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Implications of Fecal Bacteria Input from Latrine-Polluted Ponds for Wells in Sandy Aquifers

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Abstract

Ponds receiving latrine effluents may serve as sources of fecal contamination to shallow aquifers tapped by millions of tube-wells in Bangladesh. To test this hypothesis, transects of monitoring wells radiating away from four ponds were installed in a shallow sandy aquifer underlying a densely populated village and monitored for 14 months. Two of the ponds extended to medium sand. Another pond was sited within silty sand and the last in silt. The fecal indicator bacterium *E. coli* was rarely detected along the transects during the dry season and was only detected near the ponds extending to medium sand up to 7 m away during the monsoon. A log-linear decline in *E. coli* and Bacteroidales concentrations with distance along the transects in the early monsoon indicates that ponds excavated in medium sand were the likely source of contamination. Spatial removal rates ranged from 0.5-1.3 $\log_{10}$/m. After the ponds were artificially filled with groundwater to simulate the impact of a rain storm, *E. coli* levels increased near a pond recently excavated in medium sand, but no others. These observations show that adjacent sediment grain-size and how recently a pond was excavated influence how much fecal contamination ponds receiving latrine effluents contribute to neighboring groundwater.

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Supporting Information. This information is available free of charge via the Internet at http://pubs.acs.org/.

Keywords

bacteria; removal rates; setback distances; tube-wells; latrine effluent; Bangladesh

1. Introduction

Fecal contamination has been reported in many shallow sandy aquifers throughout the world[1-3]. This finding is often contrary to expectations based on laboratory-scale column experiments, which routinely demonstrate high bacterial removal rates (m^{-1}) in fine and medium sand[4-7]. Field studies, however, show that bacteria are often transported greater distances than predicted by column experiments[2,8-11]. Relatively few microbial transport studies in sand aquifers have been carried out in the field. Such research has included the injection of a microbial tracer solution in an uncontaminated aquifer at depth[2,9,12,13], an engineered wastewater infiltration basin subjected to constant loading [14,15], and an aquifer being evaluated for protection via a riverbank filtration[16]. Findings from these studies might not be applicable to microbial transport in other aquifer settings, especially in developing countries, where there are abundant sources of fecal contamination, the depth of the water table varies widely through the year, and numerous shallow wells are the main source of drinking water [17,18].

In rural Bangladesh, the population density is high, sanitation is poor, and diarrheal disease morbidity[19,20] is very high, causing an estimated 11 % of all deaths[21]. The majority of villagers obtain their drinking water from shallow tube wells in sand aquifers so at least part of the high level of diarrheal disease might be attributable to exposure to fecal-contaminated well water[22-24]. Two recent studies observed frequent occurrence of the fecal indicator bacterium (FIB) *E. coli* in tube-well water, particularly during the monsoon[18,23]. It was not clear, however, whether *E. coli* entered the aquifer through vertical infiltration from near-surface sources, like latrines, through lateral transport from fecal-contaminated ponds, which are very common in the villages[17], or from other sources, like contaminated water occasionally used to prime handpumps[25].

Ponds are typically excavated for foundation material and are ubiquitous in rural Bangladesh. The existence of a strong hydrologic connection between ponds and shallow aquifers in the Bengal Basin is unclear according to the contrasting findings of two previous studies based on stable isotopes in groundwater. One study reported that ponds in West Bengal had no impact on the isotopic composition of groundwater during the dry season[26] whereas the other study suggested that ponds in Bangladesh were responsible for the majority of labile organic matter introduced into aquifers[27]. The discrepancy could reflect local differences in seasonal and geologic features of the specific study sites that affect hydrologic connectivity.

This study investigates the connection between ponds and aquifers in the context of the potential transfer of microbial pathogens from contaminated ponds. The study was carried out in a typical village of Bangladesh where a previous study identified persistent *E. coli* in 33 private tube-wells[18]. The spatial distribution of FIB was determined by a culture-based method for *E. coli* and a DNA-based assays for *E. coli* and Bacteriodales[28], a bacterium that constitutes up to 10% of fecal mass[29], along transects radiating from several ponds dug in sediment with varying grain sizes. The study findings have implications for safe setback distances from ponds in rural Bangladesh and for understanding of microbial transport in fine and medium sand aquifers more generally.

2. Setting and Methods

2.1 Site Description and Hydrogeology

Bangladesh experiences a dry season from November through April and a monsoon from May through October with the vast majority of annual precipitation occurring during the monsoon. The study site was the village of Char Para (23.796° N, 90.629° E), or Site K[30]. Char Para is underlain by a 25 m thick unconfined sandy aquifer, bounded by a 7 m thick silt layer below[30], covers an area of 30 hectares and houses approximately 1500[17]. A survey conducted in 2009 recorded the locations of 177 latrines, 43 ponds and 144 private tube-wells within the village boundaries[17].

Nine transects consisting of 5 to 6 monitoring wells (4 to 5 same depth shallow wells and one well approximately 3 m deeper) were installed in June 2008 in lines approximately 5-7 m long radiating away from four ponds receiving latrine effluent (Fig. 1; Fig. 2). These are also known as latrine ponds[17]. Transects and wells are labeled according to Δx.y.δ, where Δ is either T or W, referring to the transect or well, respectively, x numerically references the pond (1-4), y refers to the transect adjacent to pond x (1-3), and δ refers to a well in transect y (a-e for shallow wells, and z for the single deeper well). Transect T1.1 was installed as a line of wells between ponds 1 and 3 (Fig. 1; Fig. 2).

A total of 47 wells were developed near the four ponds along nine transects (Fig. 1) using the traditional hand-flapper method: a manual mud circulation method that quickly penetrates the loose, wet floodplain deposits throughout the Bengal Basin[31]. The annulus between the PVC casing (5.1 cm dia.) and the borehole was sealed with cement grout from ground surface to the top of the sand pack, which starts 0.7 m above the 1.5 m screened interval and extends to the borehole base. Wells were developed and sampled with a battery-powered submersible pump. Shallow and deep well depths varied from 5.5 to 7.9 m and 8.5 to 10.9 m, respectively. Pond water levels were monitored with “L-shaped” piezometers consisting of a vertical steel pipe in the adjacent bank connected to a horizontal pipe extending into the pond center.

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 undisturbed core samples were collected continuously from 3 m below the surface to the bottom of the borehole, using manual direct push coring methods with an AMS 424.45 core sampler (AMS, American Falls, Idaho, USA). For each 0.3 m core, silt layers were identified at sub-centimeter resolution, and then excluded from dry sieving for grain-size distribution. Logarithmic interpolation was used to obtain the tenth percentile (d_{10}), median (d_{50}) and sixtieth percentile (d_{60}) grain diameters[32] and the uniformity coefficient ($U=d_{60}/d_{10}$) was calculated for sand samples from each core.

Water levels were measured using an electric water-level tape (Dipper-T, Heron Instruments Inc., Burlington, Ontario, Canada) and pressure transducers (Levelogger Model 3001, Solinst Canada Ltd., Georgetown, Ontario, Canada). Manual water-level monitoring of all wells and ponds was performed at minimum once a week from June 11 to July 20, 2008 and once monthly through November, 2009 thereafter. Pneumatic rising head slug tests were performed in triplicate to measure the hydraulic conductivity of aquifer sediments using a pressure transducer. Lateral average linear groundwater velocities were calculated based on hydraulic gradients between the closest and furthest monitoring well from each pond and the average conductivity of shallow wells within each transect, assuming an effective porosity of 0.4. Shallow (8-30 m) groundwater temperature in Bangladesh is typically 26 °C year-round[33].

2.2 E. coli Monitoring

Monthly *E. coli* monitoring was performed on the wells closest and furthest from a pond along eight transects (excluding T1.1) from September 2008 through May 2009. T1.1 was monitored monthly from June through October 2009 only.

All tubing and pumps were soaked in cleaning solution consisting of powdered Chlorox (5 g) and TWEEN-80 (5 mL) (T164-500, Fischer Scientific) in approx. 5 L of well water prior to sampling each transect. The cleaning solution was cycled through the tubing for 5 minutes, followed by rinsing with 10 L of a 5 g of sodium thiosulfate solution (S446-3, Fisher Scientific) for 2 minutes to consume any remaining dissolved Chlorox. To ensure that only water from the aquifer adjacent to each monitoring well was sampled, 60 to 100 liters were purged at 5-10 L per minute from each monitoring well; representing approximately three wellbore volumes[28].

Intensive monitoring was performed approximately once a week on one transect per pond (T1.1, 2.1, 3.1, 4.1) during the early 2009 monsoon (June 11 to July 20). During the first week of intensive monitoring, sampling was performed under natural gradient flow conditions, before water levels were manipulated (as described below).

2.3 Field-scale Infiltration Experiment

In order to simulate a major rainfall event, the volumes of ponds 2, 3, and 4 were increased by 11-29% by raising the water level by 0.2-0.6 m with groundwater from the slightly deeper (~10 m) wells in two of the adjacent transects (Supp. Table 1). After filling, water from pond 3 was channeled into pond 1, which was initially dry.

2.4 Chemical and Microbial Measurements

Pond water and groundwater samples were collected in 20 mL scintillation vials. Samples from all four ponds and transects were analyzed, at minimum, once prior and post artificial pond filling. After acidification to 1% HCl for at least one week to ensure re-dissolution of any iron oxide precipitate, samples were analyzed for Na, Mg, S, Ca, Fe, and As using high-resolution inductively coupled plasma mass spectrometry (HR ICP-MS)[18].

Culturable *E. coli* was measured using the MPN based Colilert™ test kit (IDEXX Laboratories, Inc.). Duplicate 100 mL groundwater samples were collected in sterile containers and measured in a lab within 8 hours of sample collection. Concentrations of *E. coli* were determined by combining the numbers of discrete positive wells in each replicate tray[28,34]. The minimum detection limit (MDL) of the Colilert test kit for a single analysis is 1 MPN/100 mL. By combining duplicate trays, however, the detection limit is lowered statistically to 0.5 MPN/100 mL[34].

For enumeration of fecal indicator bacteria (FIB) genes, 6 (+/− 2) L of groundwater and 0.2 (+/− 0.1) L of pond water were filtered onto 0.22 μm nitrocellulose filters. The filters were removed from the plastic housing, placed in sterile petri dishes, frozen and transported on dry ice 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 Bacteroidales using assays and laboratory methods as described previously[28]. The gene targets for the *E. coli* (herein referred to as *mE. coli*) and Bacteroidales assays were the 23S rRNA gene and the 16S rRNA gene, respectively[35-40]. Before performing the PCR assays, DNA samples were diluted to 5-10 ng/μL total DNA to avoid PCR inhibition. Sample and assay values were

calculated as copies/μL of total extracted DNA and converted to copies/100 mL based on the volume of water filtered. The MDL was determined from the standard curve to be 20 copies per qPCR reaction. Due to differences in the initial volume of water filtered, the MDL varied between samples[17] with a geometric mean MDL of 40 copies/100 mL for the groundwater samples. In all pond water samples, gene concentrations exceeded the MDL by several orders of magnitude.

The most complete set of microbiological and chemical parameters were measured during the intensive monitoring period (June/July 2009) along transects T1.1, T2.1, T3.1 and T4 (Fig. 1). Results from five other transects are presented in the Supplementary Material to document the range of hydrogeologic, microbiological, and chemical conditions surrounding each of the four ponds.

3. Results

3.1 Grain-size of Sediment Around Ponds

The installation of transects of monitoring wells indicated that medium aquifer sand extends to the banks of ponds 1 and 2 (Fig. 2a, b). The bottom of recently excavated pond 1 was also sandy, whereas, the bottom of pond 2 was silty. Pond 3 was excavated within a ~2-m silt layer and was not similarly connected to the aquifer (Fig. 2c). A few thin silt layers were encountered while installing monitoring wells along T1.1 and T2.1, including one laterally continuous, 20 cm thick silt later in T2.1. With one exception, the measured hydraulic conductivities of the sands around ponds 1, 2, and 3 ranged from 1.1 to 4.7×10^{-4} m/s (Supp. Table 2). The setting of pond 4 was homogeneous fine silty sand (Fig. 2d) of lower hydraulic conductivity ($0.2\text{-}0.9\times10^{-4}$ m/s).

3.2 Groundwater Levels and Hydraulic Gradients Before the Intervention

The ground surface elevation varied by no more than 1 m within Char Para (excluding ponds). All well and pond elevations were referenced to the ground surface near monitoring well KW-12.1 (Fig. 1)[41]. Monthly measurements at this location indicated a water table rising within 1.5 m of the ground surface in September 2008 and 2009 and dropping to approximately 4 m below ground towards the end of the 2009 dry season (Fig. 3d). Continuous water level measurements between July and November 2009 also indicated smaller daily to weekly fluctuations. On a single day, the elevation of the unconfined water table throughout the village varied from 4 to 70 cm (unpublished results). These fluctuations were related to irrigation pumping in nearby fields, river stage, and local rainfall events, however only weak gradients (<0.003) were observed across a 300 m transect of wells extending south from KW-12.1 to the river (Fig. 1)[41]. Throughout the early monsoon (June/July 2009) the groundwater level remained well below each pond base (Fig. 2a-d; Fig. 3d).

3.3 Distribution of Fecal Indicator Bacteria Before the Intervention

Concentrations of cultured *E. coli* were high ($10^3\text{-}10^6$ MPN/100 mL; Supp. Table 3) in ponds 2, 3, and 4 throughout the study period. No time series data were available for pond 1 because it was excavated only during the 2009 dry season and did not yet contain standing water in June 2009. In spite of the proximity of a strong potential source, the transect well closest to ponds 3 and 4 contained much lower levels of cultured *E. coli* from September 2008 through June 2009, always <10 MPN/100 mL and often <1 MPN/100 mL, especially during the monsoon (Fig. 3c; Supp. Fig. 1). In contrast, cultured *E. coli* concentrations exceeded 10 MPN/100 mL in the monitoring well closest to ponds 1 and 2 in T1.1 and T2.1, respectively, in June 2009 before they were filled (Fig. 3c). Such high *E. coli* concentrations were not found in T2.2 and T2.3 (Supp. Fig. 1). The pond and transect data combined indicate a log-linear decrease in cultured *E. coli* concentrations with distance from the ponds

before the intervention until the detection limit was reached (Fig. 4). Similarly, concentrations of *mE. coli* and Bacteroidales genes declined logarithmically with distance, however the greater relative abundance of FIB genes to cultured *E. coli* permitted the detection of this impact further into the aquifer. Further, whereas *E. coli* and *mE. coli* were not detected in the slightly deeper well deeper in T2.1, representing a reduction of 4-1og$_{10}$ across 3 m of sediment, Bacteroidales genes were present at 10,000 copies/100 mL. Pre-intervention molecular data was not available for the well in T1.1.

3.4 Groundwater Levels and Hydraulic Gradients After the Intervention

While initially flat within ±0.003 in June 2009, lateral hydraulic gradients along transects T1.1 and T2.1 radiating from ponds 1 and 2 increased to 0.05 and 0.01, respectively, during pond filling by 0.63 and 0.16 m (Fig. 3a), with corresponding Darcy velocities based on measured hydraulic conductivities of 4.0 and 0.5 m/day. In contrast, lateral hydraulic gradients along transects radiating from ponds 3 and 4 generally remained within ±0.003 while pond levels were raised by 0.53 and 0.16 m, respectively (Fig. 3a). Vertical hydraulic gradients measured between paired shallow and deeper transect wells were similar to lateral gradients (unpublished results). There was no response in hydraulic gradient to the filling of pond 3 along T1.1 either, even though this transect extends from pond 1 to pond 3 (Fig. 1). Surprisingly, there was an increase in hydraulic gradient along T3.1 while filling pond 1 (Fig. 3a). Pond 1 was dry again only 24 hours after filling.

3.5 Distribution of Fecal Indicator Bacteria After the Intervention

For ponds 2 and 3, modest reductions in cultured *E. coli* concentrations were fairly consistent with the extent of dilution expected from raising the pond level (Supp. Table 1). For pond 4, however, the decline in cultured *E. coli* concentrations from 10^6 to 10^4 MPN/100 mL after pumping suggests incomplete mixing of the original pond water with the pumped groundwater.

With the exception of cultured *E. coli* along T1.1 radiating from pond 1, pond filling did not produce substantial changes in FIB concentrations (Fig. 4). In T1.1 cultured *E. coli* penetrated significantly further into the aquifer than before filling pond 1, remaining well above the detection limit at least 7 m from the edge of the pond (Fig. 4a). In contrast only slight increases were observed in molecular *E. coli* and Bacteroidales.

Cultured *E. coli* was not detected in the deeper well of T1.1 (T1.1z) after filling pond 1 (Fig. 4a) whereas high concentrations of both *mE. coli* and Bacteroidales genes (10^4 and 10^5 copies/100 mL respectively) were detected (Fig. 4b-c). In contrast, T2.1z contained at least 2-log$_{10}$ lower concentration of each FIB than its paired shallow well (Fig. 4d-f).

3.6 Hydrochemistry

An increase in Ca concentrations with distance was the only pattern systematically observed along the transects of wells radiating from all four ponds before the intervention (Supp. Table 4). No detectable changes in groundwater composition were observed after filling the ponds, with the exception of pond 1 where Ca and Mg concentrations along the transect were homogenized to the level measured in the pond one day after filling (Supp. Fig. 2). Concentrations of dissolved Fe in groundwater gradually increased with distance to ~ 30 mg/L along the transects at ponds 2 and 4, but generally remained <5 mg/L at ponds 1 and 3.

4. Discussion

4.1 Ponds as Sources of Fecal Contamination to Aquifers in Bangladesh

Our observations demonstrate that latrine ponds can be significant sources of fecal-contaminated recharge to wells in shallow unconfined aquifers, although the degree and extent of contamination varies depending on the season and sediment grain-size adjacent to the pond. Three independent FIB assays indicated that two of the four latrine ponds were point sources of fecal contamination to the adjacent aquifer. In the shallow aquifer, FIB concentrations (MPN or copies/100 mL) declined with lateral distance from ponds 1 and 2 under natural early monsoon conditions (Fig. 4a-f). FIB concentrations from the slightly deeper well along each transect indicates that vertical removal is more efficient than lateral removal, especially where substantial horizontal silt layers are present as in T2.1 (Fig. 2b). Silt-rich layers were observed at the base of ponds (except pond 1), which may inhibit aquifer recharge when pond levels are low during the dry season[26]. This is supported by substantial increases in *E. coli* concentrations in the aquifer adjacent to recently excavated pond 1 but not in the aquifer near pond 2.

4.2 Hydrologic Connection between Ponds and Aquifers

The increase in lateral hydraulic gradients in the aquifer adjacent to ponds during artificial pond filling provided a reliable indication of where high concentrations of FIB would be found in the aquifer. Rapid transport of pond water into the aquifer was confirmed at pond 1 by the change in groundwater Ca concentrations one day after filling. Our data also indicate that the hydrologic connection between ponds and aquifers is affected by the grain-size of pond and aquifer sediment (Fig. 2). Several studies conducted with columns of packed sand reported evidence for a critical threshold in the colloid (like bacteria) size to median grain-size ratio where removal rate greatly increased due to small changes in the median grain diameter of the sand[4,7,42,43]. The sensitivity of FIB transport to sediment grain-size in the present study demonstrates this phenomenon at the field scale.

4.3 Fitted Spatial Removal Rates

Removal of bacteria through porous media may be described by a simple logarithmic spatial reduction equation first proposed by Iwasaki[44]:

$$C(x) = C_{x=0} 10^{-\lambda x} \quad \text{Equation (1)}$$

where $C(x)$ is the bacteria concentration at x distance from the input source, $C_{x=0}$ is the input concentration at the source, and λ is the removal rate. Equation (1) was fit to $\log_{10}$-standardized concentrations ($C(x)/C_{x=0}$) of each fecal bacteria indicator in groundwater using linear regression to obtain λ with 95% CI's. The interpretation of λ in the present study has some limitations because equation (1) is one-dimensional and therefore ignores dilution effects due to three dimensional spreading of the FIB plume in the sediments near a pond. This removal model also implicitly assumes the population of a given FIB is homogeneous with respect to λ[6]. Measuring λ along a log-linear slope along the length of a flow path assumes steady-state and first-order attachment, a prediction of colloid filtration theory (CFT)[45], and die-off kinetics[11]. Processes such as straining[5], clogging[14,15] and variable saturation along the length of the flow path[46], however, lead to hyper-exponential concentration-distance curves[11].

Spatial removal rates for T1.1 and T2.1 (Fig. 4) were fitted in two different ways to equation (1). The first calculates λ along the pathway between the pond base to aquifer, using the pond as the initial concentration ($C_{x=0}$), with correlation coefficients (R^2) ranging from 0.95 to 0.99. The second approach fits λ_{gw} along the aquifer flow path only, where $C_{x=0}$ is the

concentration in the well closest to the pond, and results in R^2 values ranging from 0.68 to 0.96. The fit of λ to FIB concentrations indicates a consistent log-linear decline with distance through the aquifer, agreeing with CFT[45] and first-order die-off kinetics.

The fitted λ values were used to calculate expected 7-$\log_{10}$ (99.99999%) removal distances. This is the predicted safe setback distance where *E. coli* from a latrine pond (~10^6 MPN/100 mL) will be removed to below the acceptable drinking water limit of 1 MPN/100 mL used in many countries[47]. Under natural early monsoon conditions, removal distances decrease in the order Bacteroidales > *mE. coli* > *E. coli* (Fig. 4a-f; Table 1), suggesting that Bacteroidales travels further than *mE. coli* and *E. coli*. After the simulated rainfall event, 7-$\log_{10}$ removal distances tended to equalize, with the exception that cultured *E. coli* in T2.1 was still removed across a shorter distance than *mE. coli* and Bacteroidales (Fig. 4d-f). The initial order may reflect different persistence times of the FIB in the aquifer, whereas the disappearance of this order shortly after a recent influx of contaminated pond water suggests similar removal rates.

In each case, the post-filling λ_{gw} fitted to FIB concentrations along the saturated flow pathway tends to be lower than measured from the pond through the aquifer (λ) (Fig. 4a-f), probably because bacteria must pass vertically through a partially saturated zone from each pond to the water table, lying 1-3 m below the pond base at the beginning of the monsoon (Fig. 2)[46]. In T2.1 7-$\log_{10}$ removal distance based on λ_{gw}, follow the same order before pond filling (Bacteroidales > *mE. coli* > *E. coli*). At T1.1, however, *E. coli* is predicted to travel further than *mE. coli* and Bacteroidales in the saturated aquifer. Following artificial pond filling, the 7-$\log_{10}$ removal distance based on λ ranged from 7 to 12 m, with cultured *E. coli* tending to be removed across a shorter distance (range 6.7-10.1 m) than *mE. coli* and Bacteroidales (9.5-12.4 m) (Table 1).

The longer transport distances of molecular markers relative to cultured *E. coli* may reflect longer persistence times in the aquifer. In their review Foppen and Schijven[48] reported average die-off rate constants of cultured *E. coli* in groundwater at 20 °C corresponding to T_{90} (time required for initial concentration reduction of 90% or 1 $\log_{10}$) of 4.6 days (range 2.6-23). In contrast, fresh water (aerobic) microcosms at 25 °C showed a T_{90} of 2.8 days for the Bacteroidales marker[49]. Another study comparing die-off rates of *E. coli* and Bacteroidales found T_{90}'s of 7.9 days and 1.6, respectively (sewage-seeded, fresh water, dark microcosms at 13 °C)[50]. Therefore, under aerobic conditions Bacteroidales genes seem to die-off faster than cultured *E. coli*, implying that the greater penetration distance of Bacteroidales is due to less efficient filtration[45] or straining[5]. Thus Bacteroidales may be a useful relatively conservative indicator to determine safe setback distances for drinking wells.

The spatial removal rates measured in the present study (0.5-1.3 $\log_{10}$/m) are igher than in two previous transport experiments (0.2-0.6 $\log_{10}$/m) conducted in medium and with constant Darcy velocities ranging from 0.5 to 1.7 m/day[12,14]. This may result from contaminated water entering the aquifer in pulses rather than under constant flow conditions. Peak Darcy velocities of 4.0 and 0.5 m/day after pond filling in T1.1 and T2.1 respectively, were short lived (<24 hrs) (Fig. 3), with typical velocities <0.2 m/day. If the average reported die-off constant for *E. coli* in groundwater is used[48] with peak velocities (Fig. 4), the calculated λ parameters resulting from die-off alone are 0.05 and 0.4 for T1.1 and T2.1, respectively. This indicates that diminished advection after pond levels readjusted coupled with die-off contributed substantially to λ.

4.4 Implications for Microbial Drinking Water Quality

Only the transect radiating from the freshly excavated pond showed a rapid response to an artificial increase in pond level, suggesting that older ponds, which typically have accumulated a lining of finer grained sediments, provide a measure of protection against rapid microbial contamination of shallow groundwater. On the other hand, pre-intervention FIB gradients along transects from both sandy ponds showed that chronic contamination through pond walls may be widespread. Surface runoff during the early monsoon may overtop silt layers at the pond base, at which time microbial contaminants enter the local shallow aquifer of even more established ponds. Infiltration of fecal contamination into the aquifer is most likely during the early monsoon because the head gradient is favorable. Later in August, this head gradient disappears as ground and pond water tables equalize (Fig. 3d).

Previous monitoring has shown that as many as 70% of tube-wells in any given monsoonal month contain *E. coli* in rural areas underlain by unconfined sand aquifers, including Char Para[18,23]. Rather than indicating continuous input, our data suggest that this pattern may reflect multiple inputs during the early monsoon followed by a combination of filtration, straining, transport, and die-off. The lower removal rates measured in saturated sediment suggests potential for lateral spreading of fecal bacteria once sand layers are contaminated. On the basis of Bacteroidales, the FIB that appears to be transported furthest, installing tube-wells at least 13 m away from ponds might decrease the risk of exposure to fecal contamination from the aquifer. This should be interpreted with caution because bacterial removal rates were measured at only four sites and tended to be lower in the saturated aquifer. It is likely that coarser sediment exists elsewhere in Char Para and in other villages where this distance may not be sufficient to remove fecal bacteria. It is also likely that removal rates will vary substantially for fecal viruses, which typically differ from fecal bacteria in terms of transport behavior and occurrence in aquifers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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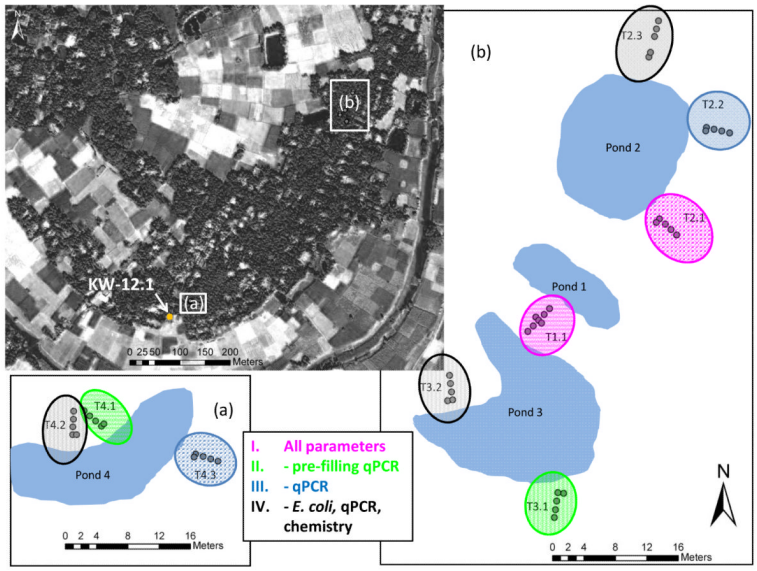

Figure 1.

Relative locations of four latrine ponds (shaded area) and transects wells (grey dots) within Site K. All shallow wells within a given transect were spaced exactly 1 m apart. Colored circles represent 4 groupings of transects according to breadth of parameters analyzed from the 6 week intensive monitoring period in June/July 2009. Group I transects (T1.1 and T2.1) were sampled before and after flooding for water chemistry, *mE. coli* and Bacteroidales as assessed by qPCR, and cultured *E. coli* at a minimum of once weekly. Each level thereafter indicates step-wise decreases in analyzed parameter coverage as indicated in the legend.

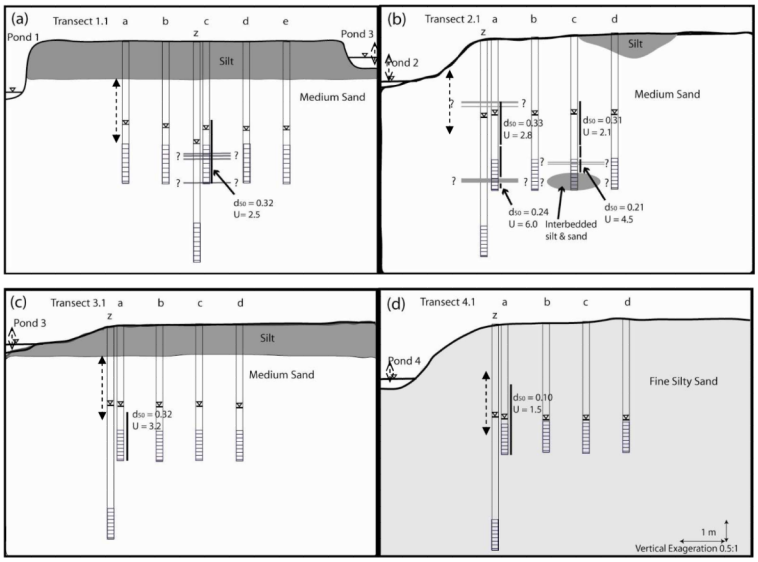

Figure 2.

Geologic cross-sections of transects. Vertical black bars represent cored sections of boreholes. Silt is indicated by dark grey shading. Median grain sizes (d_{50}) are indicated in mm and uniformity coefficients ($U=d_{60}/d_{10}$) are reported, where d_{60} and d_{10} are the grain sizes for which 60 and 10 % by mass of the sample is finer, respectively. The grad symbol indicates water levels in the wells and pond immediately after pond filling. Dashed, double headed arrows represent the maximum fluctuation in ponds and water table observed in 2009. Except during artificial pond filling, pond 1 was dry during the early monsoon, and was not monitored thereafter.

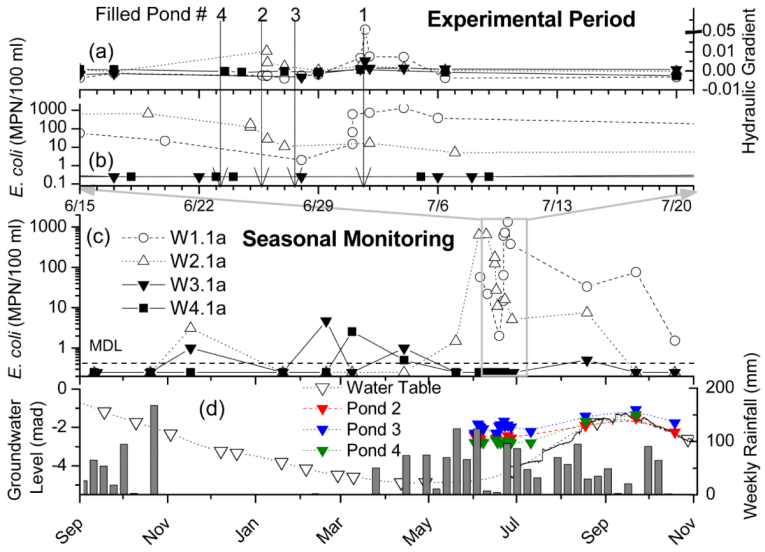

Figure 3.

E. coli and water levels adjacent to four ponds during seasonal and 6-week experimental time scales. (a) Lateral hydraulic gradients in transects away from ponds before and after artificial pond filling with pond filling dates indicated by vertical arrows. (b) *E. coli* concentrations measured at a minimum weekly during pre- and post-pond filling. (c) Seasonal *E. coli* concentration in the closest well to each pond (dotted horizontal line reflects detection limit of 0.5 MPN/100 mL). Monitoring of T1.1 began on 06/11/09. (d) Weekly precipitation (vertical columns), measured in Matlab (50 km south of Site K), accompanied by manual groundwater levels (hollow inverted triangles), relative to local ground surface, measured at well KW-12.1. Continuous water levels were available from W1.1a (solid black line). Pond levels (colored inverted triangles) were measured at a minimum monthly from 06/11/09 through 11/01/09.

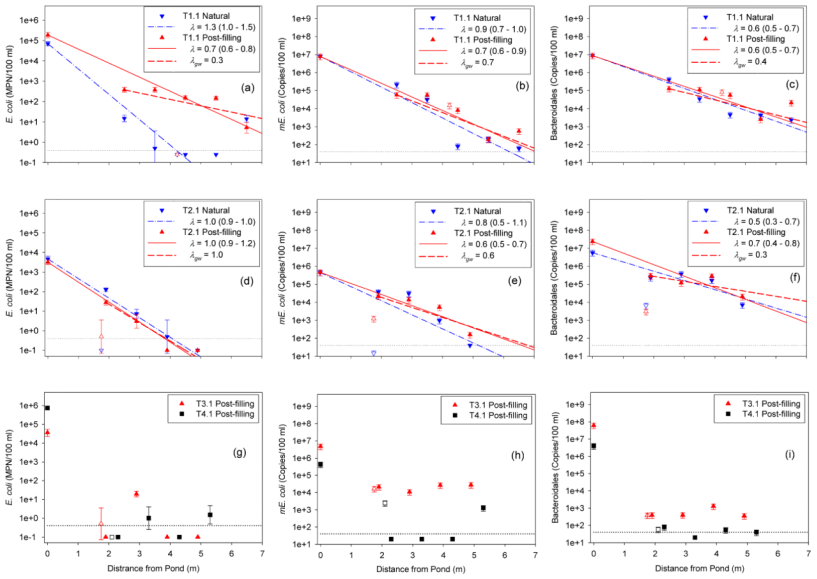

Figure 4.

Fecal bacteria concentrations in transects with lateral distance from ponds, measured before and after pond filling. Panels a, b and c represent concentrations of *E. coli*, *mE. coli* and Bacteroidales respectively, measured in pond 1 and within T1.1. Panels d, e and f represent FIB's measured in pond 2 and within T2.1. Panels g, h and I represent FIB's measured in both ponds 3 and 4 and within T3.1 and T4.1. Filled symbols represent ponds and shallow wells; open symbols represent the deeper well within each transect. Lines represent the model fitted with linear regression using equation (1). λ indicates the spatial removal rate when pond water is used as the initial concentration ($C_{x=0}$), whereas λ_{gw} (dashed line) indicates the removal rate regressed on the groundwater concentration only (the closest well to the pond becomes $C_{x=0}$). Post-filling concentrations for T1.1, T2.1, T3.1 and T4.1 are reported for 5, 1, 11 and 3 days respectively, after filling. The horizontal dotted line represents the MDL for each assay.

Table 1

Fitted spatial removal rates (λ) and 7-$\log_{10}$ removal distances (with associated 95% C.I.'s) of FIB away from ponds 1 and 2 under natural and post-filling conditions. 95% C.I.'s are not presented for λ_{gw} due to the small number of data points (n) between the first and last monitoring well in each transect. λ represents the removal rate ($\log_{10}$/m) fitted to concentration profiles extending from the pond edge to the most distal monitoring well, whereas λ_{gw} represents a fit to concentration profiles extending from the closest well to the pond to the most distal. The 7-$\log_{10}$ removal distances based on λ_{gw} are hypothetical and shown for comparison to λ only, since *E. coli* was never detected in a well in this study higher than 10^3 567 MPN/100 mL.

			Natural Early Monsoon Conditions			Post-filling Conditions					
Transect	**Geologic Media**	**FIB**	**λ(m^{-1})**	**n**	**7-$\log_{10}$ Removal Distance (m)**	**λ(m^{-1})**	**n**	**7-$\log_{10}$ Removal Distance (m)**	**λ_{gw} (m^{-1})**	**n**	**7-$\log_{10}$ Removal Distance (m)**
1.1	Medium Sand	*E. coli*	1.3 (1.0 - 1.5)	6	5.6 (4.7 - 7.0)	0.7 (0.6 - 0.8)	6	10.1 (8.5 - 12.4)	0.3	5	23.0
1.1		*mE. coli*	0.9 (0.7 - 1.0)		8.1 (7.0 - 9.5)	0.7 (0.6 - 0.9)		9.5 (8.1 - 11.5)	0.7		10.7
1.1		Bacteroidales	0.6 (0.5 - 0.7)		11.5 (10.1 - 13.4)	0.6 (0.5 - 0.7)		12.4 (10.1 - 14.6)	0.4		16.1
2.1	Medium Sand	*E. coli*	1.0 (0.9 - 1.0)	5	7.3 (6.7 - 7.7)	1.0 (0.9 - 1.2)	5	6.7 (6.0 - 8.1)	1.0	4	7.3
2.1		*mE. coli*	0.8 (0.5 - 1.1)		8.9 (6.4 - 14.6)	0.6 (0.5 - 0.7)		11.5 (9.5 - 14.6)	0.6		12.4
2.1		Bacteroidales	0.5 (0.3 - 0.7)		13.4 (10.7 - 20.1)	0.7 (0.4 - 0.8)		10.7 (8.5 - 16.1)	0.3		26.8