On-farm treatment of wastewater used for vegetable irrigation: bacteria and virus removal in small ponds in Accra, Ghana

Andrea I. Silverman, Mark O. Akrong, Pay Drechsel and Kara L. Nelson

ABSTRACT

Many urban farmers in Accra collect irrigation water from streams and open drains, which they store in small, on-farm ponds before use. Given that this water can be highly contaminated with wastewater, another potential role of the ponds is to disinfect irrigation water prior to use. To better understand the factors influencing bacteria and virus removal in these small ponds, we investigated the removal of culturable fecal indicator bacteria (*Escherichia coli* and enterococci) and coliphage (F+ and somatic coliphage) in a single batch of water stored for 3 days. Sunlight exposure was found to be important for removal. Bacteria and coliphage removal rates were faster in shallow sun-exposed water than in deeper water, due to sunlight attenuation with depth. Bacteria removal rates varied depending on solar irradiation, and correlations between total daily UVB fluence and bacteria removal rates were observed. Coliphage removal was observed in sun-exposed water but not in dark controls that allowed for sedimentation, further highlighting the importance of sunlight-mediated processes. These small ponds appear to have similar disinfection processes to larger-scale waste stabilization ponds, but can have more efficient inactivation due to their shallow depth and operation as batch reactors. Design and management recommendations for on-farm ponds are discussed.

Key words | disinfection, sunlight inactivation, urban agriculture, waste stabilization ponds, wastewater irrigation

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INTRODUCTION

Urban agriculture in Ghana's capital city, Accra, is an important example of wastewater-irrigated agriculture practised across the sub-region (Drechsel *et al.* 2006). Due to limited financial resources, institutional capacity, accountability and maintenance of existing facilities, the majority of wastewater treatment facilities in the city are not in operation (Murray & Drechsel 2011). As a result, untreated greywater and sewage (collectively referred to here as 'wastewater') flow directly and indirectly into open drains and streams, which are the primary sources of water used by farmers in Accra to irrigate vegetables. Irrigation waters used by urban farmers in Ghana were found to contain fecal indicator bacteria at concentrations that exceed the limit suggested by the World Health Organization (WHO) for the use of wastewater in agriculture (Mensah *et al.* 2001; Keraita *et al.* 2003; Amoah *et al.* 2005; Silverman *et al.* 2013), as well as human viruses at concentrations that could present health risks (Silverman *et al.* 2013). The use of wastewater in irrigated agriculture can be beneficial to both farmers and municipalities (Drechsel *et al.* 2006; Lydecker & Drechsel 2010), and on-farm wastewater treatment has been suggested as a way to reduce health risks associated with the practice (Keraita *et al.* 2008a; WHO 2006; Cofie *et al.* 2010).

Farmers in Accra often irrigate leafy vegetables manually using watering cans. To reduce the distance they must carry heavy watering cans between the water source and their crop beds, many farmers dig small reservoirs within

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or adjacent to their farm plots (typical volumes range from 2 to 5 m^3) into which they pump and then store irrigation water (Drechsel *et al.* 2006; Keraita *et al.* 2008a; Reymond *et al.* 2009); it is from these small ponds that farmers fill watering cans for overhead irrigation. Irrigation frequency varies with climate and is usually twice a day in Accra during the dry season, while as little as three times a week in cooler locations, such as Addis Ababa. Keraita *et al.* (2008a) and Reymond *et al.* (2009) reported that on-farm ponds in Ghana can reduce thermotolerant coliform and helminth egg concentrations, leading some to suggest that small on-farm ponds should be included as a component of the WHO's multi-barrier approach to reduce health risks associated with wastewater irrigation (WHO 2006; Cofie *et al.* 2010), especially if modified for higher efficiency disinfection (Keraita *et al.* 2010). Similar to larger waste stabilization ponds (WSPs) that are designed to treat wastewater at a low cost and with minimal maintenance, it is hypothesized that small ponds reduce pathogen and fecal indicator organism concentrations through predation, sedimentation and inactivation by sunlight-mediated processes (Mayo 1995; Davies-Colley 2005).

This study builds upon research by Keraita *et al.* (2008a) and Reymond *et al.* (2009) by investigating the removal of two additional groups of fecal indicator bacteria (*Escherichia coli* and enterococci) and two viral indicators (somatic and F+ coliphage) in a typical on-farm pond in Accra. The goals of this study were to: (1) better understand the factors that influence bacteria and virus removal within the pond; and (2) determine microorganism inactivation rates. Based on the results of this and previous studies, we provide recommendations for the design and operation of on-farm treatment ponds.

METHODS

Experimental design and sampling

Two independent experiments were conducted with slightly different sampling methodologies. Both experiments were conducted using a farmer-dug pond located on an urban farm in Accra's Airport Residential neighbourhood, where up to two dozen vegetable farmers share approximately a dozen ponds on approximately 3 ha of land. The experimental pond was typical of those used by urban farmers in Ghana (Keraita *et al.* 2008a; Reymond *et al.* 2009) and was used regularly by farmers to store and fetch irrigation water. The pond was approximately 3 m in diameter by 0.5 m deep; a pond of this size can hold around 3 days of irrigation water, considering plot sizes and irrigation frequency in Accra. Both experiments were conducted over the course of 3 days; the pond was filled at 18:00 the night before the experiments began with stream water augmented with effluent from a nearby, non-functioning wastewater treatment facility (approximately 3.5% of pond volume). Pond water sat undisturbed for 12 h prior to the start of experiments, during which time viruses and bacteria associated with large particles may have settled out of the water column. The pond had no inlet or outlet, and was operated as a batch reactor with no manual mixing. While farmers typically use the ponds continuously, the farmers did not add water to or collect water from the pond during the course of the experiments presented here, and the water was not disturbed in any way. Experiments were conducted in August 2010 and September 2011.

In Experiment 1, samples were collected from two regions of the pond (from: (1) a sun-exposed, open water section where samples were collected approximately 0.5 m from the pond edge; and (2) a sun-blocked section) and at two depths in each region: 10 cm below the pond surface (shallow samples) and 10 cm above the pond sediment (deep samples); a diagram of the pond and sampling locations is presented in Figure 1. The sun-blocked section consisted of a covered, 40-cm diameter plastic pipe inserted into the pond to create a column of water with similar characteristics

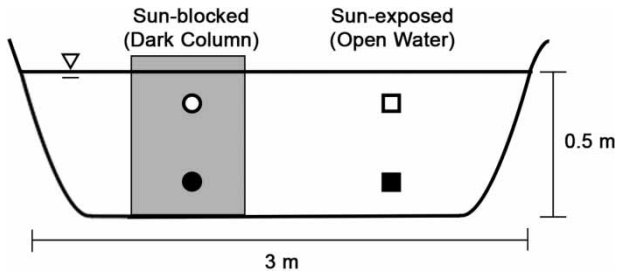

Figure 1 | Diagram of the small, on-farm pond used for experiments. Open and closed symbols indicate locations of shallow and deep samples, respectively, collected in Experiment 1.

to those of the rest of the pond, but without exposure to sunlight; there was headspace between the water surface and the cover of the column, and the cover was not airtight, which allowed for oxygen exchange at the water surface. Dark control samples collected from this 'dark column' were analyzed to help distinguish mechanisms of bacteria and virus removal: the dark column eliminated the potential for sunlight-mediated inactivation, but retained the possibility of removal due to sedimentation and predation. However, there is a possibility that the conditions within the dark column were not exactly the same as those in the rest of the pond (e.g. temperature, mixing and the effect of air movement above the pond), which present a potential to enhance sedimentation compared to the rest of the pond. Deep-water samples were collected using sampling ports installed in the pond, which consisted of L-shaped plastic pipes that allowed water collection at a consistent depth without disruption of pond stratification. The top of the sampling ports extended out of the top of the pond to allow for the insertion of sampling tubes, and the bottom was located 10 cm above the bottom of the pond. The dark column and sample ports were installed after filling the pond. The sun-exposed region of the pond was sampled every 4 hours starting at sunrise and ending at sunset (at 06:00, 10:00, 14:00 and 18:00). The dark column was sampled at 06:00 and 18:00. Samples were collected using a hand-operated vacuum pump connected to sterile PVC tubing and polypropylene collection bottles; two tube volumes were pumped and discarded before collecting each sample.

In Experiment 2, water was collected from the pond in a manner that sampled the entire water column, and the sun-blocked section was not included. Samples were collected using a bucket that was sterilized with 70% ethanol, wiped dry and rinsed with pond water before use. The bucket was used to scoop water from the pond in a manner that collected water from a cross-section of the pond depth; an effort was made not to resuspend sediments. Samples were collected every 4 hours starting at sunrise and ending at sunset (at 06:00, 10:00, 14:00 and 18:00), over the course of a 3-day period. Samples were poured into acid-washed, opaque, plastic cubitainers for transport.

For both experiments, samples were placed on ice in the dark, transported to the laboratory and analyzed immediately. A negative control consisting of sterile deionized water was analyzed each day for all microbiological assays.

Bacteria analyses

All water samples were analyzed for *E. coli* and enterococci concentrations by membrane filtration with 47-mm diameter, 0.45-μm pore size, mixed cellulose ester HA filters (Millipore). *E. coli* concentrations were quantified by plating filters on mI agar (BD) and incubating for 24 h at 37 °C. Enterococci concentrations were quantified by plating filters on mEI agar (BD) and incubating for 24 h at 41 °C. *E. coli* and enterococci plates were enumerated as colony forming units (CFUs).

Coliphage analyses

Water samples collected at 06:00 and 18:00 during Experiment 1 were concentrated for coliphage enumeration using membrane filtration with 47-mm diameter, 0.45-μm pore size, mixed cellulose ester HA filters. Before filtration, water samples were amended with $MgCl_2$ (0.05 M final concentration) and held for 5 min to facilitate virus adsorption to filters (Lukasik *et al.* 2000). Between 15 and 150 mL of sample was filtered, depending on water turbidity and filter clogging. Filters were preserved until elution by freezing at −20 °C on 300 μL of 50% glycerol (1:1 vol/vol with phosphate buffered saline).

Coliphage were eluted from filters by adding 3% beef extract (pH 9; 30 g/L beef extract, 30 mL/L Tween 80, 0.3 M NaCl) and swirling for 10 min. Filter eluent was assayed for coliphage using the double agar layer (DAL) method with 100 and 1-mL sample inoculums, a modified Luria Bertani (LB) top agar (0.75% wt/vol) and bottom agar (1.5% wt/vol), and appropriate hosts and antibiotics. F+ coliphage were assayed using *E. coli* F_{amp} host bacteria with ampicillin and streptomycin antibiotics (0.0015 g/L of each); somatic coliphage were assayed using *E. coli* CN13 host with nalidixic acid (0.01 g/L). Modified LB consists of: bacto agar (0.75% or 1.5% wt/vol; BD), 10 g/L bacto tryptone (BD), 0.137 M NaCl, 1 g/L yeast extract (EMD Chemicals), 0.0055 M dextrose (EMD Chemicals) and 0.002 M $CaCl_2$ (Fisher Scientific). Eluted filters were plated, face down, on top agar augmented with 0.3% Tween 80. Plates were incubated at 37 °C for 18–24 h and enumerated as plaque forming units (PFUs).

Total phage concentrations were calculated by adding counts from the DAL and filter plates. Coliphage samples were concentrated and plated in duplicate.

Environmental parameters

Total UVB irradiance (280–320 nm) was measured every 15–30 min during daylight hours using a hand-held meter (Solartech, Inc.); total daily UVB fluence was calculated as the sum of UVB irradiance multiplied by the time between measurements. Pond water absorbance was measured at the beginning of the experiment using a UV-visible spectrophotometer (Jenway model 6705 spectrophotometer); absorbance was measured at 280, 300, 320, 360, 400, 550 and 700 nm wavelengths. The depth of 99% light attenuation at each wavelength was calculated using the Beer–Lambert law. During Experiment 2, pH and dissolved oxygen (DO) concentration were measured using portable meters (Oakton pHTestr20 and Thermo-Orion 830A, respectively).

Data analysis

First-order, observed removal rate constants (k_{obs}) for each organism and sampling location were calculated for each day between the hours of 06:00 and 18:00. k_{obs} was calculated as the negative slope of the linear regression trend line of $\ln(C_t/C_o)$ versus time (t), where C_o is the concentration of a particular bacteria or coliphage measured at 06:00 and C_t is the concentration at t. Bacteria k_{obs} in the sun-exposed section of the pond were calculated using four data points, while dark column k_{obs} were calculated using two data points. Unless noted otherwise, average k_{obs} over the course of each 3-day experiment was calculated as the mean of three daily k_{obs}. Statistical analysis and graphs were made using GraphPad Prism (v6.0c).

RESULTS AND DISCUSSION

Environmental and pond water characteristics

Experiments were conducted during the rainy season in southern Ghana (August 2010 and September 2011); however, there was no precipitation during experiments. Cloud cover ranged from partly cloudy to overcast. UVB irradiation measured at the pond surface is presented in Figure 5. The pond water was green in colour, and the pH and DO concentration in the sunlit region of the pond increased from an average of 7.9 and 3.4 mg/L, respectively, at 06:00 to an average of 9.6 and 19.4 mg/L, respectively, at 14:00, indicating algae presence and high rates of photosynthesis. Absorbance measurements indicated that the pond water attenuated light: 99% of 300 and 550 nm light was attenuated at depths of 2.9 and 7.4 cm, respectively (Figure 2).

Bacteria removal

During Experiment 1, bacteria concentrations were observed to decrease faster in the sun-exposed section of the pond than in the dark column (Figure 3). The overall decrease in concentration of bacteria in the sun-exposed section over the course of 3 days in Experiment 1 was 1.7 log for *E. coli* and 1.8 log for enterococci. Overall bacteria removal in the dark column was 0.95 log for *E. coli* and

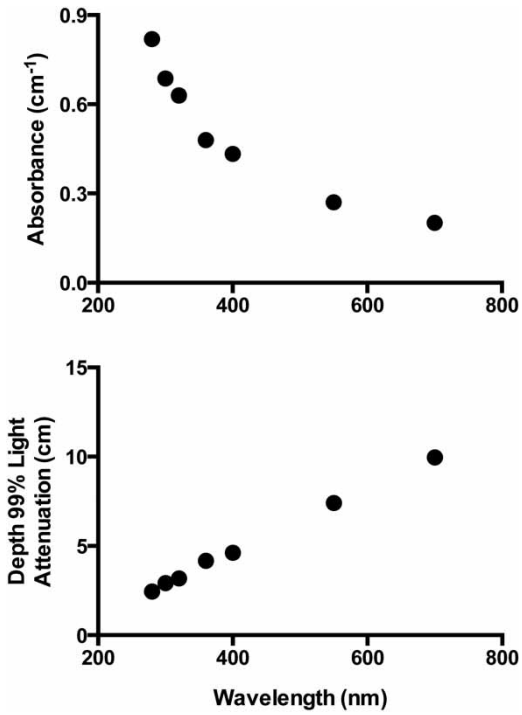

Figure 2 | Pond water absorbance and depths of 99% light attenuation.

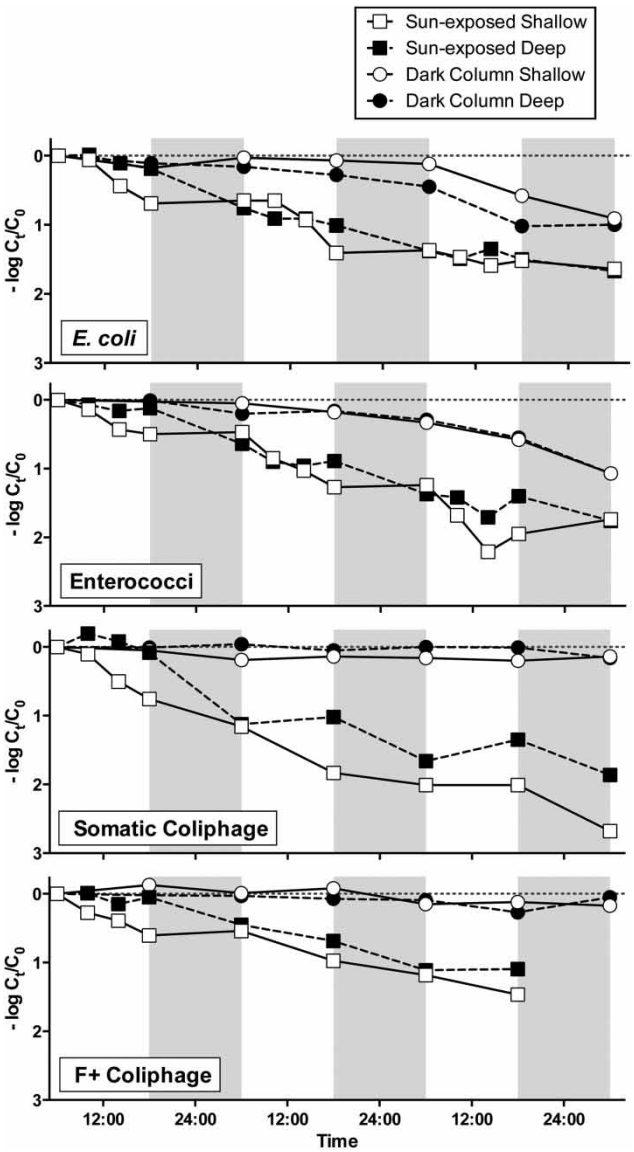

Figure 3 | Bacteria and coliphage removal measured at each sampling location during Experiment 1. Shaded areas represent night-time.

1.1 log for enterococci, which account for only 20% of the bacteria removal observed in the sun-exposed pond water. Little bacteria removal occurred in the dark column until the third day and night.

The dark column was designed to allow sedimentation to occur but not sunlight exposure; enhanced rates of bacteria removal in the sun-exposed section are attributed to sunlight-mediated inactivation, while all bacteria removal in the dark column is attributed to dark processes such as predation, sedimentation and loss of viability due to environmental stress. Sunlight-mediated inactivation can occur through endogenous processes (where photons absorbed by microorganisms cause direct, inactivating damage) or exogenous processes (where photons absorbed by water constituents, i.e. photosensitizers, lead to the formation of highly reactive molecules, e.g. reactive oxygen species, which inactivate microorganisms through oxidation reactions) (Davies-Colley *et al.* 1999); both endogenous and exogenous sunlight inactivation mechanisms are expected to occur in this system (Curtis *et al.* 1992; Davies-Colley *et al.* 1999; Kadir & Nelson 2014). Elevated pH has been found to increase sunlight-mediated inactivation of *E. coli* but not enterococci (Curtis *et al.* 1992; Davies-Colley *et al.* 1999), and elevated DO has been found to increase sunlight-mediated inactivation of both bacteria (Davies-Colley *et al.* 1999; Kadir & Nelson 2014). The high pH and DO measured in this system are attributed to algal photosynthesis during sunlight exposure; it is therefore appropriate to include pH- and DO-enhanced inactivation as sunlight-mediated processes. We were unable to measure pH and DO in the dark column; it is likely that the dark column had lower pH and DO due to a lack of photosynthesis.

While the overall rates of bacteria removal observed in the shallow and deep regions of the sun-exposed pond were similar, the trend during daylight hours was different at the two depths (Figures 3 and 4). For the shallow samples, the greatest decrease in bacteria concentrations occurred during the day, with little to no decrease at night. For the deep sun-exposed samples, the decrease in bacteria concentrations was slower during the day than in the shallow samples, followed by an additional decrease overnight, as measured the following morning. The most likely explanation for these

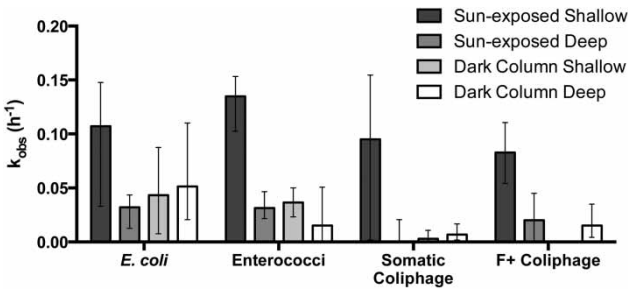

Figure 4 | Average first-order observed removal rate constants (k_{obs}; h^{-1}) measured during the day for each microorganism at each sample location during Experiment 1 ($n = 3$). k_{obs} were calculated between the hours of 6:00 and 18:00. Error bars indicate the range of k_{obs} values.

trends is that the pond was thermally stratified during the day, followed by whole pond mixing overnight. Water sampled from the top and bottom of the pond had different colours and smells in the afternoon, suggesting stratification. During the day, a combination of light attenuation and thermal stratification may have confined sunlight disinfection to the top layer of the water column; similar results were observed in a larger scale WSP by Mayo (1989, 1995). At night, apparent bacteria removal in the deep samples may have been a result of deep water mixing with shallow water, which contained lower bacteria concentrations; the fact that bacteria concentrations at the top and bottom of the water column were similar at 06:00 supports this hypothesis.

The effect of sunlight was also observed when comparing the inactivation rates reported above with those from Experiment 2, which was conducted during a period with greater insolation (Figure 5). There was more bacterial inactivation during the second experiment, with an overall 3.2 and 2.7-log reduction of *E. coli* and enterococci, respectively, over the course of the 3-day experiment. After pooling daily k_{obs} from both experiments, a correlation was observed between the *E. coli* and enterococci k_{obs} measured each day between 06:00 and 18:00 (average of sunlit shallow and deep inactivation rates for Experiment 1 data) and that day's total UVB fluence (Figures 6(a) and 6(b); $R^2 = 0.94$ for *E. coli* and 0.79 for enterococci, $n = 6$). Note that doubling the total daily UVB fluence more than doubled k_{obs}; this suggests that bacterial inactivation was not due to *direct* sunlight inactivation mechanisms alone. Ponds are complex systems, and a hypothesis as to why we observed a greater increase in k_{obs} is that exogenous sunlight inactivation mechanisms or other sunlight-mediated processes added to bacterial inactivation; for example, greater sunlight intensity would result in higher water temperatures, a greater concentration of reactive oxygen species (which can cause oxidative damage to some microorganisms), and higher pH and DO in algae-laden waters, all of which could affect *E. coli* and enterococci k_{obs} (Davies-Colley *et al.* 1999; Kadir & Nelson 2014).

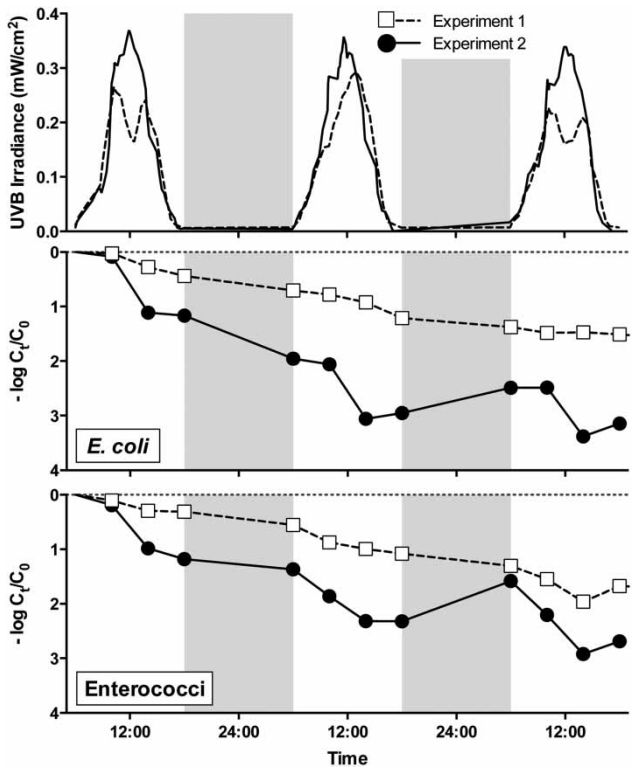

Figure 5 | Comparison of sunlight-UVB irradiance, and *E. coli* and enterococci removal during two independent experiments. Experiment 1 data (also presented in Figure 3) are denoted by a dotted line and open squares, and represent average removal measured in the sun-exposed region of the pond (average of removal measured in shallow and deep sampling locations). Experiment 2 data are denoted by a solid line and filled circles, and represent depth-average removal measured in sun-exposed pond water.

Solar irradiance changes throughout the year. The total daily UVB fluence (i.e. UVB irradiance integrated over time) for the 21st day of each month in Accra under clear sky conditions is presented in Figure 6(c); hourly irradiance spectra were predicted by the SMARTS radiative transfer model (Gueymard 2005) as global horizontal irradiance. Under clear sky conditions, solar irradiance (and therefore inactivation rates) in Accra (which is close to the equator) would be highest in March and September (when the sun is directly over the equator), and lowest in December and June (when the sun is directly over the Tropics of Capricorn and Cancer, respectively); however, other climatic conditions – such as cloud cover, air pollution and atmospheric dust (e.g. from the seasonal harmattan wind) – would also act to decrease irradiance and therefore microorganism k_{obs}.

Keraita *et al.* (2008a) studied fecal coliform removal from small farmer-dug ponds in Kumasi, Ghana and found an average k_{obs} of 1.6 d^{-1} during the first 6 days of treatment during the dry season. This value falls within the two, 3-day average *E. coli* k_{obs} measured in the present study: 1.5 and 2.8 d^{-1} (calculated as the negative slope of $\ln(C_t/C_o)$ versus time (d) using all *E. coli* data points for each 3-day

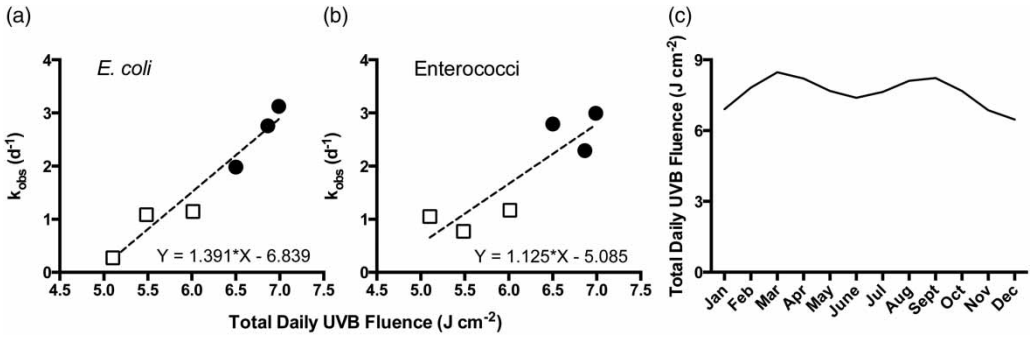

Figure 6 | (a) and (b) Daytime k_{obs} (measured between 6:00 and 18:00) plotted versus total UVB fluence measured on that day. Each point represents data obtained on 1 day; data are from Experiments 1 (open squares) and 2 (filled circles). *E. coli* $R^2 = 0.94$; enterococci $R^2 = 0.79$. (c) Total UVB fluence predicted for the 21st day of each month by the SMARTS radiative transfer model (Gueymard 2005); for reference, Experiments 1 and 2 were conducted in August and September, respectively.

experiment; Experiment 1 combined $\ln(C_t/C_o)$ data from the top and bottom of the pond). Keraita *et al.* (2008a) also found more fecal coliform removal in the dry season than the wet season, which they attributed to greater insolation during the dry season.

In a review of 186 full-size WSPs, von Sperling (2005) found a median range (25–75 percentile) of overall coliform removal (i.e. removal from inlet to outlet) of 1.4–2.3 log units for primary facultative ponds and 0.5–1.7 log units for maturation ponds. The overall reductions in *E. coli* concentration observed in the present study were 1.7 and 3.2 log units for Experiments 1 and 2, respectively, which fall within and exceed the range for primary facultative ponds (the influent water quality of the experimental ponds more closely resembles that of primary facultative ponds than maturation ponds). Full-scale WSPs have hydraulic residence times (HRTs) in the order of weeks (Shilton & Walmsley 2005), as compared to 3 days for the on-farm ponds; given that k_{obs} is calculated as removal over time, full-scale WSPs have much lower average k_{obs} than small on-farm ponds. The faster observed inactivation rates in on-farm ponds are likely due to shallower depth and operation as batch reactors, which have more efficient kinetics than conventional WSPs. Conventional WSPs are designed to have a constant flow and to approximate either completely mixed or plug flow reactors (PFR). Although an ideal PFR has the same kinetic efficiency as a batch reactor, real WSPs experience dispersion, short-circuiting and dead space, all of which decrease the overall removal.

The 2006 WHO *Guidelines for the Safe Use of Wastewater, Excreta and Greywater in Agriculture* recommend that irrigation water contains no more than 10^3 *E. coli*/100 mL for the irrigation of root crops and 10^4 *E. coli*/100 mL for the irrigation of leafy crops (WHO 2006). The ability of small ponds to disinfect contaminated irrigation water to an extent that meets the WHO recommendation depends on k_{obs}, the HRT and the initial *E. coli* concentration (which in turn depends on the water source). The *E. coli* concentrations measured at the end of each experiment were quite different: the final *E. coli* concentration for Experiment 1 was 8.2×10^4 CFU/100 mL while that of Experiment 2 was 82 CFU/100 mL, due to a lower initial *E. coli* concentration and faster inactivation rate. All natural treatment systems have variability in their treatment efficiency; while variable treatment efficiency in small ponds can result in a final *E. coli* concentration greater than the WHO recommendation, the ponds can still play a role in the multi-barrier approach to reducing risks from wastewater irrigation (WHO 2006), as suggested by Amoah *et al.* (2011). Pond design recommendations to improve treatment efficiency are provided below.

Coliphage removal

Previous evaluations of small on-farm treatment ponds (Keraita *et al.* 2008a; Reymond *et al.* 2009) did not investigate virus removal. In the present study, we observed a 2.0 log and 1.5 log reduction of somatic and F+ coliphage, respectively, in the shallow sun-exposed section of the pond by 18:00 on the third day of Experiment 1; the total reduction was 1.4 log and 1.1 log for somatic and F+ coliphage, respectively, in samples collected from the deep

sun-exposed section. However, the bulk of the coliphage removal observed in the deep sun-exposed section occurred at night, with insignificant inactivation occurring during the day (Figures 3 and 4). As with the trend seen for bacteria removal, this could be due to thermal stratification of the pond during the day and mixing at night. Interestingly, a reduction in coliphage concentrations was also seen in the shallow sun-exposed section at night (nights 1, 2 and 3 for somatic coliphage and night 2 for F+ coliphage). Two possible explanations for this finding are continued inactivation or removal of the viruses at night, or error or variability in virus concentration measurements given that measurements at night were based on only two time points.

Insignificant coliphage removal was observed in the dark column, indicating that sunlight was essential for coliphage removal and dark processes (e.g. sedimentation and predation) did not contribute.

CONCLUSIONS AND IMPLICATIONS FOR POND MANAGEMENT

Conclusions from this and previous research can be used to make suggestions for management of small on-farm ponds to maximize disinfection. We found that sunlight is important for reducing bacteria and virus concentrations, and faster inactivation rates were measured in water at the pond surface compared with deeper water. Other researchers also found fecal coliform inactivation rates to be slower in deeper WSPs (Sarikaya *et al.* 1987; Saqqar & Pescod 1991; Mayo 1995; Pearson *et al.* 1995; von Sperling 1999, 2005). Therefore, for disinfection of bacteria and viruses for which removal is dominated by sunlight inactivation, ponds should be shallow to allow for light penetration, though not less than 0.4 m deep to avoid growth of emergent plants (Davies-Colley 2005) and allow water collection using watering cans. Additionally, shallow ponds, such as those studied here, are less likely to become permanently stratified (i.e. thermally stratified during the day with no mixing at night), which would isolate deeper waters and limit sunlight disinfection. As an example, deep water-storage reservoirs, such as those used in Israel to store wastewater for irrigation, have limited treatment efficiency compared with conventional WSP (Dor *et al.* 1987; Juanico & Shelef 1994), partially due to their depth (e.g. 5.5–15 m deep) and permanent stratification (Liran *et al.* 1994).

To reduce pond surface shading and maximize sunlight exposure, ponds should be kept clear of emergent and floating plants, and vegetation growing on pond embankments should be cut frequently. While floating vegetation, such as duckweed and water lettuce, can aid in nutrient removal, this is not necessary for irrigation water; nitrate concentrations measured by Keraita *et al.* (2008a) in on-farm ponds that did not contain floating plants, for example, were in the range suggested by the Food and Agriculture Organization of the United Nations for vegetable irrigation (Ayers & Westcot 1985). Emergent and floating vegetation have been observed to reduce rates of bacteria removal (Pearson *et al.* 1995; Awuah *et al.* 2004), and can provide a habitat for insect larvae, such as mosquitos (Lloyd 2005). Given the concern that urban agriculture and WSPs can increase the risk of malaria and other vector-borne diseases (Drechsel *et al.* 2006 and ref. within), efforts must be made to reduce vector habitats, especially in regions endemic with diseases such as malaria, dengue fever and schistosomiasis.

Conversely, suspended algae can be beneficial for pond disinfection. When exposed to sunlight, algae photosynthesis increases pond water pH and DO concentration, which can increase microorganism inactivation rates (Davies-Colley *et al.* 1999). Algae may also be a source of photosensitizers that cause exogenous sunlight inactivation, to which enterococci and F+ RNA coliphage are susceptible (Davies-Colley *et al.* 1999; Kadir & Nelson 2014; Sinton *et al.* 2002). While we observed algae growth in ponds in this study, Reymond *et al.* (2009) did not, possibly due to the use of a different water source (i.e. drain water versus contaminated stream water) or HRT (e.g. algae growth may be prevented if the HRT is too short to allow an algae population to establish itself). More research into the conditions that promote algae growth in small ponds, and the effect of algae on disinfection, is needed.

As observed in the comparison between small on-farm ponds and full-scale WSPs, operating ponds as batch reactors (as opposed to flow-through systems) can lead to higher microorganism inactivation. However, the operation of just one batch reactor (Figure 7, Scheme 1) is difficult, given that farmers in hot climates use the water stored

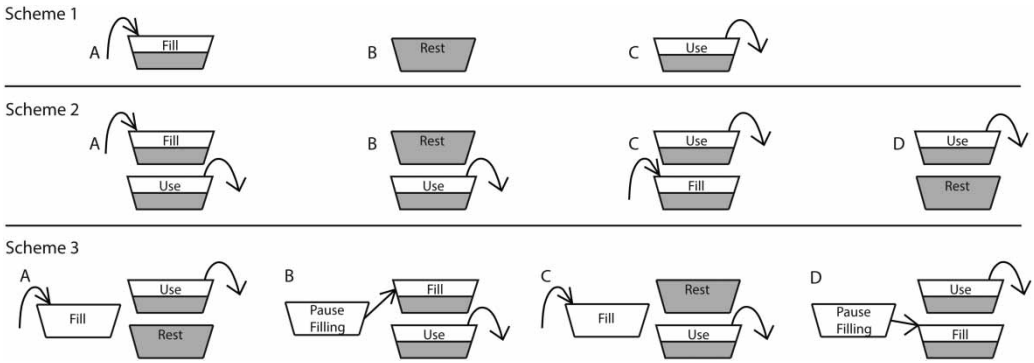

Figure 7 | Options for treatment pond configuration and operation. For each scheme, A to D illustrate the order of operation.

within ponds every day. This problem can be overcome if farmers have more than one pond and operate them in a fill-rest-use cycle, as suggested by Cofie *et al.* (2010) and Mara *et al.* (2010; Figure 7, Scheme 2).

An additional challenge with small batch reactors that rely on solar radiation for bacteria and virus disinfection, and have relatively short HRT (e.g. in the order of days), is that they can produce treated irrigation water of variable quality; this was demonstrated by variability in daily k_{obs} values measured in this study. Longer retention times can help reduce variability in final water quality and can be achieved by increasing the pond volume via the surface area (not depth). Pond volumes can be increased by making individual ponds larger, or by combining a few farmers' small ponds into larger shared ponds, which should be operated in a fill-rest-use cycle. However, larger ponds that are shared among a group may not be located conveniently for all farmers, which is a disadvantage given that the original purpose of the ponds was to reduce walking distances between water source and farmers' cropping beds.

In addition to the risks posed by bacterial and viral pathogens, helminths are important etiologies of disease associated with wastewater irrigation (e.g. Blumenthal *et al.* 2001; Blumenthal & Peasey 2002; Ensink *et al.* 2005). While we did not measure helminth egg concentrations or removal in the current study, Keraita *et al.* (2008a) found helminth egg removal in farmer-dug ponds to be similar during the wet and dry seasons, indicating sedimentation as the main removal mechanism; the researchers also found that pond water reached a helminth egg concentration of <1 egg/L (i.e. the WHO recommended limit) within 3 days. Helminth eggs and other pathogens that settle out of the water column accumulate in the pond sediment (Nelson 2003; Keraita *et al.* 2008a), and can be resuspended when farmers collect irrigation water (Keraita *et al.* 2008a). While Keraita *et al.* (2008a) suggested the use of deeper ponds to prevent sediment re-suspension during water collection (e.g. when farmers enter ponds to collect water, or if watering cans touch the sediment in shallow water), deeper ponds would result in lower depth-averaged rates of sunlight disinfection. An alternative option is to build a pond upstream of the parallel batch reactors in Scheme 2, with the express purpose of allowing sedimentation (Figure 7, Scheme 3): the pond should be designed with a long enough HRT to allow for complete removal of helminth eggs before the water enters the second pond. The two-phase system presented in Scheme 3 promotes two treatment mechanisms (i.e. sedimentation for helminths and sunlight disinfection for bacteria and viruses). If Scheme 3 is properly designed and managed, so that all sedimentation occurs before water enters the second ponds, only the single upstream sedimentation pond would require desludging. However, we realize that this scheme may not be a feasible option due to increased labour and land requirements. If digging multiple ponds is not an option, we recommend determining whether bacteria and viruses or helminths are the greater water treatment priority in a particular setting, and designing the pond to meet that need.

No matter which treatment scheme is deemed most appropriate, overarching challenges with on-farm wastewater treatment include limited land availability, insecure land tenure, and lack of incentives for farmers to accept

more labour or change current irrigation practices (Keraita *et al*. 2008b; Flynn-Dapaah 2002). Most farmers do not have formal land tenure (Flynn-Dapaah 2002), and many do not consider the health risk associated with wastewater irrigation to be a priority (Keraita *et al*. 2008b); these factors reduce farmers' ability and desire to make monetary or labour investments into water treatment infrastructure, whether additional ponds or sediment removal. Keraita *et al*. (2008b) suggested two routes that could be taken to provide incentives for farmers to participate in on-farm wastewater treatment: (1) market incentives that could include 'higher economic returns for safer vegetables which could be achieved through the establishment of distinct marketing channels (and monitoring) of safer produce'; and (2) 'institutional support from government institutions like provision of extension services in exotic vegetable farming, loans, awards and land tenure security' in exchange for farmer support of on-farm irrigation water treatment. A next step would be to target specific incentive routes for on-farm wastewater treatment and implement pilot projects to test if interventions work from institutional, management and water treatment perspectives.

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