using quantitative PCR to track sources of fecal pollution affecting Santa Monica bay, California. *Applied & Environmental Microbiology* **2006**, *72*(2), 1604-1612.

Pruss, A.; Kay, D.; Fewtrell, L.; Bartram, J. Estimating the Burden of Disease from Water, Sanitation, and Hygiene at a Global Level. *Environmental Health Perspectives* **2002**, *110*(5), 537-542.

Radloff, K. A.; Cheng, Z; Rahman, M. W.; Ahmed, K. M.; Mailloux, B. J.; Juhl, A. R.; Schlosser, P.; van Geen, A. Mobilization of arsenic during one-year incubations of grey aquifer sands from Araihazar, Bangladesh. *Environmental Science & Technology* **2007** *41*(10), 3639-3645.

Rudolph, D. L.; Barry, D. A. J.; Goss, M. J. Contamination in Ontario farmstead domestic wells and its association with agriculture: 2. Results from multilevel monitoring well installations. *Journal of Contaminant Hydrology* **1998**, *32*(3-4), 295-311.

Schijven, J. F.; Medema, G.; Vogelaar, A J.; Hassanizadeh, S. M. Removal of microorganisms by deep well injection. *Journal of Contaminant Hydrology* **2000**, *44*(3-4), 301-327.

Scott, T. M.; Rose, J. B.; Jenkins, T. M.; Farrah, S. R.; Lukasik, J. Microbial source tracking: current methodology and future directions. *Applied and Environmental Microbiology* **2002**, *68*(2), 5796-5803.

Sengupta, S.; McArthur, J. M.; Sarkar, A.; Leng, M. J.; Ravenscroft, P.; Howarth, R. J. Banerjee, D. M. Do ponds cause Arsenic-Pollution of groundwater in the Bengal Basin? An answer from West Bengal. *Environmental Science & Technology* **2008**, *42*(14), 5156-5164.

Sinton, L. W.; Hall, C. H.; Lynch, P. A.; Davies-Colley, R. J. Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saline waters. Applied and Environmental Microbiology, **2002**, *68*(3), 1122-1131.

Taylor, R.; Cronin, A.; Pedley, S.; Barker, J.; Atkinson, T. The implications of groundwater velocity variations on microbial transport and wellhead protection - review of field evidence. *Fems Microbiology Ecology* **2004** *49*(1), 17-26.

Toride, N., Leij, F.J., van Genuchten, M.Th. The CXTFIT Code for Estimating Transport Parameters from Laboratory or Field Tracer Experiments, Version 2.0. Research Report No. 137, U.S. Salinity Laboratory, USDA, ARS, Riverside, California, 1995.

van Geen, A.; Ahmed, K. M.; Akita, Y.; Alam, Md. J.; Culligan, P. J.; Feighery, J.; Ferguson, A.; Emch, M.; Escamilla, V.; Knappett, P.; Layton, A. C.; Mailloux, B. J.; McKay, L. D.; Mey, J. L.; Serre, M. L.; Streatfield, P. K.; Wu, J.; Yunus; Md. Seasonal contamination of shallow tubewells in Bangladesh with the fecal indicator *E. coli*: Impacts of population density and hydrogeology. In Preparation for *Environmental Science & Technology*.

United States Geological Survey, National Water-Quality Assessment Program Principal Aquifers, S. S. Embrey and D. L. Runkle. Microbial Quality of the Nation's Ground-Water Resources, 1993-2004, Scientific Investigations Report 2006-5290, U.S. Department of the Interior, 2006.

Yates, M. V. 2007. Classical indicators in the 21st century – Far and beyond the coliform. *Water Environment Research* **2007**, *79*(3), 279-286.

Zhuang, J.; Jin, Y.; Flury, M. Comparison of Hanford Colloids and Kaolinite Transport in Porous Media. *Vadose Zone Journal* **2004**, *3*, 395-402.

Zhuang, J.; McCarthy, J.F.; Tyner, J.S.; Perfect, E.; Flury, M. In Situ Colloid Mobilization in Hanford Sediments under Unsaturated Transient Flow Conditions: Effect of Irrigation Pattern. *Environmental Science & Technology* **2007**, *41*, 3199-3204.

Zhuang, J.; Tyner, J.S.; Perfect, E. Colloid transport and remobilization in porous media during infiltration and drainage. *Journal of Hydrology* **2009**, *377*, 112-119.

CHAPTER V - CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Two years of monthly *E. coli* monitoring from over fifty-five wells throughout the sandy village of Char Para (Site K) revealed extensive fecal contamination of private wells with 30 to 70% of wells testing positive for *E. coli*. The installation and monthly sampling of sealed monitoring wells confirmed that the shallow aquifer, in which private wells are emplaced, is broadly contaminated, although sealed monitoring wells tended to have less contamination than unsealed private wells (Appendix V). This may be partly due to differences in usage of the private wells which are pumped many times daily, compared to the monitoring wells which are typically sampled once a month. It was demonstrated that widespread unsanitary latrines, leaking effluent onto the open ground, contaminate ponds and create point sources of fecal contamination to the aquifers. Using molecular fecal source tracking and GIS-based comparisons of fecal bacteria concentrations to human population density in the vicinity of the ponds, this fecal contamination was shown to be primarily human in origin. Some of these ponds were shown to discharge fecal bacteria into the ground during the early monsoon with *E. coli* and *Bacteroides* moving up to 12 m into the adjacent aquifer. Since the bacteria may persist in oligotrophic groundwater for months, pond discharge may explain much of the widespread fecal contamination in the shallow aquifer during the wet season. Standing water throughout the village at the end of the wet season, however, especially in the vicinity of private wells without seals, may contribute to the vertical movement of bacteria along the annuli of private wells or through the thin vadose zone and into the saturated aquifer.

To the author's knowledge, this dissertation represents the first time that culture-based and molecular-based (DNA) measurements were performed concurrently to evaluate the

transport of fecal bacteria through an aquifer. Molecular-based methods indicated more widespread fecal contamination, laterally and vertically, in the sandy aquifer than that indicated by cultured *E. coli* alone.

The broader scientific implications of these studies are that, in contrast to the results of many previously published column experiments which suggest that bacterial removal in fine sand (d_{50} = 0.2 - 0.3 mm) should be rapid over short distances, fecal bacteria were shown to move substantial distances through fine deltaic sand aquifers in Bangladesh. Therefore, the hypothesis that switching to low Arsenic wells may increase exposure to waterborne pathogens due to the placement of these wells in areas of the aquifer with rapid recharge rates (Leber et al., 2010; van Geen et al., In Prep), seems plausible. The possibility that bacteria are also moving along the outer annuli of private wells during the wet season may result in relatively high concentrations of *E. coli* even in private wells that are overlain by silt deposits due to preferential transport along macropores.

It remains unclear what impact the observed concentrations of culturable *E. coli* have on the health of Bangladeshi people, since: 1) *E. coli* is only a fecal indicator bacteria and not a pathogen; and 2) microbial drinking water quality is only one of several important factors, including sanitation and hygiene, in lowering diarrheal disease. It is even less clear how to interpret observed concentrations of *E. coli* and *Bacteroides* genomes, since molecular fecal bacteria enumeration methods have yet to be tested in epidemiologic, dose-response studies. Ongoing public health studies in the region of Matlab where *E. coli* detection prevalence in

tubewells as well as pathogens are being compared to household diarrheal disease rates, will address this.

Although fecal bacteria were observed to move through the shallow aquifer it is still unclear which environmental source of human feces (latrines v. ponds) and pathways (saturated zone, vadose zone, annulus of tubewells) predominantly impact fecal contamination of private tubewells. Sealed monitoring wells which were only pumped once a month demonstrated the presence of *E. coli* in the aquifer, but these wells tended to contain detectable levels of *E. coli* less frequently than in private wells, leaving open the possibility that annular flow along the outside of private well casings and/or frequent pumping are the primary pathway for contamination. Several simple experiments would help address these questions:

- To test the hypothesis that *flow along the outside of private well casings are responsible for contamination*, several private wells could be installed with proper seals from the ground surface down to the top of the screened interval next to existing private wells (unsealed). Weekly monitoring could be performed on 10 sealed wells and 10 paired, unsealed private wells from the early monsoon (May) through late monsoon (August). Households will be encouraged to use both sources of water to keep daily pumped volumes approximately equal. If *E. coli* is detected more frequently in the unsealed wells than the sealed wells, then the outer casing of private wells will be implicated as pathways.
    - Two experiments could be performed to test the hypothesis that *contamination sources are local and infiltrate vertically through the vadose zone*. Five sealed private

wells could be installed with an above ground simple sprinkler system which simulates monsoonal rainfalls in the dry season. If the simulated local (<5 m radius) rainfall causes *E. coli* to increase in the wells to levels observed in the same wells during the monsoon, it will show that local, vertical infiltration is an important transport pathway. A second, complimentary experiment is the use of an impermeable plastic sheet buried under the soil (10 cm depth) within a radius of 5 m surrounding five sealed private wells. These wells should be monitored for *E. coli* during the wet season and *E. coli* concentrations and prevalence should be statistically compared to sealed wells without an impermeable soil cover. If the wells with impermeable soil covers are less contaminated during the early monsoon, than neighboring wells (or than the same wells the year before), this would demonstrate the impact of vertical transport from local sources on the levels of *E. coli* in the aquifer.

In a culture where latrines, drinking water wells and ponds are clustered together closely out of convenience or necessity to maintain hygiene, these findings suggest that ongoing improvements in the management of human fecal waste and improved placement and construction of private wells may substantially reduce human exposure to waterborne pathogens. Since broad fecal contamination exists in unconfined, shallow aquifers the gains from the leading Arsenic mitigation option of well switching need to be carefully balanced with the risk of increased consumption of waterborne pathogens.

APPENDICES

APPENDIX II - ULTRAFILTRATION

Table A-II-1. Physical and chemical parameters and microbial concentrations in nine wells where ultrafiltration was performed.

Well ID (sample date)	EDTA Added (Yes/No)	Fe (ppm)	Temperature (C°)	Electrical Conductivity (mS/cm)	Dissolved Oxygen (ppm)	pH	ORP‡	S (ppm)	*E. coli* (MPN/100 ml)	Total Coliforms (MPN/100 ml)	*mE. coli* (copies/ 100 ml)	*Bacteroides* (copies/100 ml)	Adenovirus (copies/100 ml)
KW-12.1	No	0.5	26.1	0.298	0.2	6.5	NR†	13	190	67684	3,555,776	5,170,656	49,886
KW-24	No	3.5	25.3	0.218	0.3	6.4	NR	6	6350	17258	1,016,760	6,848,717	39,318
KW-25	No	10	26.1	0.957	0.3	6.3	57	26	101	10864	1,776,242	6,207,012	10,889
KW-30	No	8.8	25.8	0.461	0.5	6.6	42	19	6535	655473	14,645,632	4,766,286	24,774,500
UTK-1	No	0.6	25.7	0.540	1.0	7.2	112	29	<1	12597	577,404	2,998,392	183,746
UTK-30	No	9	27.4	0.440	0.8	6.7	-33	21	<1	2036	37,442	1,486,628	103,929
UTK-31	No	7.6	26.1	0.355	0.8	6.5	-22	19	<1	219	158,886	1,020,115	169,937
UTK-7 (Jun 12)	No	6.2	25.8	0.572	0.5	6.4	59	16	508	23825	1,556,360	2,379,136	634,109
UTK-8	No	3.8	25.4	0.446	1.1	7.2	98	9	218	35649	613,076	5,578,944	55,437
KW-12.1	Yes	0.5	26.1	0.298	0.2	6.5	NR	13	174	11307	336,577	381,460	317,580
KW-24	Yes	3.5	25.3	0.218	0.3	6.4	NR	6	7931	22019	PCR Inhibition		
KW-25	Yes	10	26.1	0.957	0.3	6.3	57	26	13	13387	<72,000	1,343,211	823,996
KW-30	Yes	8.8	25.8	0.461	0.5	6.6	42	19	6982	382850	13,055,710	715,005	11,006,345
UTK-1	Yes	0.6	25.7	0.540	1.0	7.2	112	29	136	14368	1,941,221	3,083,056	6,214,853
UTK-30	Yes	9	27.4	0.440	0.8	6.7	-33	21	<1	6567	197,361	11,934,418	<14,400
UTK-31	Yes	7.6	26.1	0.355	0.8	6.5	-22	19	362	23946	1,816,098	18,173,099	423,083
UTK-7 (Jun 12)	Yes	6.2	25.8	0.572	0.5	6.4	59	16	146	16483	325,685	<24,000	280,694
UTK-7 (Jun 11)	Yes	6.2	25.8	0.572	0.5	6.4	59	16	586	<1	215,839	<58,437	<58,437
UTK-8	Yes	3.8	25.4	0.446	1.1	7.2	98	9	87	7119	291,986	1,205,220	447,185

‡ Oxidative Reductive Potential

† Not Reported

APPENDIX III – POND MICROBIOLOGY

Table A-III-1. Information recorded on each surveyed pond.

Field Observation	**Units**
Village-wide Survey	
Household location	lat/long degrees
Household population	count
Latrine location	lat/long degrees
Latrine type	sanitary/unsanitary
Pond location	lat/long degrees
Pond owner's name	-
Information Collected Within Each Pond Basin	
Deepest Depth of Pond	m
Long axis of pond	m
Short axis of pond	m
Designated Purpose	latrine/fishing/bathing
Number of Sanitary Latrines	count
Number of Unsanitary Latrines	count
Number of Cows	count
pH	-
Temperature	˚C
Electrical Conductivity	µs/cm
Dissolved Oxygen	mg/L

Table A-III-2. Real-time PCR assays used to detect *E. coli*, and *Bacteroides* rRNA genes and the Adenovirus hexon gene, the primers and probe used for each assay, and the annealing temperature used for each assay.

Assay name (target organism)	**Primer/probe name and sequence (5'–3')**	**Size (bp) of product**	**Annealing temp (°C)**	**Reference**
EC23S (*E. coli*)	EC23Sf, 5' GAG CCT GAA TCA GTG TGT GTG 3' EC23Sr, 5' ATT TTT GTG TAC GGG GCT GT 3' EC23Srv1bhq, 5' -(FAM)CGC CTT TCC AGA CGC TTC CAC (BHQ-1)- 3'	78	55	Modified from (*25*), (*26*)
AllBac (all *Bacteroides*)	AllBac296f, 5'-GAGAGGAAGGTCCCCCAC-3' AllBac412r, 5'-CGCTACTTGGCTGGTTCAG-3' AllBac375Bhqr, 5'-(FAM)CCATTGACCAATATTCCTCACTGCTGCCT(BHQ-1)-3'	106	60	(*15*)
HuBac	mHuBac563f, 5'-ATTGGGTTTAAAGGGAGCGTAG-3' mHuBac694r, 5'-CTACACCACGAATTCCGCC-3' mHuBac594Taq, 5'-(FAM)TAAGTCAGTTGTGAAAGTTTGCGGCTC(BHQ-1)-3'	131	69	(*15*)
HF183-like	GBAC34f 5' CGC TAG CTA CAG GCT TAA CAC 3' GBAC313r,5' GTG GGG GAC CTT CCT CTC 3' SerH285bhq, 5' (FAM)ATCCATCGTTGACTAGGTGGGCCGTTA(BHQ-1)-3'	279	60	Modified from (*14*), (*12*)
BoBac	CBac367f, 5'-GAAG(G/A)CTGAACCAGCCAAGTA-3' CBAC467r, 5'-GCTTATTCATACGGTACATACAAG-3' CBAC402 Bhq, 5'-(FAM)TGAAGGATGAAGGTTCTATGGATTGAAACTT(BHQ-1)-3'	100	57	(*15*)
Adeno (Adenovirus 40/41 hexon gene)	AV40/41-117f, 5'- CAGCCTGGGGAACAAGTTCAG 3' AV40/41-258r, 5' -CAGCGTAAAGCGCACTTTGTAA 3' AV40/41-157BHQ, 5' -(FAM)ACCCACGATGTAACCACAGACAGGTC (BHQ-1)-3'	141	60	(*22*)

Table A-III-3. Summary of data sets for 6 bacterial and viral markers.

Marker	Method Detection Limit	Number Above Detection Limit
E. coli	100 CFU/100 ml[a]	43
mE. coli	<1 copy/ng extracted DNA[b] equivalent to a geometric mean 8,374 copies/100 ml[c]	39
Bacteroides		43
Human *Bacteroides*		36
Bovine *Bacteroides*		24
Adenovirus		41

[a] *E. coli* method detection limit was constant based on a 1:100 dilution of pond water

[b] The method detection limit for all molecular assays was 1 copy/ng. However the amount of DNA extracted varied between samples

[c] Molecular detection limits were converted to copies/100 ml based on DNA concentration, volume DNA extraction and volume of water filtered. The geometric mean of this data set was 8,374 copies/100 ml

Table A-III-4. Chemical and Microbiological Information on 43 ponds within Site K.

Pond ID	Temperature (˚C)	Elelectrical Conductivity (mS/cm)	Dissolved Oxygen (mg/L)	*E. coli* (MPN/100 ml)	*mE. coli* (copies/100 ml)	*Bacteroides* (copies/100 ml)	Human *Bacteroides* (copies/100 ml)	Bovine Bacteroides (copies/100 ml)	Adenovirus (copies/100 ml)
KP-01	30.91	0.144	0.50	3.32E+03	4.95E+05	8.58E+05	2.87E+05	1.42E+04	2.57E+06
KP-02	29.60	0.144	0.66	7.86E+04	1.54E+06	6.03E+06	4.07E+06	6.96E+05	1.66E+05
KP-04	28.48	0.257	0.39	6.21E+03	1.81E+06	3.09E+07	1.31E+05	1.08E+05	2.60E+04
KP-05	29.00	0.301	0.38	5.59E+04	3.39E+06	1.74E+08	BDL	9.62E+04	6.39E+04
KP-06	26.84	0.130	0.26	2.03E+02	1.13E+05	1.68E+06	BDL	BDL	3.18E+04
KP-08	27.89	0.372	0.19	2.75E+05	5.93E+06	9.91E+07	1.52E+06	1.17E+05	BDL
KP-10	27.60	1.340	0.38	5.62E+05	5.64E+06	1.89E+08	8.45E+06	6.96E+06	6.21E+04
KP-14	25.96	0.162	0.29	1.00E+02	1.47E+04	6.77E+05	8.63E+04	3.34E+03	2.46E+04
KP-15	29.00	0.214	0.58	1.09E+04	4.37E+05	1.04E+07	1.37E+05	BDL	1.26E+05
KP-16	27.61	0.652	0.43	9.69E+05	2.26E+07	1.07E+09	4.26E+07	BDL	3.45E+05
KP-17	27.04	0.545	0.52	3.50E+04	6.80E+06	8.08E+07	2.39E+07	5.53E+05	3.57E+05
KP-18	26.99	0.141	0.30	1.93E+03	8.05E+04	1.18E+06	2.30E+05	BDL	1.32E+04
KP-19	27.63	0.651	0.25	2.30E+04	1.39E+06	9.23E+07	8.19E+05	7.33E+04	7.64E+06
KP-20	26.12	0.408	0.17	1.28E+04	5.91E+05	6.00E+06	6.10E+05	1.21E+05	8.83E+06
KP-21	26.79	0.449	0.20	1.42E+04	6.13E+05	3.61E+07	7.97E+05	2.65E+05	2.64E+05
KP-22	27.26	0.473	0.17	1.85E+04	BDL†	3.30E+06	1.09E+06	1.80E+05	1.46E+04
KP-23	29.60	0.208	0.68	5.50E+03	2.27E+05	6.69E+06	9.13E+05	7.22E+04	2.06E+05
KP-24	27.62	0.288	0.38	2.47E+03	3.06E+05	1.01E+07	2.29E+06	2.28E+04	8.92E+05
KP-25	30.12	0.227	0.40	2.64E+05	5.19E+06	3.16E+06	7.97E+05	1.94E+05	BDL
KP-26	27.57	0.406	0.21	1.77E+05	1.00E+06	3.98E+07	6.62E+05	5.01E+04	5.86E+04
KP-27	33.49	0.134	0.86	1.47E+04	4.72E+05	4.09E+06	1.53E+05	1.54E+05	2.09E+05
KP-28	31.19	0.296	1.51	2.01E+02	5.80E+05	7.54E+06	BDL	3.24E+04	6.31E+05
KP-29	32.71	0.302	1.06	7.11E+02	1.07E+05	3.03E+06	5.86E+04	BDL	7.57E+04
KP-30	29.84	0.167	0.84	6.21E+04	3.38E+06	8.97E+07	6.80E+07	5.99E+06	5.03E+05
KP-33	27.43	0.402	0.13	3.09E+05	7.40E+06	1.18E+08	3.22E+06	1.27E+05	1.87E+05
KP-34	27.57	0.132	0.17	1.12E+03	1.68E+04	1.65E+07	4.26E+05	BDL	9.03E+05
KP-36	32.03	0.092	0.77	2.73E+03	7.76E+05	6.32E+07	1.71E+05	7.08E+03	7.12E+04
KP-38	28.01	0.172	0.20	2.33E+03	BDL	4.28E+06	BDL	BDL	4.20E+04
KP-39	29.25	0.112	0.38	4.51E+03	8.05E+04	6.18E+06	6.07E+05	6.44E+04	5.05E+04
KP-40	30.77	0.171	0.52	1.00E+02	BDL	1.49E+05	BDL	BDL	1.84E+05
KP-41	30.41	0.113	0.92	3.10E+02	2.59E+04	1.17E+06	BDL	BDL	7.99E+04
KP-42	28.41	0.237	0.30	1.36E+03	1.46E+05	7.48E+05	6.12E+05	1.10E+04	7.59E+04
KP-43	28.87	0.240	0.34	6.28E+04	2.21E+05	9.77E+06	1.56E+06	2.12E+05	4.93E+05
KP-44	31.35	0.319	0.83	6.06E+03	2.54E+04	1.18E+07	3.27E+06	1.46E+06	5.03E+04
KP-45	26.94	0.248	0.23	2.66E+03	1.49E+04	2.44E+06	1.92E+05	BDL	3.56E+04
KP-46	27.71	0.270	0.55	5.79E+02	7.68E+04	1.20E+06	2.15E+05	2.56E+04	6.57E+04
KP-47	28.90	1.700	0.61	7.38E+04	2.85E+06	2.58E+07	5.82E+06	9.31E+04	4.61E+05
KP-48	32.40	0.253	0.83	7.51E+02	6.27E+03	5.12E+06	4.12E+05	4.40E+03	9.43E+04
KP-49	27.53	0.147	0.39	1.05E+04	3.70E+04	2.58E+06	2.40E+06	1.82E+04	9.47E+04
KP-50	29.44	0.979	0.18	3.55E+03	1.86E+06	1.13E+07	1.75E+06	8.32E+04	1.04E+05
KP-51	31.03	0.220	0.24	1.00E+02	2.58E+05	3.40E+06	BDL	BDL	1.02E+05
KP-53	28.50	0.661	0.17	5.93E+03	3.23E+07	7.06E+06	1.34E+04	1.39E+04	3.79E+05
KP-54	29.27	0.183	0.46	4.11E+03	2.50E+04	4.37E+06	4.35E+04	5.45E+03	1.75E+05

† Below Detection Limit

Table A-III-5. Physical Properties of 43 ponds within Site K.

Pond ID	Latitude	Longitude	Designated Pond Use	Population within 45 m of pond	Unsanitary Latrines*	Sanitary Latrines*	Number of Cows	Depth†	Long Axis (ft)	Short Axis (ft)	Surface Area (ft^2)	Volume (ft^3)
KP-01	90.63020	23.79480	fish/bathing	45	0	0	1	2.0	60	25	1,178	1,178
KP-02	90.63072	23.79545	no use	38	1	0	2	2.0	69	25	1,355	1,355
KP-04	90.63175	23.79845	latrine	23	1	0	4	2.0	43	43	1,452	1,452
KP-05	90.63155	23.79802	latrine	53	5	2	3	3.5	46	46	1,662	2,908
KP-06	90.63025	23.79636	no use	49	0	0	1	4.0	40	25	785	1,571
KP-08	90.62846	23.79572	latrine	75	2	2	2	4.0	25	22	432	864
KP-10	90.62877	23.79467	latrine	58	3	3	3	3.0	60	10	471	707
KP-14	90.62914	23.79493	no use	28	0	0	0	1.6	25	25	481	385
KP-15	90.63165	23.79826	no use	38	5	2	3	2.0	36	23	650	650
KP-16	90.62832	23.79556	latrine	77	3	0	6	0.5	18	13	184	46
KP-17	90.62644	23.79567	no use	41	2	0	5	1.8	27	17	360	315
KP-18	90.62645	23.79581	no use	29	0	0	1	1.0	39	24	711	355
KP-19	90.62646	23.79534	latrine	42	2	0	2	0.7	26	18	357	119
KP-20	90.62667	23.79528	latrine	35	4	0	0	1.0	39	23	705	352
KP-21	90.62791	23.79483	latrine	51	3	2	7	2.0	80	22	1,382	1,382
KP-22	90.62916	23.79521	latrine	50	5	5	3	4.0	60	33	1,555	3,110
KP-23	90.63102	23.79531	fish/bathing	0	0	0	1	4.0	86	82	5,539	11,077
KP-24	90.63040	23.79662	fish/bathing	37	0	1	2	2.0	27	17	360	360
KP-25	90.62997	23.79691	no use	32	1	1	1	1.0	45	28	990	495
KP-26	90.63077	23.79763	no use	61	3	0	0	2.0	NA	NA	NA	NA
KP-27	90.63100	23.79779	fish/bathing	84	0	0	1	4.0	130	89	9,087	18,174
KP-28	90.63059	23.79826	fish/bathing	12	0	0	1	2.5	102	83	6,649	8,311
KP-29	90.63038	23.79895	fish/bathing	0	0	0	1	7.0	152	101	12,057	42,201
KP-30	90.62815	23.79594	latrine	32	1	0	0	0.7	33	18	467	155
KP-33	90.62861	23.79585	latrine	83	3	1	3	NA	69	16	867	NA
KP-34	90.62829	23.79632	no use	24	1	2	0	1.0	30	9	212	106
KP-36	90.63253	23.79787	fish/bathing	12	1	0	5	8.0	126	68	6,729	26,917
KP-38	90.62749	23.79721	no use	35	0	1	0	1.0	20	20	314	157
KP-39	90.62710	23.79743	no use	9	0	1	0	2.0	46	38	1,373	1,373
KP-40	90.62705	23.79774	fish/bathing	9	1	3	0	4.0	43	27	912	1,824
KP-41	90.62667	23.79823	fish/bathing	5	4	1	3	6.0	94	60	4,430	13,289
KP-42	90.63163	23.79656	latrine	33	2	1	0	1.5	28	28	616	462
KP-43	90.63152	23.79630	no use	28	1	0	0	2.0	95	18	1,343	1,343
KP-44	90.63208	23.79731	no use	46	0	0	0	2.0	47	47	1,735	1,735
KP-45	90.63305	23.79836	fish/bathing	12	0	0	1	5.0	76	51	3,044	7,611
KP-46	23.79865	90.63120	no use	12	0	1	1	2.0	19	19	269	269
KP-47	90.63208	23.79870	no use	39	0	0	1	1.0	22	22	380	190
KP-48	90.63220	23.79888	no use	31	3	4	2	NA	23	23	415	NA
KP-49	90.63112	23.79758	fish/bathing	126	1	1	0	4.0	115	89	8,039	16,077
KP-50	90.62976	23.79581	no use	27	2	1	0	2.0	40	24	754	754
KP-51	90.62928	23.79558	latrine	50	0	0	0	2.0	17	17	227	227
KP-53	90.62889	23.79630	latrine	77	6	0	3	1.5	41	28	902	676
KP-54	90.62917	23.79635	no use	19	1	2	4	2.5	64	33	1,659	2,073

* Assessed in field by counting within the pond drainage basin

† Assessed by measuring deepest depth of pond

Figure A-III-1. Relative amounts of fecal contamination from Human or Bovine sources. Blue circles represent ponds where human or bovine *Bacteroides* were not detected.

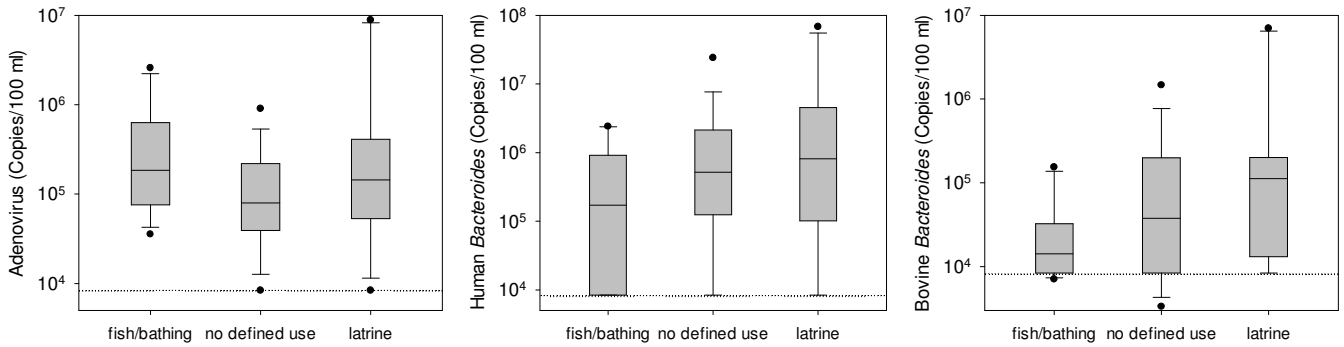


Figure A-III-2. Log-transformed concentrations (copies/100 ml) of 3 markers at Site K. The center line represents the median, upper and lower bounds of the box are the 75th and 25th percentile and whiskers represent the extent of the data. Outliers are represented by dots. The number of ponds were 43, with a maximum of 11 fish/bathing, 16 latrine and 16 ponds with no defined use in each category. Non-detects are included in this analysis for molecular assays as the geometric mean detection limit 8,374 copies/100 ml, indicated by the doted line.

Summary of Adenovirus Findings

Although it was widely present at high concentrations, the pathogen Adenovirus did not vary with pond type. This may result from the fact that excretion of pathogens from human hosts requires a current or recent infection (*6-7*). Poor correlations are typically found between fecal indicators and all viral pathogens (*8*). Although not all of the serotypes of Adenovirus are associated with gastrointestinal disease, its prevalence in human feces has led to the suggestion that it can be used as a viral human fecal indicator (*9-10*). Adenovirus is known to be the most prevalent human enteric virus found in sewage. Excretion of Adenovirus after a diarrheal infection is known to last only 14 days (*7*), and thus the presence of Adenovirus in ponds may be sporadic, possibly explaining the poor correlations typically found between Adenovirus and fecal indicator bacteria (*11*). The highest level of Adenovirus (10^6 copies/ml) was detected in a large commercial fishing pond, at least 100 m away from latrines or livestock.

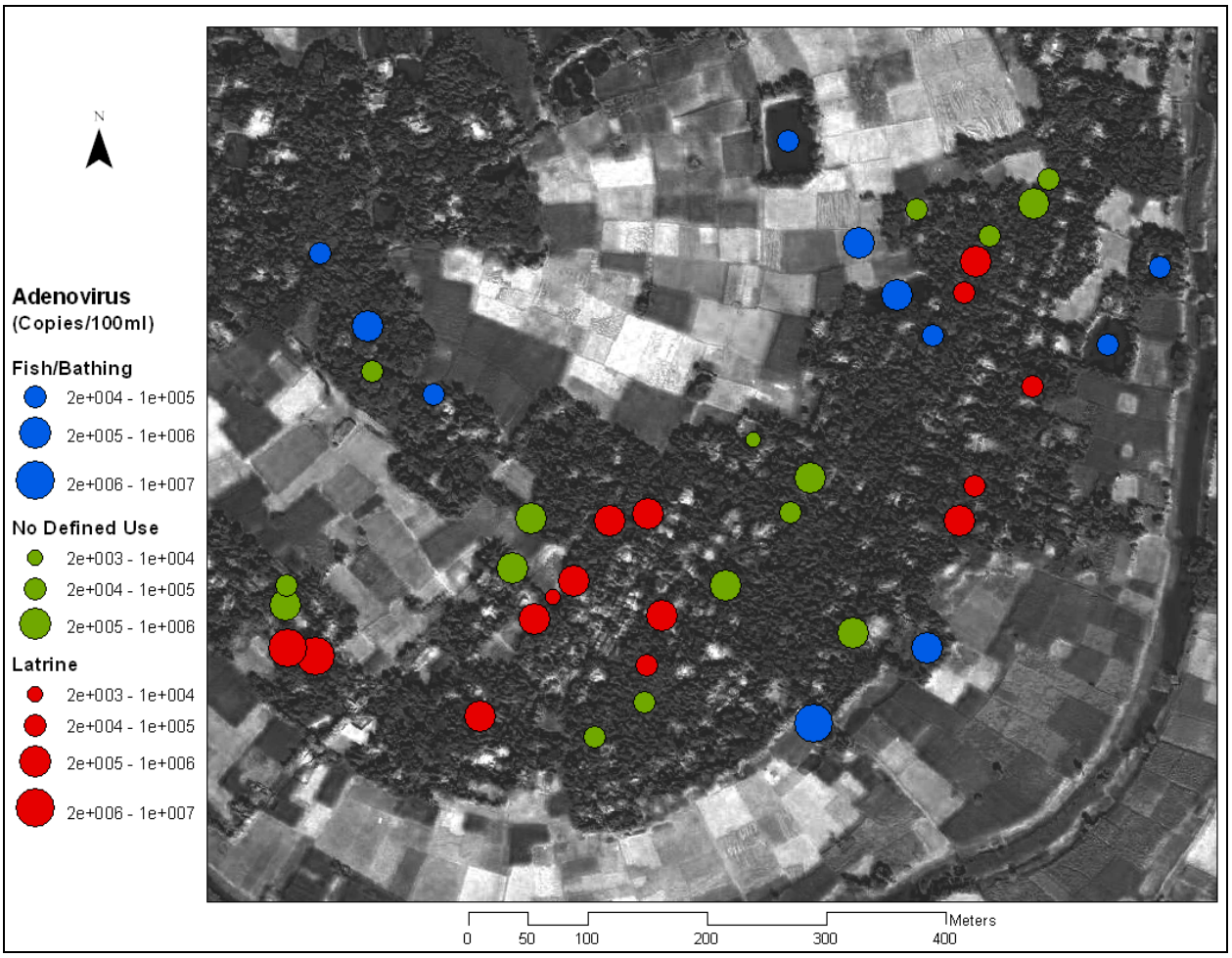


Figure A-III-3. Concentrations of Adenovirus in each pond classified by pond type.

Table A-III-6. Locations and Ages of Sanitary Latrines in Site K as of June 30, 2009.

Latrine ID†	Longitude	Latitude	Age (years)	Latrine ID†	Longitude	Latitude	Age (years)
1	90.63156	23.79808	1.0	92	90.62883	23.79482	30.0
4	90.63144	23.79782	10.0	93	90.62903	23.79509	50.0
5	90.63153	23.79779	2.0	99	90.62915	23.79551	1.0
7	90.63180	23.79826	5.0	100	90.62916	23.79551	6.0
10	90.63214	23.79838	1.0	101	90.62922	23.79555	7.0
10	90.63241	23.79818	1.0	105	90.63012	23.79544	6.0
10	90.63252	23.79816	1.0	105	90.63010	23.79542	6.0
11	90.63246	23.79854	6.0	107	90.63050	23.79564	6.0
13	90.63041	23.79775	2.0	116	90.63195	23.79707	2.0
14	90.63000	23.79595	4.0	117	90.63197	23.79699	8.0
14	90.63012	23.79638	0.2	118	90.63219	23.79704	4.0
16	90.62972	23.79572	6.0	121	90.63235	23.79630	4.0
19	90.63041	23.79554	6.0	123	90.63218	23.79877	20.0
22	90.63144	23.79626	8.0	126	90.62660	23.79819	20.0
25	90.62983	23.79490	8.0	137	90.62814	23.79564	0.2
28	90.62865	23.79450	4.0	141	90.62892	23.79613	3.0
28	90.62872	23.79448	2.0	148	90.63118	23.79849	1.0
30	90.62796	23.79462	23.0	149	90.63133	23.79828	1.0
31	90.62843	23.79522	6.0	150	90.63079	23.79644	0.3
32	90.62909	23.79495	5.0	152	90.62645	23.79534	2.0
39	90.62782	23.79709	3.0	153	90.62665	23.79574	2.0
42	90.62774	23.79530	5.0	154	90.62664	23.79520	20.0
44	90.62775	23.79483	7.0	155	90.62653	23.79510	2.0
45	90.62727	23.79507	5.0	200	90.63050	23.79536	1.0
46	90.62693	23.79507	3.0	211	90.62794	23.79432	5.0
47	90.62654	23.79535	10.0	301	90.63204	23.79781	3.0
64	90.63032	23.79693	0.5	301	90.63187	23.79773	3.0
66	90.63015	23.79676	1.0	602	90.63196	23.79756	2.0
67	90.62998	23.79685	15.0	902	90.63213	23.79836	4.0
68	90.63031	23.79658	4.0	3302	90.62962	23.79502	0.0
69	90.62767	23.79724	3.0	3801	90.62792	23.79699	13.0
70	90.62741	23.79713	12.0	3802	90.62765	23.79678	1.0
71	90.62742	23.79709	10.0	11002	90.63028	23.79585	3.0
76	90.62795	23.79589	16.0	13201	90.62854	23.79563	6.0
81	90.62730	23.79500	7.0	15101	90.62616	23.79573	1.0
82	90.62745	23.79497	3.0	15102	90.62637	23.79577	25.0
83	90.62723	23.79494	2.0	charpara mosque	90.63186	23.79669	NR‡
86	90.62861	23.79416	2.0	inside house	90.62563	23.79638	2.0
88	90.62880	23.79449	5.0	nafia textile	90.63161	23.79655	6.0
91	90.62897	23.79447	7.0	nurjahan textile	90.63086	23.79722	NR

† ID system by Veronica Escamilla

‡ Not Reported

Table A-III-7. Locations and ages of Unsanitary Latrines at Site K as of June 30, 2009.

Latrine ID†	Longitude	Latitude	Age (years)	Latrine ID†	Longitude	Latitude	Age (years)
002	90.63172	23.79807	2.0	097	90.62913	23.79545	2.0
007	90.63202	23.79811	8.0	098	90.62914	23.79540	13.0
008	90.63221	23.79807	4.0	103	90.63052	23.79504	15.0
012	90.63081	23.79780	2.0	106	90.63003	23.79569	5.0
014	90.63016	23.79637	0.3	108	90.63057	23.79584	5.0
014	90.63016	23.79636	4.0	109	90.63045	23.79577	0.3
015	90.62930	23.79597	10.0	111	90.63008	23.79593	3.0
015	90.62937	23.79602	2.0	112	90.63225	23.79776	6.0
017	90.62976	23.79570	6.0	113	90.63221	23.79772	12.0
018	90.63054	23.79512	5.0	115	90.63207	23.79734	8.0
023	90.63151	23.79632	4.0	119	90.63227	23.79667	2.0
024	90.63142	23.79619	7.0	120	90.63187	23.79660	15.0
025	90.63003	23.79485	8.0	122	90.63229	23.79913	20.0
026	90.62968	23.79465	25.0	124	90.62740	23.79681	4.0
027	90.62994	23.79472	8.0	126	90.62659	23.79813	20.0
028	90.62867	23.79449	4.0	127	90.62870	23.79590	1.0
031	90.62822	23.79498	6.0	128	90.62871	23.79587	8.0
035	90.62845	23.79635	1.0	129	90.62863	23.79577	3.0
036	90.62906	23.79678	5.0	130	90.62857	23.79571	3.0
037	90.62808	23.79679	15.0	131	90.62849	23.79560	6.0
040	90.62777	23.79714	8.0	133	90.62850	23.79542	6.0
041	90.62807	23.79599	7.0	135	90.62717	23.79775	0.3
043	90.62794	23.79517	1.0	135	90.62701	23.79768	15.0
048	90.62949	23.79495	20.0	136	90.62826	23.79554	12.0
051	90.62948	23.79436	4.0	138	90.62829	23.79547	50.0
051	90.62950	23.79442	3.0	139	90.62877	23.79621	25.0
053	90.63081	23.79766	0.3	140	90.62851	23.79648	30.0
054	90.63074	23.79761	20.0	142	90.62889	23.79611	7.0
055	90.63072	23.79779	5.0	143	90.62899	23.79611	3.0
056	90.63080	23.79771	12.0	144	90.62904	23.79620	6.0
057	90.63104	23.79754	1.0	146	90.62912	23.79634	3.0
058	90.63108	23.79736	3.0	147	90.62892	23.79643	3.0
059	90.63133	23.79743	16.0	00301	90.63171	23.79791	9.0
060	90.63146	23.79748	15.0	00302	90.63166	23.79775	1.0
061	90.63155	23.79753	5.0	00601	90.63199	23.79744	5.0
062	90.63017	23.79666	6.0	00901	90.63202	23.79841	10.0

Unsanitary Latrines Continued…							
063	90.63042	23.79689	10.0	2901	90.62877	23.79454	44.0
063	90.63041	23.79673	2.0	03301	90.62965	23.79507	3.0
065	90.63000	23.79704	0.3	03301	90.62955	23.79493	3.0
074	90.62835	23.79680	3.0	04901	90.62925	23.79482	1.0
075	90.62864	23.79678	4.0	04902	90.62935	23.79487	6.0
078	90.62805	23.79504	20.0	08501	90.62664	23.79538	0.1
079	90.62794	23.79479	0.0	08502	90.62669	23.79531	10.0
080	90.62794	23.79476	3.0	10201	90.62915	23.79566	2.0
084	90.62669	23.79574	1.0	10202	90.62920	23.79565	20.0
087	90.62856	23.79425	30.0	10401	90.63025	23.79508	3.0
094	90.62904	23.79514	2.0	10402	90.63024	23.79524	5.0
095	90.62907	23.79529	60.0	11001	90.63027	23.79580	0.2
096	90.62912	23.79537	3.0	13202	90.62854	23.79566	3.0

† ID system by Veronica Escamilla

Table A-III-8. Pond KP-01 Monthly Monitoring Data.

Date	Total Coliforms (MPN/100 ml)	*E. coli* (MPN/100 ml)	Temp (˚C)	Electrical Conductivity (mS/cm)	Dissolved Oxygen (ppm)	pH	ORP*	Sulfate (ppm)
2/27/08	6,508	100	22.3	0.16	10.3	7.7	-99.1	NS
3/27/08	286,142	97,318	31.8	0.34	5.4	8.3	-118.4	14.6
4/24/08	125,082	7,866	37.2	0.33	2.8	8.0	-127.8	8.0
6/20/08	100,297	3,321	30.9	0.14	0.5	7.1	112.0	NS
7/28/08	724,713	100	32.2	0.23	0.9	9.7	545.5	NS
8/27/08	3,777	254	31.4	0.23	1.4	7.9	330.1	1.0
9/22/08	54,512	681	29.7	0.23	1.3	8.8	256.1	0.0
10/30/08	25,767	253	25.8	0.22	0.9	6.8	231.2	4.0
11/23/08	116,803	866,440	23.6	0.23	0.0	8.1	127.4	5.0
1/28/09	410,580	198,900	22.5	0.13	0.2	8.0	480.2	18.0
2/15/09	Dry							
3/15/09	Dry							
4/23/09	Dry							
5/29/09	63,255	5,791	30.1	0.11	1.0	8.3	267.3	65.0
7/20/09	275,510	20,658	30.2	0.09	2.21	NA	154.3	39
8/27/09	365,400	35,784	29.7	0.09	1.68	NA	98.6	36
10/2/09	579,430	176,095	31.7	0.21	1.27	8.2	-30.6	10

* Oxidative Reductive Potential

Exceeded Maximum Detection Limit

NS - Not Sampled

NA - Not Available due to malfunctioning probe

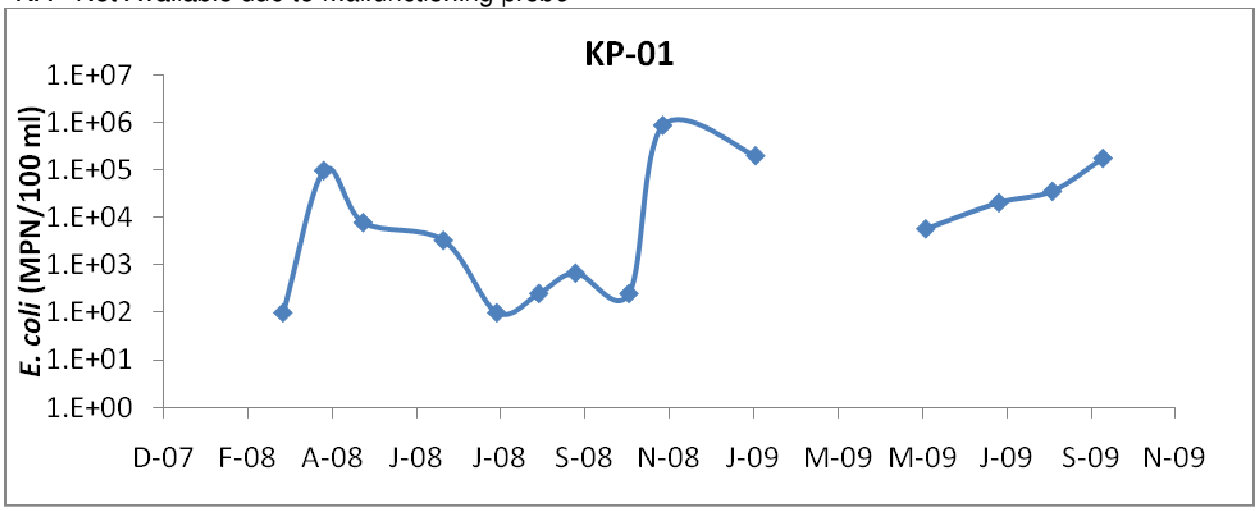


Table A-III-9. Pond KP-04 Monthly Monitoring Data.

Date	Total Coliforms (MPN/100 ml)	*E. coli* (MPN/100 ml)	Temperature (˚C)	Electrical Conductivity (mS/cm)	Dissolved Oxygen (ppm)	pH	ORP*	Sulfate (ppm)
2/27/08	18,571	2,624	19.6	0.27	15.2	7.7	-108.1	NS
3/27/08	18,942	1,277	27.5	0.45	2.1	7.8	-102.3	8.5
4/24/08	89,522	26,158	31.2	0.62	2.3	7.5	-97.5	7.7
6/20/08	168,207	6,207	28.5	0.26	0.4	7.4	170.0	NS
7/28/08	729,992	5,351	29.1	0.16	0.5	7.3	564.1	NS
8/27/08	69,394	13,934	27.7	0.25	0.8	7.0	320.5	9.0
9/22/08	422,875	79,953	27.5	0.29	0.6	7.7	240.4	8.0
10/30/08	43,721	1,555	23.4	0.24	0.9	6.7	259.2	10.0
11/23/08	20,143	816	20.3	0.28	0.0	6.0	213.2	3.1
1/28/09	68,596	1,591	20.8	0.37	1.2	7.4	227.7	29.0
2/15/09	Dry							
3/15/09	Dry							
4/23/09	2,419,600	2,419,600	28.2	0.34	1.0	8.2	195.8	77.0
5/29/09	76,885	5,611	29.6	0.49	1.7	9.2	233.6	63.0
7/20/09	770,100	33,518	28.0	0.11	1.3	NA	2.35.8	71
8/27/09	134,145	9,992	31.3	0.18	2.1	NA	155.8	52
10/1/09	23,022	1,623	27.7	0.22	1.5	8.1	-3.7	4

* Oxidative Reductive Potential

Exceeded Maximum Detection Limit

NS - Not Sampled

NA - Not Available due to malfunctioning probe

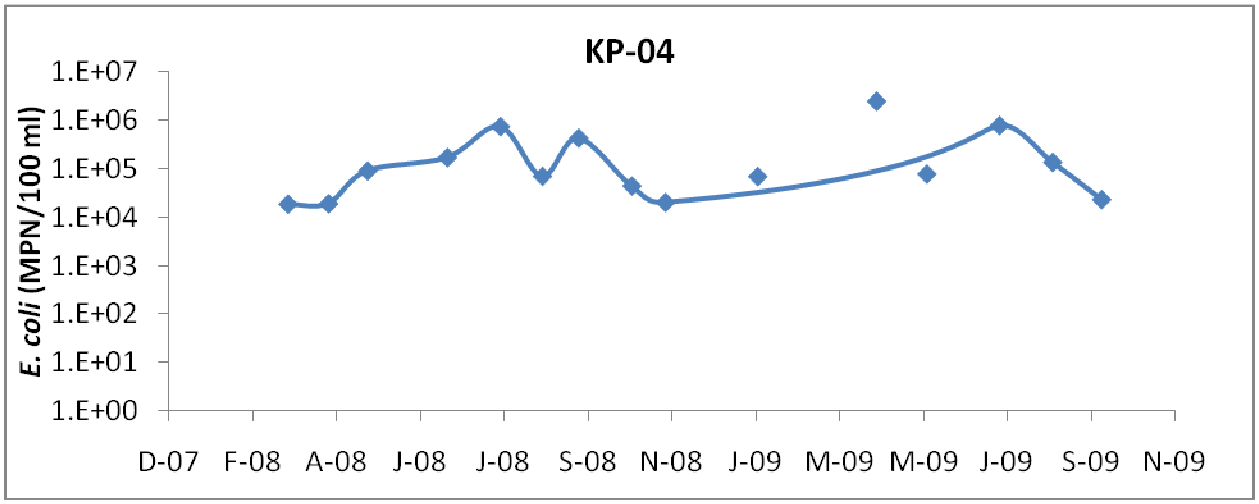


Table A-III-10. Pond KP-05 Monthly Monitoring Data.

Date	Total Coliforms (MPN/100 ml)	*E. coli* (MPN/100 ml)	Temperature (˚C)	Electrical Conductivity (mS/cm)	Dissolved Oxygen (ppm)	pH	ORP*	Sulfate (ppm)
2/27/08	38,185	54,480	18.9	0.50	12.5	7.4	-93.3	NS
3/27/08	868,625	79,045	28.5	0.82	4.3	7.8	-103.2	11.6
4/24/08	25,607	944	32.5	0.39	2.3	7.7	-11.8	7.5
6/20/08	623,167	55,853	29.0	0.30	0.4	7.5	355.0	NS
7/28/08	692,830	9,140	29.7	0.16	0.6	6.8	569.2	NS
8/27/08	126,628	43,023	28.4	0.09	0.8	7.0	324.1	12.0
9/22/08	448,140	65,996	27.7	0.28	0.5	7.8	232.2	11.0
10/30/08	184,820	77,042	24.4	0.27	0.7	7.0	255.1	10.0
11/23/08	613,140	57,450	22.4	0.36	0.0	7.5	242.5	3.8
1/28/09	2,419,600	2,419,600	21.2	0.40	1.0	8.2	268.1	49.0
2/15/09	Dry							
3/15/09	Dry							
4/23/09	980,390	142,090	28.0	0.48	1.2	7.9	128.7	77.0
5/29/09	1,046,240	726,990	31.1	0.43	1.2	8.7	202.5	70.0
7/20/09	547,500	47,281	27.4	0.28	1.5	NA	189.6	65
8/27/09	307,590	62,516	28.5	0.23	1.7	NA	210.5	45
10/1/09	97,799	14,649	29.3	0.20	1.3	8.0	-116.9	12

* Oxidative Reductive Potential

Exceeded Maximum Detection Limit

NS - Not Sampled

NA - Not Available due to malfunctioning probe

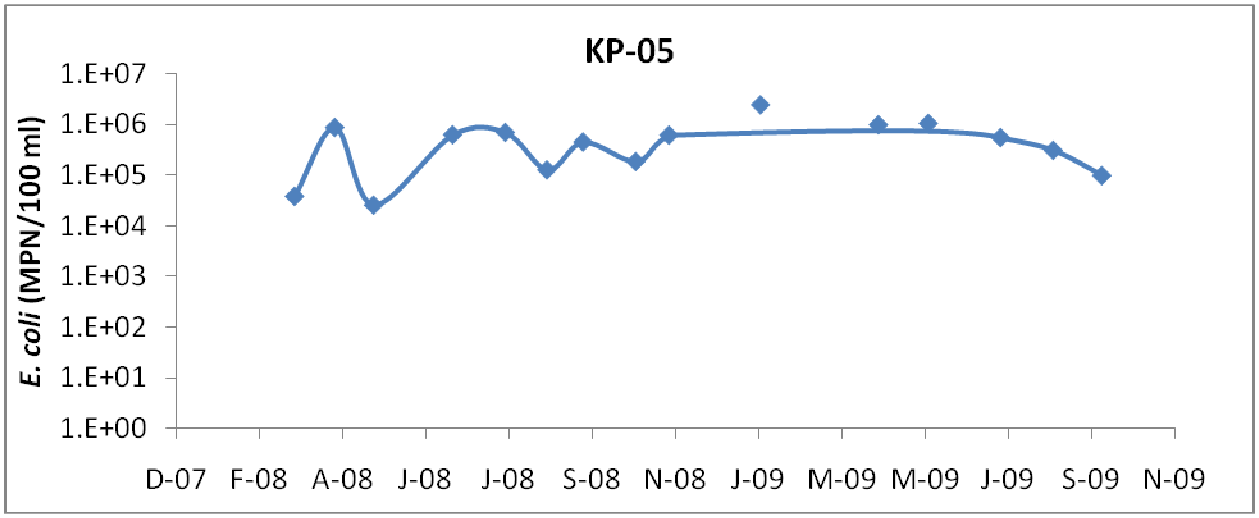


Table A-III-11. Pond KP-06 Monthly Monitoring Data.

Date	Total Coliforms (MPN/100 ml)	*E. coli* (MPN/100 ml)	Temperature (˚C)	Electrical Conductivity (mS/cm)	Dissolved Oxygen (ppm)	pH	ORP*	Sulfate (ppm)
2/27/08	26,806	3,830	18.6	0.19	7.8	7.3	-104.7	NS
3/27/08	2,202,930	109,207	26.3	0.44	2.2	7.5	-98.9	12.2
4/24/08	495,005	21,642	29.2	0.35	1.9	7.6	-104.3	7.6
6/20/08	16,154	203	26.8	0.13	0.3	6.7	28.7	NS
7/28/08	1,948,500	78,146	28.3	0.16	0.3	7.6	590.1	NS
8/27/08	108,852	57,586	28.0	0.03	1.1	5.7	475.1	12.0
9/22/08	903,270	150,298	27.3	0.19	0.9	7.9	261.4	14.0
10/30/08	18,696	4,316	22.2	0.12	0.8	7.8	125.0	12.0
11/23/08	59,822	16,247	21.2	0.14	0.0	7.8	413.2	3.4
1/28/09	139,133	78,526	20.0	0.12	0.9	7.5	112.9	31.0
2/15/09	Dry							
3/15/09	Dry							
4/23/09	1,732,890	365,400	27.8	0.27	1.0	9.1	114.5	74.0
5/29/09	2,419,600	686,670	28.0	0.12	2.1	8.8	250.8	56.0
7/20/09	325,540	40,539	28.1	0.08	2.0	NA	255.3	77
8/27/09	770,100	217,280	27.1	0.07	3.1	NA	122.9	70
10/1/09	2,419,600	435,170	27.5	0.13	1.0	7.6	-296.2	14

* Oxidative Reductive Potential

Exceeded Maximum Detection Limit

NS - Not Sampled

NA - Not Available due to malfunctioning probe

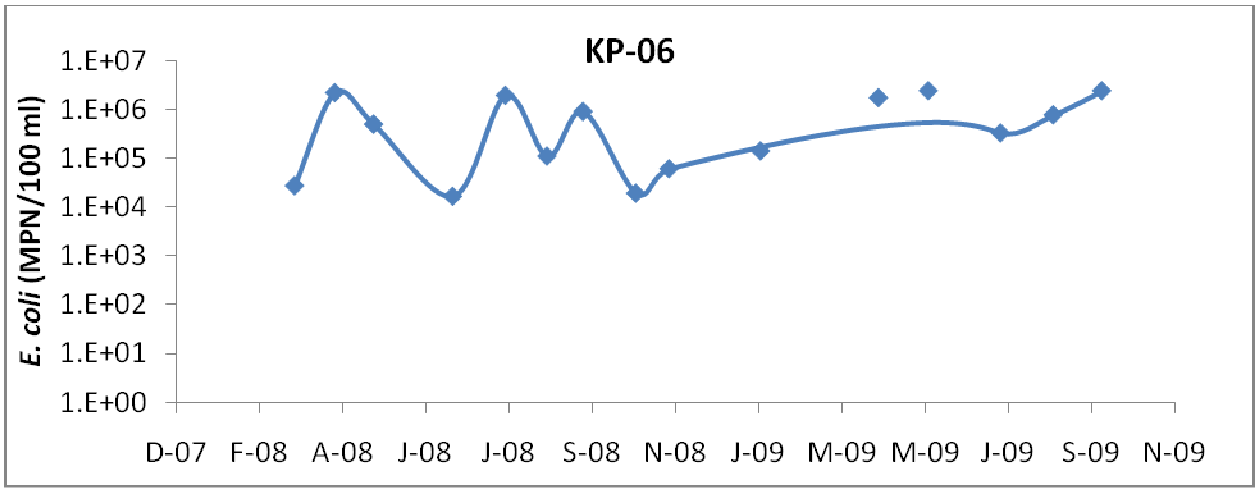


Table A-III-12. Pond KP-07 Monthly Monitoring Data.

Date	Total Coliforms (MPN/100 ml)	*E. coli* (MPN/100 ml)	Temperature (˚C)	Electrical Conductivity (µs/cm)	Dissolved Oxygen (ppm)	pH	ORP*	Sulfate (ppm)
2/27/08	94,280	1,355	19.6	0.39	5.6	7.1	-72.8	NS
3/27/08	328,165	13,611	27.7	0.67	7.7	7.3	-105.7	13.4
4/24/08	134,503	5,867	29.7	0.45	2.2	7.5	-97.6	7.5
6/20/08	87,970	4,723	27.4	0.23	0.1	6.7	-4.3	NS
7/28/08	1,584,700	98,941	29.4	0.20	0.4	7.5	557.4	NS
8/27/08	491,335	152,073	29.4	0.27	1.0	6.3	435.2	19.0
9/22/08	866,440	138,466	27.7	0.34	0.7	7.7	259.3	13.0
10/30/08	30,581	9,365	29.7	0.19	1.0	7.9	88.9	12.0
11/23/08	237,070	69,896	21.3	0.09	0.1	7.4	401.2	10.3
1/28/09	1,986,290	866,440	20.7	0.25	1.0	8.0	222.4	77.0
2/15/09	Dry							
3/15/09	Dry							
4/23/09	206,092	19,366	28.2	0.42	2.1	8.8	124.3	53.0
5/29/09	2,419,600	224,400	28.8	0.12	1.9	8.4	245.7	43.0
7/20/09	920,840	160,712	27.6	0.33	1.5	NA	201.8	47
8/27/09	1,299,650	269,345	27.9	0.07	1.6	NA	236.1	77
10/1/09	8,835	2,011	27.8	0.15	1.5	7.8	-121.5	15

* Oxidative Reductive Potential

Exceeded Maximum Detection Limit

NS - Not Sampled

NA - Not Available due to malfunctioning probe

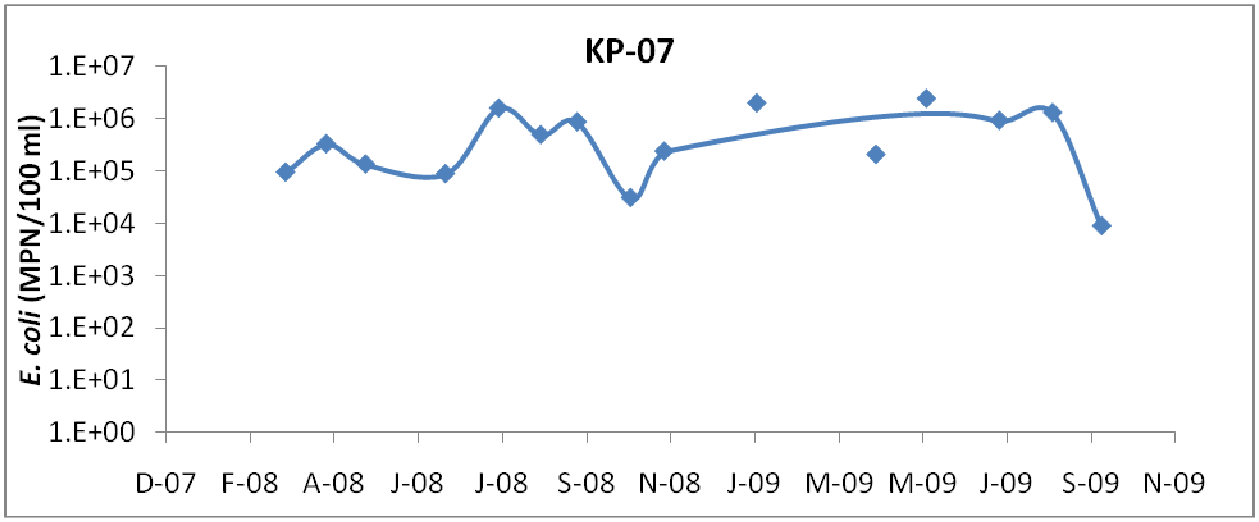


Table A-III-13. Pond KP-10 Monthly Monitoring Data.

Date	Total Coliforms (MPN/100 ml)	*E. coli* (MPN/100 ml)	Temp (˚C)	Electrical Conductivity (mS/cm)	Dissolved Oxygen (ppm)	pH	ORP*	Sulfate (ppm)
2/27/08	NS							
3/27/08	NS							
4/24/08	NS							
6/20/08	1,468,617	561,690	27.6	1.34	0.4	7.3	37.1	NS
7/28/08	511,540	41,716	30.3	0.33	1.1	9.6	540.2	NS
8/27/08	26,805	2,439	29.6	0.45	1.1	7.2	359.2	5.0
9/22/08	686,670	52,328	27.7	0.55	1.0	7.8	278.6	8.0
10/30/08	64,190	26,485	32.6	0.29	0.9	7.9	87.6	13.0
11/23/08	1,203,330	866,440	20.7	0.78	0.0	6.9	343.2	8.8
1/28/09	2,419,600	2,419,600	21.1	0.24	0.9	8.0	978.3	37.0
2/15/09	Dry							
3/15/09	Dry							
4/23/09	Dry							
5/29/09	Dry							
7/20/09	344,800	114,005	29.1	0.41	2.1	NA	225.2	55
8/27/09	336,261	93,704	28.1	0.08	2.5	NA	145.2	77
10/2/09	110,407	17,034	29.1	0.30	1.9	7.8	-60.4	15

* Oxidative Reductive Potential

Exceeded Maximum Detection Limit

NS - Not Sampled

NA - Not Available due to malfunctioning probe

Literature Cited In Appendix III

(1) Smith, G.J.; Helf, M.; Nesbet, C.; Betita, H. A.; Meek, J.; Ferre, F. Fast and accurate method for quantitating *E. coli* host-cell DNA contamination in plasmid DNA preparations. *Biotechniques* **1999**, *26*(3), 518.

(2) Layton, A.; Williams, D.; Sayler, G. S.; McKay, L. D. *Proceedings of the 13th Annual Tennessee Water Resources Symposium*, Tennessee Section of the American Water Resources Association, 2003.

(3) Layton, A.; McKay, L. D.; Williams, D.; Garrett, V.; Gentry, R.; Sayler, G. Development of *Bacteroides* 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water. *Applied & Environmental Microbiology* **2006** *72*(6), 4214-4224.

(4) Bernhard, A.E.; Field, K.G. Identification of nonpoint sources of fecal pollution in coastal waters by using host-specific 16S ribosomal DNA genetic markers from fecal anaerobes. *Applied and Environmental Microbiology* **2000**, *66*(4), 1587-1594.

(5) Rajal, V. B.; McSwain, B. S.; Thompson, D. E.; Leutenegger, C. M.; Wuertz, S. Molecular quantitative analysis of human viruses in California storm water. *Water Research* **2007**, *41*(*19*), 4287-4298.

(6) Kotloff, K.L.; Losonsky, G. A.; Morris, J. G. Jr.; Wasserman, S. S.; Singh-Naz, N.; Levine, M. M. Enteric Adenovirus Infection and Childhood Diarrhea - an Epidemiologic-Study in 3 Clinical Settings. *Pediatrics* **1989**, *84*(2), 219-225.

(7) Van, R.; Wun, C. C.; Oryan, M. L.; Matson, D. O.; Jackson, L.; Pickering, L. K. Outbreaks of Human Enteric Adenovirus Type-40 and Type-41 in Houston Day-Care-Centers. *J. Pediatrics* **1992**, *120*(4), 516-521.

(8) Wilkes, G.; Edge, T.; Gannon, V.; Jokinen, C.; Lyautey, E.; Medeiros, D.; Neumann, N.; Ruecker, N.; Topp, E.; Lapen, D. R. Seasonal Relationships Among Indicator Bacteria, Pathogenic Bacteria, Cryptosporidium Oocysts, Giardia Cysts, and Hydrological Inidices for Surface Waters Within an Agricultural Landscape, *Water Research* **2009**, *43*, 2209-2223.

(9) Castignolles, N., Petit, F.; Mendel, I.; Simon, L.; Cattolico, L.; Buffet-Janvresse, C. Detection of Adenovirus in the waters of the Seine River estuary by nested-PCR. *Molecular and Cellular Probes* **1998**, *12*(3), 175-180.

(10) Wyn-Jones, A. P.; Sellwood, J. Enteric viruses in the aquatic environment. *J. App. Microbiol.* **2001**, *91*, 945-962.

(11) Lee, C.; Lee, S.H.; Han, E.; Kim, S.J. Use of cell culture-PCR assay based on combination of A549 and BGMK cell lines and molecular identification as a tool to monitor infectious andenoviruses and enteroviruses in river water. *Applied and Environmental Microbiology* **2004**, *70*(11), 6695-6705.

APPENDIX IV – TRANSECT EXPERIMENTS

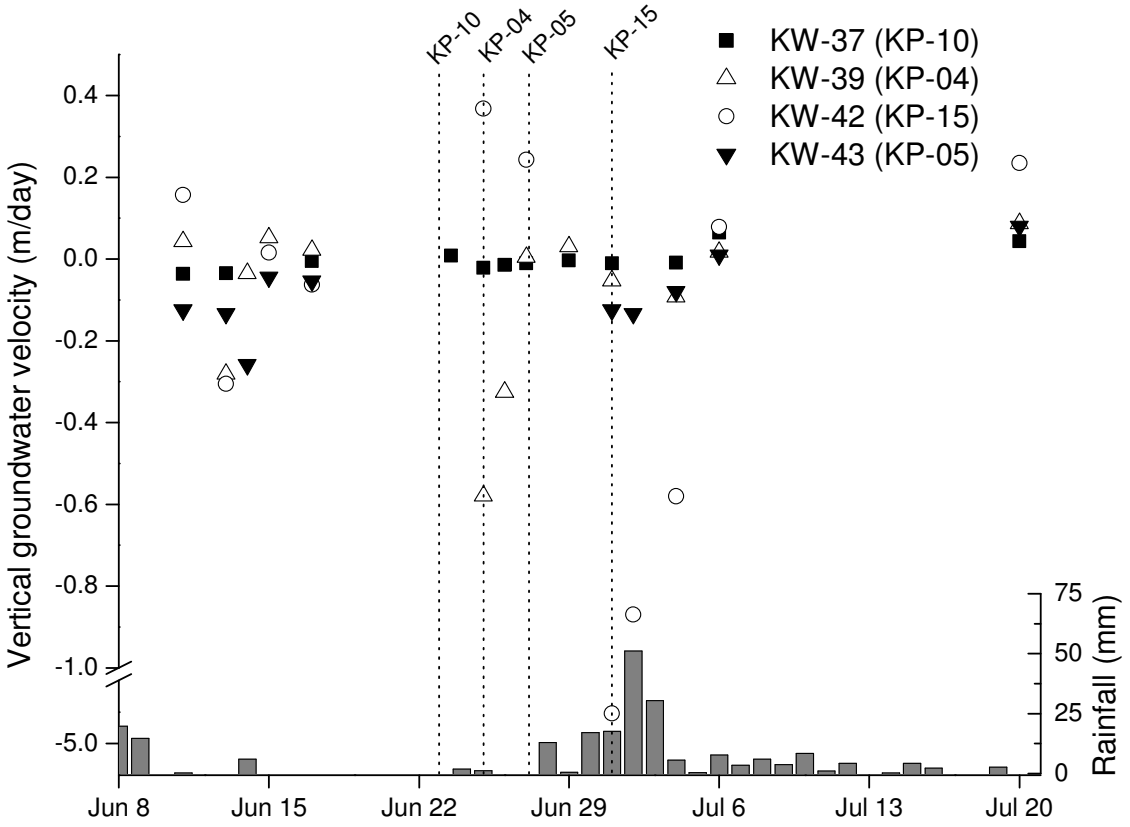


Figure A-IV-1. Response of Vertical Groundwater velocities in transects to natural precipitation events and artificial pond flooding. Flooding dates are indicated with labeled vertical dotted lines. Precipitation histogram shows daily rainfall for Dhaka 25 Km west of Site K. Vertical Darcy velocities were calculated assuming an anisotropy factor of 10 (K_x/K_z). Downward velocities are negative.

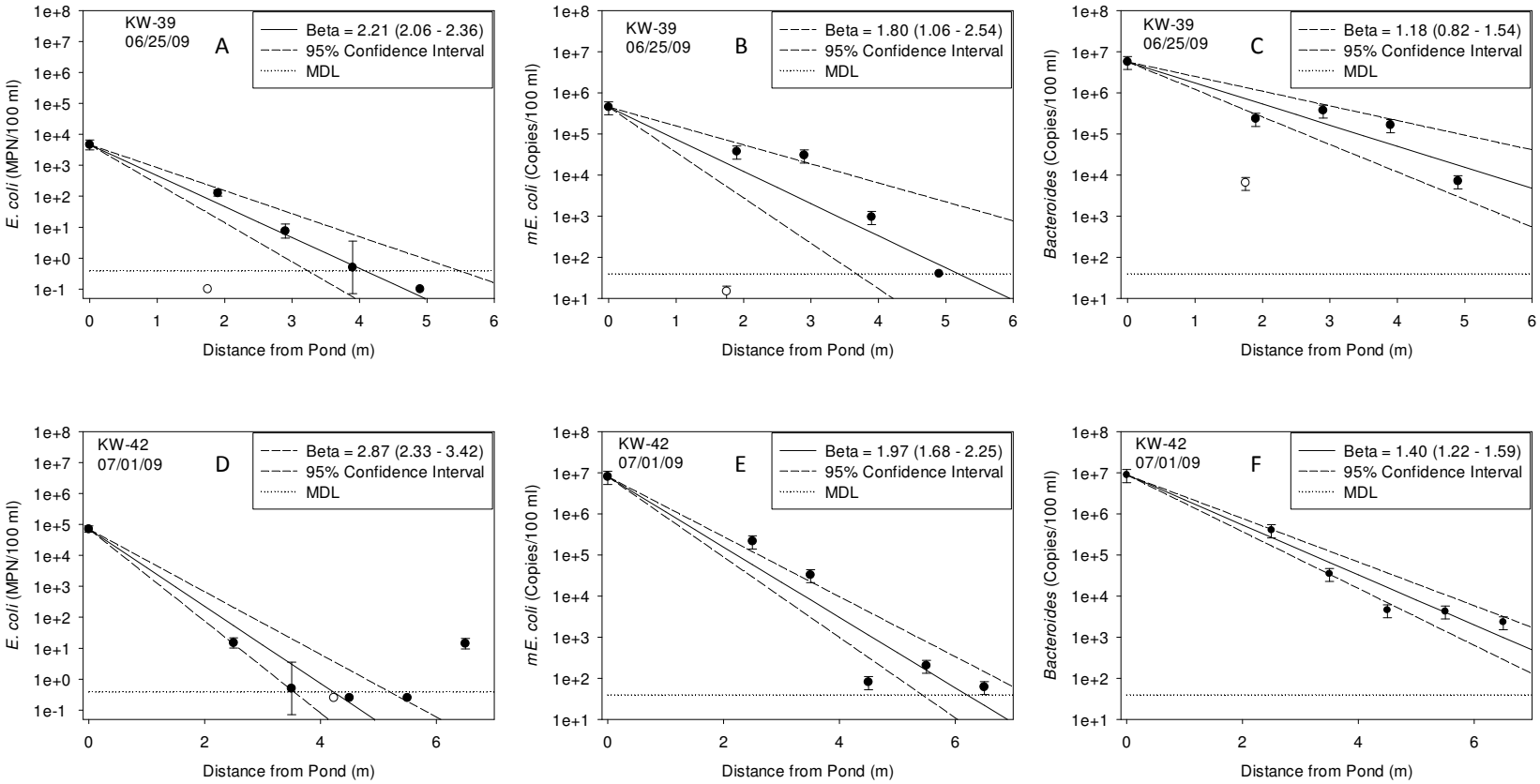


Figure A-IV-2. Pre-flooding bacterial concentrations in transects KW-39 and -42 with lateral distance from pond KP-04 and -15 respectively. Black filled symbols represent pond and shallow wells, and hollow symbols represent deep wells. The deep well in KW-42 was not sampled for molecular markers at this event. The solid line represents the curve fitted with linear regression.

Table A-IV-1. Drilling Logs for Transect KW-36.

Depth Below Ground Surface (ft bgs)	(m bgs)	2	a	b	c	d
1	0.3	sand, med-fine, silty, brown	no sample	sand, fine, silty, brown	sand, very fine, silty, brown	no sample
2	0.6					
3	0.9					
4	1.2					
5	1.5					
6	1.8					
7	2.1	sand, fine, silty, brown	no sample	same as above	same as above	
8	2.4					
9	2.7					
10	3.0					sand, fine, silty, brown
11	3.4	sand, fine, silty, grey-brown	sand, fine, silty, brown	sand, very fine, silty, grey-brown	sand, fine, silty, grey	sand, very fine, silty, grey
12	3.7					
13	4.0					
14	4.3					
15	4.6					
16	4.9	sand, fine, silty, grey	sand, fine, silty, grey	sand, very fine, silty, grey	same as above	silt, sandy, grey
17	5.2					
18	5.5					
19	5.8					
20	6.1					
21	6.4	silt, cohesive, grey		silt, cohesive, grey		
22	6.7					
23	7.0					
24	7.3					
25	7.6					
26	7.9	sand, coarse, grey				
27	8.2					
28	8.5					
29	8.8					
30	9.1					
31	9.5	same as above				
32	9.8					
33	10.1					
34	10.4					
35	10.7					

Table A-IV-2. Drilling Logs for Transect KW-37.

Depth Below Ground Surface (ft bgs)	(m bgs)	2	a	b	c	d
1	0.3					
2	0.6			sand, med-fine, trace silt, brown		
3	0.9					
4	1.2					
5	1.5	sand, very fine, silty, brown	not sampled			not sampled
6	1.8					
7	2.1					
8	2.4			sand, fine, silty, brown	sand, fine, silty, brown	
9	2.7					
10	3.0					
11	3.4					sand, fine, silty, brown
12	3.7	sand, fine, silty, grey-brown	sand, very fine, silty, brown	sand, fine, silty, grey		
13	4.0					
14	4.3					
15	4.6					sand, very fine, silty, grey (19.4 ft)
16	4.9					
17	5.2	sand, fine, some silt, grey	sand, fine, silty, grey	same as above	sand, fine, silty, grey	
18	5.5					
19	5.8					
20	6.1					silt, grey
21	6.4			same as		sand, very
22	6.7					
23	7.0	same as above				
24	7.3					
25	7.6					
26	7.9		sand, med,	sand, med,		sand, med,
27	8.2					
28	8.5					
29	8.8					
30	9.1					
31	9.5	sand, med, trace silt, grey-brown				
32	9.8					
33	10.1					
34	10.4					
35	10.7					
36	11.0					
37	11.3					

Table A-IV-3. Drilling Logs for Transect KW-38.

Depth Below Ground Surface (ft bgs)	(m bgs)	2	a	b	c	d
1	0.3	sand, fine, silty, brown	no sample	sand, fine, silty, brown	sand, fine-very fine, silty brown	no sample
1.5	0.5					
2	0.6					
2.5	0.8					
3	0.9					
3.5	1.1					
4	1.2					
4.5	1.4					
5	1.5					
5.5	1.7					
6	1.8					
6.5	2.0					
7	2.1					
7.5	2.3					
8	2.4					
8.5	2.6					
9	2.7					
9.5	2.9					
10	3.0					
10.5	3.2	sand, fine, silty, grey	sand, fine, silty, brown	sand, very fine, silty, grey-brown	sand, very fine, silty, grey-brown	sand, very fine, silty, grey
11	3.4					
11.5	3.5					
12	3.7		sand, fine, silty, grey			
12.5	3.8					
13	4.0					
13.5	4.1					
14	4.3		lost core			
14.5	4.4					
15	4.6					sand, very fine, silty, grey
15.5	4.7					
16	4.9		silt, sandy, grey			
16.5	5.0					
17	5.2			sand, med-fine, silty, grey	sand, very fine, silty, grey	sand, fine-med, silty, grey
17.5	5.3					
18	5.5		sand, fine-med, silty, grey			
18.5	5.6					
19	5.8					
19.5	5.9					
20	6.1					
21	6.4	sand, coarse, trace silt, grey-brown				
22	6.7					
23	7.0					
24	7.3					
25	7.6					
26	7.9					
27	8.2					
28	8.5					
29	8.8					
30	9.1					
31	9.5					
32	9.8					
33	10.1					
34	10.4					
35	10.7					

Table A-IV-4. Drilling Logs for Transect KW-39.

Depth Below Ground Surface (ft bgs)	(m bgs)	2	a	b	c	d
1	0.3					
1.5	0.5	no log available				
2	0.6					
2.5	0.8		sand, coarse, trace silt, brown	sand, coarse, silty, brown		silt, clayey, brown-grey
3	0.9					
3.5	1.1					
4	1.2					
4.5	1.4				sand, med-coarse, silty, brown	
5	1.5					
5.5	1.7					
6	1.8					
6.5	2.0					
7	2.1					
7.5	2.3		sand, very fine, silty, brown	same as above		sand, coarse, silty, brown
8	2.4					
8.5	2.6					
9	2.7					
9.5	2.9					
10	3.0					
10.5	3.2					
11	3.4					
11.5	3.5		sand, coarse, trace silt, brown			
12	3.7			sand, coarse, silty, grey-brown	sand, med, silty, brown	sand, coarse, silty, brown
12.5	3.8					
13	4.0					
13.5	4.1					
14	4.3					
14.5	4.4					
15	4.6					
15.5	4.7					
16	4.9				sand, med, silty, grey w/ dark brown organic matter at 20 ft	
16.5	5.0					
17	5.2					
17.5	5.3					sand, coarse, silty, brown
18	5.5		sand, coarse, grey			
18.5	5.6					
19	5.8					
19.5	5.9			sand, coarse, silty, grey		
20	6.1					
20.5	6.3				sand, fine, grey, w/ organic matter at 21.5 ft	
21	6.4					
21.5	6.6					
22	6.7					
22.5	6.9		sand, fine, silty, grey		silt, sandy,	sand, coarse, silty, grey
23	7.0					
23.5	7.2		sand,		sand,	
24	7.3		sand, very		fine,	
24.5	7.5					
25	7.6					

Table A-IV-5. Drilling Logs for Transect KW-40.

Depth Below Ground Surface (ft bgs)	(m bgs)	2	a	b	c	d
1	0.3	silt, clayey, plastic, brown	no sample	silt, sandy, low plasticity brown	silt, sandy, brown	silt, trace sand, motelled, medium plasticity, grey-brown
2	0.6					
3	0.9					
4	1.2					
5	1.5					
6	1.8					
7	2.1					
8	2.4					
9	2.7					
10	3.0					
10.5	3.2		silt, sandy,			
11	3.4					
11.5	3.5					
12	3.7		sand, silty, med, brown	sand, coarse, silty, brown	sand, coarse-med, silty, brown	no sample
12.5	3.8					
13	4.0					
13.5	4.1					
14	4.3					
14.5	4.4	sand, coarse, some silt, brown				
15	4.6					
15.5	4.7		sand, some silt, med-coarse, brown			sand, med, silty, brown
16	4.9					
16.5	5.0					
17	5.2			sand, coarse, silty, grey-brown		
17.5	5.3					
18	5.5					silt, sandy, grey
18.5	5.6					
19	5.8				sand, very fine, silty, grey	
19.5	5.9					
20	6.1					
20.5	6.3		sand, coarse, silty, grey			
21	6.4					sand, fine, silty, grey
21.5	6.6					
22	6.7	sand, coarse, trace silt, grey-brown		sand, coarse, silty, grey		
22.5	6.9					
23	7.0					
23.5	7.2					
24	7.3		silt, grey			
24.5	7.5					
25	7.6					
25.5	7.8					
26	7.9	sand, med, some silt, grey				
27	8.2					
28	8.5					
29	8.8					
30	9.1					
30.5	9.3	sand, coarse, trace silt, grey				
31	9.5					
32	9.8					
33	10.1					
34	10.4					

Table A-IV-6. Drilling Logs for Transect KW-41.

Depth Below Ground Surface (ft bgs)	(m bgs)	2	a	b	c	d
1	0.3					
1.5	0.5					
2	0.6					
2.5	0.8			clay, high plasticity, brown	clay, high plasticity, brown	clay, high plasticity, brown
3	0.9					
3.5	1.1					
4	1.2					
4.5	1.4					
5	1.5		sand, coarse, silty, brown			
5.5	1.7					
6	1.8					
6.5	2.0					
7	2.1					sand, coarse, silty, brown
7.5	2.3	sand, coarse, silty, brown				
8	2.4					
8.5	2.6					
9	2.7					
9.5	2.9			sand, coarse-med, silty, brown		
10	3.0					
10.5	3.2					
11	3.4					
11.5	3.5					
12	3.7		sand, med-coarse, silty, brown			sand, coarse-med, silty, brown-reddish brown
12.5	3.8				sand, coarse-med, silty, brown	
13	4.0					
13.5	4.1					
14	4.3					
14.5	4.4					
15	4.6					
15.5	4.7					
16	4.9		sand, med-coarse, silty, grey			sand, coarse-med, silty, grey (to 17.4 ft) sand, very
16.5	5.0					
17	5.2			sand, coarse-med, silty, grey		
17.5	5.3					
18	5.5					
18.5	5.6					
19	5.8		silt, trace			sand, coarse, some silt, grey
19.5	5.9	sand, coarse, silty, grey				
20	6.1					
20.5	6.3					
21	6.4					
21.5	6.6					silt, sand,
22	6.7					
23	7.0					
24	7.3					
25	7.6					
26	7.9					

Table A-IV-7. Drilling Logs for Transect KW-42.

Depth Below Ground Surface (ft bgs)	(m bgs)	2	a	b	e	d	c
1	0.3	clay, trace silt, plastic, brown	clay, trace silt, high plasticity, brown	clay, trace silt, high plasticity, brown	CH, clay, brown	CH, clay, trace silt, med-high plasticity, brown	CH, clay, trace silt, med-high plasticity, brown
1.5	0.5						
2	0.6						
2.5	0.8						
3	0.9						
3.5	1.1						
4	1.2						
4.5	1.4						
5	1.5						
5.5	1.7	sand, trace silt, coarse, brown	sand, trace silt, coarse, brown	sand, trace silt, coarse, brown	SP, sand, silty, sub-rounded, brown	SP, sand, med-coarse, sub-rounded, brown	SP, sand, med-coarse, sub-rounded, brown
6	1.8						
6.5	2.0						
7	2.1						
7.5	2.3						
8	2.4						
8.5	2.6						
9	2.7						
9.5	2.9						
10	3.0						
10.5	3.2				SP, sand, med-coarse, sub-rounded, brown	SP, sand, same as above, grey	
11	3.4						
11.5	3.5						
12	3.7						
12.5	3.8						
13	4.0						
13.5	4.1				SP, sand, med-coarse, sub-rounded, grey (9x 1 cm thick dark brown/black organic/Fe staining 14-17, no odor)		
14	4.3						
14.5	4.4						
15	4.6						
15.5	4.7	sand, trace silt, coarse, grey	sand, trace silt, coarse, grey	sand, trace silt, coarse, grey			SP, same as above, grey
16	4.9						
16.5	5.0						
17	5.2						
17.5	5.3						
18	5.5						
19	5.8						
20	6.1						
21	6.4						
22	6.7						
23	7.0						
24	7.3						
25	7.6						
26	7.9						
27	8.2						
28	8.5						

Table A-IV-8. Drilling Logs for Transect KW-43.

Depth Below Ground Surface (ft bgs)	(m bgs)	2	a	b	c	d
1	0.3	CH, clay, med-high plasticity, brown	CH, clay, high plasticity, brown	No Sample	CH, clay, med-high plasticity, brown	CH, clay, med-high, high plasticity, brown
2	0.6					
3	0.9					
4	1.2					
5	1.5					
6	1.8	SP, sand, med-coarse, silty, sub-rounded, brown	SP, sand, med-coarse, sub-rounded, brown		SP, sand, med-coarse, silty, brown	SP, sand, med-coarse, silty, brown
7	2.1					
8	2.4					
9	2.7					
10	3.0					
11	3.4					
12	3.7					
13	4.0					
14	4.3					
15	4.6					
16	4.9				SP, sand, med-coarse, silty, light grey-brown	
17	5.2					
18	5.5					
18.5	5.6					
19	5.8		SP, sand, med-coarse, sub-rounded, grey			SP, sand, med-coarse, silty, grey
20	6.1	SP, sand, med-coarse, silty, grey				
21	6.4					
22	6.7					
23	7.0					
24	7.3					
25	7.6					
26	7.9	SP, sand, med, silty, grey				
27	8.2					
28	8.5					
29	8.8					
30	9.1	SP, sand, fine-med, silty, grey				
31	9.5					
32	9.8					

Table A-IV-9. Drilling Logs for Transect KW-44.

Depth Below Ground Surface (ft bgs)	(m bgs)	2	a	b	c	d
1	0.3	CH, clay, med-high plasticity, brown	clay, silty, cohesive, brown, chunks	CH, clay, med-high plasticity, brown	CH, clay, med-high plasticity, brown	
2	0.6					
3	0.9					
4	1.2					
5	1.5					
6	1.8	SP, sand, med-coarse, silty, brown	sand, coarse, brown	SP, sand, med-coarse, silty, brown	SP, sand, med-coarse, silty, brown	
7	2.1					
8	2.4					
9	2.7					
10	3.0					
11	3.4					
12	3.7					
13	4.0					
14	4.3					
15	4.6					
16	4.9				SP, sand, med-coarse, silty, grey	
17	5.2		sand, coarse, brown			
18	5.5					
19	5.8					
20	6.1			SP, sand, med-coarse, silty, brown		
21	6.4	same as above, grey	sand, coarse, grey		same as above, dark brown peat at 21 ft	
22	6.7					
23	7.0					
24	7.3					
25	7.6					
26	7.9	SP, sand, med-fine, silty, grey				
27	8.2					
28	8.5					
29	8.8					
30	9.1					
31	9.5	SP, sand, med-coarse, silty, grey				
32	9.8					
33	10.1					
34	10.4					
35	10.7					

Grain Size Analyses from Transect wells from Chapter IV

The following equation was used to logarithmically interpolate between points on the grain size distribution curves to calculate d_{10}, d_{50} and d_{60} values.

$$\ln x = \ln x_o + \left(\frac{\ln x_1 - \ln x_o}{y_1 - y_o}\right)(y - y_o)$$

Table A-IV-10. Summary of Cored Intervals for Grain Size Analysis.

Well ID	Cored Interval (ft bgs)
KW-36.1a	10 to 20
KW-37.1a	10 to 21
KW-39.1a	10 to 24
KW-39.1c	10 to 24
KW-40.1a	10 to 16
KW-41.1a	10 to 19
KW-41.1d	10 to 22
KW-42.1e	10 to 18
KW-43.1a	14 to 23
KW-44.1d	10 to 25

Table A-IV-11. Sediment Sieving Results for KW-36.1a.

Sieve Size (mm)	10-11 ft bgs	11-12 ft bgs	12-13 ft bgs	13-14 ft bgs	14-15 ft bgs	15-16 ft bgs	16-17 ft bgs	17-18 ft bgs	19-20 ft bgs		
1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1		
0.5	0.5	0.2	0.4	0.8	0.1	0.1	0.1	0.2	0.1		
0.25	0.5	0.2	1.6	2.3	1.6	0.5	0.7	2.1	0.1		
0.106	25.9	18.4	77.9	53.9	62.8	43.6	72.2	52.2	46		
0.053	28.6	31.8	27.1	34.2	31.2	10.2	21.4	23.7	51.6		
<0.053	5.5	5.5	5.6	7	5	1.1	4.1	10	2.1		
Total Mass (g)	**61.2**	**56.3**	**112.7**	**98.3**	**100.8**	**55.6**	**98.6**	**88.3**	**100.0**		
Percent Finer											
1	99.7	99.6	99.9	99.9	99.9	99.8	99.9	99.9	99.9		
0.5	98.9	99.3	99.6	99.1	99.8	99.6	99.8	99.7	99.8		
0.25	98.0	98.9	98.1	96.7	98.2	98.7	99.1	97.3	99.7		
0.106	55.7	66.3	29.0	41.9	35.9	20.3	25.9	38.2	53.7		
0.053	9.0	9.8	5.0	7.1	5.0	2.0	4.2	11.3	2.1		
<0.053	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**Average**	**St Dev**
d10	*0.05*	*0.05*	*0.06*	*0.06*	*0.06*	*0.07*	*0.06*	*0.05*	*0.06*	*0.06*	*0.01*
d50	*0.10*	*0.09*	*0.14*	*0.12*	*0.13*	*0.15*	*0.14*	*0.13*	*0.10*	*0.12*	*0.02*
d60	*0.12*	*0.10*	*0.16*	*0.14*	*0.15*	*0.16*	*0.16*	*0.15*	*0.12*	*0.14*	*0.02*
U	*2.15*	*1.85*	*2.54*	*2.51*	*2.49*	*2.28*	*2.48*	*2.84*	*2.02*	*2.35*	*0.30*

Table A-IV-12. Sediment Sieving Results for KW-37.1a.

Sieve Size (mm)	10-11 ft bgs	11-12 ft bgs	12-13 ft bgs	13-14 ft bgs	14-15 ft bgs	15-16 ft bgs	16-17 ft bgs	17-18 ft bgs	18-19 ft bgs	19-20 ft bgs	20-21 ft bgs		
1	0.1	0.4	0.1	0.3	0.5	0.4	0.3	0.4	0.5	0.5	0.1		
0.5	0.2	1.3	0.2	0.6	0.9	0.7	0.5	0.6	0.6	0.5	0.4		
0.25	1.1	3.4	2.2	3.7	5	3.1	3.1	2.2	3.2	5.3	8.7		
0.106	29.6	50	37.1	58	46.9	42.2	51.7	61.4	49.1	64.9	67.4		
0.053	33.1	47.7	41.2	33.7	44.6	33.9	38.9	16.3	8.1	5.5	11.2		
<0.053	5.2	7.4	3	6.1	2.3	5.9	9.1	4.9	1.8	2.4	4.9		
Total Mass (g)	**69.3**	**110.2**	**83.8**	**102.4**	**100.2**	**86.2**	**103.6**	**85.8**	**63.3**	**79.1**	**92.7**		
Percent Finer													
1	99.9	99.6	99.9	99.7	99.5	99.5	99.7	99.5	99.2	99.4	99.9		
0.5	99.6	98.5	99.6	99.1	98.6	98.7	99.2	98.8	98.3	98.7	99.5		
0.25	98.0	95.4	97.0	95.5	93.6	95.1	96.2	96.3	93.2	92.0	90.1		
0.106	55.3	50.0	52.7	38.9	46.8	46.2	46.3	24.7	15.6	10.0	17.4		
0.053	7.5	6.7	3.6	6.0	2.3	6.8	8.8	5.7	2.8	3.0	5.3		
<0.053	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**Average**	**St Dev**
d10	*0.05*	*0.06*	*0.06*	*0.06*	*0.06*	*0.06*	*0.05*	*0.06*	*0.08*	*0.11*	*0.07*	*0.06*	*0.02*
d50	*0.10*	*0.11*	*0.10*	*0.13*	*0.11*	*0.11*	*0.11*	*0.14*	*0.16*	*0.16*	*0.16*	*0.13*	*0.02*
d60	*0.12*	*0.13*	*0.12*	*0.15*	*0.14*	*0.14*	*0.13*	*0.16*	*0.17*	*0.18*	*0.18*	*0.15*	*0.02*
U	*2.12*	*2.29*	*2.10*	*2.53*	*2.26*	*2.41*	*2.47*	*2.61*	*2.22*	*1.68*	*2.52*	*2.29*	*0.26*

Table A-IV-13. Sediment Sieving Results for KW-39.1a.

Sieve Size (mm)	10-11 ft bgs	11-12 ft bgs	12-13 ft bgs	13-14 ft bgs	14-15 ft bgs	15-16 ft bgs	16-17 ft bgs	17-18 ft bgs	18-19 ft bgs	19-20 ft bgs	20-21 ft bgs	21-22 ft bgs	22-23 ft bgs	23-24 ft bgs		
1	0.2	0.1	0.0	0.2	0.2	0.7	0.4	0.5	0.1	0.7	0.2	0.0	0.1	0.2		
0.5	2.0	1.9	1.4	0.7	1.3	1.8	1.6	1.4	2.8	4.2	2.1	0.1	0.5	1.7		
0.25	51.4	49.5	72.9	44.4	76.1	85.8	52.5	64.3	70.4	66.4	58.0	8.7	6.4	34.0		
0.106	9.5	8.6	14.0	10.1	15.9	16.7	10.5	16.2	26.3	26.9	26.8	6.9	30.7	16.2		
0.053	3.3	2.3	4.9	3.7	7.7	8.4	4.5	9.6	18.4	14.7	17.5	4.6	60.8	15.5		
<0.053	3.3	2.0	2.4	1.6	2.6	2.3	3.2	4.2	5.6	4.9	7.1	1.5	9.5	3.2		
Total Mass (g)	**69.7**	**64.3**	**95.6**	**60.7**	**104.0**	**115.5**	**72.8**	**96.1**	**123.6**	**117.7**	**111.7**	**21.8**	**108.0**	**70.8**		
Percent Finer																
1	99.7	99.9	100.0	99.8	99.8	99.4	99.5	99.5	100.0	99.4	99.8	100.0	99.9	99.7		
0.5	96.8	96.9	98.5	98.6	98.5	97.9	97.3	98.1	97.7	95.9	97.9	99.5	99.4	97.3		
0.25	23.1	20.1	22.2	25.4	25.3	23.6	25.1	31.2	40.7	39.5	46.0	59.8	93.5	49.3		
0.106	9.4	6.6	7.6	8.7	10.0	9.2	10.6	14.3	19.4	16.6	22.0	28.0	65.1	26.4		
0.053	4.7	3.1	2.5	2.7	2.5	2.0	4.5	4.4	4.6	4.1	6.4	6.9	8.8	4.5		
<0.053	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**Average**	**St Dev**
d10	*0.11*	*0.13*	*0.12*	*0.11*	*0.11*	*0.11*	*0.10*	*0.08*	*0.07*	*0.07*	*0.06*	*Silt*	*Silt*	*0.06*	*0.09*	*0.02*
d50	*0.32*	*0.33*	*0.32*	*0.32*	*0.32*	*0.32*	*0.32*	*0.30*	*0.28*	*0.28*	*0.26*			*0.25*	*0.30*	*0.03*
d60	*0.35*	*0.36*	*0.35*	*0.35*	*0.35*	*0.35*	*0.35*	*0.34*	*0.32*	*0.32*	*0.30*			*0.29*	*0.34*	*0.02*
U	*3.21*	*2.73*	*2.89*	*3.07*	*3.27*	*3.16*	*3.54*	*4.31*	*4.63*	*4.38*	*4.84*			*4.63*	*3.72*	*0.77*

Table A-IV-14. Sediment Sieving Results for KW-39.1c.

Sieve Size (mm)	10-11 ft bgs	11-12 ft bgs	14-15 ft bgs	15-16 ft bgs	16-17 ft bgs	17-18 ft bgs	18-19 ft bgs	19-20 ft bgs	20-21 ft bgs	21-22 ft bgs	22-23 ft bgs	23-24 ft bgs		
1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	2.7	0.1	0.2		
0.5	1.5	1.3	0.3	0.5	0.5	1.9	2	2.3	2	2.7	2	0.7		
0.25	48.5	42.5	24.8	39	40	73	53	52.7	38.5	35	3.4	8		
0.106	10.5	9.6	7.7	9.6	11	19.5	16	22.1	34.5	28.5	26.1	27.3		
0.053	2.2	2.1	2.5	2.5	5	12.5	9.5	15	27	17.8	36.8	29.9		
<0.053	1.5	1.2	1.3	1	1.6	3	3	5	8.7	4.5	7.8	11.2		
Total Mass (g)	**64.3**	**56.8**	**36.7**	**52.7**	**58.2**	**110.0**	**83.6**	**97.2**	**110.9**	**91.2**	**76.2**	**77.3**		
Percent Finer														
1	99.8	99.8	99.7	99.8	99.8	99.9	99.9	99.9	99.8	97.0	99.9	99.7		
0.5	97.5	97.5	98.9	98.9	99.0	98.2	97.5	97.5	98.0	94.1	97.2	98.8		
0.25	22.1	22.7	31.3	24.9	30.2	31.8	34.1	43.3	63.3	55.7	92.8	88.5		
0.106	5.8	5.8	10.4	6.6	11.3	14.1	15.0	20.6	32.2	24.5	58.5	53.2		
0.053	2.3	2.1	3.5	1.9	2.7	2.7	3.6	5.1	7.8	4.9	10.2	14.5		
<0.053	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**Average**	**St Dev**
d10	*0.13*	*0.13*	*0.10*	*0.12*	*0.10*	*0.08*	*0.08*	*0.07*	*0.06*	*0.06*	*Silt*	*Silt*	*0.09*	*0.03*
d50	*0.32*	*0.32*	*0.30*	*0.32*	*0.31*	*0.30*	*0.30*	*0.27*	*0.17*	*0.21*			*0.28*	*0.05*
d60	*0.35*	*0.35*	*0.34*	*0.35*	*0.34*	*0.34*	*0.33*	*0.31*	*0.23*	*0.27*			*0.32*	*0.04*
U	*2.67*	*2.69*	*3.21*	*2.80*	*3.55*	*4.06*	*4.23*	*4.69*	*4.05*	*4.26*			*3.62*	*0.74*

Table A-IV-15. Sediment Sieving Results for KW-40.1a.

Sieve Size (mm)	**10-11 ft bgs**	**11-12 ft bgs**	**12-13 ft bgs**	**13-14 ft bgs**	**14-15 ft bgs**	**15-16 ft bgs**		
1	0.1	0.1	0.1	0.1	0.1	0.1		
0.5	1.9	1.3	1.1	1.3	3.3	1.5		
0.25	15.6	38.2	49	52.5	87.6	59.5		
0.106	10	9.6	10	9.7	17.7	14.1		
0.053	17.7	6.6	3.9	5.7	13.5	15.1		
<0.053	8.9	2.3	2.5	3.5	5.4	5.2		
Total Mass (g)	**54.2**	**58.1**	**66.6**	**72.8**	**127.6**	**95.5**		
Percent Finer								
1	99.8	99.8	99.8	99.9	99.9	99.9		
0.5	96.3	97.6	98.2	98.1	97.3	98.3		
0.25	67.5	31.8	24.6	26.0	28.7	36.0		
0.106	49.1	15.3	9.6	12.6	14.8	21.3		
0.053	16.4	4.0	3.8	4.8	4.2	5.4		
<0.053	0.0	0.0	0.0	0.0	0.0	0.0	**Average**	**St Dev**
d10	*0.02*	*0.08*	*0.11*	*0.08*	*0.08*	*0.06*	*0.08*	*0.02*
d50	*0.16*	*0.30*	*0.32*	*0.31*	*0.31*	*0.29*	*0.31*	*0.01*
d60	*0.21*	*0.34*	*0.35*	*0.35*	*0.34*	*0.33*	*0.34*	*0.01*
U	*12.11*	*4.39*	*3.14*	*4.13*	*4.43*	*5.04*	*4.23*	*0.69*

Table A-IV-16. Sediment Sieving Results for KW-41.1a.

Sieve Size (mm)	**10-11 ft bgs**	**11-12 ft bgs**	**12-13 ft bgs**	**13-14 ft bgs**	**14-15 ft bgs**	**15-16 ft bgs**	**16-17 ft bgs**	**17-18 ft bgs**	**18-19 ft bgs**		
1	0.1	0.1	0.1	0.1	0.1	0.4	0.1	0.1	0.1		
0.5	1.8	1.4	0.8	1.1	1	3.8	2	0.7	0.7		
0.25	52.5	54.6	69.4	43.9	45.4	60.8	21.8	9.9	10.7		
0.106	13.8	24	26.5	16.7	28.5	33.7	37.4	25.9	41.4		
0.053	4.7	13.3	14.7	11.4	15	18.6	26.8	20.8	19.1		
<0.053	1.5	2.1	2.3	2.4	2.2	2.5	2.1	3.1	3.4		
Total Mass (g)	**74.4**	**95.5**	**113.8**	**75.6**	**92.2**	**119.8**	**90.2**	**60.5**	**75.4**		
Percent Finer											
1	99.9	99.9	99.9	99.9	99.9	99.7	99.9	99.8	99.9		
0.5	97.4	98.4	99.2	98.4	98.8	96.5	97.7	98.7	98.9		
0.25	26.9	41.3	38.2	40.3	49.6	45.7	73.5	82.3	84.7		
0.106	8.3	16.1	14.9	18.3	18.7	17.6	32.0	39.5	29.8		
0.053	2.0	2.2	2.0	3.2	2.4	2.1	2.3	5.1	4.5		
<0.053	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**Average**	**St Dev**
d10	*0.11*	*0.08*	*0.08*	*0.07*	*0.07*	*0.08*	*0.06*	*0.06*	*0.06*	*0.08*	*0.02*
d50	*0.31*	*0.28*	*0.29*	*0.28*	*0.25*	*0.26*	*0.15*	*0.13*	*0.15*	*0.23*	*0.07*
d60	*0.35*	*0.31*	*0.32*	*0.32*	*0.29*	*0.30*	*0.19*	*0.16*	*0.17*	*0.27*	*0.07*
U	*3.02*	*4.02*	*3.94*	*4.36*	*3.95*	*4.03*	*2.98*	*2.73*	*2.76*	*3.53*	*0.64*

Table A-IV-17. Sediment Sieving Results for KW-41.1d.

Sieve Size (mm)	10-11 ft bgs	11-12 ft bgs	12-13 ft bgs	13-14 ft bgs	14-15 ft bgs	15-16 ft bgs	16-17 ft bgs	17-18 ft bgs	18-19 ft bgs	19-20 ft bgs	20-21 ft bgs	21-22 ft bgs		
1	0.2	0.1	0.1	0.1	0.1	0.1	0.2	0.3	0.2	0.2	0.1	0.1		
0.5	1.1	2.6	0.9	0.6	0.9	0.6	1.9	1.4	1.8	2.4	1.3	0.2		
0.25	41.3	63.7	36.2	51.4	51.2	45.3	48.4	19.9	44.2	42.3	32.9	7		
0.106	14.3	6.7	15.9	27.4	17.7	15.7	29.3	25.8	15.7	29.1	47.9	56.3		
0.053	5.7	4	4.2	9.6	5.6	7	15	17.5	6.8	12.4	19.5	30.5		
<0.053	3.3	2.3	1.8	2.4	2.1	2.2	2.6	3.2	3.9	4.6	5.7	10.3		
Total Mass (g)	**65.9**	**79.4**	**59.1**	**91.5**	**77.6**	**70.9**	**97.4**	**68.1**	**72.6**	**91.0**	**107.4**	**104.4**		
Percent Finer														
1	99.7	99.9	99.8	99.9	99.9	99.9	99.8	99.6	99.7	99.8	99.9	99.9		
0.5	98.0	96.6	98.3	99.2	98.7	99.0	97.8	97.5	97.2	97.1	98.7	99.7		
0.25	35.4	16.4	37.1	43.1	32.7	35.1	48.2	68.3	36.4	50.7	68.1	93.0		
0.106	13.7	7.9	10.2	13.1	9.9	13.0	18.1	30.4	14.7	18.7	23.5	39.1		
0.053	5.0	2.9	3.0	2.6	2.7	3.1	2.7	4.7	5.4	5.1	5.3	9.9		
<0.053	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**Average**	**St Dev**
d10	*0.09*	*0.14*	*0.10*	*0.09*	*0.11*	*0.09*	*0.07*	*0.06*	*0.07*	*0.07*	*0.06*	*0.05*	*0.09*	*0.02*
d50	*0.29*	*0.33*	*0.29*	*0.27*	*0.30*	*0.29*	*0.26*	*0.17*	*0.43*	*0.25*	*0.18*	*0.13*	*0.29*	*0.07*
d60	*0.33*	*0.36*	*0.32*	*0.31*	*0.33*	*0.33*	*0.35*	*0.21*	*0.64*	*0.32*	*0.21*	*0.15*	*0.35*	*0.12*
U	*3.58*	*2.59*	*3.10*	*3.57*	*3.12*	*3.81*	*4.75*	*3.39*	*8.55*	*4.71*	*3.38*	*2.78*	*4.05*	*1.79*

Table A-IV-18. Sediment Sieving Results for KW-42.1e.

Sieve Size (mm)	10-11 ft bgs	11-12 ft bgs	12-13 ft bgs	13-14 ft bgs	14-15 ft bgs	15-16 ft bgs	16-17 ft bgs	17-18 ft bgs		
1	0	0.1	0.1	0.1	0.2	0.1	0.3	0.1		
0.5	0.6	1.3	2.5	1.6	4.9	3.5	4.5	3.8		
0.25	57.9	45.4	63.9	65	74.8	62.6	84.6	67.5		
0.106	8.7	8.6	8.9	10.4	15.1	6	14.2	14.5		
0.053	3.6	3.7	4.4	5.8	7.6	3	9.2	8.2		
<0.053	0.4	0.7	0.5	1.1	2.5	1	3.2	1.4		
Total Mass (g)	**71.2**	**59.8**	**80.3**	**84.0**	**105.1**	**76.2**	**116.0**	**95.5**		
Percent Finer										
1	100.0	99.8	99.9	99.9	99.8	99.9	99.7	99.9		
0.5	99.2	97.7	96.8	98.0	95.1	95.3	95.9	95.9		
0.25	17.8	21.7	17.2	20.6	24.0	13.1	22.9	25.2		
0.106	5.6	7.4	6.1	8.2	9.6	5.2	10.7	10.1		
0.053	0.6	1.2	0.6	1.3	2.4	1.3	2.8	1.5		
<0.053	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**Average**	**St Dev**
d10	*0.14*	*0.12*	*0.14*	*0.12*	*0.11*	*0.18*	*0.10*	*0.11*	*0.13*	*0.03*
d50	*0.33*	*0.32*	*0.33*	*0.33*	*0.32*	*0.34*	*0.32*	*0.32*	*0.33*	*0.01*
d60	*0.36*	*0.35*	*0.36*	*0.36*	*0.36*	*0.37*	*0.36*	*0.35*	*0.36*	*0.01*
U	*2.48*	*2.86*	*2.53*	*2.97*	*3.27*	*2.09*	*3.56*	*3.33*	*2.89*	*0.50*

Table A-IV-19. Sediment Sieving Results for KW-43.1a.

Sieve Size (mm)	**14-15 ft bgs**	**15-16 ft bgs**	**16-17 ft bgs**	**17-18 ft bgs**	**18-19 ft bgs**	**19-20 ft bgs**	**20-21 ft bgs**	**21-22 ft bgs**	**22-23 ft bgs**		
1	0.2	0.1	0.1	0.1	0.1	0.3	0.1	0.1	0.1		
0.5	0.8	0.4	1.4	1.3	0.5	0.3	0.1	1.1	2.3		
0.25	46.9	36.3	80.1	84.3	50.1	57.8	52.4	56.4	42.7		
0.106	13.6	9.2	17.5	21.8	13	16.1	11	12.6	8.2		
0.053	4.2	3.5	7.3	6.7	5.5	3.5	5.4	5.6	3.5		
<0.053	2	1.4	3	2.5	0.6	1	2.6	2	1		
Total Mass (g)	**67.7**	**50.9**	**109.4**	**116.7**	**69.8**	**79.0**	**71.6**	**77.8**	**57.8**		
Percent Finer											
1	99.7	99.8	99.9	99.9	99.9	99.6	99.9	99.9	99.8		
0.5	98.5	99.0	98.6	98.8	99.1	99.2	99.7	98.5	95.8		
0.25	29.2	27.7	25.4	26.6	27.4	26.1	26.5	26.0	22.0		
0.106	9.2	9.6	9.4	7.9	8.7	5.7	11.2	9.8	7.8		
0.053	3.0	2.8	2.7	2.1	0.9	1.3	3.6	2.6	1.7		
<0.053	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**Average**	**St Dev**
d10	*0.11*	*0.11*	*0.11*	*0.12*	*0.11*	*0.13*	*0.10*	*0.11*	*0.12*	*0.11*	*0.01*
d50	*0.31*	*0.31*	*0.32*	*0.31*	*0.31*	*0.31*	*0.31*	*0.31*	*0.33*	*0.31*	*0.00*
d60	*0.34*	*0.34*	*0.35*	*0.34*	*0.34*	*0.34*	*0.34*	*0.35*	*0.36*	*0.35*	*0.00*
U	*3.09*	*3.17*	*3.17*	*2.95*	*3.05*	*2.71*	*3.46*	*3.23*	*2.95*	*3.09*	*0.21*

Table A-IV-20. Sediment Sieving Results for KW-44.1d.

Sieve Size (mm)	**10-11 ft bgs**	**11-12 ft bgs**	**12-13 ft bgs**	**13-14 ft bgs**	**14-15 ft bgs**	**15-16 ft bgs**	**16-17 ft bgs**	**17-18 ft bgs**	**18-19 ft bgs**	**19-20 ft bgs**	**20-21 ft bgs**	**21-22 ft bgs**	**22-23 ft bgs**	**23-24 ft bgs**	**24-25 ft bgs**		
1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.2	0.2	1	0.2	0.4		
0.5	0.9	1.2	1	0.5	0.4	1.7	2.3	1.8	2.9	3.1	1.4	2.9	2.6	0.9	2		
0.25	16.4	16.9	21.1	48.6	22.9	39.9	32.6	20.1	35.6	44.6	24.9	43.9	33.3	37.6	33.4		
0.106	4.6	6.2	4	11.6	5.3	8.7	6.6	8.9	10.3	14.4	8.9	17.1	11.6	32.3	13.5		
0.053	2.8	3	2	5.6	4.7	5.8	5.2	5.7	7.9	8.7	5.5	10.7	8.2	8.4	4.5		
<0.053	0.3	1.6	1.2	2.9	1.7	2.7	2.2	2.4	4.8	6.4	2.2	5.1	3.7	4	2.7		
Total Mass (g)	**25.1**	**29.0**	**29.4**	**69.3**	**35.1**	**58.9**	**49.1**	**39.1**	**61.7**	**77.3**	**43.1**	**79.9**	**60.4**	**83.4**	**56.5**		
Percent Finer																	
1	99.6	99.7	99.7	99.9	99.7	99.8	99.6	99.5	99.7	99.9	99.5	99.7	98.3	99.8	99.3		
0.5	96.0	95.5	96.3	99.1	98.6	96.9	94.9	94.9	95.0	95.9	96.3	96.1	94.0	98.7	95.8		
0.25	30.7	37.2	24.5	29.0	33.3	29.2	28.5	43.5	37.3	38.2	38.5	41.2	38.9	53.6	36.6		
0.106	12.4	15.9	10.9	12.3	18.2	14.4	15.1	20.7	20.6	19.5	17.9	19.8	19.7	14.9	12.7		
0.053	1.2	5.5	4.1	4.2	4.8	4.6	4.5	6.1	7.8	8.3	5.1	6.4	6.1	4.8	4.8		
<0.053	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	**Average**	**St Dev**
d10	*0.09*	*0.07*	*0.10*	*0.09*	*0.07*	*0.08*	*0.08*	*0.06*	*0.06*	*0.06*	*0.07*	*0.06*	*0.06*	*0.08*	*0.08*	*0.07*	*0.01*
d50	*0.31*	*0.29*	*0.32*	*0.31*	*0.30*	*0.31*	*0.31*	*0.27*	*0.29*	*0.29*	*0.29*	*0.28*	*0.29*	*0.24*	*0.29*	*0.29*	*0.02*
d60	*0.34*	*0.33*	*0.35*	*0.34*	*0.33*	*0.34*	*0.35*	*0.31*	*0.33*	*0.32*	*0.32*	*0.32*	*0.33*	*0.28*	*0.33*	*0.33*	*0.02*
U	*3.73*	*4.58*	*3.64*	*3.89*	*4.79*	*4.42*	*4.57*	*4.91*	*5.50*	*5.52*	*4.68*	*4.96*	*5.05*	*3.64*	*3.94*	*4.52*	*0.63*

Table A-IV-21. Major Cations in Ponds Before and After Artificial Filling.

Sample ID	Date of Sample	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)	Si (ppm)	S (ppm)	P (ppm)	Mn (ppm)
Detection Limit		0.1950	0.0160	0.0160	0.0260	0.0020	0.0340	0.0110	0.0030	0.0002
KP-04	26-Jun-09	16.5	8.88	54.97	19.01	1.44	31.90	2.55	0.419	0.6621
KP-04	27-Jun-09	16.4	9.93	54.82	18.14	0.47	32.34	2.57	0.306	0.7856
KP-04	02-Jul-09	8.2	14.25	17.02	7.38	3.83	18.85	2.62	0.959	0.2289
KP-04	07-Jul-09	5.5	13.59	12.01	4.54	3.43	15.00	1.97	1.657	0.4603
KP-04	25-Jun-09	16.4	8.90	53.97	18.63	3.25	30.94	2.51	0.827	0.7536
KP-05	06-Jul-09	8.0	12.97	13.13	5.75	4.13	19.58	1.46	0.816	0.5454
KP-05	08-Jul-09	8.0	13.18	13.80	5.54	4.32	18.82	1.27	0.923	0.6514
KP-05	22-Jun-09	11.3	17.34	13.86	6.26	10.19	26.49	2.06	1.796	0.6168
KP-05	28-Jun-09	11.7	10.46	19.20	8.21	8.04	23.35	1.83	0.679	1.2021
KP-10	26-Jun-09	37.5	28.43	42.25	18.28	3.30	31.82	4.67	0.559	1.8930

Table A-IV-22. Trace Metals in Ponds Before and After Artificial Filling.

Sample ID	Date of Sample	Ni (ppb)	As (ppb)	Mo (ppb)	Ba (ppb)	U (ppb)	Pb (ppb)	Cd (ppb)	Sb (ppb)
Detection Limit		1.4400	0.0320	0.0120	0.0760	0.0010	0.0500	0.0110	0.0080
KP-04	26-Jun-09	8	70.82	1.82	131.2	0.435	3.21	0.04	0.037
KP-04	27-Jun-09	1	52.59	1.90	47.4	0.680	1.53	BD	0.017
KP-04	02-Jul-09	29	14.05	0.53	41.3	0.508	5.85	0.04	0.183
KP-04	07-Jul-09	24	24.35	0.73	35.3	0.307	4.08	0.04	0.165
KP-04	25-Jun-09	20	97.35	1.86	76.0	0.235	2.63	BD	0.035
KP-05	06-Jul-09	25	6.74	0.26	40.4	0.432	3.53	BD	0.111
KP-05	08-Jul-09	25	9.25	0.34	37.6	0.404	3.60	0.03	0.126
KP-05	22-Jun-09	68	6.87	0.25	79.1	1.009	9.30	0.09	0.113
KP-05	28-Jun-09	42	9.59	0.39	52.4	0.144	1.19	BD	0.032
KP-10	26-Jun-09	19	13.62	0.75	93.2	0.651	5.88	0.21	0.260

Table A-IV-23. Major Cations in Deep Transect and Private Wells Before and After Artificial Pond Filling.

Sample ID	Date of Sample	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)	Si (ppm)	S (ppm)	P (ppm)	Mn (ppm)
Detection Limit		0.1950	0.0160	0.0160	0.0260	0.0020	0.0340	0.0110	0.0030	0.0002
UTK-32	26-Jun-09	9.3	17.43	40.66	15.49	6.04	30.19	2.59	0.127	3.1804
UTK-32	27-Jun-09	9.8	17.59	40.44	15.87	6.09	31.14	2.74	0.138	3.4111
UTK-32	25-Jun-09	9.9	18.10	42.18	16.13	4.70	30.73	2.97	0.117	3.2948
UTK-33	08-Jul-09	9.3	4.23	22.52	9.46	17.28	23.18	5.89	0.297	1.4562
UTK-33	28-Jun-09	13.4	5.52	26.63	11.93	20.71	25.90	7.96	0.375	1.5386
KW-36.2	26-Jun-09	18.5	9.96	33.93	12.47	21.66	26.22	3.16	0.974	1.9135
KW-36.2	09-Jul-09	15.7	8.02	27.06	11.60	18.16	27.43	3.11	0.986	1.6916
KW-36.2	13-Jun-09	17.4	6.17	39.29	18.31	19.81	27.46	3.47	0.974	2.2592
KW-37.2	24-Jun-09	24.9	25.39	43.05	15.97	50.26	19.09	0.81	0.597	3.0761
KW-37.2	09-Jul-09	38.5	33.07	50.65	16.10	51.27	18.69	0.73	0.555	3.0655
KW-37.2	23-Jun-09	26.6	27.94	47.97	18.28	44.55	18.71	0.57	0.577	3.9083
KW-39.2	26-Jun-09	13.6	7.59	57.17	18.39	6.31	29.89	0.93	1.351	0.8660
KW-39.2	27-Jun-09	14.6	7.58	56.01	19.52	6.72	31.83	1.06	1.391	0.9315
KW-39.2	02-Jul-09	14.0	7.26	56.28	20.55	6.44	31.66	1.96	1.341	0.8975
KW-39.2	07-Jul-09	13.9	7.52	58.72	20.24	6.64	31.21	2.74	1.324	0.9340
KW-39.2	19-Jun-09	14.4	7.44	53.54	19.05	6.74	31.24	0.74	1.355	0.9071
KW-41.2	27-Jun-09	18.0	8.23	51.21	19.85	11.89	32.30	2.49	1.503	0.6922
KW-41.2	08-Jul-09	15.9	7.81	49.87	21.46	7.22	33.51	1.86	1.676	0.5760
KW-41.2	20-Jun-09	16.4	9.49	47.87	20.52	9.17	31.94	1.03	1.627	0.5811
KW-42.2	02-Jul-09	7.9	3.65	24.61	11.68	28.76	30.59	2.45	0.994	2.0314
KW-42.2	06-Jul-09	7.8	3.19	20.84	9.95	24.43	28.54	2.94	0.984	1.8558
KW-42.2	15-Jun-09	8.2	2.83	16.33	7.74	24.51	28.81	0.80	1.246	0.7430
KW-43.2	08-Jul-09	13.1	6.82	26.07	10.39	6.47	28.28	2.56	0.267	2.4704
KW-43.2	22-Jun-09	7.4	4.59	23.07	8.76	3.29	25.88	2.23	0.137	2.3820

Table A-IV-24. Trace Metals in Deep Transect and Private Wells Before and After Artificial Pond Filling.

Sample ID	Date of Sample	Ni (ppb)	As (ppb)	Mo (ppb)	Ba (ppb)	U (ppb)	Pb (ppb)	Cd (ppb)	Sb (ppb)
Detection Limit		1.4400	0.0320	0.0120	0.0760	0.0010	0.0500	0.0110	0.0080
UTK-32	26-Jun-09	39	4.01	1.55	86.7	1.540	2.16	0.03	0.079
UTK-32	27-Jun-09	39	4.93	2.49	89.6	1.694	1.08	0.05	0.127
UTK-32	25-Jun-09	28	3.98	2.10	89.0	1.798	2.58	0.06	0.047
UTK-33	08-Jul-09	88	7.20	0.28	83.3	0.009	0.33	BD	0.069
UTK-33	28-Jun-09	103	9.03	0.34	109.9	0.013	0.30	BD	0.114
KW-36.2	26-Jun-09	121	24.71	0.38	214.7	0.005	0.33	BD	BD
KW-36.2	09-Jul-09	95	23.18	0.33	174.0	0.007	0.05	BD	BD
KW-36.2	13-Jun-09	108	24.59	0.37	181.3	0.009	0.29	BD	BD
KW-37.2	24-Jun-09	290	16.97	0.39	424.0	0.004	0.05	BD	BD
KW-37.2	09-Jul-09	310	19.37	0.51	502.9	0.022	3.47	0.02	0.044
KW-37.2	23-Jun-09	338	17.24	0.27	484.4	0.003	0.19	BD	BD
KW-39.2	26-Jun-09	36	114.03	2.17	117.6	0.007	1.91	BD	BD
KW-39.2	27-Jun-09	37	120.97	2.34	123.0	0.007	1.30	BD	BD
KW-39.2	02-Jul-09	37	130.60	2.39	121.6	0.008	1.29	BD	BD
KW-39.2	07-Jul-09	39	130.12	2.08	126.1	0.005	0.41	BD	BD
KW-39.2	19-Jun-09	37	116.90	2.41	114.9	0.017	3.96	0.01	BD
KW-41.2	27-Jun-09	64	99.95	1.59	135.2	0.006	0.42	BD	BD
KW-41.2	08-Jul-09	32	116.04	1.84	121.0	0.008	1.23	BD	BD
KW-41.2	20-Jun-09	49	74.68	1.90	133.8	0.003	0.80	BD	BD
KW-42.2	02-Jul-09	157	13.12	0.17	138.3	0.081	4.41	0.19	0.074
KW-42.2	06-Jul-09	119	12.15	0.15	114.2	0.002	0.52	BD	BD
KW-42.2	15-Jun-09	130	9.74	0.09	94.9	0.002	0.62	BD	BD
KW-43.2	08-Jul-09	31	17.92	0.39	66.4	0.025	0.32	BD	BD
KW-43.2	22-Jun-09	16	5.51	0.11	50.7	0.014	1.39	BD	BD

Table A-IV-25. Major Cations in Shallow Transect Wells Before and After Artificial Pond Filling.

Sample ID	Date of Sample	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)	Si (ppm)	S (ppm)	P (ppm)	Mn (ppm)
Detection Limit		0.1950	0.0160	0.0160	0.0260	0.0020	0.0340	0.0110	0.0030	0.0002
KW-36.1a	26-Jun-09	19.5	4.61	51.95	28.61	19.74	32.71	8.44	0.706	2.4542
KW-36.1a	09-Jul-09	19.9	3.73	46.68	22.09	13.90	21.72	9.74	0.616	2.0993
KW-36.1a	13-Jun-09	14.5	3.53	40.35	21.89	10.98	24.67	5.21	0.575	1.8421
KW-36.1b	26-Jun-09	16.0	5.47	33.14	26.08	26.48	47.49	4.53	0.623	1.6720
KW-36.1b	09-Jul-09	16.0	3.50	37.57	22.51	13.79	24.01	4.03	0.398	1.7760
KW-36.1b	13-Jun-09	16.7	3.71	42.77	23.99	9.17	22.42	9.94	0.197	1.6345
KW-36.1c	26-Jun-09	15.9	3.62	41.31	23.51	21.12	24.83	9.86	0.703	1.6229
KW-36.1c	09-Jul-09	15.1	3.45	34.92	20.62	18.37	24.57	4.81	0.659	1.5817
KW-36.1d	26-Jun-09	16.2	3.52	40.45	25.83	23.01	22.57	13.07	0.430	1.0440
KW-36.1d	09-Jul-09	16.3	3.33	32.06	21.67	19.00	22.79	7.16	0.439	1.1932
KW-36.1d	12-Jun-09	16.6	3.69	45.84	26.77	25.64	22.98	14.38	0.434	1.3935
KW-37.1a	24-Jun-09	31.5	7.89	15.22	6.10	12.84	12.95	7.60	0.075	1.5990
KW-37.1a	09-Jul-09	22.1	6.09	10.51	4.91	7.39	12.88	6.51	0.053	1.2784
KW-37.1a	23-Jun-09	31.2	6.79	13.88	5.88	11.09	13.16	7.55	0.067	1.6085
KW-37.1b	24-Jun-09	29.0	7.34	18.58	7.93	16.86	15.01	10.70	0.097	1.5977
KW-37.1b	09-Jul-09	28.6	5.91	17.73	7.29	12.24	14.76	8.96	0.109	1.3933
KW-37.1b	23-Jun-09	28.6	5.73	14.69	7.09	11.51	14.77	9.16	0.093	1.3573
KW-37.1c	24-Jun-09	20.1	4.48	25.34	12.25	27.79	17.60	15.63	0.263	1.8965
KW-37.1c	09-Jul-09	30.1	5.15	20.77	10.68	22.12	17.15	8.87	0.231	1.5312
KW-37.1d	24-Jun-09	21.6	4.32	35.08	21.52	22.49	18.60	31.08	0.052	2.6163
KW-37.1d	09-Jul-09	21.7	3.49	23.20	11.20	6.05	17.91	12.99	0.035	1.6287
KW-37.1d	23-Jun-09	21.4	4.51	38.13	23.56	17.28	18.06	33.27	0.039	2.8622
KW-39.1a	26-Jun-09	9.6	11.20	20.40	7.46	12.08	19.51	3.55	0.836	3.2004
KW-39.1a	27-Jun-09	11.5	11.82	24.22	8.39	14.45	21.15	3.90	0.934	2.9150
KW-39.1a	02-Jul-09	12.8	11.97	25.76	8.99	16.17	21.38	3.49	1.002	2.8828
KW-39.1a	07-Jul-09	13.1	14.14	25.44	8.55	15.69	19.98	3.71	0.966	2.8880
KW-39.1a	19-Jun-09	9.3	8.93	21.79	9.53	15.53	22.94	6.64	0.995	3.4390
KW-39.1b	26-Jun-09	8.6	9.20	21.43	7.79	14.93	19.83	4.15	0.927	3.1021
KW-39.1b	27-Jun-09	9.9	10.75	25.15	8.92	16.68	20.89	4.24	0.875	2.9846
KW-39.1b	02-Jul-09	11.4	10.15	28.24	9.69	18.49	21.73	4.78	0.867	2.7530
KW-39.1b	07-Jul-09	13.2	10.68	27.28	9.65	18.31	21.65	4.55	0.934	2.7867
KW-39.1b	19-Jun-09	10.2	9.03	28.79	11.43	20.57	24.98	10.00	0.938	2.9101
KW-39.1c	26-Jun-09	8.9	9.29	26.57	10.31	19.86	24.23	8.36	0.974	3.3555
KW-39.1c	27-Jun-09	9.5	8.94	22.61	11.46	19.68	24.63	7.09	1.039	2.8650
KW-39.1c	02-Jul-09	9.2	9.11	22.58	9.89	18.16	24.38	5.93	1.008	2.8512
KW-39.1c	07-Jul-09	10.6	11.90	27.94	10.43	20.70	22.99	4.77	0.896	3.0650
KW-39.1c	19-Jun-09	8.3	10.89	33.93	12.89	25.64	26.61	6.24	0.950	4.0616
KW-39.1d	26-Jun-09	8.6	6.00	36.30	15.68	26.72	29.33	5.20	0.903	2.4278

Table A-IV-25. Continued…

Sample ID	Date of Sample	Na (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)	Si (ppm)	S (ppm)	P (ppm)	Mn (ppm)
Detection Limit		0.1950	0.0160	0.0160	0.0260	0.0020	0.0340	0.0110	0.0030	0.0002
KW-39.1d	27-Jun-09	8.8	6.16	35.35	15.77	27.00	29.98	4.85	0.871	2.4932
KW-39.1d	02-Jul-09	8.6	6.09	35.58	16.01	27.04	29.66	5.41	0.902	2.4347
KW-39.1d	07-Jul-09	8.7	6.10	35.58	15.74	26.36	29.81	5.44	0.875	2.3417
KW-41.1a	27-Jun-09	13.7	3.65	29.34	13.76	14.16	21.68	6.53	0.127	0.3035
KW-41.1a	08-Jul-09	18.3	3.77	30.00	15.18	17.68	20.42	7.70	0.132	0.3436
KW-41.1a	20-Jun-09	12.0	3.90	32.88	15.54	18.19	21.18	7.80	0.140	0.4162
KW-41.1b	27-Jun-09	31.9	4.33	32.00	15.62	11.37	21.89	7.98	0.090	0.3138
KW-41.1b	08-Jul-09	38.4	4.30	28.74	13.52	11.74	20.71	7.37	0.097	0.3018
KW-41.1b	20-Jun-09	37.2	5.00	35.99	18.44	19.16	21.70	11.15	0.115	0.4887
KW-41.1c	27-Jun-09	43.1	4.55	55.10	15.60	5.30	23.45	8.06	0.235	0.3753
KW-41.1c	08-Jul-09	52.9	4.68	46.88	13.90	5.85	21.73	6.80	0.210	0.3604
KW-41.1c	20-Jun-09	43.3	4.64	57.31	16.30	6.32	22.72	10.11	0.234	0.3726
KW-41.1d	27-Jun-09	26.1	8.01	32.82	18.27	22.60	22.02	6.80	0.361	0.5356
KW-41.1d	08-Jul-09	35.1	7.92	30.74	19.62	21.99	22.51	7.16	0.379	0.5376
KW-41.1d	20-Jun-09	30.0	8.47	38.25	20.79	26.16	22.55	9.04	0.383	0.6641
KW-42.1a	02-Jul-09	5.5	2.91	13.87	5.66	1.23	16.48	3.03	0.024	0.1785
KW-42.1a	06-Jul-09	8.6	5.82	12.37	5.49	4.07	13.37	3.41	0.032	0.7971
KW-42.1a	15-Jun-09	5.1	3.43	7.27	3.72	3.65	17.36	3.85	0.047	0.5666
KW-42.1b	06-Jul-09	9.3	5.36	11.49	4.83	5.04	13.72	3.95	0.048	0.5687
KW-42.1b	15-Jun-09	7.4	3.90	13.54	5.77	6.00	18.75	5.54	0.065	0.7046
KW-42.1c	02-Jul-09	6.3	2.68	15.12	6.76	3.04	16.99	4.45	0.025	0.3881
KW-42.1c	06-Jul-09	6.8	3.19	14.50	7.17	2.22	17.34	3.48	BD	0.2969
KW-42.1c	15-Jun-09	7.9	2.90	27.77	13.61	2.60	18.65	0.46	0.048	0.3006
KW-42.1d	02-Jul-09	5.6	2.65	9.95	4.48	1.21	15.47	3.67	0.022	0.2529
KW-42.1d	06-Jul-09	8.6	3.16	13.37	5.55	2.19	15.14	3.45	0.012	0.4463
KW-42.1d	15-Jun-09	7.7	3.63	21.86	10.87	2.46	18.30	1.29	0.023	0.5104
KW-42.1e	06-Jul-09	9.1	3.60	12.92	5.26	1.54	14.27	3.06	0.026	0.1938
KW-42.1e	15-Jun-09	7.7	3.09	19.05	8.77	1.90	16.72	3.33	0.031	0.2255
KW-43.1a	08-Jul-09	13.5	17.98	28.04	7.04	0.27	28.77	2.26	0.024	2.3257
KW-43.1a	22-Jun-09	8.6	14.52	18.72	4.58	0.29	26.89	1.80	0.024	1.4282
KW-43.1a	28-Jun-09	8.9	14.30	18.33	4.53	0.26	26.83	1.57	BD	1.5097
KW-43.1b	08-Jul-09	10.3	18.55	23.03	5.90	0.24	30.26	1.11	0.024	1.8161
KW-43.1b	22-Jun-09	8.0	17.75	20.18	5.30	0.27	28.01	1.39	0.030	1.6927
KW-43.1b	28-Jun-09	9.8	20.07	36.61	9.69	0.83	26.92	0.50	0.043	2.9177
KW-43.1c	08-Jul-09	13.2	21.15	26.16	7.21	0.32	33.13	1.96	0.021	2.0500
KW-43.1c	22-Jun-09	10.3	22.31	26.18	7.01	0.26	31.45	0.41	0.028	1.8176
KW-43.1c	28-Jun-09	10.2	22.10	26.62	6.81	0.25	30.23	0.52	BD	1.7759
KW-43.1d	08-Jul-09	12.8	21.30	36.06	9.35	1.18	29.56	0.28	0.038	2.8449
KW-43.1d	22-Jun-09	9.3	19.11	34.07	8.95	0.78	28.30	0.50	0.044	3.5851
KW-43.1d	28-Jun-09	8.7	17.53	18.88	5.54	0.39	28.37	1.40	0.022	1.6653

Table A-IV-26. Trace Metals in Shallow Transect Wells Before and After Artificial Pond Filling.

Sample ID	Date of Sample	Ni (ppb)	As (ppb)	Mo (ppb)	Ba (ppb)	U (ppb)	Pb (ppb)	Cd (ppb)	Sb (ppb)
Detection Limit		1.4400	0.0320	0.0120	0.0760	0.0010	0.0500	0.0110	0.0080
KW-36.1a	26-Jun-09	108	13.13	0.20	152.3	0.280	4.57	0.02	0.043
KW-36.1a	09-Jul-09	77	10.52	0.19	119.8	0.013	0.18	BD	BD
KW-36.1a	13-Jun-09	64	10.83	0.18	98.9	0.072	8.75	0.02	0.018
KW-36.1b	26-Jun-09	155	5.82	0.07	159.0	1.284	15.67	0.07	0.036
KW-36.1b	09-Jul-09	74	9.08	0.15	103.4	0.114	54.87	0.07	0.024
KW-36.1b	13-Jun-09	51	7.41	0.25	114.1	0.377	146.43	0.02	0.035
KW-36.1c	26-Jun-09	124	11.98	0.24	112.9	0.072	4.43	BD	0.020
KW-36.1c	09-Jul-09	100	11.43	0.28	103.9	0.060	3.09	BD	BD
KW-36.1d	26-Jun-09	124	9.66	0.04	133.4	0.019	1.01	BD	BD
KW-36.1d	09-Jul-09	97	9.20	0.29	109.0	0.011	0.20	BD	BD
KW-36.1d	12-Jun-09	144	10.10	0.32	144.0	0.030	0.62	0.02	0.022
KW-37.1a	24-Jun-09	76	3.71	0.29	89.6	0.027	0.43	BD	BD
KW-37.1a	09-Jul-09	40	3.72	0.10	58.5	0.018	0.05	BD	BD
KW-37.1a	23-Jun-09	62	3.44	0.14	81.3	0.026	0.80	BD	BD
KW-37.1b	24-Jun-09	94	5.07	0.40	103.9	0.025	0.96	BD	BD
KW-37.1b	09-Jul-09	63	6.01	0.26	79.4	0.033	0.59	BD	BD
KW-37.1b	23-Jun-09	56	4.94	0.23	75.3	0.026	1.78	BD	BD
KW-37.1c	24-Jun-09	152	7.21	0.11	119.5	0.039	0.34	BD	BD
KW-37.1c	09-Jul-09	118	6.10	0.23	124.9	0.052	4.37	0.02	0.048
KW-37.1d	24-Jun-09	113	1.19	0.06	143.6	0.081	0.33	BD	BD
KW-37.1d	09-Jul-09	32	1.26	0.06	89.7	0.078	4.17	0.02	0.036
KW-37.1d	23-Jun-09	92	0.94	0.10	147.3	0.068	0.76	0.03	BD
KW-39.1a	26-Jun-09	66	26.06	0.55	155.2	0.014	2.93	BD	BD
KW-39.1a	27-Jun-09	80	30.32	0.47	185.3	0.005	1.63	BD	BD
KW-39.1a	02-Jul-09	94	31.91	0.48	193.6	0.002	0.52	BD	BD
KW-39.1a	07-Jul-09	87	27.91	0.86	202.9	0.002	0.72	BD	BD
KW-39.1a	19-Jun-09	80	36.64	1.04	149.2	0.023	3.62	0.03	0.014
KW-39.1b	26-Jun-09	80	29.85	0.82	149.4	0.009	1.83	BD	BD
KW-39.1b	27-Jun-09	94	31.72	0.58	186.4	0.006	1.43	BD	BD
KW-39.1b	02-Jul-09	102	33.59	0.55	187.6	0.005	0.81	BD	BD
KW-39.1b	07-Jul-09	99	32.69	0.50	200.9	BD	0.68	BD	BD
KW-39.1b	19-Jun-09	111	41.82	1.05	184.9	0.020	3.34	0.02	0.021
KW-39.1c	26-Jun-09	113	35.57	1.44	163.5	0.022	1.70	BD	BD
KW-39.1c	27-Jun-09	99	33.48	1.45	154.0	0.012	1.23	0.01	BD
KW-39.1c	02-Jul-09	93	34.50	1.25	147.8	0.013	1.56	BD	BD
KW-39.1c	07-Jul-09	111	32.61	0.77	189.9	0.010	1.55	BD	BD
KW-39.1c	19-Jun-09	138	38.53	0.88	209.6	0.038	3.40	BD	BD
KW-39.1d	26-Jun-09	144	28.05	0.51	192.1	0.054	1.50	BD	BD

Table A-IV-26. Continued…

Sample ID	Date of Sample	Ni (ppb)	As (ppb)	Mo (ppb)	Ba (ppb)	U (ppb)	Pb (ppb)	Cd (ppb)	Sb (ppb)
Detection Limit		1.4400	0.0320	0.0120	0.0760	0.0010	0.0500	0.0110	0.0080
KW-39.1d	27-Jun-09	143	29.12	0.48	194.1	0.043	2.00	BD	BD
KW-39.1d	02-Jul-09	145	27.50	0.43	190.2	0.038	0.87	BD	BD
KW-39.1d	07-Jul-09	138	27.48	0.45	191.7	0.031	0.57	BD	BD
KW-41.1a	27-Jun-09	81	1.74	0.04	93.2	0.042	0.75	0.01	BD
KW-41.1a	08-Jul-09	93	2.43	0.05	108.1	0.063	2.03	0.05	0.020
KW-41.1a	20-Jun-09	99	2.24	0.01	102.1	0.058	0.90	BD	BD
KW-41.1b	27-Jun-09	60	1.98	0.05	87.4	0.128	1.10	BD	BD
KW-41.1b	08-Jul-09	64	2.13	0.11	82.9	0.139	1.90	BD	0.019
KW-41.1b	20-Jun-09	98	2.32	0.07	111.3	0.220	3.80	BD	BD
KW-41.1c	27-Jun-09	29	3.77	0.47	55.3	0.268	0.76	BD	0.037
KW-41.1c	08-Jul-09	35	3.59	0.71	58.3	0.273	15.45	BD	0.071
KW-41.1c	20-Jun-09	33	3.68	0.60	58.4	0.406	0.58	BD	0.043
KW-41.1d	27-Jun-09	118	4.06	0.09	163.4	0.023	2.21	BD	0.018
KW-41.1d	08-Jul-09	108	4.12	0.04	168.2	0.018	17.68	BD	BD
KW-41.1d	20-Jun-09	144	3.85	0.01	187.9	0.010	0.55	BD	BD
KW-42.1a	02-Jul-09	13	1.37	0.13	27.2	0.043	2.36	BD	0.057
KW-42.1a	06-Jul-09	24	5.33	0.14	45.8	0.045	0.51	BD	0.023
KW-42.1a	15-Jun-09	18	5.55	0.17	29.4	0.027	1.27	BD	0.020
KW-42.1b	06-Jul-09	26	12.57	0.15	46.4	0.039	3.48	BD	BD
KW-42.1b	15-Jun-09	35	4.00	0.22	44.8	0.023	1.14	BD	BD
KW-42.1c	02-Jul-09	15	0.91	0.06	39.9	0.034	1.08	BD	BD
KW-42.1c	06-Jul-09	10	1.16	0.07	40.8	0.032	1.29	BD	BD
KW-42.1c	15-Jun-09	13	1.08	0.06	50.3	0.187	2.88	BD	BD
KW-42.1d	02-Jul-09	5	0.31	0.07	22.7	0.036	1.01	BD	BD
KW-42.1d	06-Jul-09	14	2.66	0.09	34.0	0.045	0.96	BD	BD
KW-42.1d	15-Jun-09	12	0.69	0.02	48.4	0.099	1.45	0.02	BD
KW-42.1e	06-Jul-09	10	4.26	0.07	28.6	0.039	1.04	BD	BD
KW-42.1e	15-Jun-09	11	0.55	0.04	35.2	0.035	2.43	BD	BD
KW-43.1a	08-Jul-09	7	0.79	0.09	65.1	0.084	1.21	0.11	BD
KW-43.1a	22-Jun-09	3	0.99	0.14	39.4	0.058	1.00	0.02	BD
KW-43.1a	28-Jun-09	6	1.30	0.14	39.4	0.055	0.62	0.03	BD
KW-43.1b	08-Jul-09	3	0.41	0.21	61.4	0.143	1.56	0.08	BD
KW-43.1b	22-Jun-09	4	0.36	0.38	50.5	0.117	1.01	BD	BD
KW-43.1b	28-Jun-09	10	0.60	0.33	98.1	1.063	0.45	0.02	BD
KW-43.1c	08-Jul-09	3	0.40	0.30	71.7	0.151	0.94	0.03	BD
KW-43.1c	22-Jun-09	2	0.39	0.41	65.7	0.237	1.14	BD	BD
KW-43.1c	28-Jun-09	2	0.21	0.45	62.6	0.248	0.57	0.03	BD
KW-43.1d	08-Jul-09	9	0.89	0.11	109.7	0.624	0.28	0.03	BD
KW-43.1d	22-Jun-09	4	0.79	0.29	89.9	0.816	1.01	BD	BD
KW-43.1d	28-Jun-09	3	0.36	0.29	50.4	0.106	1.04	BD	BD

Table A-IV-27. Electrical Conductivity Monthly Monitoring Results from Shallow Transect Monitoring wells (mS/cm). "NS" refers to wells that were not sampled.

Well ID	8/26/08	9/20/08	10/29/08	11/22/08	1/29/09	2/25/09	3/16/09	4/22/09	5/27/09	7/19/09	8/26/09	9/30/09	10/27/09	Average
KW-36.1 a	0.49	0.44	NS	0.44	0.57	0.70	NS	dry	dry	0.50	0.30	0.71	0.70	0.54
KW-36.1 d	0.43	0.44	NS	0.50	0.54	NS	0.91	dry	dry	0.54	0.56	0.65	0.62	0.58
KW-37.1 a	0.33	0.31	NS	0.38	0.47	0.46	0.86	dry	1.43	0.26	0.44	0.20	0.21	0.49
KW-37.1 d	0.28	0.35	NS	0.40	0.42	0.44	0.82	dry	1.29	0.42	0.32	0.17	0.15	0.46
KW-38.1 a	0.28	0.40	NS	0.50	0.46	0.54	0.93	dry	dry	0.58	0.43	0.48	0.42	0.50
KW-38.1 d	0.53	0.43	NS	0.38	0.48	0.47	0.79	1.59	1.62	0.39	0.47	0.21	0.17	0.63
KW-39.1 a	0.46	0.55	NS	0.48	0.64	0.56	0.81	0.74	0.78	0.40	0.41	0.43	0.55	0.57
KW-39.1 c	0.52	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.40	0.40	0.42	0.44
KW-39.1 d (27)	0.43	0.70	0.57	0.65	0.66	0.68	1.15	1.60	1.73	NS	NS	NS	NS	0.91
KW-40.1 a	0.57	0.51	NS	0.10	0.45	NS	NS	1.59	1.00	0.59	0.59	0.35	NS	0.64
KW-40.1 d	0.53	0.53	NS	0.38	0.44	NS	NS	1.69	0.88	0.52	0.50	0.33	NS	0.65
KW-41.1 a	0.40	0.34	NS	0.38	0.58	0.35	NS	1.32	1.18	0.51	0.54	0.35	0.25	0.56
KW-41.1 d	0.44	0.41	NS	0.45	0.51	0.05	NS	1.54	1.42	0.52	0.60	0.42	0.42	0.62
KW-43.1 a	0.45	0.42	NS	0.45	0.74	0.03	NS	0.57	0.88	0.34	0.27	0.39	0.31	0.44
KW-43.1 d	0.45	0.42	NS	0.44	0.59	0.39	NS	0.40	0.54	0.39	0.35	0.47	0.45	0.44
KW-44.1 a (28)	0.38	0.35	0.37	0.39	0.50	0.50	1.03	0.40	0.86	NS	NS	NS	NS	0.53
KW-44.1 d	0.35	0.38	NS	0.37	0.55	0.46	0.93	0.50	0.58	0.33	0.37	0.35	0.35	0.46

Table A-IV-28. *E. coli* Monthly Monitoring Results from Shallow Transect Monitoring wells at Site K (MPN/100 ml). Concentrations are based upon duplicate 100 ml samples (except 11/25/08 when only single 100 ml samples were taken). "0.3" is the estimated detection limit and indicates no *E. coli* was detected in either 100 ml sample. "NS" refers to wells that were not sampled.

Well ID	9/20/08	10/29/08	11/25/08	1/29/09	2/26/09	3/16/09	4/25/09	5/25/09	6/28/09	7/19/09	8/27/09	9/30/09	10/27/09
KW-36.1d	0.3	0.3	0.3	0.3	0.3	0.3	NS	NS	NS	1.5	0.5	0.3	0.3
KW-37.1a	0.3	0.3	0.3	0.3	0.3	2.6	NS	0.5	NS	0.3	0.3	0.3	0.3
KW-37.1d	0.3	0.3	0.3	0.3	0.3	28.3	NS	4.7	NS	1.0	2.0	0.3	5.8
KW-38.1a	0.3	0.3	0.3	0.3	0.3	0.3	NS	NS	NS	0.3	0.3	0.3	0.5
KW-38.1d	0.3	0.3	10.9	0.3	0.3	6.3	0.5	1.0	NS	0.3	0.3	7.4	2.6
KW-39.1a	0.3	0.3	3.1	0.3	0.3	0.3	0.3	1.5	NS	5.8	7.5	0.3	0.3
KW-39.1d (27)	0.3	1.0	0.3	0.3	0.3	5.2	0.3	1.0	NS	0.3	0.3	NS	NS
KW-40.1a	0.3	0.3	0.3	0.3	0.3	4.3	1.0	2.0	NS	2.6	46.8	1.5	0.3
KW-40.1d	0.3	0.3	5.3	0.3	0.3	1.5	13.3	0.3	NS	5.8	9.8	0.3	0.3
KW-41.1a	0.3	0.3	2.0	0.3	0.3	0.5	1.0	1.5	NS	0.3	0.3	0.3	0.3
KW-41.1d	0.3	0.3	0.3	0.3	1.0	1.0	0.5	0.3	NS	0.3	0.3	0.3	0.3
KW-43.1a	0.3	0.3	1.0	0.3	9.8	0.3	1.0	0.3	NS	0.5	0.5	0.3	0.3
KW-43.1d	0.3	0.3	6.2	0.3	0.3	0.3	0.3	0.5	NS	0.3	0.3	0.3	0.3
KW-44.1a (28)	0.3	0.3	1.0	0.3	0.3	0.5	0.5	2.0	0.3	0.3	0.3	NS	NS
KW-44.1d	0.3	0.3	1.0	0.3	0.3	0.3	0.5	0.3	NS	0.3	0.3	0.5	0.5

Table A-IV-29. Water Levels from Transects Surrounding Pond KP-10. Levels indicate depth of water table below top of casing (m).

	KL-10	KW-36.2	KW-36.1a	KW-36.1b	KW-36.1c	KW-36.1d	KW-37.2	KW-37.1a	KW-37.1b	KW-37.1c	KW-37.1d	KW-38.2	KW-38.1a	KW-38.1b	KW-38.1c	KW-38.1d
6/11/09	2.150	4.656	4.604	4.641	4.683	4.579	4.853	4.769	4.79	4.869	4.847	4.64	4.585	4.705	4.792	4.784
6/12/09	NA	4.645	4.586	4.634	4.582	4.566	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6/13/09	2.190	4.616	4.574	4.616	4.565	4.551	4.815	4.732	4.747	4.824	4.806	4.603	4.540	4.646	4.742	4.739
6/14/09	2.291	4.579	4.546	4.584	4.531	4.516	4.780	4.075	4.272	4.795	4.772	4.568	4.509	4.629	4.712	4.705
6/15/09	2.374	4.559	4.532	4.567	4.514	4.501	4.752	4.689	4.708	4.778	4.758	4.549	4.492	4.619	4.704	4.688
6/17/09	dry	4.536	4.516	4.549	4.495	4.485	4.741	4.675	4.693	4.765	4.744	4.529	4.475	4.621	4.682	4.665
6/23/09	NA	4.511	4.501	4.528	4.474	4.459	4.713	4.655	4.674	4.744	4.721	4.501	4.451	4.605	4.611	4.643
6/24/09	NA	NA	4.501	4.525	4.480	4.460	4.728	4.657	4.674	4.749	4.722	4.509	4.455	4.610	4.667	4.646
6/25/09 11:00 PM	2.295	4.505	4.472	4.507	4.454	4.439	4.713	4.638	4.657	4.726	4.705	4.495	4.442	4.598	4.656	4.634
6/25/09 5:00 PM	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6/26/09	2.388	4.504	4.472	4.504	4.451	4.437	4.706	4.635	4.655	4.723	4.702	4.492	4.438	4.593	4.652	4.632
6/27/09	dry	4.500	4.473	4.505	4.451	4.436	4.703	4.634	4.654	4.723	4.700	4.489	4.437	4.652	4.650	4.630
6/28/09	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6/29/09	dry	4.498	4.450	4.509	4.454	4.438	4.702	4.637	4.658	4.725	4.702	4.489	4.427	4.592	4.648	4.631
7/1/09 11:00 AM	NA	4.486	4.463	4.494	4.441	4.426	4.689	4.620	4.643	4.703	4.689	4.479	4.425	4.580	4.636	4.616
7/1/09 6:00 PM	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
7/2/09	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
7/4/09	2.183	4.411	4.397	4.435	4.399	4.366	4.610	4.542	4.567	4.632	4.609	4.397	4.345	4.497	4.557	4.539
7/6/09	2.301	4.353	4.344	4.383	4.328	4.313	4.517	4.491	4.510	4.581	4.556	4.343	4.291	4.437	4.504	4.481
7/20/09	dry	4.047	4.053	4.085	4.032	4.014	4.253	4.215	4.237	4.304	4.274	4.04	3.996	4.146	4.207	4.194
8/27/09	1.642	1.944	2.002	2.031	1.981	1.966	2.151	2.106	2.133	2.214	2.179	1.936	1.911	2.061	2.13	2.094
10/1/09	1.241	1.616	1.741	1.748	1.696	1.682	1.958	1.884	1.915	1.972	1.951	1.738	1.684	1.833	1.861	1.88
10/28/09	1.986	2.519	2.481	2.504	2.452	2.435	2.712	2.649	2.673	2.739	2.714	2.502	2.449	2.607	2.564	2.463

Table A-IV-30. Water Levels from Transects Surrounding Pond KP-04. Levels indicate depth of water table below top of casing (m).

	KL-04	KW-39.2	KW-39.1a	KW-39.1b	KW-39.1c	KW-39.1d	KW-40.2	KW-40.1a	KW-40.1b	KW-40.1c	KW-40.1d	KW-41.2	KW-41.1a	KW-41.1b	KW-41.1c	KW-41.1d
6/11/09	2.979	4.37	4.296	4.336	4.403	4.54	5.004	5.204	4.998	4.981	4.991	3.642	3.774	4.125	4.445	4.582
6/12/09	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6/13/09	2.978	4.357	4.209	4.256	4.332	4.493	4.985	4.954	4.942	4.952	4.956	3.6	3.727	4.181	4.402	4.543
6/14/09	2.982	4.383	4.291	4.331	4.401	4.539	5.306	5.107	4.998	4.989	4.991	3.633	3.767	4.119	4.431	4.578
6/15/09	2.961	4.367	4.295	4.325	4.405	4.546	5.021	5.008	4.992	4.976	4.973	3.612	3.761	4.114	4.431	4.554
6/17/09	2.985	4.380	4.301	4.345	4.410	4.549	4.979	4.999	4.986	4.963	4.957	NA	3.767	4.119	4.435	4.574
6/23/09	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6/24/09	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6/25/09 11:00 PM	NA	4.293	4.082	4.132	4.218	4.359	NA	4.899	4.905	4.964	4.979	NA	3.732	4.509	4.374	4.518
6/25/09 5:00 PM	2.821	NA	NA	4.147	4.217	4.358	NA	4.884	4.877	4.921	4.933	3.549	3.654	4.008	4.328	4.469
6/26/09	NA	4.278	4.125	4.172	4.243	4.384	4.943	4.874	4.874	4.885	4.895	3.535	3.645	4.001	4.317	4.459
6/27/09	2.980	4.242	4.164	4.206	4.275	4.417	4.892	4.902	4.890	4.875	4.867	3.508	3.644	3.999	4.314	4.453
6/28/09	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6/29/09	2.984	4.241	4.169	4.212	4.281	4.416	4.889	4.905	4.893	4.873	4.863	3.506	3.646	3.997	4.313	4.451
7/1/09 11:00 AM	2.988	4.217	4.126	4.169	4.238	4.379	4.870	4.873	4.861	4.851	4.845	3.482	3.617	3.972	4.286	4.426
7/1/09 6:00 PM	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
7/2/09	2.965	4.191	4.088	4.132	4.201	4.339	4.839	4.832	4.822	4.818	4.814	3.457	3.586	3.941	4.261	4.398
7/4/09	2.875	4.124	4.024	4.098	4.137	4.275	4.773	4.768	4.756	4.751	4.749	3.394	3.526	3.881	4.205	4.339
7/6/09	2.984	4.087	4.012	4.056	4.124	4.259	4.729	4.745	4.734	4.716	4.708	3.352	3.490	3.845	4.160	4.300
7/20/09	2.987	3.737	3.678	3.718	3.787	3.923	4.384	4.419	4.412	4.381	4.361	3	3.146	3.492	3.812	3.948
8/27/09	1.859	1.652	2.665	1.641	1.708	1.604	2.305	2.336	2.276	2.289	2.331	0.908	1.065	1.435	2.111	2.265
10/1/09	1.536	1.616	1.501	1.539	1.608	1.747	2.279	2.232	2.234	2.233	2.245	0.881	0.992	1.356	1.672	1.816
10/28/09	NM	2.427	2.309	2.348	2.414	2.554	3.074	3.033	3.029	3.026	3.041	1.679	1.801	2.155	2.459	2.607

Table A-IV-31. Water Levels from Transects Surrounding Pond KP-05. Levels indicate depth of water table below top of casing (m).

	KL-05	KW-42.0	KW-42.2	KW-42.1a	KW-42.1b	KW-42.1e	KW-42.1d	KW-42.1c	KW-43.2	KW-43.1a	KW-43.1b	KW-43.1c	KW-43.1d	KW-44.2	KW-44.1a	KW-44.1b	KW-44.1c	KW-44.1d
6/11/09	2.35	dry	4.01	4.03	4.027	4.02	4.001	4.006	3.946	3.951	4.002	4.702	4.506	4.524	4.538	4.518	4.539	4.597
6/12/09	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6/13/09	1.871	dry	3.994	3.955	3.963	3.959	3.941	3.948	3.944	3.948	4	4.73	4.59	4.548	4.549	4.529	4.552	4.499
6/14/09	1.956	dry	4.302	4.004	4.008	3.991	3.985	4.004	3.972	3.962	4.204	4.904	4.801	4.584	4.586	4.568	4.592	4.567
6/15/09	2.035	dry	3.989	3.991	3.99	3.982	3.962	3.961	3.964	3.978	4.027	4.098	4.084	4.581	4.593	4.574	4.601	4.546
6/17/09	2.116	dry	4.039	4.031	4.033	4.021	4.005	4.011	3.952	3.965	4.019	4.088	4.073	4.568	4.583	4.565	4.565	4.521
6/23/09	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6/24/09	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6/25/09 11:00 PM	2.352	dry	3.895	3.942	3.939	3.939	3.912	3.916	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6/25/09 5:00 PM	NA	NA	3.883	3.93	3.929	3.924	3.902	3.905	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6/26/09	NA	dry	3.912	3.924	3.925	3.913	3.895	3.899	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6/27/09	2.347	dry	3.885	3.916	3.911	3.905	3.884	3.885	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6/28/09	1.99	dry	pumped	3.945	3.943	3.938	3.917	3.92	pumped	3.886	3.936	4.004	3.985	4.462	4.471	4.451	4.484	4.412
6/29/09	1.917	dry	pumped	3.905	3.906	3.899	3.879	3.882	pumped	3.857	3.908	3.977	3.962	4.424	4.437	4.418	4.439	4.378
7/1/09 11:00 AM	1.719	dry	3.845	3.767	3.787	3.782	3.768	3.779	3.795	3.805	3.858	3.93	3.917	4.381	4.389	4.371	4.392	4.333
7/1/09 6:00 PM	1.926	dry	3.816	3.19	3.327	3.361	3.391	3.378	3.779	3.777	3.837	3.906	3.902	4.365	4.353	4.331	4.351	4.301
7/2/09	1.946	dry	3.812	3.701	3.712	3.712	3.702	3.716	3.761	3.758	3.811	3.884	3.871	4.344	4.349	4.329	4.340	4.295
7/4/09	1.925	dry	3.752	3.678	3.697	3.693	3.678	3.692	3.703	3.706	3.764	3.829	3.819	4.289	4.298	4.278	4.299	4.241
7/6/09	2.022	dry	3.730	3.74	3.740	3.733	3.712	3.71	3.676	3.689	3.745	3.815	3.801	4.263	4.279	4.26	4.284	4.221
7/20/09	2.256	dry	3.399	3.429	3.431	3.424	3.404	3.401	3.334	3.355	3.408	3.477	3.466	3.933	3.95	3.931	3.954	3.891
8/27/09	1.461	1.359	1.315	1.329	1.331	1.335	1.313	1.299	1.247	1.291	1.334	1.394	1.378	NA	1.815	NA	NA	1.869
10/1/09	1.119	1.218	1.211	1.196	1.201	1.197	1.177	1.168	1.145	1.152	1.204	1.272	1.258	NA	1.737	NA	NA	1.689
10/28/09	1.802	2.041	2.049	2.017	2.021	2.021	2.001	1.999	1.975	1.977	2.029	2.097	2.082	NA	2.586	NA	NA	2.569

Table A-IV-32. Elevations of Tops of Casings of Transect Wells and L-piezometers (m above datum). The datum corresponds to that set by Karrie Radloff for Site K.

Well ID	Adjusted Tops (mad)	Well ID	Adjusted Tops (mad)
KL-04	0.189	KW-40.1c	0.658
KL-05	0.049	KW-40.1d	0.654
KL-10	-0.256	KW-40.2	0.669
KL-15	-0.014	KW-40.2	0.669
KW-36.1a	0.042	KW-41.1a	-0.583
KW-36.1b	0.067	KW-41.1b	-0.228
KW-36.1c	0.016	KW-41.1c	0.087
KW-36.1d	0.000	KW-41.1d	0.226
KW-36.2	0.045	KW-41.2	-0.667
KW-37.1a	0.190	KW-42.0	-0.330
KW-37.1b	0.222	KW-42.1a	-0.358
KW-37.1c	0.280	KW-42.1b	-0.352
KW-37.1c	0.280	KW-42.1c	-0.373
KW-37.1d	0.257	KW-42.1d	-0.373
KW-37.2	0.253	KW-42.1e	-0.353
KW-38.1a	-0.012	KW-42.2	-0.358
KW-38.1b	0.147	KW-43.1a	-0.413
KW-38.1c	0.206	KW-43.1b	-0.360
KW-38.1d	0.184	KW-43.1c	-0.292
KW-38.2	0.034	KW-43.1d	-0.304
KW-39.1a	-0.065	KW-43.2	-0.425
KW-39.1b	-0.030	KW-44.1a (28)	0.183
KW-39.1c	0.036	KW-44.1b	0.183
KW-39.1d (27)	0.181	KW-44.1c	0.184
KW-39.2	0.014	KW-44.1d	0.130
KW-40.1a	0.667	KW-44.1d	0.130
KW-40.1b	0.664	KW-44.2	0.172

APPENDIX V – VILLAGE-SCALE FECAL CONTAMINATION MONITORING

This section is in an outline of a manuscript in preparation for publication.

Factors Influencing the Spatial and Temporal Distribution of Fecal Contamination in a Sandy Aquifer in Bangladesh

Abstract

Over fifty groundwater wells were monitored monthly for *E. coli* in a sandy aquifer underlying a village in Bangladesh over two years. Monthly *E. coli* prevalence varied from 30 to 70%, peaking in the wet season in both years (2008 and 2009). Precipitation was found to be the predominant temporal influence on *E. coli* prevalence in both private wells and monitoring wells, however, several other potential factors were tested to explain the spatial distribution of *E. coli* and sulphate within each month and across months. These include: water levels, surficial geology, ground elevation, well depth, chemistry (Low v. High Ionic Strength), well construction, proximity of ponds and latrines and population density. Private well construction and/or frequent pumping were found to result in significantly ($p<0.05$) more frequent *E. coli* detections than properly sealed monitoring wells which were pumped only during monthly sampling. Population and latrine density was found to significantly ($p<0.05$) influence sulphate concentrations and the ionic strength of private tubewells.

Table A-V-1. Classification of Well Types at Site K.

Well Type (Notation)	Seal (y/n)	Pumping Frequency	Count
Private (P)	n	daily	37
Monitoring (M)	n	monthly	6
Monitoring (MS)	y	monthly	11
		Total	*54*

Table A-V-2. Depths of Site K Private and Monitoring Wells. Private well depths are approximate (+/- 2 m) whereas reported monitoring well depths are exact (+/- 1 cm). “bgs” refers to below ground surface.

Private Wells		Monitoring Wells	
Well No	Depth (m bgs)	Well No	Depth (m bgs)
UTK-01	9.1	KW-12.1	7.5
UTK-02	10.7	KW-12.2	9.9
UTK-03	10.7	KW-12.3	14.8
UTK-04	8.4	KW-20.1	7.4
UTK-05	15.2	KW-20.2	10.7
UTK-06	7.6	KW-20.3	14.1
UTK-07	7.6	KW-23	7.2
UTK-08	16.8	KW-24	11.7
UTK-09	30.5	KW-25	15.4
UTK-10	7.6	KW-26	7.2
UTK-11	7.6	KW-27	7.5
UTK-12	NA	KW-28	7.7
UTK-13	7.6	KW-29	8.7
UTK-14	11.4	KW-30	13.5
UTK-15	8.4	KW-34	7.5
UTK-16	7.6	KW-35	7.7
UTK-17	7.6		
UTK-18	15.2		
UTK-20	9.1		
UTK-21	12.2		
UTK-22	9.1		
UTK-23	12.2		
UTK-24	9.1		
UTK-25	6.1		
UTK-26	7.6		
UTK-27	9.1		
UTK-28	7.6		
UTK-29	7.6		
UTK-30	13.7		
UTK-32	7.6		
UTK-34	7.6		
UTK-35	NA		
UTK-36	NA		
UTK-37	NA		

Table A-V-3. Elevations of Tops of Casings of Monitoring Wells (m above datum). The datum corresponds to that set by Karrie Radloff for Site K.

Well ID	Adjusted Tops (mad)
KW-12.0	0.671
KW-12.1	0.685
KW-12.2	0.633
KW-12.3	0.706
KW-20.0a	0.071
KW-20.0b	0.053
KW-20.1	0.045
KW-20.2	0.075
KW-20.3	0.105
KW-23	-0.165
KW-24	0.067
KW-25	0.577
KW-26	-0.472
KW-29	0.484
KW-30	-0.098
KW-31	-0.39
KW-32	0.294
KW-33	0.355
KW-34	-0.338
KW-35	-0.972

Table A-V-4. Measured Hydraulic Conductivities of Site K Monitoring Wells.

Well ID	K Trial 1 (m/s)	K Trial 2 (m/s)	K Trial 3 (m/s)	Average K (m/s)	CV*	Average K for Transect (m/s)	CV* for Transect
KW-23	1.80E-04	1.86E-04	1.95E-04	1.9E-04	4		
KW-24	6.01E-05	5.32E-05		5.7E-05	9		
KW-25	bad data						
KW-26	8.69E-05	8.29E-05		8.5E-05	3		
KW-29	8.62E-05	8.95E-05	9.39E-05	9.0E-05	4		
KW-30	1.49E-04	1.59E-04	1.66E-04	1.6E-04	5		
KW-36.1a	1.71E-05	1.70E-05	1.68E-05	1.7E-05	1		
KW-36.1b	1.96E-05	2.12E-05	2.30E-05	2.1E-05	8		
KW-36.1c	3.14E-05	2.55E-05	2.45E-05	2.7E-05	14		
KW-36.1d	4.61E-05	5.30E-05	4.97E-05	5.0E-05	7	2.87E-05	50.5
KW-36.2	3.52E-04	3.68E-04	3.89E-04	3.7E-04	5		
KW-37.1a	8.79E-05	9.04E-05	8.71E-05	8.8E-05	2		
KW-37.1b	8.67E-05	8.51E-05		8.6E-05	1		
KW-37.1c	6.49E-05	6.70E-05	6.82E-05	6.7E-05	3		
KW-37.1d	8.07E-05	8.62E-05	8.27E-05	8.3E-05	3	8.11E-05	12.1
KW-37.2	4.05E-04	3.96E-04	3.88E-04	4.0E-04	2		
KW-38.1a	4.46E-05	4.58E-05	4.37E-05	4.5E-05	2		
KW-38.1b	5.20E-05	5.15E-05	5.67E-05	5.3E-05	5		
KW-38.1c	7.29E-05	7.72E-05	7.92E-05	7.6E-05	4		
KW-38.1d	7.62E-05	7.62E-05	7.27E-05	7.5E-05	3	6.24E-05	25.4
KW-38.2	1.85E-04	1.91E-04	1.85E-04	1.9E-04	2		
KW-39.1a	2.06E-04	2.35E-04	2.41E-04	2.3E-04	8		
KW-39.1b	1.97E-04	2.13E-04	2.14E-04	2.1E-04	5		
KW-39.1c	2.54E-04	2.67E-04	2.77E-04	2.7E-04	4		
KW-39.1d (27)	1.09E-04	1.11E-04	1.13E-04	1.1E-04	2	2.03E-04	32.4
KW-39.2	1.41E-04	1.62E-04		1.5E-04	10		
KW-40.1a	2.64E-04	2.45E-04	2.41E-04	2.5E-04	5		
KW-40.1b	5.92E-05	6.03E-05	6.71E-05	6.2E-05	7		
KW-40.1c	4.27E-05	4.57E-05	4.33E-05	4.4E-05	4		
KW-40.1d	6.09E-05	5.52E-05	5.56E-05	5.7E-05	6	1.03E-04	95.0
KW-40.2	1.16E-04	9.29E-05	1.21E-04	1.1E-04	13		
KW-41.1a	2.85E-04	2.53E-04	2.64E-04	2.7E-04	6		
KW-41.1b	2.77E-04	2.79E-04	2.50E-04	2.7E-04	6		

*Coefficient of Variation

Table A-V-4 continued…

Well ID	K Trial 1 (m/s)	K Trial 2 (m/s)	K Trial 3 (m/s)	Average K (m/s)	CV*	Average K for Transect (m/s)	CV* for Transect
KW-41.1c	poor results						
KW-41.1d	2.66E-04	2.88E-04	2.87E-04	2.8E-04	5	2.72E-04	2.7
KW-41.2	1.34E-04	1.36E-04	1.41E-04	1.4E-04	2		
KW-42.1a	3.36E-04	3.41E-04	3.34E-04	3.4E-04	1		
KW-42.1b	3.55E-04	3.68E-04	3.79E-04	3.7E-04	3		
KW-42.1c	3.40E-04	3.43E-04	3.56E-04	3.5E-04	2		
KW-42.1d	4.45E-04	4.64E-04	4.52E-04	4.5E-04	2		
KW-42.1e	3.15E-04	3.14E-04	3.00E-04	3.1E-04	3	3.63E-04	15.2
KW-42.2	3.24E-04	3.35E-04	3.45E-04	3.3E-04	3		
KW-43.1a	4.35E-04	4.02E-04	3.66E-04	4.0E-04	9		
KW-43.1b	4.62E-04	4.92E-04	4.56E-04	4.7E-04	4		
KW-43.1c	3.30E-04	3.45E-04	3.45E-04	3.4E-04	3		
KW-43.1d	4.35E-04	4.36E-04	4.41E-04	4.4E-04	1	4.12E-04	13.5
KW-43.2	2.63E-04	2.93E-04	2.86E-04	2.8E-04	6		
KW-44.1a (28)	2.73E-04	2.64E-04	2.62E-04	2.7E-04	2		
KW-44.1b	2.76E-04	3.03E-04	3.10E-04	3.0E-04	6		
KW-44.1c	2.59E-04	3.29E-04	3.30E-04	3.1E-04	13		
KW-44.1d	3.13E-04	3.17E-04	3.30E-04	3.2E-04	3	2.97E-04	7.6
KW-44.2	3.44E-04	3.71E-04	3.59E-04	3.6E-04	4		

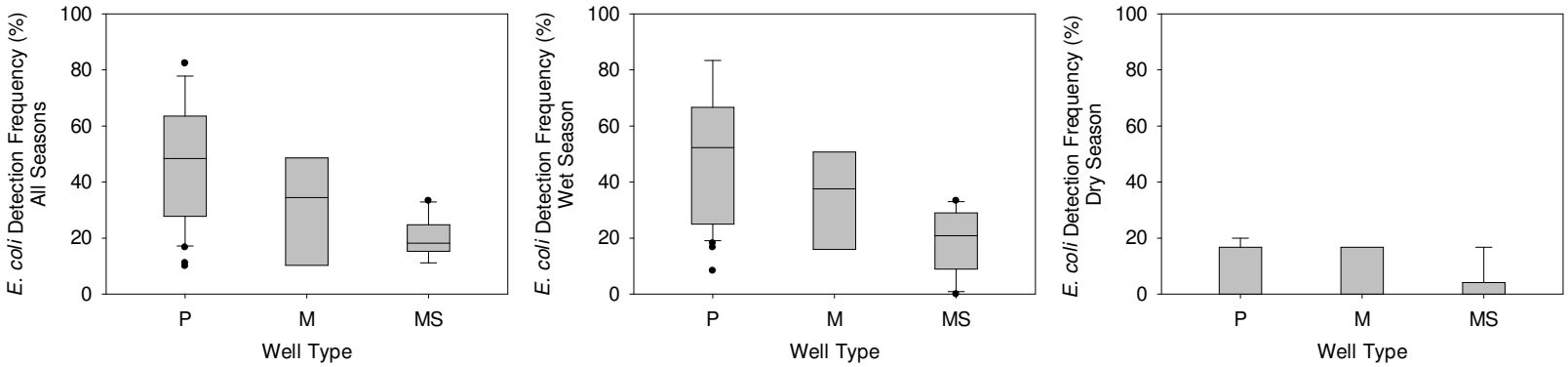


Figure A-V-1. Detection Frequencies of *E. coli* in monthly monitored private (P), monitoring (M), and sealed monitoring (MS) wells. Sampling was carried out from April 2008 through November 2009. The number of wells with at least three months of monthly data in each season were 34, 6 and 10 for P, M and MS respectively. There were a total of 12 possible wet season sampling events and 6 dry season months.

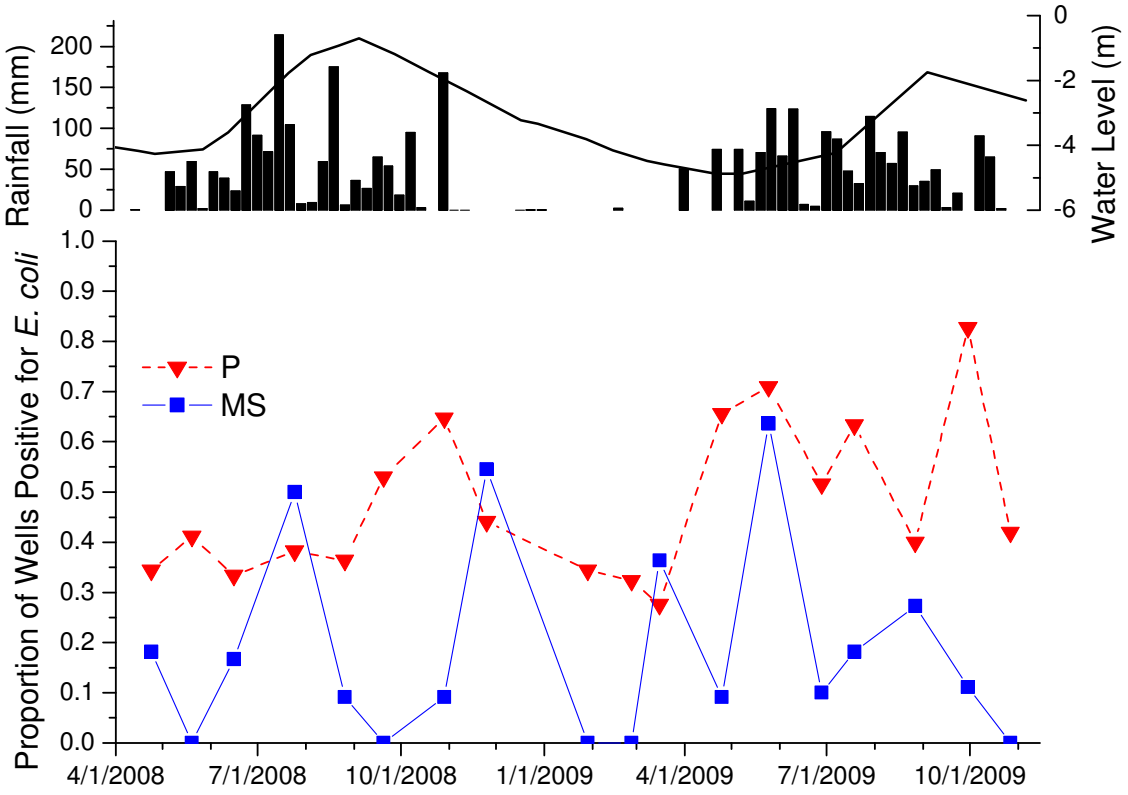


Figure A-V-2. Monthly proportion of private (P) and sealed monitoring wells (MS) testing positive for *E. coli*. Weekly precipitation is shown for Matlab (50 Km south of Site K). Manual groundwater levels are displayed at Site K (black line) from 01/01/08 through 11/11/09 whereas continuous water levels (dashed line) were available from 07/10/09 through 11/11/09.

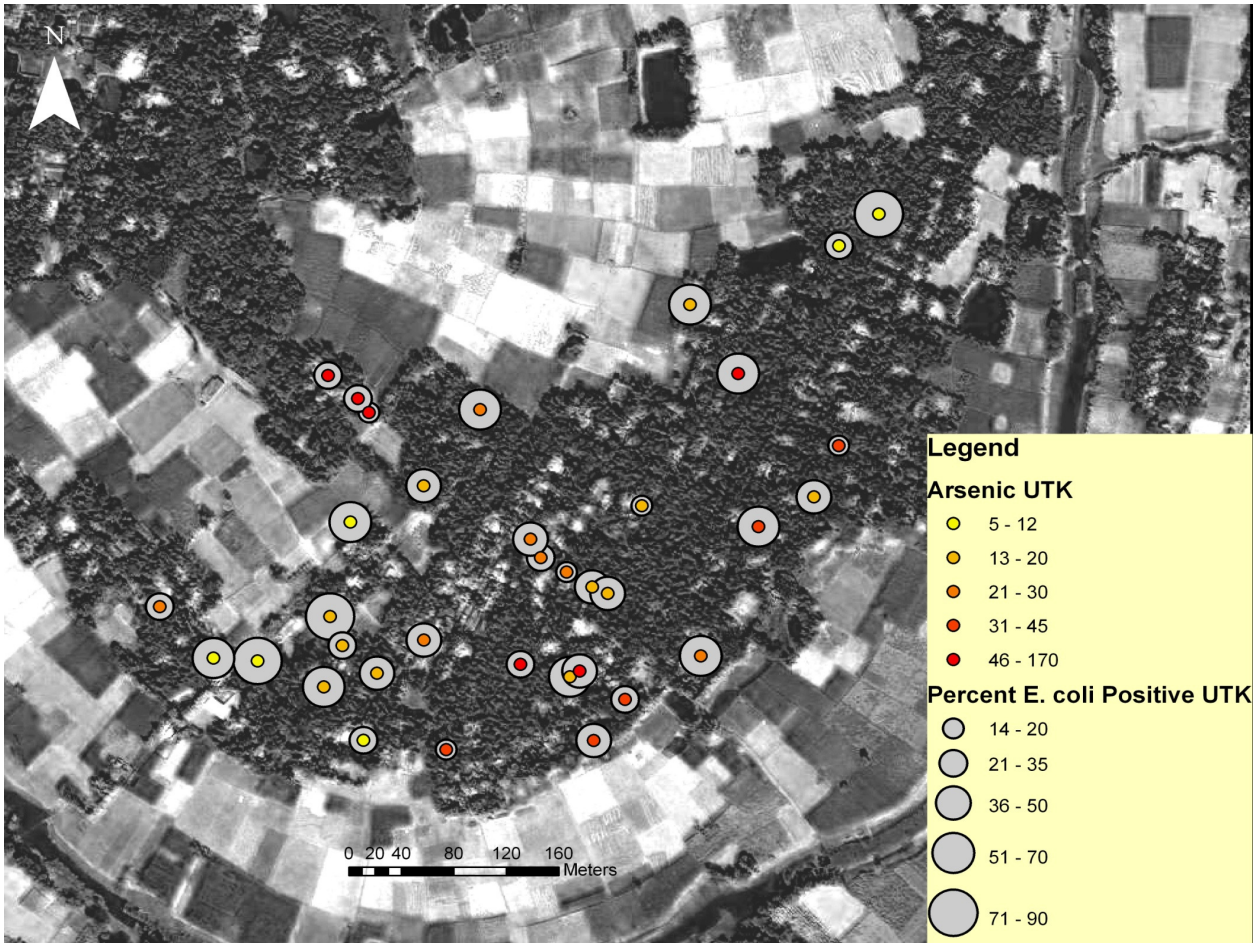


Figure A-V-3. Spatial Distribution of Arsenic and *E. coli* in wells that were monitored monthly for *E. coli* and Total Coliforms from January 15, 2008 through November 30, 2009.

Table A-V-5. Locations, Arsenic concentrations and detected *E. coli* frequency in monthly monitored private wells at Site K (Jan/08 through Nov/09)

ID	Longitude	Latitude	Average Arsenic (ppm)*	% Postive for *E. coli*	% Postive for *E. coli* Wet Season†	% Postive for *E. coli* Dry Season‡	Total Sampling Events (01/01/08 - 11/30/09)
UTK-01	90.628154	23.794961	17.3	41	62	0	22
UTK-02	90.628501	23.795204	22.2	41	46	11	22
UTK-03	90.629225	23.795041	74.8	23	23	11	22
UTK-04	90.629596	23.794957	17.4	59	77	11	22
UTK-05	90.630013	23.794801	33.6	32	31	33	22
UTK-06	90.629670	23.794999	86.0	36	46	0	22
UTK-07	90.629752	23.795605	17.9	47	50	22	19
UTK-08	90.630113	23.796194	17.9	19	17	22	21
UTK-09	90.630817	23.797154	84.8	53	57	13	15
UTK-10	90.630448	23.797643	19.5	59	69	11	22
UTK-11	90.628061	23.794477	11.5	32	38	11	22
UTK-12	90.627795	23.795362	12.9	82	85	44	22
UTK-13	90.627936	23.796043	11.6	64	69	22	22
UTK-14	90.628479	23.796312	15.1	45	62	11	22
UTK-15	90.629870	23.795559	14.3	41	54	0	22
UTK-16	90.629558	23.795709	22.5	23	31	0	22
UTK-17	90.629363	23.795809	25.8	21	17	25	14
UTK-18	90.629285	23.795941	29.9	41	54	22	22
UTK-20	90.627892	23.795154	16.2	32	38	0	22
UTK-21	90.627260	23.795034	5.5	77	77	44	22
UTK-22	90.627757	23.794856	14.3	67	85	0	21
UTK-23	90.626930	23.795050	6.5	52	58	33	21
UTK-24	90.626521	23.795414	29.4	36	62	0	22
UTK-25	90.628682	23.794420	43.1	14	8	22	22
UTK-26	90.629784	23.794501	44.6	52	69	0	21
UTK-27	90.630573	23.795121	21.2	57	62	38	21
UTK-28	90.630988	23.796056	33.0	63	69	33	19
UTK-29	90.631399	23.796277	18.2	47	63	0	17
UTK-30	90.631577	23.796647	37.7	14	23	0	22
UTK-32	90.631847	23.798316	4.6	81	77	50	21
UTK-33	90.631553	23.798084	9.8	29	36	0	17
UTK-34	90.628890	23.796865	22.7	55	58	25	20
UTK-35	90.628061	23.796832	78.1	16	23	0	19
UTK-36	90.627975	23.796931	75.4	26	23	17	19
UTK-37	90.627750	23.797094	169.7	26	23	0	19

* Averaged between two sampling dates (Aug/08 and Mar/09) and analyzed using ICP-MS

† Wet season defined as May through November

‡ Dry season defined as December through April

Table A-V-6. Locations, Arsenic concentrations and detected *E. coli* frequency in monthly monitored monitoring wells at Site K (Jan/08 through Nov/09)

ID	Longitude	Latitude	Average Arsenic (ppm)*	% Postive for *E. coli*	% Postive for *E. coli* Wet Season†	% Postive for *E. coli* Dry Season‡	Total Sampling Events (01/01/08 - 11/30/09)
KW-12.1	90.628310	23.794841	11.4	47	31	33	19
KW-12.2	90.628310	23.794841	39.7	32	38	17	19
KW-12.3	90.628310	23.794841	49.3	63	62	17	19
KW-20.1	90.628790	23.794735	16.7	17	17	17	18
KW-20.2	90.628790	23.794735	20.9	44	42	33	18
KW-20.3	90.628790	23.794735	38.1	11	8	17	18
KW-23	90.628210	23.796389	44.9	17	15	20	18
KW-24	90.629856	23.795684	28.4	33	38	20	18
KW-25	90.629412	23.795870	33.1	18	17	0	17
KW-26	90.628361	23.795639	5.0	19	27	0	16
KW-27	90.631779	23.798248	25.4	23	25	0	13
KW-28	90.631528	23.798050	7.7	29	22	0	14
KW-29	90.630730	23.795111	23.6	24	31	0	17
KW-30	90.630174	23.796329	22.8	17	23	0	18
KW-34	90.627502	23.795633	11.1	11	15	0	18
KW-35	90.625863	23.796423	45.6	11	8	0	18

* Averaged between two sampling dates (Aug/08 and Mar/09) and analyzed using ICP-MS

† Wet season defined as May through November

‡ Dry season defined as December through April

Table A-V-7. Concentration of Major Cations and Arsenic in Private Wells. "NA" refers to samples that were not analyzed.

Well ID	P (ppm) Aug/08	P (ppm) Mar/09	S (ppm) Aug/08	S (ppm) Mar/09	Mn (ppm) Aug/08	Mn (ppm) Mar/09	Fe (ppm) Aug/08	Fe (ppm) Mar/09	As (ppb) Aug/08	As (ppb) Mar/09
UTK-01	0.0	0.0	11.8	6.6	0.7	0.5	0.2	0.9	16	16
UTK-02	0.0	0.0	10.5	10.6	0.1	0.2	0.2	0.1	17	15
UTK-03	0.5	0.4	2.9	2.0	0.7	3.0	6.6	12.0	44	56
UTK-04	0.5	0.6	14.6	29.6	1.3	1.7	0.1	0.0	17	18
UTK-05	1.0	0.5	5.5	7.9	0.8	1.3	15.2	10.8	30	27
UTK-06	1.4	1.3	2.6	3.7	0.8	1.5	4.7	5.6	103	105
UTK-07	0.1	0.1	9.7	11.2	0.8	0.2	9.3	1.8	19	18
UTK-08	NA	0.8	NA	0.6	NA	1.4	NA	7.7	NA	37
UTK-09	1.1	0.6	0.1	3.4	1.0	0.9	6.5	7.8	161	69
UTK-10	0.9	0.3	3.1	16.3	0.4	0.7	7.1	4.9	30	17
UTK-11	0.0	0.0	9.7	5.3	0.0	0.0	0.5	0.1	11	9
UTK-12	0.0	0.0	12.2	13.1	0.1	0.2	0.2	0.1	10	9
UTK-13	0.1	0.1	4.9	2.2	0.1	0.1	5.0	2.3	11	12
UTK-14	0.4	0.3	4.6	10.4	0.8	1.0	19.2	15.5	17	16
UTK-15	0.1	0.1	1.4	2.9	0.3	0.2	4.8	3.3	14	14
UTK-16	0.6	0.4	8.6	2.7	1.6	0.8	20.5	13.5	24	21
UTK-17	0.2	0.1	2.3	7.8	1.2	0.5	12.2	7.9	30	22
UTK-18	0.4	0.3	8.2	10.0	1.7	1.6	8.2	6.3	27	26
UTK-19	NA	0.0	NA	10.0	NA	0.7	NA	0.6	NA	6
UTK-20	0.0	0.0	5.4	5.5	0.5	0.4	0.3	0.2	6	5
UTK-21	0.0	0.0	17.1	10.8	0.5	0.8	0.9	0.4	5	5
UTK-22	0.1	0.0	7.3	11.3	0.8	1.2	2.1	1.4	24	9
UTK-23	0.1	0.0	0.5	1.0	0.1	0.1	2.4	0.9	8	9
UTK-24	0.5	0.2	5.9	10.1	2.9	3.2	15.6	6.9	49	36
UTK-25	1.1	0.7	18.3	18.9	3.3	3.2	17.7	13.0	78	57
UTK-26	0.5	0.3	1.8	6.7	0.8	0.7	4.5	7.3	53	33
UTK-27	0.1	0.0	7.5	16.5	0.1	0.2	1.5	0.4	4	3
UTK-28	0.1	0.1	10.6	16.4	0.7	1.0	7.5	11.0	9	11
UTK-29	0.0	0.0	4.4	5.6	0.3	0.1	1.2	0.4	4	3
UTK-30	0.8	NA	3.8	NA	1.2	NA	13.5	NA	72	NA
UTK-31	0.7	NA	7.0	NA	0.2	NA	17.6	NA	20	NA
UTK-32	0.1	0.1	35.6	1.2	2.3	2.7	5.0	3.5	6	7
UTK-33	0.4	NA	3.0	NA	1.0	NA	13.7	NA	13	NA
UTK-34	1.2	0.7	2.1	3.8	0.9	0.5	5.9	3.6	28	18
UTK-35	0.9	0.5	1.5	3.3	1.3	1.4	20.3	13.1	90	66
UTK-36	1.0	0.7	2.3	2.5	1.1	1.0	16.0	12.4	84	67
UTK-37	2.0	0.9	0.1	0.1	1.5	1.3	9.1	6.2	181	158

Table A-V-8. Concentration of Major Cations and Arsenic in Monitoring Wells. “NA” refers to samples that were not analyzed.

Well ID	P (ppm) Aug/08	P (ppm) Mar/09	S (ppm) Aug/08	S (ppm) Mar/09	Mn (ppm) Aug/08	Mn (ppm) Mar/09	Fe (ppm) Aug/08	Fe (ppm) Mar/09	As (ppb) Aug/08	As (ppb) Mar/09
KW-12.0	0.0	NA	4.0	NA	0.0	NA	0.2	NA	2	NA
KW-12.1	0.1	0.0	6.5	4.6	0.2	0.1	0.7	0.3	3	3
KW-12.2	0.3	0.4	6.1	13.0	1.6	1.9	8.8	11.1	30	20
KW-12.3	0.6	0.5	7.0	9.5	2.7	1.2	10.1	10.4	83	49
KW-20.1	0.8	0.6	6.0	14.3	0.8	1.1	17.6	22.5	20	16
KW-20.2	0.7	0.8	3.2	5.7	0.5	0.7	16.1	21.7	13	13
KW-20.3	0.7	0.9	1.5	1.3	1.2	0.7	31.0	19.9	27	29
KW-23	0.2	0.1	1.0	8.3	1.3	1.4	2.4	1.7	54	49
KW-24	1.0	0.5	0.2	3.5	1.6	0.4	4.6	2.3	56	36
KW-25	0.2	NA	16.1	NA	2.2	NA	19.6	NA	39	NA
KW-26	0.0	0.0	1.3	0.2	0.0	0.1	0.0	0.3	2	1
KW-27	0.7	0.9	5.4	4.2	1.6	1.6	21.3	25.9	25	27
KW-28	0.4	NA	0.8	NA	1.7	NA	14.1	NA	8	NA
KW-29	0.9	0.5	0.9	26.0	0.3	0.7	6.6	14.4	11	8
KW-30	0.9	0.6	2.5	0.8	0.8	0.8	11.7	10.9	29	26
KW-34	0.3	0.3	0.8	0.4	0.5	0.6	5.9	10.3	8	7
KW-35	0.3	0.2	0.4	1.3	1.9	1.8	2.6	3.6	36	36

Table A-V-9. Electrical Conductivity Monthly Monitoring Results from Private Wells at Site K (mS/cm). “NS” refers to wells that were not sampled.

Well ID	2/27/08	3/27/08	4/24/08	5/20/08	6/16/08	7/25/08	8/26/08	9/20/08	10/29/08	11/22/08	1/29/09	2/25/09	3/16/09	4/22/09	5/27/09	7/19/09	8/26/09	9/30/09	10/27/09
UTK-01	0.57	0.56	0.54	0.65	0.55	0.57	0.69	0.63	0.68	0.64	0.84	0.59	0.87	1.32	1.50	0.76	0.92	0.75	0.78
UTK-02	0.76	0.81	0.79	0.95	0.74	0.61	0.70	0.66	0.82	0.94	1.10	0.99	1.22	1.65	1.66	0.64	0.61	0.45	0.55
UTK-03	0.25	0.23	0.20	0.24	0.21	0.16	0.20	0.16	0.31	0.30	0.04	0.45	0.56	0.89	1.29	0.23	0.21	0.21	0.26
UTK-04	1.50	1.66	1.79	1.90	1.52	1.24	1.13	1.08	0.96	1.83	1.82	1.73	2.15	1.91	2.33	1.32	1.07	0.91	1.03
UTK-05	0.33	0.50	0.55	0.59	0.52	0.51	0.28	0.46	0.51	0.61	0.60	0.71	0.85	1.20	1.48	0.47	0.30	0.25	0.32
UTK-06	0.55	0.62	0.72	0.76	0.51	0.37	0.37	0.32	0.11	0.65	0.70	0.62	0.70	1.20	1.54	0.49	0.41	0.30	0.30
UTK-07	0.79	0.86	0.91	0.98	0.57	0.51	0.69	0.94	1.00	1.33	1.15	1.03	1.18	1.59	1.65	NS	NS	NS	0.40
UTK-08	0.35	0.40	0.43	0.53	0.40	0.45	NS	0.45	0.39	0.52	0.57	0.57	0.76	1.35	1.83	0.49	0.47	0.45	0.45
UTK-09	0.55	0.47	0.50	0.63	0.46	0.41	0.33	0.46	0.39	0.75	0.73	0.61	1.04	NS	NS	NS	NS	NS	NS
UTK-10	0.23	0.34	0.40	0.53	0.36	0.41	0.27	0.31	0.31	0.31	0.47	0.12	0.90	1.00	1.47	0.30	0.27	0.26	0.26
UTK-11	0.30	0.31	0.33	0.48	0.61	0.67	0.68	0.05	0.65	0.04	0.51	0.58	0.58	1.00	1.63	0.75	0.79	0.64	0.75
UTK-12	0.98	0.91	0.77	0.85	0.61	0.50	0.50	0.49	0.35	0.22	0.33	0.41	1.02	1.41	2.38	0.58	0.59	0.51	0.55
UTK-13	0.31	0.26	0.22	0.29	0.25	0.23	0.26	0.44	0.24	0.44	0.46	0.26	0.51	0.87	1.86	0.22	0.23	0.22	0.26
UTK-14	0.80	0.79	0.59	0.71	0.60	0.46	0.54	0.56	0.44	0.85	0.05	0.87	1.13	1.34	2.23	0.54	0.41	0.41	0.43
UTK-15	0.38	0.44	0.43	0.45	0.37	0.32	0.32	0.29	0.27	0.31	0.44	0.47	0.60	0.92	1.42	0.27	0.26	0.24	0.26
UTK-16	0.60	0.65	0.62	0.75	0.60	0.56	0.56	0.55	0.41	0.78	0.74	0.67	0.85	1.29	1.89	0.47	0.38	0.37	0.43
UTK-17	0.32	0.30	NS	NS	NS	0.37	0.17	0.42	0.30	0.49	0.58	0.55	0.66	1.06	NS	NS	NS	NS	NS
UTK-18	0.53	0.55	0.59	0.72	0.60	0.57	0.59	0.57	0.11	0.93	0.93	0.78	1.01	1.21	2.06	0.53	0.71	0.66	0.63
UTK-20	0.38	0.46	0.45	0.55	0.46	0.43	0.45	0.41	0.28	0.56	0.61	0.54	0.76	1.19	2.15	0.51	0.48	0.45	0.50
UTK-21	1.08	1.45	1.39	1.25	0.25	0.79	0.79	0.55	0.31	0.48	0.43	0.52	0.98	1.46	2.54	0.42	0.36	0.36	0.37
UTK-22	0.49	0.57	0.58	0.69	0.50	0.42	0.46	0.46	0.33	0.26	NS	0.62	0.86	1.24	2.17	0.79	0.27	0.78	0.90
UTK-23	0.12	0.16	0.17	0.22	0.18	0.16	0.23	0.23	0.15	0.23	0.06	0.23	0.40	0.82	1.86	0.18	0.18	NS	0.17
UTK-24	0.60	0.67	0.72	0.78	0.60	0.51	0.49	0.47	0.33	0.62	0.60	0.25	1.05	1.43	2.43	0.61	0.57	0.51	0.54
UTK-25	0.85	0.96	1.29	1.53	1.34	1.19	1.36	0.98	0.48	1.12	1.45	1.44	1.57	1.63	2.79	1.18	0.95	0.94	0.93
UTK-26	0.82	NS	0.72	0.56	0.35	0.19	0.26	0.24	0.28	0.38	0.53	0.69	0.73	0.98	1.83	0.29	0.24	0.22	0.23
UTK-27	0.60	0.56	0.50	0.48	0.26	0.25	0.35	0.34	0.10	0.64	NS	0.80	0.98	1.35	1.78	0.34	0.21	0.19	0.22
UTK-28	0.87	NS	NS	1.22	0.91	0.81	0.81	0.69	0.69	0.88	0.09	1.08	1.35	NS	2.79	0.71	0.51	0.48	0.55
UTK-29	0.37	0.30	0.24	0.28	0.24	0.27	0.36	0.33	0.45	0.51	0.62	0.65	0.56	0.97	2.00	NS	NS	NS	NS
UTK-30	0.48	0.54	0.55	0.58	0.42	0.37	0.43	0.41	0.48	0.68	0.77	0.76	0.85	1.24	2.17	0.32	0.31	0.28	0.29
UTK-31	0.50	0.53	0.54	0.54	0.36	0.35	0.36	0.26	0.27	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
UTK-32	0.29	0.36	0.36	0.43	0.36	0.34	0.91	0.80	0.58	0.55	0.69	0.72	0.99	1.35	2.09	0.45	0.46	0.41	0.52
UTK-33	0.33	0.39	0.29	0.43	0.30	0.31	0.38	0.35	NS	NS	NS	NS	NS	1.16	2.03	0.39	0.33	0.21	0.24
UTK-34	0.29	0.37	0.39	0.50	0.43	0.43	0.48	0.49	0.55	0.63	0.73	0.60	1.01	1.27	NS	0.50	0.48	0.44	0.46
UTK-35	0.35	0.39	0.39	0.47	0.40	0.37	0.43	0.45	0.51	0.62	0.65	0.51	0.89	1.16	1.87	0.40	0.37	0.35	0.42
UTK-36	0.39	0.48	0.50	0.62	0.51	0.40	0.15	0.44	0.44	0.52	0.57	0.56	0.94	1.26	2.15	0.43	0.41	0.38	0.40
UTK-37	0.46	0.56	0.55	0.63	0.54	0.48	0.50	0.52	0.52	0.70	0.68	0.61	0.97	1.31	2.12	0.50	0.49	0.45	0.47

Table A-V-10. Electrical Conductivity Monthly Monitoring Results from Monitoring Wells at Site K (mS/cm).

Well ID	2/27/08	3/27/08	4/24/08	5/20/08	6/16/08	7/25/08	8/26/08	9/20/08	10/29/08	11/22/08	1/29/09	2/25/09	3/16/09	4/22/09	5/27/09	7/19/09	8/26/09	9/30/09	10/27/09
KW-12.1	NS	NS	0.32	0.36	0.30	0.43	0.44	0.35	0.32	0.33	0.14	0.08	1.50	1.60	1.63	0.29	0.30	0.29	0.38
KW-12.2	NS	NS	0.40	0.48	0.40	0.54	0.47	0.43	0.22	0.66	0.84	0.87	1.37	1.54	1.53	0.47	0.24	0.35	0.38
KW-12.3	NS	NS	0.44	0.55	0.40	0.58	0.49	0.44	0.32	0.62	0.18	0.74	1.30	1.53	1.59	0.34	0.34	0.38	0.39
KW-20.1	NS	NS	0.45	0.53	NS	0.39	0.35	0.33	0.38	0.35	0.56	0.63	1.06	1.57	1.64	0.33	0.42	0.26	0.31
KW-20.2	NS	NS	0.21	0.29	NS	0.26	0.27	0.30	0.37	0.39	0.41	0.45	0.79	1.50	1.41	0.32	0.40	0.32	0.35
KW-20.3	NS	NS	0.53	0.44	NS	0.41	0.39	0.33	0.31	0.34	0.41	0.42	0.82	1.50	1.72	0.40	0.27	0.43	0.60
KW-23	NS	NS	0.36	0.43	0.33	0.40	0.34	0.33	0.38	0.41	0.50	0.56	1.37	1.35	1.43	0.33	0.34	0.32	0.31
KW-24	NS	NS	0.22	0.30	0.22	0.29	0.26	0.25	0.21	0.35	0.05	0.07	0.96	1.37	1.29	0.32	0.40	0.24	0.25
KW-25	NS	NS	0.89	0.98	NS	1.13	0.75	0.84	0.71	0.95	0.07	0.97	0.78	0.71	0.93	1.19	0.34	0.95	0.34
KW-26	NS	NS	0.53	0.62	NS	NS	0.40	0.35	0.44	0.48	0.37	0.07	1.51	1.42	0.52	0.30	0.64	0.59	0.75
KW-27	NS	NS	0.45	0.49	NS	NS	0.43	0.70	0.57	0.65	0.66	0.68	1.15	1.60	1.73	NS	NS	NS	NS
KW-28	NS	NS	0.51	0.71	NS	NS	0.38	0.35	0.37	0.39	0.50	0.50	1.03	0.40	0.86	NS	NS	NS	NS
KW-29	NS	NS	0.59	0.39	0.24	0.30	0.25	0.24	0.29	0.41	NS	0.04	1.48	1.53	0.99	0.20	1.00	0.21	0.23
KW-30	NS	NS	0.50	0.58	0.43	0.50	0.39	0.32	0.35	0.44	0.06	0.56	0.97	0.78	0.77	0.36	0.41	0.38	0.38
KW-33	NS	NS	0.56	0.59	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
KW-34	NS	NS	0.34	0.43	0.29	0.36	0.29	0.28	0.30	0.39	0.44	0.50	1.57	1.48	1.58	0.26	0.43	0.22	0.21
KW-35	NS	NS	0.18	0.18	0.15	0.24	0.19	0.20	0.36	0.38	0.03	0.29	1.40	1.39	1.21	0.17	0.33	0.14	0.20

Table A-V-11. *E. coli* Monthly Monitoring Results from Private Wells at Site K (MPN/100 ml). Concentrations are based upon duplicate 100 ml samples (except 11/25/08 when only single 100 ml samples were taken). "0.3" is the estimated detection limit and indicates no *E. coli* was detected in either 100 ml sample. "NS" refers to wells that were not sampled.

Well ID	4/24/08	5/20/08	6/16/08	7/25/08	8/26/08	9/20/08	10/29/08	11/25/08	1/29/09	2/26/09	3/16/09	4/25/09	5/25/09	6/28/09	7/19/09	8/27/09	9/30/09	10/27/09
UTK-01	0.3	0.3	0.3	570.3	3.1	1.5	0.3	0.3	0.3	0.3	0.5	0.3	0.5	21.6	3.6	0.3	4.7	3.1
UTK-02	0.3	0.3	0.3	0.3	0.3	0.3	1.5	0.3	15.6	0.3	0.3	3.1	0.5	2.0	138.3	0.5	6.4	0.3
UTK-03	2.0	0.3	0.3	0.3	0.3	0.3	2.0	0.3	0.3	0.3	0.3	23.3	0.3	0.3	31.5	0.3	3.6	0.3
UTK-04	0.3	0.3	4.3	1.0	0.3	71.0	3.6	0.3	0.3	0.3	0.5	1.5	1.5	4.3	8.1	0.5	2.0	2.5
UTK-05	2.0	0.3	0.3	0.3	0.3	0.3	4.7	3.0	0.3	0.3	0.3	0.3	0.3	0.3	6.9	0.3	3.6	0.3
UTK-06	0.3	5.2	0.3	0.3	0.3	1.0	2419.6	12.2	0.3	1.0	0.3	2.0	0.5	0.3	0.3	0.3	0.5	0.3
UTK-07	0.3	0.3	2.0	0.3	0.3	2.0	5.3	8.3	1.0	0.3	0.3	17.4	8.1	0.3	NS	NS	NS	0.3
UTK-08	4.7	0.3	0.3	0.3	NS	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	4.7	1.0
UTK-09	40.3	0.3	12.7	0.3	1.0	0.3	3.6	18.3	296.4	3.1	2.0	NS	NS	NS	NS	NS	NS	NS
UTK-10	4.3	236.4	0.3	0.3	0.3	2.6	0.3	1.0	22.3	0.3	2.6	210.9	1.0	11.4	0.5	1.0	0.5	139.3
UTK-11	0.3	0.3	3.0	1.0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1035.8	3.1	0.5	0.3	0.3	5.8	0.3
UTK-12	0.3	1.5	3.0	0.3	244.5	237.8	10.3	1.0	0.3	1.0	4.3	2.0	338.7	2.0	0.5	2.0	151.7	0.3
UTK-13	0.3	1.0	0.3	0.3	4.3	2.5	0.3	5.3	11.8	0.3	0.5	3.1	1.5	0.3	1.5	7.3	1.0	19.4
UTK-14	0.3	2.0	1.0	6.2	0.3	0.3	1986.3	0.3	0.3	0.3	0.3	558.7	0.5	243.3	1.0	0.3	1.5	0.3
UTK-15	0.3	0.3	0.3	0.3	1.0	0.3	23.7	0.3	2.0	0.3	0.3	6.9	1.0	14.7	7.5	0.3	0.5	0.5
UTK-16	0.3	0.3	0.3	0.3	0.3	0.3	816.4	0.3	0.3	0.3	0.3	0.5	0.5	0.3	0.3	0.3	0.5	36.5
UTK-17	NS	0.3	NS	0.3	0.3	0.3	2.0	0.3	0.3	0.3	0.3	0.3	NS	NS	NS	NS	NS	NS
UTK-18	0.3	0.3	1.0	0.3	0.3	0.3	5.2	4.7	0.3	0.3	0.3	0.3	19.4	36.5	0.3	1.0	0.3	1.5
UTK-20	0.3	0.3	0.3	111.4	3.1	0.3	0.3	1.5	0.3	0.3	2.0	1.0	6.9	0.3	0.3	1.0	0.3	0.3
UTK-21	39.3	1.0	2.5	3.6	18.6	34.7	3.6	12.6	2.5	5.3	0.3	4.7	4.3	2455.6	1.0	0.3	0.3	0.3
UTK-22	0.3	1.0	0.3	1.0	10.5	15.3	0.3	10.5	NS	12.8	1.0	56.0	0.5	1.5	5.8	6.9	52.5	2082.7
UTK-23	3.1	13.8	0.3	2.0	0.3	2.5	0.3	1.0	0.3	0.3	0.3	4.7	2.0	2.5	2.6	0.3	NS	0.3
UTK-24	0.3	28.7	2.0	1203.3	0.3	16.7	0.3	0.3	0.3	0.3	0.3	0.3	44.8	0.3	0.3	0.5	0.5	0.5
UTK-25	1.5	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	2.6	0.3	0.3	0.3
UTK-26	0.3	1.0	0.3	1.0	0.3	4.1	3.1	1.0	0.3	1.0	0.3	4.3	0.3	0.3	1.0	0.5	1.0	0.5
UTK-27	1.5	17.3	1.0	0.3	4.7	4.3	1.0	1.0	NS	1.0	0.3	0.3	0.3	36.7	0.3	0.3	1.5	0.3
UTK-28	NS	10.9	0.3	1.0	1.0	21.7	1.0	0.3	0.3	7.4	0.3	NS	1.5	5.7	12.2	2.0	0.3	0.3
UTK-29	0.3	0.3	0.3	1.0	1.0	2.0	1.0	0.3	1062.1	4.3	0.3	0.5	4.3	NS	NS	NS	NS	NS
UTK-30	0.3	0.3	0.3	0.3	0.3	1.0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.5	0.3	1.0	0.3
UTK-32	2.0	1.5	1.5	0.3	0.3	3.1	10.3	0.3	8.6	10.3	NS	499.4	45.4	0.5	8.1	42.3	51.6	229.4
UTK-34	8.7	1.5	0.3	0.3	2.5	2.0	10.5	0.3	0.3	1.0	NS	3.6	NS	0.3	1.0	0.3	1.5	0.5
UTK-35	0.3	0.3	0.3	1.0	0.3	0.3	2.0	0.3	0.3	0.3	NS	0.3	0.3	0.3	0.3	0.3	1.0	0.3
UTK-36	0.3	0.3	0.3	0.3	0.3	0.3	121.0	0.3	5.2	0.3	NS	0.3	2.0	0.3	0.3	0.3	3.6	0.3
UTK-37	0.3	0.3	0.3	0.3	0.3	0.3	0.3	22.6	1.0	0.3	NS	2.0	0.3	1.0	0.3	0.3	0.5	0.3

Table A-V-12. *E. coli* Monthly Monitoring Results from Monitoring Wells at Site K (MPN/100 ml). Concentrations are based upon duplicate 100 ml samples (except 11/25/08 when only single 100 ml samples were taken). "0.3" is the estimated detection limit and indicates no *E. coli* was detected in either 100 ml sample. "NS" refers to wells that were not sampled.

Well ID	4/24/08	5/20/08	6/16/08	7/25/08	8/26/08	9/20/08	10/29/08	11/25/08	1/29/09	2/26/09	3/16/09	4/25/09	5/25/09	6/28/09	7/19/09	8/27/09	9/30/09	10/27/09
KW-12.1	72.5	0.3	4.1	2.0	1.0	0.3	0.3	0.3	0.3	2419.6	130.0	24.7	0.3	0.3	0.3	0.3	0.3	0.5
KW-12.2	0.3	0.3	1.0	32.8	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1.5	0.5	0.3	1.5
KW-12.3	0.3	94.3	0.3	281.3	0.3	0.3	1.5	0.3	0.3	2.5	0.5	65.5	0.3	10.3	6.3	10.4	255.1	1075.7
KW-20.1	0.3	0.3	NS	28.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	2.0	0.3	0.3	0.3	0.3
KW-20.2	6.9	536.3	NS	148.8	4.2	0.3	1.5	0.3	0.3	0.3	0.5	0.3	0.3	0.3	0.3	0.3	0.3	1.5
KW-20.3	0.3	0.3	NS	56.7	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
KW-23	1.0	0.3	0.3	3.3	0.3	0.3	0.3	1.0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
KW-24	2.0	0.3	0.3	5.2	0.3	0.3	0.3	7.5	0.3	0.3	0.3	0.3	0.5	0.3	1.5	1.0	0.3	0.3
KW-25	0.3	0.3	NS	0.3	0.3	0.3	0.3	15.8	0.3	0.3	0.5	0.3	2.6	0.3	0.3	0.3	0.3	0.3
KW-26	0.3	0.3	NS	NS	1.0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	9.8	0.5	0.3	0.3	0.3	0.3
KW-27	0.3	0.3	NS	NS	0.3	0.3	1.0	0.3	0.3	0.3	5.2	0.3	1.0	NS	0.3	0.3	NS	NS
KW-28	0.3	0.3	NS	NS	0.3	0.3	0.3	1.0	0.3	0.3	0.5	0.5	2.0	0.3	0.3	0.3	NS	NS
KW-29	0.3	0.3	0.3	244.5	0.3	0.3	0.3	59.4	NS	0.3	0.3	0.3	0.3	0.3	1.5	11.6	0.3	0.3
KW-30	0.3	0.3	497.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	2.6	0.3	0.3	1.5	0.3	0.3
KW-34	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	4.7	0.3	0.3	0.3	5.8	0.3
KW-35	0.3	0.3	0.3	0.3	0.3	0.3	0.3	1.0	0.3	0.3	1.0	0.3	0.3	0.3	0.3	0.3	0.3	0.3

VITA

Peter Knappett was born in Chapleau, Ontario, Canada in 1977. He attended Muskoka Christian School through the eighth grade after which he attended Bracebridge and Muskoka Lakes Secondary School. After graduation in 1996 he was accepted into the University of Waterloo and enrolled in the Earth Sciences Honours undergraduate program. During this degree he completed several internships in municipal government, environmental and energy companies and achieved a place on the Dean's honours list upon graduation. Mr. Knappett's senior thesis was on the extraction of radioactive carbon from spent ion exchange resins used in nuclear reactors. After his undergraduate degree he completed one year of research on a scholarship from the German Federal Government at the Center for Environmental Research in Leipzig on the detection of waterborne pathogens in surface and ground waters in central Europe. From 2002 to 2003 Mr. Knappett worked as a hydrogeologist for the Environmental Consulting Company Conestoga-Rovers and Associates in Waterloo, Ontario. In 2003 he began his masters in Civil Engineering at the University of Waterloo where he studied virus and bacteria transport through saturated sand aquifers. After his masters degree in 2005 he completed a research project on nitrate contamination in aquifers in southern Germany and Austria while at the University of Stuttgart. In January, 2006 he began his PhD at the University of Tennessee in Knoxville. During his PhD he travelled several times to Bangladesh for field work related to his dissertation, part of a larger NIH/NSF-funded project in collaboration with Columbia University, University of Tennessee, University of North Carolina at Chapel Hill and Dhaka University. In June 2010 Mr. Knappett will begin a post doctoral Marie Curie research fellowship at the Helmholtz Center for Environmental Health in Munich, Germany.