Removal of indigenous coliphages and enteric viruses during riverbank filtration from highly polluted river water in Delhi (India)

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ABSTRACT

Emerging countries frequently afflicted by waterborne diseases require safe and cost-efficient production of drinking water, a task that is becoming more challenging as many rivers carry a high degree of pollution. A study was conducted on the banks of the Yamuna River, Delhi, India, to ascertain if riverbank filtration (RBF) can significantly improve the quality of the highly polluted surface water in terms of virus removal (coliphages, enteric viruses). Human adenoviruses and noroviruses, both present in the Yamuna River in the range of 10^5 genomes/100 mL, were undetectable after 50 m infiltration and approximately 119 days of underground passage. Indigenous somatic coliphages, used as surrogates of human pathogenic viruses, underwent approximately 5 $\log_{10}$ removal after only 3.8 m of RBF. The initial removal after 1 m was 3.3 $\log_{10}$, and the removal between 1 and 2.4 m and between 2.4 and 3.8 m was 0.7 $\log_{10}$ each. RBF is therefore an excellent candidate to improve the water situation in emerging countries with respect to virus removal.

Key words | coliphages, drinking water, enteric viruses, polluted rivers, riverbank filtration

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INTRODUCTION

Episodes of waterborne diseases are frequent and recurrent in emerging countries with poor sanitary conditions that affect drinking water. Waterborne epidemics might affect thousands of persons, like the hepatitis E epidemics of Kanpur, India (Naik *et al.* 1992) and Delhi, India (Dennis 1959; Ramalingaswami & Purcell 1988), with 79,000 and 25,000 ill persons, respectively. Both epidemics occurred after drinking water treatment had suffered a failure or proved insufficient, allowing contaminated drinking water to reach the consumers. To improve the consequences of water shortage and poor sanitary conditions, systems for obtaining drinking water are needed which are effective, reliable and cost-efficient.

In the course of riverbank filtration (RBF), surface water undergoes a passage through the confining banks of a river (or lake) and is obtained again in wells located at a certain distance from the riverbank (Eckert & Irmscher 2006). During the underground passage, the quality of the infiltrated river water (bank filtrate) improves thanks, among others things, to filtration, sorption of pollutants to soil particles, and physical and microbial degradation of the pollutants and pathogens present in the water. RBF has been successfully practised in Europe for more than 100 years (Richert 1900; Hiemstra *et al.* 2003; Eckert & Irmscher 2006), and remains the major potable water supply

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technology in many densely populated areas, such as the city of Berlin, which gets at least 70% of its drinking water through RBF and artificial recharge of groundwater with surface water (Massmann *et al.* 2007).

The Yamuna River, originating in the Himalayas, and flowing towards its confluence with the Ganges, enters the metropolitan area of Delhi through the northern part of the city (Figure 1). In the northern part of Delhi, a well field (Figure 1) situated along the Yamuna River is abstracting around 24 million gallons per day (MGD) (approximately 100 million L/day, which corresponds to 4% of the total water supply) of groundwater for the municipal water supply (Rao *et al.* 2006, 2007). At this stage, having not yet entered the densely populated area, the Yamuna River is only moderately polluted (up to 20 faecal streptococci per 100 mL; CPCB 2006, Lorenzen *et al.* 2010). The river flows further south into the densely populated area of the city, where it becomes severely contaminated by mostly untreated sewage (Walia & Mehra 1998; Singh 2001; Agarwal *et al.* 2006; Haberman 2006). In the Yamuna River in central Delhi, the concentration of faecal coliforms is approximately 10^7 most probable number (MPN)/100 mL (Sprenger *et al.* 2008), and the concentration of faecal streptococci is up to 5,000 MPN/100 mL (CPCB 2006; Lorenzen *et al.* 2010). In this stretch, which covers about 2% of the total length of the river, the Yamuna receives around 70% of its total pollution load (CSE 2007). Around 200 million litres of raw sewage and 20 million litres of industrial waste reach the river every day, reducing it to a sewage run-off (UNDP 2006). Some authors consider the water quality of the Yamuna River in central Delhi to be among the worst of all rivers in India (Haberman 2006).

Most countries practising RBF generally accept that soil passage lasting a certain period of time will reduce viral pathogens present in the surface water to tolerable concentration levels and render the water suitable for consumption. However, the large period of time statutorily required for this reduction – depending on the country, around 50 days (Chave *et al.* 2006) – makes it difficult to investigate the final degree of removal occurring after the prescribed infiltration time, since the virus concentrations drop under detection levels well before this time is reached. Instead, numerous studies have looked at the removals taking place at lesser distances. Removal of poliovirus 1 was 2.7 $\log_{10}$ units after 0.75 m infiltration in columns (Landry *et al.* 1980). Poliovirus 1, echo 1 and echo 29 became undetectable after 1.6 m column infiltration (Lance *et al.* 1982). Removal of bacteriophage MS2 was 8.2

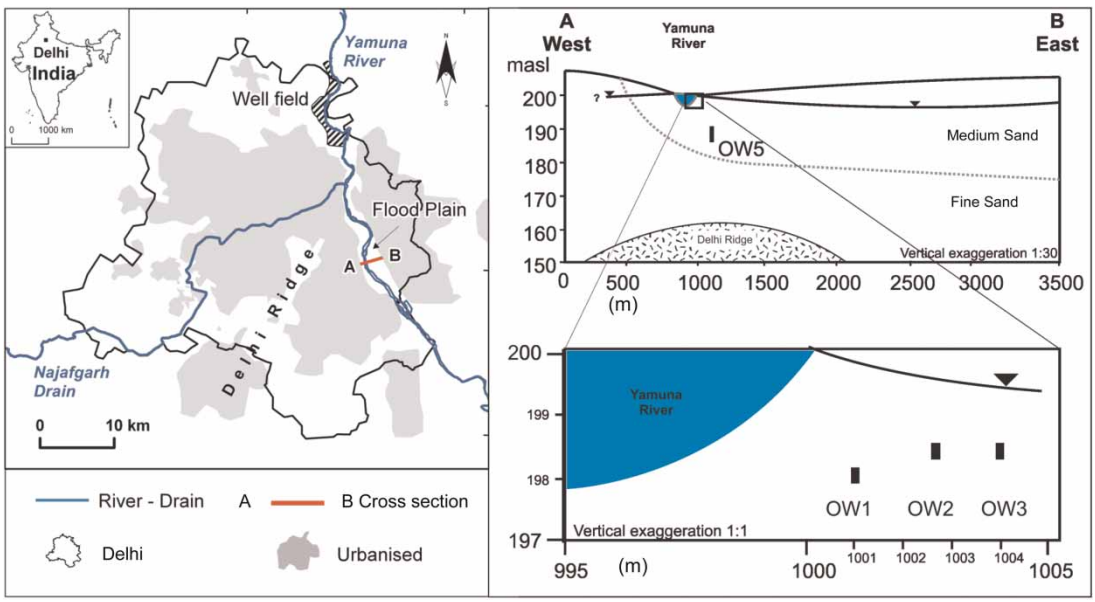

Figure 1 | Location map of the study area (left) and cross sectional view with observation wells OW1, OW2, OW3 and OW5 (right), masl = metres above sea level, (▾), groundwater level, vertical bars indicate position and lengths of observation well filter screen.

$\log_{10}$ units after 38 m infiltration in an aquifer. From these 8.2 $\log_{10}$ units, 6 $\log_{10}$ units removal was observed during the first 8 m (Schijven *et al.* 2000; for an exhaustive review of soil filtration performance, see Pang 2009). This paper studies the removal of viral (somatic coliphages, enteric viruses) contaminants during similar distances of underground passage adjacent to the river Yamuna, to investigate if the high degree of organic and faecal pollution of this river still allows the viral removal otherwise observed with RBF.

MATERIALS AND METHODS

Study area description

The study area, located on the eastern banks of the Yamuna River in central Delhi, is depicted in Figure 1. The left side of the figure shows the location of the field site within the city, marked 'B'. The right side of the figure outlines with two sketches, drawn to different scales, the field site and the position of the four observation wells (see below) with respect to the river.

Construction of observation wells. Sampling procedure and chemical analysis

Groundwater observation wells at the field site were constructed with short filter screens at specific depths. One observation well (OW5) at 50 m distance from the river was drilled to 10 m depth. Another three observation wells (OW1, OW2, OW3) were constructed at 1, 2.4 and 3.8 m distance from the river shore and fitted with poly(vinyl chloride) (PVC) filter screens with an effective mesh of ca. 1 mm at depths of 2, 1.5 and 1.5 m, respectively. The observation wells OW1, OW2, OW3 and OW5 were in line and perpendicular to the Yamuna River, forming a transect.

Sediment samples were obtained and analysed for grain size and determination of organic carbon content at the laboratories of Free University of Berlin. Soil grain composition was measured by sieving according to DIN 18123 (1996), and the fraction of organic carbon was determined by ignition loss according to DIN 18128 (2002). Weight loss on ignition was determined after drying at 105 °C followed by ignition at 550 °C. Weight loss is expressed as fraction of total weight. Iron content was measured by aqua regia digestion according to DIN ISO 11466 (1997).

River water was collected directly into the sampling bottles at approximately 30 cm below the water surface close to the shore line, using an extension pole. Groundwater was abstracted with a submersible 12 V pump. On-site parameters (pH, temperature, electrical conductivity and dissolved oxygen) were measured with Eutech Cyberscan (Metex Corporation, Buffalo, New York, USA) devices in a flow-through cell from 30 cm depth in the Yamuna or from the bottom of the observation wells. After on-site parameters were stable and at least three static water volumes of the observation wells had been pumped out, the water samples were taken and stored in 20 mL polypropylene bottles. All samples for ion determination were filtered on site with 0.2 μm acetate cellulose filters. The sample for cation measurements was acidified to pH 2 with ultra-pure HNO_3 and one bottle of each sample (not acidified) was kept for anion determinations. Alkalinity, as HCO_3^-, was determined by HCl titration in the field using a Merck (Darmstadt, Germany) Acidity test. Ammonium content was determined on site by Merck Aquaquant colometric tests. Water samples were analysed for Ca, Mg and NO_3^- by ion chromatography (Dionex DX 500). Dissolved organic carbon (DOC) was analysed by a DOC-analyser in the laboratory (Shimadzu).

Analysis of enteric pathogenic viruses

The concentrations of four human-pathogenic viruses were analysed in surface water and groundwater samples (OW5). The native water of the river and the OW5 concentrate (see below) was analysed for four enteric viruses: adenovirus (HAdV), norovirus (NoV), hepatitis A virus (HAV) and hepatitis E virus (HEV). HAdV and NoV were assayed quantitatively using quantitative-polymerase chain reaction (qPCR) and reverse transcription quantitative-PCR (RT-qPCR) respectively. The two hepatitis viruses were not assayed quantitatively, since previous studies had shown very low concentrations in polluted waters (Rodriguez-Manzano *et al.* 2010, 2012). For this reason both viruses were analysed using non-quantitative PCR assays, which allow a higher sensitivity.

River water

Water of the Yamuna, containing high concentrations of viruses, was processed without being concentrated. The water samples were transferred into steam-sterilized (100 °C, 2 hours) plastic bottles, which were placed into a dark box containing cooling elements and transported to the laboratory in Delhi, where they were stored at −80 °C, and then transported by air to the microbiological laboratories of the University of Barcelona and the Federal Environmental Agency in Berlin.

Concentration of viruses from 10 L samples

The water from OW5 was concentrated in order to render the overall detection procedure more sensitive. All groundwater samples were taken with a previously disinfected pump. One day before sampling, the pump, the hose and the electric wires were submerged into a solution of 1% sodium hypochlorite. In order to neutralize this disinfection solution, the whole pumping equipment was then put into a solution of 1% sodium thiosulphate for 2 hours. At both steps the pump was operated for at least 30 min in order to sterilize/neutralize (flush out possible contamination residues from the inner mechanics). For the concentration procedure, 10 L of water was abstracted from OW5 and concentrated as described by Wyn-Jones *et al.* (2011). Briefly, the sample was passed through a glass-wool column to bind the viruses. The viruses were then eluted from the glass wool by slow (20–30 min) passage of 200 mL 3% (w/v) beef extract at pH 9.5 in 0.05 M glycine buffer through the column. The eluate was flocculated by the addition of 1 M and 0.1 M HCl until the pH reached 3.5. The resultant protein floc, containing virus, was deposited by centrifugation at 7,500 × *g* for 30 min, and dissolved to a final volume of 10 mL phosphate-buffered saline. The concentrates were stored at −80 °C and then transported by air to the microbiological laboratories of the University of Barcelona and the Federal Environmental Agency in Berlin.

Analysis of enteric viruses, positive controls

The positive controls used for HEV analysis were 10% faecal suspensions obtained from rhesus monkeys experimentally infected with a strain isolated from a sewage sample collected in Barcelona (Pina *et al.* 1998). Human adenovirus 2 (HAdV2), isolated from a clinical sample and used as positive control for the determinations of human adenoviruses, was grown on A549 cells propagated in Eagle's minimum essential medium (EMEM) supplemented with 1% glutamine, 50 mg/mL gentamicin and 5% (growth medium) or 2% (maintenance medium) of heat-inactivated foetal bovine serum. Viral suspensions were stored at −80 °C until use. Norovirus GGII (NoV GGII), used as positive control, was contained in diluted suspensions from faecal clinical samples kindly provided by Dr Annika Allard, Umea University, Sweden. The positive control for the detection of HAV was provided by the manufacturer of the kit.

Extraction of nucleic acids of enteric viruses from the water concentrates

Nucleic acids, both DNA and RNA, were extracted from 5 mL volumes of sample concentrate using the NucliSens® miniMAGTM system (Biomérieux, France) according to manufacturer's instructions, with slight modifications comprising centrifugation at 1,500 × *g* for 2 min after addition of the silica suspension to reduce cross-contamination. The final 100 µL nucleic acid extract was centrifuged at 13,000 × *g* for 1 min to pelletize any remaining traces of silica which could inhibit downstream RT-PCR reactions. The supernatant was then transferred to a clean microfuge tube and was stored at −80 °C if not used immediately.

Semi-nested RT-PCR for the detection of HEV

The presence of genomic RNA of HEV was analysed by semi-nested RT-PCR to amplify a fragment within the open reading frame 2 of the genome using the degenerated primers described by Erker *et al.* (1999) and the OneStep RT-PCR Kit (Qiagen, Hilden, Germany). Five microlitres of the extracted nucleic acids or a tenfold dilution was tested by RT-PCR in a total reaction volume of 50 µL containing 1xOneStep Qiagen Buffer, 2 µL of Qiagen OneStep Enzyme Mix, 400 µM of each dNTP, 10 units of ribonuclease inhibitor (Applied Biosystems, Foster City, CA, USA), and 25 pmol of each outer primer (HEVORF2con-a1 and HEVORF2con-s1). After 30 min at 50 °C, the reaction

was heated at 95 °C for 15 min, followed by 35 cycles at 94 °C for 20 s, annealing at 55 °C for 30 s, and extension at 72 °C for 20 s. All amplifications were completed with a 10 min, 72 °C extension period. The second round of amplification was performed with 30 cycles under the same conditions described. Standard precautions and controls were applied when performing these assays: all samples were run in quadruplicate (two replicates per dilution), and positive and negative controls were included.

Nested RT-PCR for the detection of HAV

The presence of HAV in the samples from river and bank filtrates were tested by a semi-quantitative nested RT-PCR using the HAVGene Detection Kit from Mediagnost (Reutlingen, Germany), which amplifies a 780 bp fragment (first round of amplification) and a 311 bp fragment (second round of amplification) with a detection limit of about 10 infectious particles per mL. Amplifications were performed according to the manufacturer's instructions. Amplification products were visualized on a 1.5% agarose gel in tris(hydroxymethyl)aminomethane/borate/ethylenediamine tetraacetic acid (TBE) buffer.

Quantitative real-time PCR (qPCR) and RT-qPCR of HAdV and NoV

For the specific detection and quantification of HAdV genomes, 10 μL of the 10-fold and 100-fold dilutions of every DNA extraction were also assayed. Amplification was performed in a 25 μL reaction mixture with the PCR Master Mix (Applied Biosystems). The reaction contained 10 μL of a DNA sample or 10 μL of a quantified plasmid DNA, 1xTaqMan Master Mix, and the corresponding primers and TaqMan probes at their corresponding concentrations (Hernroth *et al.* 2002). An ABI Prism 7000 sequence detection system (Applied Biosystems, Life Technologies GmbH, Darmstadt, Germany) was used by the Federal Environmental Agency to perform the qPCR and RT-qPCR. The University of Barcelona used a MX3000P detector system (Stratagene, La Jolla, CA, USA).

After activation of the uracil N glycosilase contained in the core mix (2 min at 50 °C) and activation of the AmpliTaq Gold for 10 min at 95 °C, 40 cycles (15 s at 95 °C and 1 min at 60 °C) followed. All samples were run in quadruplicate, analysing two replicates of the neat and 10-fold dilution for well-water concentrate and two replicates of the 10-fold and 100-fold dilutions in river water. Positive and negative controls were included. The plasmid used as standard for quantification of HAdV was pAd41, containing the hexon region of HAdV41 in pBR322 kindly donated by Dr Annika Allard. For NoV GGII, the plasmid pNoV, containing the ORF1–ORF2 junction in pTrueBlue, was kindly donated by Dr Vinjé and Dr Jothikumar from the Centers for Disease Control and Prevention (CDC) (Atlanta, GA, USA). *Escherichia coli* JM109 cells (Promega, Madison, WI, USA) were transformed with the plasmids (pAdV41 or pNoV). The plasmids were purified from bacteria using the QIAGEN Plasmid Midi Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions, and the DNA was quantified with a GeneQuant pro (Amersham Biosciences, Piscataway, NJ, USA). To reduce the possibility of DNA contamination, the plasmids were linearized with EcoRI (pNoV) or NruI (pAd41) (Promega, Madison, WI, USA), and then purified and quantified again. Suspensions containing 10^{-2} to 10^{7} viral DNA molecules per 10 μL were made in tris(hydroxymethyl)aminomethane/ethylenediamine tetraacetic acid (TE) buffer and used as standard dilutions, then aliquoted and stored at −80 °C until use.

RT-qPCR for quantification of norovirus GGII was performed in a 25 μL reaction mixture using the QuantiTectTM Probe RT-PCR kit (Qiagen, Valencia, CA, USA). The reaction mixture contained 5 μL of nucleic acid extraction or 5 μL of a quantified plasmid cDNA, 1 μM of the JJV2F and COG2R primers and 0.1 μM of the RING2-TP probe, as described by Jothikumar *et al.* (2006). Following retrotranscription (30 min at 50 °C) and activation of the HotStarTaq (15 min at 95 °C), 45 cycles (10 s at 95 °C, 20 s at 55 °C, 15 s at 72 °C) were performed using an MX3000P sequence detection system (Stratagene, La Jolla, CA, USA).

For the detection and quantitation of specific viral genomes, 10 μL (for HAdV) and 5 μL (for HEV and NoV GGII) of neat and 10-fold dilutions of every DNA/RNA extraction were tested. These dilutions were designed to detect and reduce amplification inhibition caused by the potential presence of inhibitory substances that may interfere with the qPCR. All samples were run in quadruplicate (two replicates per dilution), and positive and negative controls were included. Known quantities of target DNA were

added to a parallel amplification reactions containing qPCR mix and the plasmid. In every assay, the amplification plots of samples and standard dilutions were compared. A sample was considered positive if it produced correct amplification curves and the quantification data were within the detection limit. The amount of DNA was defined as the average of the duplicate data obtained. The Federal Environmental Agency and the University of Barcelona used the same detection systems as described above.

Analysis of coliphages

Somatic coliphages are viruses which attack *E. coli*. Harmless to man, but otherwise of similar size and physical properties as human pathogenic viruses, they are used as surrogates for human enteric viruses. Water was transferred in steam-sterilized plastic bottles to the laboratory in Delhi, as described above. Detection analysis was always carried out on the same day. In order to prevent cross-contamination f surface water to groundwater samples, both sample types were stored separately. Sampling of coliphages was carried out on the river water and in the OW1, OW2, OW3 and OW5 observation wells, and detection was conducted according to a modified United States Environmental Protection Agency (USEPA) method (USEPA Method 1602 (2001)). Briefly, an appropriate volume of water to be analysed was made up to 100 mL with sterilized water, or in the case of the observation wells, a sample volume of 500 mL was taken. To these samples were added log-phase host bacteria, and 100 or 500 mL of double-strength molten tryptic soy agar, depending on the original sample volume, so as to make up a final volume double the sample volume. The sample was mixed thoroughly and the total volume was poured into Petri dishes. After overnight incubation, circular lysis zones (plaques) were counted and added for all plates from a single sample. The quantity of coliphage in a sample was expressed as plaque forming units (PFU)/100 mL. As a modification of the USEPA Method 1602, we used sample volumes larger than 100 mL, as indicator bacteria we used the strain *E. coli* WG5, and the Petri dishes for plating the nutrient agar, containing the indicator bacteria and the phages, were scaled-up to 22 cm diameter. These large dishes were chosen in order to test the larger sample volumes of 500 mL, avoiding previous concentration steps of the sampled water, which would have introduced an additional factor of uncertainty into the measurements. With this procedure 500 mL samples, mixed with 500 mL double concentrated nutrient agar, were directly distributed into the Petri dishes, allowing a detection limit of 0.2 PFU/100 mL.

Breakthrough of coliphages and viral pathogens from the river Yamuna to the well OW5

Monthly measurements of concentrations of somatic coliphages were carried out over a period of 8 months (nine observations), in both the river water and the bank filtrate of OW5, situated at 50 m distance and approximately 119 days of travel time from the riverbank. The travel time calculation was based on results from a vertical two-dimensional transient groundwater flow model. The model accurately simulates the spatial and temporal distribution of chloride concentration. Travel times to OW5 ranged from 107 to 137 days (mean travel time 119 days; for a detailed description of the model, see Sprenger (2011)). Beside the somatic phages, two samples, from the Yamuna and from OW5 well infiltrate, were assayed for four human pathogenic viruses frequently present in sewage-contaminated waters.

Study of the kinetics of phage removal between the river and wells OW1, OW2, OW3

In a separate series, phages were measured in the river and in the three wells OW1, OW2, and OW3, which are situated close to the riverbank. Monthly samples were taken from the river and from the wells in order to investigate the spatial kinetics of the phage removal along the filtration path.

RESULTS AND DISCUSSION

The experiments carried out sought mainly to answer two questions:

- Are pathogenic viruses or native somatic phages still detectable after the infiltrated river water has stayed approximately 119 days underground (from the shore to OW5, located at 50 m from the shore), a filtration

condition which would be considered adequate for obtaining drinking water via RBF by many countries (Chave *et al.* 2006)?

- What are the removal kinetics of native phages during infiltration through the first 4 m into the riverbank of the Yamuna?

Soil and water composition

The hydrological and hydrochemical properties of the sediment and groundwater samples from the observation wells and the aquifer are shown in Table 1. The river water and the groundwater at the study area were virtually free of dissolved oxygen, and dissolved organic carbon content was around 4 mg/L. Consequently, the redox conditions in the aquifer were anoxic. The occurrence of reduced species of iron, arsenic and nitrogen has been demonstrated previously (Lorenzen *et al.* 2010). Nitrogen occurred in the form of NH_4^+, averaging 5 mg/L. No mixing between genuine groundwater and bank filtrate could be observed in the infiltrate samples abstracted from all four observation wells, as was inferred from the virtually identical ionic composition of the infiltrate and the river water (not shown).

Table 1 | Hydrogeological and hydrochemical properties of groundwater and aquifer at the study area

Parameter	Sand	Water
Clay (%)[a]	1	
Silt (%)[a]	5	
Sand (%)[a]	94	
Gravel (%)[a]	0	
Fraction organic carbon (%)[b]	0.3–2	
Total Fe (III) (g/kg)[c]	1.4	
pH[d]		7.1 (±0.1)
Electrical conductivity (μS/cm)[d]		1,314 (±212)
Temperature (°C)[d]		21 (±3)
Dissolved oxygen (mg/L)[d,f]		0.1 (±0.00)
Nitrate (mg/L)[d]		0.4 (±0.3)
Ammonium (mg/L)[d]		5 (±0.5)
HCO_3^- (mg/L)[d]		604 (±77)
DOC (mg/L)[e]		4.4 (±1)
Ca^{2+} (mg/L)[e]		75 (±8)
Mg^{2+} (mg/L)[e]		32 (±3)

[a]Measured by sieving.
[b]Measured by ignition losses.
[c]Measured by *aqua regia* digestion.
[d]Field measurement.
[e]Laboratory measurement.
[f]Oxygen measurements were carried out four times in the river and in each of the wells OW5, OW1, OW2, and OW3.

Qualitative estimation of the breakthrough of phages and viral pathogens at 50 m from the Yamuna riverbank

In order to allow a qualitative, time-extended estimation of the breakthrough, if any, of native somatic phages from the Yamuna River to a 50 m underground distance, measurements of phages (nine observations, both in the river and in OW5) and four pathogenic viruses (one observation in the river and in OW5) were carried out in the Yamuna and in OW5.

The average concentration of coliphages in the Yamuna Oin this series of measurements was $5.3 \times 10^4 \pm 2.6 \times 10^4$ PFU/100 mL (concentration span: 3.5×10^4–9×10^4), whereas none of the samples taken from the observation well (OW5) contained coliphages above the detection limit of 0.2 PFU/100 mL. The removal of somatic coliphages may be calculated to be more than 6 $\log_{10}$ units if the volumes of all nine observations in OW5 are compounded (4,500 mL), and considering that no phage was detected in this volume. As was pointed out above, the samples extracted from all sampling wells consisted entirely of river infiltrate, without dilution with genuine groundwater.

The concentrations of HAdV and NoV found in the river water were of the same order of magnitude as somatic coliphages. The Yamuna water was positive for all four pathogenic viruses assayed, that is HAdV, NoV, HAV and HEV, whereas no viruses could be detected in the bank filtrate of OW5 (Table 2). Based on these results, the removal of enteric adenoviruses and noroviruses, expressed as [$-\log_{10}$(C/Co)], was estimated to be in the order of 10^5 $\log_{10}$ units.

Quantitative estimation of removal of the native phages with increasing distance from shore

To obtain a quantitative estimate of the removal of native viruses during underground passage, concentrations of somatic phages were determined in the river water and in three in-line observation wells located close to the river

Table 2 | Enteric virus genomes in the river water and in the bank filtrate of the OW5 observation well

Organism	Yamuna River[a]	Yamuna River[b]	Average	Standard deviation	Bank filtrate in OW5
HAdV	4.1×10^4 genome copies/100 mL	3.1×10^4 genome copies/100 mL	3.6×10^4 genome copies/100 mL	7.1×10^3 genome copies/100 mL	None in 500 mL[a,b]
NoV	5.7×10^3 genome copies/100 mL	1×10^5 genome copies/100 mL	5.4×10^4 genome copies/100 mL	6.9×10^4 genome copies/100 mL	None in 500 mL[a,b]
HAV	ND	Positive in 100 mL	–	–	None in 1000 mL[b]
HEV	Positive in 100 mL	ND	–	–	None in 1000 mL[a]

[a]University of Barcelona.
[b]Federal Environmental Agency of Germany.
ND: Not determined.

(OW1, OW2, OW3). To obtain a time-representative average, four samples were taken at monthly intervals. Somatic coliphages in the Yamuna in this series of measurements were present at an average concentration of 1.02×10^5 PFU/100 mL (Table 3). Removal of the bacteriophages was not linear with distance. During the first metre of subsurface passage the removal was in the range of 3.3 $\log_{10}$ units /m. Between the first and the second, as well as between the second and third observation well, the removal remained approximately constant with about 0.5 $\log_{10}$ units/m (Table 3). That the results were obtained with native, and not with laboratory grown, phages underlines the robustness of the results, as Pang (2009) pointed out, laboratory cultured microorganisms tend to be removed more effectively than native ones.

As Table 3 shows, the removal rate in the first metre is higher than subsequently. This frequently observed slowing down of the removal rate with increasing infiltration distance has been attributed by Schijven *et al.* (2000) partly to the transition from an oxic to an anoxic environment below ground. Van der Wielen *et al.* (2008) have also postulated that an anoxic environment reduces the removal of

Table 3 | Bacteriophage concentrations in the Yamuna River and along the transect formed by the observation wells OW1–OW3

Monitoring point	Sampling month	Coliphages (PFU/100 mL)	Coliphages: geometric mean and geometric standard deviation (PFU/100 mL)	Distance from the river (m)	Removal between adjacent wells [$-\log_{10}$(C/Co)]	Travel time (days)[a]	Pore water velocity at well screen (m/d)[a]
Yamuna River	Dec-07	76,100	102,358 (±1,011)	–	–	–	–
	Jan-08	189,600					
	Feb-08	80,000					
	Mar-08	95,100					
OW1	Dec-07	24.4	47.1 (±1.8)	1	3.3	3.5	0.4
	Jan-08	112					
	Feb-08	28					
	Mar-08	64.4					
OW2	Dec-07	5.2	7.3 (±2)	2.4	0.52	6	0.5
	Jan-08	13.6					
	Feb-08	14					
	Mar-08	2.8					
OW3	Dec-07	0.5	1.2 (±2.2)	3.8	0.49	8	0.5
	Jan-08	0.6					
	Feb-08	2.4					
	Mar-08	2.8					

[a]Based on numerical modelling (Sprenger 2011).

viruses below ground. But in our work the transition from oxic to anoxic groundwater cannot be the reason for the non-linear removal, since the water of the Yamuna itself was already anoxic (Table 1; Lorenzen *et al*. 2010), and the oxygen concentration in the observation wells never exceeded 0.1 mg/L (Table 1). The higher initial removal might be explained by the presence of superficially located sediment layers in the river bed, which may be able to better remove the coliphages than the deeper ones, and also by heterogeneities in the viral population. As in other faecally polluted rivers (Grabow (2001), and literature therein), phages in the Yamuna presumably consist of a variety of different types that differ in size, surface structure, and aggregation, properties that have been shown (Redman *et al*. 2001; Chatterjee & Gupta 2009) to greatly influence the filtration properties of microorganisms. Obviously, the phages more prone to be adsorbed will remain in the upper layers of the soil, whereas the others will penetrate further down and be removed at slower rates.

The capacity of the soil to bind viruses has been reported to be in the order of more than 10^7 PFU/g soil for polioviruses (Yeager & O'Brien 1979a, b, c). In these studies it was additionally reported that polioviruses became inactivated by 90% in water-saturated soil during a period of up to 7 days. In the two series of coliphage measurements in the Yamuna reported here, their highest concentration was 1.89×10^5/100 mL, and the highest concentration for NoV 5.4×10^4 genome copies/100 mL. Therefore, provided that the infiltration distance is sufficient and the velocity of the water is low enough to allow adsorption to take place, there is little concern that the virus-binding capacity of the soil could be exceeded even when exposed to highly polluted waters.

One could point out that, as the association of the viruses to the soil particles is reversible (Schijven & Hassanizadeh 2000; Schijven & Šimunek 2002), viruses initially associated with the soil might dissociate later from the soil particles and appear in the end-filtrate. But our study suggests that this is very improbable. Since the faecal pollution of the Yamuna upstream of our field site has persisted already for years, or tens of years (CPCB 2006; CSE 2007), we assume that the input of viruses from the river into the soil, and their output at the location of our observation wells, reached a steady state a long time ago, and that the estimation of removal of viruses at these sites is a constant and realistic one.

From a statutory standpoint, RBF, if carried out in a soil and water matrix as present in our experimental site, would fulfil the USEPA surface water and groundwater rule, which prescribes that viruses be reduced by the water treatment processes by a factor of at least 10^4, whenever the water resource is surface water or groundwater under the influence of surface water (USEPA 2006). But at the filtration distance of 4 m, RBF would not comply with the recommendations of WHO (2006) for the removal of viruses from contaminated surface in low income countries. Larger distances would be necessary to achieve the removal of more than 10 $\log_{10}$ units recommended by WHO (2006) (in cases where the concentration in the raw water is the same as in the Yamuna).

In considering the adequacy of RBF for obtaining drinking water from very contaminated sources, it must not be forgotten that conventional precipitation-coagulation-fast-filtration, a technique which is very frequently used as a first step in processing river water to drinking water, seldom reaches virus removals higher than 2 $\log_{10}$ units (Berg 1973). Thanks to the high virus removal, RBF, as an alternative technique, would make a first chlorination step unnecessary and preclude the formation of organochlorides arising after chlorination of water carrying a high organic load. Besides, RBF will be significantly cheaper to implement than other technical procedures, a very important consideration in emerging countries, which frequently lack the resources for buying, running, and maintaining fast-filtration-coagulation facilities.

CONCLUSIONS

- The capacity of RBF for eliminating viruses from a highly contaminated stream, like the investigated Yamuna River in central Delhi, is very efficient, as less than 4 m filtration distance proved sufficient for reducing indigenous somatic phages by a factor of more than 10^4, and 50 m by a factor of more than 10^6. These removal degrees are of the same range as found under conditions of cleaner, controlled surface waters. Our results show that a severe organic and faecal load with associated anoxicity, like that seen in our experimental site, is no obstacle for a high performance of RBF.

- The validity of the levels of removal observed in this work is the more reassuring, as the infiltration studies were carried out with indigenous phages, circumventing possible biases inherent to the use of artificially spiked test organisms.

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