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Dynamics of pathogens and fecal indicators during riverbank filtration in times of high and low river levels

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ABSTRACT

Riverbank filtration is an established and quantitatively important approach to mine high-quality raw water for drinking water production. Bacterial fecal indicators are routinely used to monitor hygienic raw water quality, however, their applicability in viral contamination has been questioned repeatedly. Additionally, there are concerns that the increasing frequency and intensity of meteorological and hydrological events, i.e., heavy precipitation and droughts leading to high and low river levels, may impair riverbank filtration performance. In this study, we explored the removal of adenovirus compared with several commonly used bacterial and viral water quality indicators during different river levels. In a seasonal study, water from the Rhine River, a series of groundwater monitoring wells, and a production well were regularly collected and analyzed for adenovirus, coliphages, *E. coli, C. perfringens*, coliform bacteria, the total number of prokaryotic cells (TCC), and the number of virus-like particles (TVPC) using molecular and cultivation-based assays. Additionally, basic physico-chemical parameters, including temperature, pH, dissolved organic carbon, and nutrients, were measured. The highest $\log_{10}$ reduction during the >72 m of riverbank filtration from the river channel to the production well was observed for coliforms (>3.7 $\log_{10}$), followed by *E. coli* (>3.4 $\log_{10}$), somatic coliphages (>3.1 $\log_{10}$), *C. perfringens* (>2.5 $\log_{10}$), and *F*+ coliphages (>2.1 $\log_{10}$) at high river levels. Adenovirus decreased by 1.6–3.1 log units in the first monitoring well (>32 m) and was not detected in further distant wells. The highest removal efficiency of adenovirus and most other viral and bacterial fecal indicators was achieved during high river levels, which were characterized by increased numbers of pathogens and indicators. During low river levels, coliforms and *C. perfringens* were occasionally present in raw water at the production well. Adenovirus, quantified via droplet digital PCR, correlated with *E. coli*, somatic coliphages, TCC, TVPC, pH, and DOC at high river levels. At low river levels, adenoviruses correlated with coliforms, TVPC, pH, and water travel time. We conclude that although standard fecal indicators are insufficient for assessing hygienic raw water quality, a combination of *E. coli*, coliforms and somatic coliphages can assess riverbank filtration performance in adenovirus removal. Furthermore, effects of extreme hydrological events should be studied on an event-to-event basis at high spatial and temporal resolutions. Finally, there is an urgent need for a lower limit of detection for pathogenic viruses in natural waters. Preconcentration of viral particles from larger water volumes (>100 L) constitutes a promising strategy.

1. Introduction

Groundwater is the primary water source for drinking water supply in Europe and many regions worldwide (Völker and Borchardt, 2019). When groundwater is insufficient or of low quality, surface water is used. However, since the surface water quality is generally low, purification is required. Wherever possible, natural purification processes are used instead of technical treatment. Along large rivers and lakes,

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induced riverbank filtration is a common approach for drinking water production (Kuehn and Mueller, 2000). In this study, surface water is guided through riverbed sediments and the adjacent shallow aquifer to a production well. Although riverbank filtration appears efficient, providing adequate water conditioning most of the year, extreme hydrological events, which tend to become more frequent due to climate change, can pose a serious risk to the quality of bank-filtrated water and the safe operation of drinking water supply (Sprenger et al., 2011).

Drinking water supply from surface water via riverbank filtration is widely used not only in Europe but also in North and South America and Asia (Gillefalk et al., 2018). In Germany, approximately 16% of drinking water is produced from induced riverbank filtration (Gillefalk et al., 2018). At numerous sites, riverbank filtration has a long history of more than 150 years of operation (Schubert, 2002). With ongoing industrialization and population growth, there has been widespread pollution of surface water resources. Although the establishment of high standards in wastewater treatment has improved water quality over the past decades, pollutants such as pathogenic viruses may exhibit high resistance to current water treatment techniques, which poses a continuing concern in drinking water production (Fong and Lipp, 2005).

In riverbank filtration, the reduction in the concentration of pollutants is achieved by hydrodynamic (e.g., dilution, dispersion, and diffusion), mechanical (e.g., filtering, straining, and sedimentation), physicochemical (e.g., sorption, precipitation, and redox reaction), and biological (e.g., biodegradation) processes (Jaramillo, 2011). Numerous studies have focused on the efficiency of riverbank filtration in removing organic compounds (Hamann et al., 2016; Glorian et al., 2018) and organic matter (Derx et al., 2013; Romero-Esquivel et al., 2017), as well as the immobilization of metals and other inorganic compounds (Ibrahim et al., 2015; Sandhu et al., 2019). The hygienic aspects of water quality have always been of particular concern. Recent studies have investigated the reduction of pathogens, including protozoans (e.g., *Giardia lamblia* and *Cryptosporidium parvum*; Freitas et al., 2017), pathogenic bacteria (e.g., *Clostridium sp*. and *Pseudomonas aeruginosa*; Weiss et al., 2003; Nagy-Kovács et al., 2019), and viruses (e.g., adenovirus and norovirus; Betancourt et al., 2014; Sprenger et al., 2014).

Pathogenic viruses pose a particular risk to water quality because of their persistence in the aqueous environment, resistance to disinfection methods, small colloidal size, and low infectious doses (Fong and Lipp, 2005). To date, the efficiency of riverbank filtration in removing pathogenic viruses has mainly been studied using viral surrogates or fecal indicators such as bacteriophages, rather than following the fate of human pathogenic viruses, with only a few exceptions (Betancourt et al., 2014). Reasons for using surrogates are manifold, including difficulties associated with the direct detection of pathogenic viruses (McMinn et al., 2017). Commonly used fecal indicators, such as *E. coli* and coliforms, are reliable in assessing bacterial pathogen contamination. However, their application to assess and monitor viral contamination is limited (Ashbolt et al., 2001). Even the use of bacteriophages (e.g., coliphages) as indicators for assessing water contaminated with human pathogenic viruses lack predictive power (Jofre et al., 2016). Alternatively, adenoviruses, which cause several gastroenteric diseases, have been proposed as monitoring targets (WHO, 2006). They are shed into wastewater in significant amounts, along with the feces of infected patients (Bauer et al., 2011).

Riverbank filtration systems must be considered highly vulnerable to pathogen contamination under extreme hydrological conditions. Floods and low water situations are expected to occur more frequently in Europe in the future because of climate change (Sprenger et al., 2011; Eckert et al., 2008; IPCC, 2014). Hari et al. (2020) demonstrated that the occurrence of the 2018–2019 summer drought in central Europe is unprecedented in the last 250 years. It has been repeatedly recommended to evaluate the impact of extreme hydrological events on water quality and riverbank filtration efficiency, particularly the impact on the natural attenuation of bacterial and viral pathogens (Masse-Dufresne et al., 2021).

The primary objective of this study was to investigate the dynamics in attenuation efficiency of riverbank filtration for adenoviruses, viral indicators, and other fecal and microbial indicators with respect to the impact of extreme hydrological events. We targeted the predictability of hygienic contamination via individual fecal and/or viral indicators, and its practical use for monitoring riverbank filtration efficiency. Correlations between microbiological and physico-chemical variables with a focus on water quality parameters were assessed. For 16 months, water samples were collected from the banks of the Rhine River and from various groundwater monitoring wells along an observation transect from the river to the extraction well and beyond at the Flehe waterworks in Düsseldorf, Germany. Samples were analyzed for the presence of adenoviruses, coliphages, bacterial indicators (*Escherichia coli*, coliforms, and *Clostridium perfringens*), the total number of prokaryotic cells (TCC) and virus particles (TVPC), microbial activity (ATP), dissolved organic carbon (DOC), and key physico-chemical parameters, including temperature, electrical conductivity (EC), pH, dissolved oxygen (DO), major ions, and water stable isotope ratios ($^{2}H/^{1}H$ and $^{18}O/^{16}O$).

2. Material and methods

2.1. Study site

The Flehe waterworks in the city of Duesseldorf, Germany, is located on an outer bend of the Rhine River between kilometers 731.5 and 732.1 (Fig. 1A). Here, the Rhine River is 400 m wide with a flow velocity of 1–1.4 m s^{-1}, a hydraulic gradient of 0.2 m km^{-1}, and a median discharge of 2100 $m^3 s^{-1}$ (Sharma et al., 2012). Samples were collected between January 2018 and May 2019. During this period, the mean river level was 29.5 ± 1.6 m.a.s.l. (meters above sea level; Fig. 1C). Thus, we assumed river levels above 31.1 m.a.s.l. as "high" (flood), below 27.9 m.a.s.l. as "low" (drought), and river levels between 27.9 and 31.1 m.a.s.l. as medium river levels in our study. River water quality was monitored at a nearby station (kilometer 732.1) by the local water authorities.

The hydrogeology at the site is marked by a shallow quaternary aquifer consisting of heterogeneous sand-gravel mixtures (d_{50} = 0.5–9.4 mm) with an effective porosity of 20% and a range in k_f values from 2×10^{-2} to 4×10^{-3} m s^{-1} (Schubert, 2002). The aquifer is about 20 m thick and confined below by a tertiary silty fine sand aquitard. Numerous groundwater monitoring wells surround the riverbank filtration plant. Eleven wells have been selected for our study because of their locations with increasing distance to the Rhine River and the extension to various depths, which allowed following the fate of the bank filtrate from the river to one of the production wells (PW5). PW5 is part of a well gallery located parallel to the river, which forces river water through the riverbank and the shallow aquifer (Fig. 1B). The bank filtrate obtained from PW5 was a mixture of water from the well gallery. Between the river and PW5, two sets of monitoring wells (A and B) are situated at a horizontal distance of 40.2 and 20.4 m to the production well (Fig. 1C). According to Schubert (2002), the minimum travel distance from the Rhine River to well row A is 32.2 m with river levels above 26 m.a.s.l.

Another set of monitoring wells (C) and a single well (OO8) are located opposite PW5 and the river, where land side groundwater flows toward the production well (Fig. 1). Each well row, i.e., A, B, and C, comprises three individual wells (1–3) with 1 m filter screens at different depths to allow a depth-dependent sampling (Fig. 1C). The actual pumping rate during the period of our study varied between 177 and 540 $m^3 h^{-1}$ (excluding one extreme outlier value of 39 $m^3 h^{-1}$) with a mean of 322 $m^3 h^{-1}$, excluding a shut-down period from April 10 to May 23, 2018 (Figure S1).

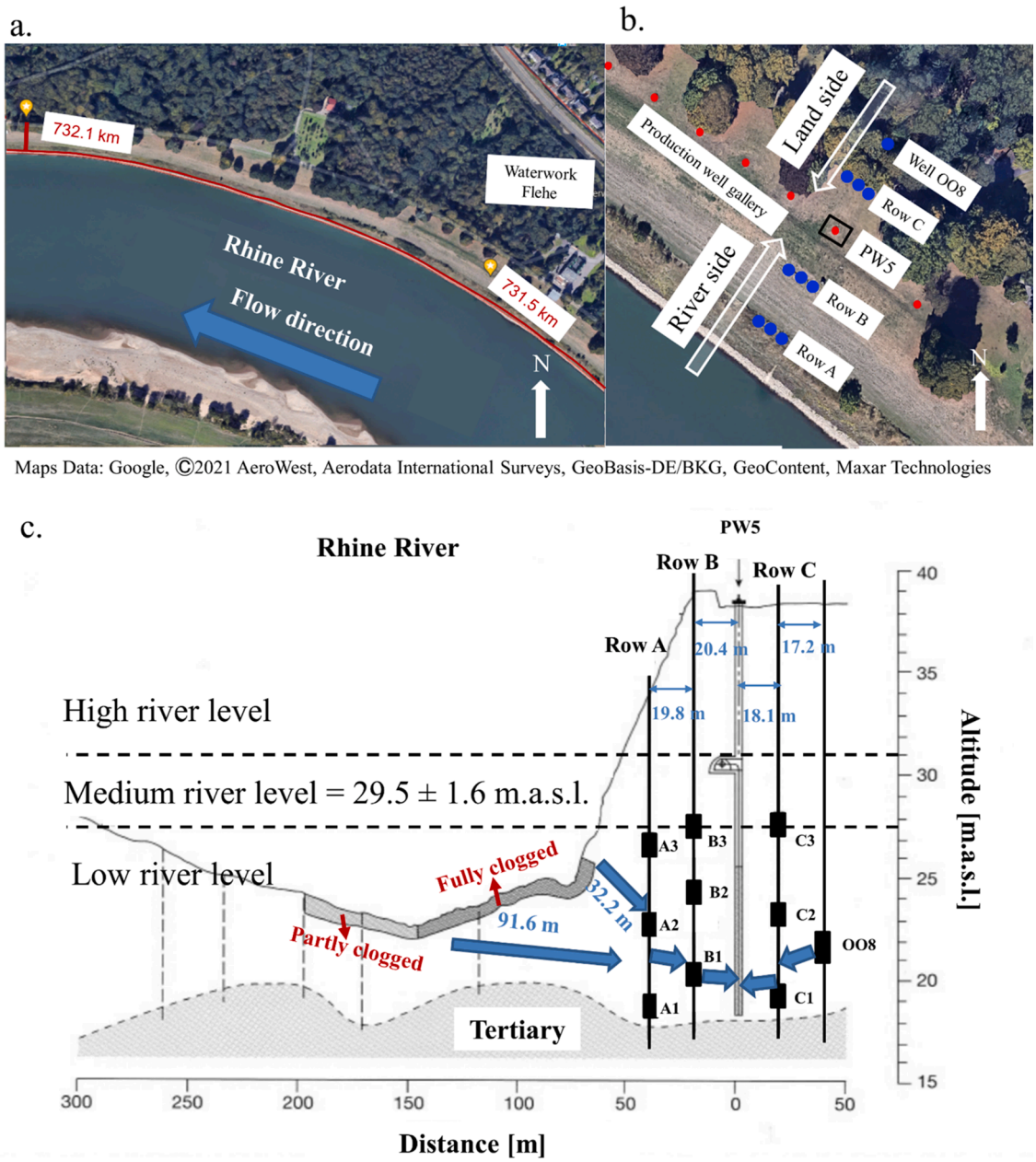

Fig. 1. (a) The Duesseldorf-Flehe waterworks; geographic location. (b) Position of sampling wells. (c) Position of the filter screens in the groundwater monitoring wells. River are categorized as high, medium, and low. Travel distances during infiltration have been calculated according to Schubert (2002).

2.2. Sampling and analysis

Water samples from the Rhine River and observation wells were collected at least once a month from January 2018 to May 2019; sampling started only in July 2018 for well PW5.

For molecular analysis and coliphage tests, 10 L water was collected from all sites in sterile high-density polyethylene carbons. Further water samples from all sites were collected in prebaked 500 mL glass bottles to analyze DOC, fecal indicators (*E. coli*, coliforms, and C. *perfringens*), and ATP. To quantify TCC and TVPC, water samples (15 mL) were fixed with glutaraldehyde (0.5%, v/v) and stored at 4 °C or put on dry ice and kept at −80 °C until further processing.

Key physico-chemical parameters such as pH, water temperature, DO, and EC were measured in situ with WTW field sensors. Groundwater levels were recorded with a level sensor, and river level information was obtained from the Federal Institute for Hydrology (https://www.bafg.de/DE/Home/homepage_node.html).

For most variables a single sample was collected and analyses in the lab were done in duplicates or triplicates (technical replicates). The coefficient of variation for the data obtained were within 10% if not mentioned otherwise.

2.2.1. Water isotopes, major ions, and DOC analysis

Water collected for stable water isotope analysis was filtered through 0.22 μm syringe filters (Merck Millipore) and then analyzed with a laser-based water stable isotope analyzer (Picarro L2130-i). A two–point calibration with laboratory reference material calibrated against VSMOW-SLAP (Vienna Standard Mean Ocean Water-Standard Light Antarctic Precipitation) was used. Each sample was measured up to nine times. Precision of the instrument (1σ) was better than 0.15 ‰ and 0.6 ‰ for $\delta^{18}O$ and $\delta^{2}H$, respectively. Results are in delta notation in ‰ relative to the Vienna Standard Mean Ocean Water.

Chemical analyses for major cations (K^{+}, Na^{+}, Ca^{2+}, and Mg^{2+}) were performed with a 7900 ICP-MS (Agilent) following DIN EN ISO

17,294–2 (2005), and major anions (Cl^-, Br^-, NO_3^-, and SO_4^{2-}) were analyzed in an ion chromatograph (ICS 1600, Dionex) following DIN EN ISO 10,304–1 (2009).

For DOC measurements, 8 mL water was filtered through a pre-rinsed 0.45 μm syringe filter (Merck Millipore), followed by acidification with HCl to pH ≤ 2. The non-purgeable organic carbon was analyzed in a TOC analyzer (Shimadzu TOC-5000A) with MQ water as blank.

2.2.2. TVPC, TCC, and ATP measurements

Water samples for TVPC were first filtered through a 0.22 μm syringe filter (Merck Millipore) and then fixed with 0.5% (v/v) glutaraldehyde and stored at −80 °C until further analysis following the protocol of Brussaard (2014). Later, samples were unfrozen and diluted appropriately with filtered (0.1 μm syringe filter) MQ water to achieve a particle concentration of approximately 10^6 virus-like particles per milliliter. Samples were then stained with 1 × SYBR Gold nucleic acid dye and incubated for 10 min at 80 °C in the dark prior to measurement.

For TCC analysis, river water and groundwater samples were fixed with 0.5% (v/v) glutaraldehyde and then stored at 4 °C for a maximum of 10 days before further processing. The samples were then diluted appropriately with filtered MQ water (0.1 μm syringe filter) to achieve a cell concentration of approximately 10^5 cells per milliliter. The samples were then stained with 10 × SYBR Green nucleic acid dye and incubated for 13 min at 37 °C in darkness prior to measurement (Bayer et al., 2016).

TCC and TVPC were quantified via flow cytometry (FC 500 CYTOMICS, Beckman Coutler) equipped with an air-cooled 488-nm Argon ion laser in biological and technical replicates. As an internal reference, fluorescent beads (Trucount) were added to each sample. Using the StemCXP Cytometer software (v2.2), the analysis and evaluation of the samples were performed. Filtered MQ (0.1 μm filter) was used as blank for these two analyses.

Intracellular ATP concentrations were derived from the measured concentrations of total and extracellular ATP in water samples. ATP analysis followed the protocol of Hammes et al. (2010) using the BacTiter-Glo Microbial Cell Viability Assay kit (Promega). Freshwater samples were analyzed within 3 days after storage at 4 °C in the dark. Additional samples collected between regular intervals were frozen for subsequent analysis. With these samples, intracellular ATP was calculated from measurements of total ATP only using the linear relationship between total ATP and extracellular ATP found in regular groundwater ($R^2 = 0.40$, $p << 0.05$) and river water ($R^2 = 0.25$, $p << 0.05$) samples. Autoclaved MQ water were used as blank for extracellular and total ATP analysis.

2.2.3. Biological indicators

The detection and enumeration of *E. coli* and coliforms were performed using the Colilert-18 method (IDEXX) according to ISO 9308–2 (1990). The detection and enumeration of *Clostridium perfringens* were performed using the membrane method according to ISO 14189 (2013). The detection limit for all indicator bacteria was 1 MPN or 1 CFU per 100 mL sample.

Analysis of somatic and *F+* coliphages from 10 L water samples followed the protocol of Binder (2013). The titer of infectious coliphages in water samples was determined using the double-agar overlay method (plaque assay; Kropinski et al., 2009). Briefly, a solid agar base (10 mL of 1.5% agar, w/v) was overlaid with a mixture of soft agar (4 mL of 0.75% agar, w/v) and 1 mL of a well-mixed solution of (diluted) sample and host bacteria (*E. coli Famp* for *F+* coliphages and *E. coli CN-13* for somatic coliphages) harvested in the logarithmic growth phase (1:1, v/v). Serial dilutions of samples were incubated at 37 °C overnight and quantified in duplicates. The detection limit for coliphages was 0.07 PFU per 100 mL.

2.2.4. Quantification of adenovirus

We quantified the free and particle-adsorbed fraction (containing viruses on and in organic and inorganic particles > 0.22 μm) of adenovirus particles in water samples. Adsorbed viruses in 10 L freshwater were first filtered onto 0.22 μm PES filters (Thermofisher). The filtrate containing the fraction of free virus particles was dedicated to a $FeCl_3$ addition (2 mg L^{-1}) to concentrate virus particles along with Fe precipitation (John et al., 2011). After ≥1 h of sedimentation, the precipitate was collected on 0.45 μm filters (Whatman). The adsorbed viruses (together with microbial biomass collected on 0.22 μm filters) and free viruses (Fe precipitate collected on 0.45 μm filters) were both frozen at −80 °C for later processing. For data analysis, virus abundance values from the free and adsorbed fraction were combined.

The AllPrep PowerViral DNA/RNA Kit (Qiagen) was used to extract adenovirus DNA. For the adsorbed adenovirus fraction, small pieces of the 0.22 μm PES filters were directly processed according to the instruction of the manufacturer. For the free adenovirus fraction, first, the iron precipitate was dissolved using an ascorbate buffer (John et al., 2011), and then, the resuspension was concentrated via Amicon® Ultra-15 Centrifugal Filter Units (Merck). The concentrate was then used for DNA extraction.

For quantitative estimation of adenovirus particles, a droplet digital PCR (ddPCR) was applied with the following details in accordance with the digital MIQE guidelines (Huggett, 2020). The detection was based on a 131-bp fragment using the primer sequences 5′-GCC-ACG-GTG-GGT-TTC-TAA-ACT-T-3′, 5′-GCC—CCA-GTG-GTC-TTA-CAT-GCA-CAT-C-3′, and a probe 5′-HEX-TGC-ACC-AGA-CCC-GGG-CTC-AGG-TAC-TCC-GA-BHQ1–3′ (Heim et al., 2003). Primers and the probe were obtained from ThermoFisher Scientific. The ddPCR was performed on the QX200 Droplet Digital PCR System (BioRad) using ddPCR Supermix for Probes (Bio-Rad Laboratories). Reactions were set up in a final volume of 20 μL. The reaction mixture consisted of ddPCR Supermix for Probes, 900 nM of each primer, 250 nM probes, 4 U HaeIII, (ThermoFisher Scientific), 1 μL template and nuclease-free water. DNA digestion was performed to improve ddPCR efficiency according to the manufactures recommendations for ddPCR Supermix (Bio–Rad Laboratories). The mixture was combined with 70 μL droplet generation oil and droplets were prepared using the Droplet Generator QX200 (Bio-Rad Laboratories). The resulting droplets were transferred to a 96-well plate and the PCR was then performed in a C1000 Touch Cycler at 95 °C for 10 min, followed by 40 cycles at 94 °C for 30 s and 60 °C for 1 min, and then, a final incubation at 98 °C for 10 min and a hold step at 4 °C until reading on the QX200 Droplet Reader (Bio-Rad Laboratories). No-template control and a positive control (human Adenovirus Type 2 strain DNA) were included in each ddPCR assay. QuantaSoft Analysis Pro (BioRad) were used to manually threshold and export the data. Analyses with a droplet number below 8000 were repeated with an additional tenfold dilution. For selected samples, up to four replicates were performed with an average coefficient of variation of 23%. All detections were performed in the same lab and with the same methods. Based on the initial sample volume, and the volume of DNA extract used in our study, a detection limit between 10.9 and 397 particles L^{-1}, was calculated; without taking into account losses during concentration and extraction.

2.3. Water travel time analysis

Mean travel times for conservative solute transport from the river to the observation wells, as influenced by the river level changes and the pumping rate, was estimated from the measured piezometric pressure heads of the observation wells. For this, we calculated the mean particle velocity in the groundwater on the basis of Darcy's law, taking the hydraulic gradient between observation wells A1 and B1:

$$v(t) = \frac{1}{n}\cdot\left(k\cdot -\frac{dh(t)}{dl}\right) = \frac{1}{n}\cdot\left(k\cdot\frac{h_{A1}(t) - h_{B1}(t)}{\Delta l_{A1/B1}}\right)$$

where $v(t)$ represents the mean particle velocity in the groundwater at

time t [m d^{-1}], n represents the effective porosity of the aquifer [–], k represents the hydraulic conductivity of the aquifer [m d^{-1}], $dh(t)/dl$ represents the hydraulic gradient at time t [–], $h_{A1}(t)$ and $h_{B1}(t)$ represent piezometric pressure heads at time t at the observation wells A1 and B1 [m], and $\Delta l_{A1/B1}$ represents the distance between wells A1 and B1 [m]. Following the modeling results of Sharma et al. (2012) for the same field site, we assumed $n = 0.2$ and $k = 1.59 \times 10^{-3}$ m s^{-1}.

Assuming flow is only in the direction perpendicular to the river, the mean travel time $T(s,t)$ from the river to an observation well at a distance s from the river and time t can be calculated using the following equations:

$$s = \int_{t-T(s,t)}^{t} v(t')dt' = V(t) - V(t - T(s,t)),\ V(t) = \int v(t)dt,$$

$v(t)$ is assumed to be stepwise constant based on the hydraulic gradients available at the sampling dates. Then, the integral $V(t)$ can be given with

$$V(t) = \left(\sum_{i=0}^{j-1}(v_i \cdot dt_i)\right) + v_j \cdot \left(\left(\sum_{i=0}^{j} dt_i\right) - t\right),\ t > \sum_{i=0}^{j-1} dt_i \text{ and } t \le \sum_{i=0}^{j} dt_i.$$

Then,

$$s = V(t) - V(t - T(s,t))$$

$$s = v_k \cdot \left(\left(\sum_{i=0}^{k+1} dt_i\right) - (t - T(s,t))\right) + \left(\sum_{i=k+1}^{j-1}(v_i \cdot dt_i)\right) + v_j \cdot \left(\left(\sum_{i=0}^{j} dt_i\right) - t\right)$$

$T(s,t)$ can be calculated by finding k∈[0,1,…, N], where N represents the highest timestep number so that these two equations are satisfied:

$$s > \left(\sum_{i=k+1}^{j-1}(v_i \cdot dt_i)\right) + v_j \cdot \left(\left(\sum_{i=k}^{j} dt_i\right) - t\right),$$

$$s < \left(\sum_{i=k}^{j-1}(v_i \cdot dt_i)\right) + v_j \cdot \left(\left(\sum_{i=k}^{j} dt_i\right) - t\right).$$

Then,

$$T(s,t) = \frac{s - \left(\sum_{i=k+1}^{j-1}(v_i \cdot dt_i)\right) + v_j \cdot \left(\left(\sum_{i=0}^{j} dt_i\right) - t\right)}{v_k} + t - \sum_{i=0}^{k+1} dt_i.$$

Note that $T(s,t)$ is only an estimate for the travel time since (i) the distance between the river and the observation wells slightly varies over time due to river level changes, (ii) flow will have a vertical component close to the river groundwater, (iii) our limited sampling frequency prevent us from fully capturing the dynamically changing the hydraulic gradient, (iv) and spatial heterogeneities in n and k are neglected.

The mean travel time T was calculated for observation wells at A (s = 40 m), B (s = 60 m), and PW5 (s = 80 m) with the river as a reference for the riverside groundwater flow. Using the hydraulic gradient between observation wells C and OO8, T is also calculated for observation wells C (s = 17 m from OO8) and PW5 (s = 35 m from OO8).

During the shut-off of PW5 in Spring 2018, the river level and the groundwater level at well OO8 indicate that natural effluent conditions have returned; thus, no travel time could be calculated. Excluding the switch-off of PW5, the attenuation efficiency of riverbank filtration for viral and bacterial indicators was evaluated for three hydrological situations, i.e., the high, medium, and low river levels. These hydrological events were categorized by river levels, as described in Section 2.1, whereas the water samples from the observation wells were categorized according to the hydrological event at the commencement of its underground passage considering the estimated mean travel time (Figure S2).

2.4. Statistical analysis

All statistics were implemented with software packages in the R environment (R Foundation; http://www.r-project.org/), with the significance level set as α = 0.05. Differences and similarities in abiotic and biotic variables within and between the individual well rows were tested through Kruskal–Wallis tests ("kruska.test") and ANOVA ("oneway.test"). Spearman rank correlation matrices (using "Hmisc" package) were used to explore relationships between biological (abundance of viral and bacterial indicators, ATP, TCC, and TVPC), and chemical (ion concentrations) and physico-chemical factors (pH, DO, EC, and temperature). The log reduction of biological indicators during riverbank filtration was calculated by comparing the composition of groundwater and river water at different hydrological events (Figure S2). Principal component analysis (PCA) was performed, considering the selected biological variables and physico-chemical parameters, using the "prcomp" function.

3. Results

3.1. Water level dynamics

The river level during the 16-month sampling period showed a typical seasonality with high levels in winter and low levels in summer (Fig. 2). The highest levels were monitored at the beginning of the field study with 33 meter above sea level (m.a.s.l). Lowest river levels (<27.3 m.a.s.l.) were recorded in late October 2018. The groundwater levels in the different observation wells were very similar but dampened dynamic, as those in the river (Fig. 2). The river level was always above the groundwater levels indicating infiltrating conditions, except for the shutdown period of the waterworks facility.

3.2. Bank filtration water travel times

Travel times of the infiltrated river water are strongly related to the river level (Fig. 3). Extended travel times occur when the river level is receding over a longer period, for example, during the regression of the high river level. Short travel times are caused by sudden river level increases, as observed in winter 2018/2019. When PW5 was shut-off for maintenance, the direction of water flow between the river and the production well could have reversed, resulting in groundwater discharge to the river and negative water flow velocities. When river levels are medium to low, which was the case for most of the year, groundwater from the land side (area opposite to the river with respect to the extraction well) flows toward PW5, passing well OO8 and row C (Fig. 3). During high river levels, river water infiltrates far beyond the production well, reversing the water flow direction at the land side.

3.3. Water stable isotope signatures

The $\delta^{18}O$ values, as well as $\delta^{2}H$ values (Figure S3) in river water, remained constant throughout the year, with only small variations (−9.5 ± 0.3) (Fig. 4a). Compared with surface water, groundwater-specific isotopic signatures at OO8 and row C showed higher values from March to November 2018 (Fig. 2). Samples taken at OO8 and row C during high water levels showed similar isotope ratios to river water. At low river levels, only water in rows A, B, and PW5 contained river water, as depicted from the isotope ratios, whereas wells opposite to the production well (C, OO8) contained land side groundwater.

3.4. Dynamics in physico-chemical conditions

The river water temperature showed a seasonality with low values down to 3.9 °C in winter and high temperatures up to 25.7 °C in summer

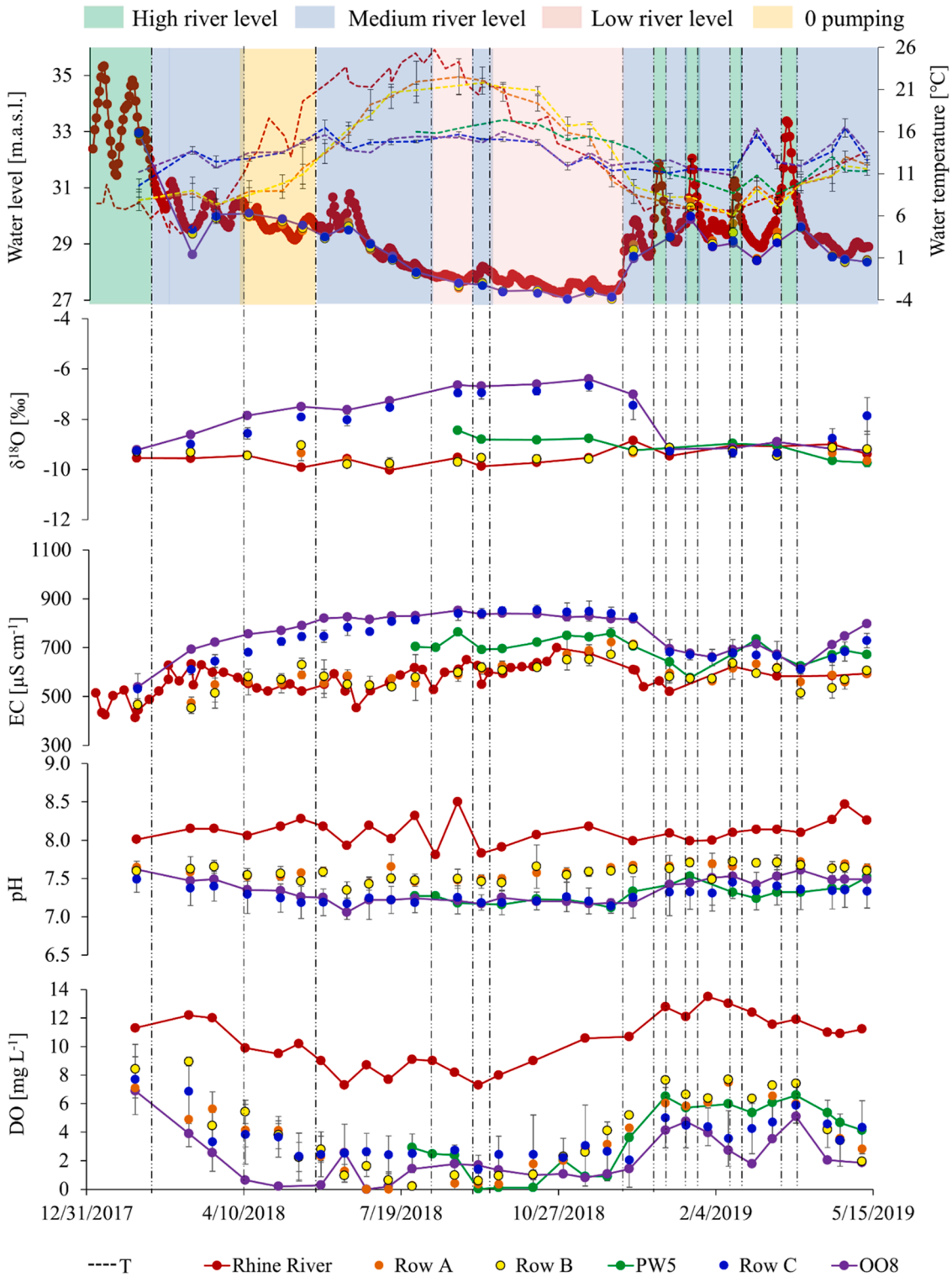

Fig. 2. Seasonal dynamics of water levels, $^{18}O/^{16}O$ water signature, and selected physico-chemical variables, as monitored in the Rhine River and at different observation wells. Error bars depict the variance of individual wells within one well row (e.g., A1, A2, and A3). Datapoints of the selected sites (river, PW5, and OO8) are connected with a line. T = river or groundwater temperature.

(Fig. 2). The mean river water temperature during the period of sampling was 14.2 °C ± 7.1 °C. The groundwater temperature at OO8 varied between 10.8 °C and 16.6 °C (13.7 °C ± 1.6 °C). At PW5, as recorded between July 2018 and May 2019, the water temperature ranged between 8.6 °C and 17.4 °C with a mean of 13.4 °C ± 3.0 °C.

EC in the river water showed no significant variation (Fig. 2) with a mean value of 566 ± 61 µS cm^{-1}. Groundwater in OO8 had its minimum of 539 µS cm^{-1} during high river levels and its highest values (up to 852 µS cm^{-1}) when disconnected from the river during medium and low water levels. The overall pattern of EC in water from the observation wells was consistent with stable water isotope analysis results. The EC pattern was also consistent with selected ions, exemplarily displayed for Ca^{2+} and SO_4^{2-} (Figure S4).

Generally, pH values did not show strong fluctuations during the

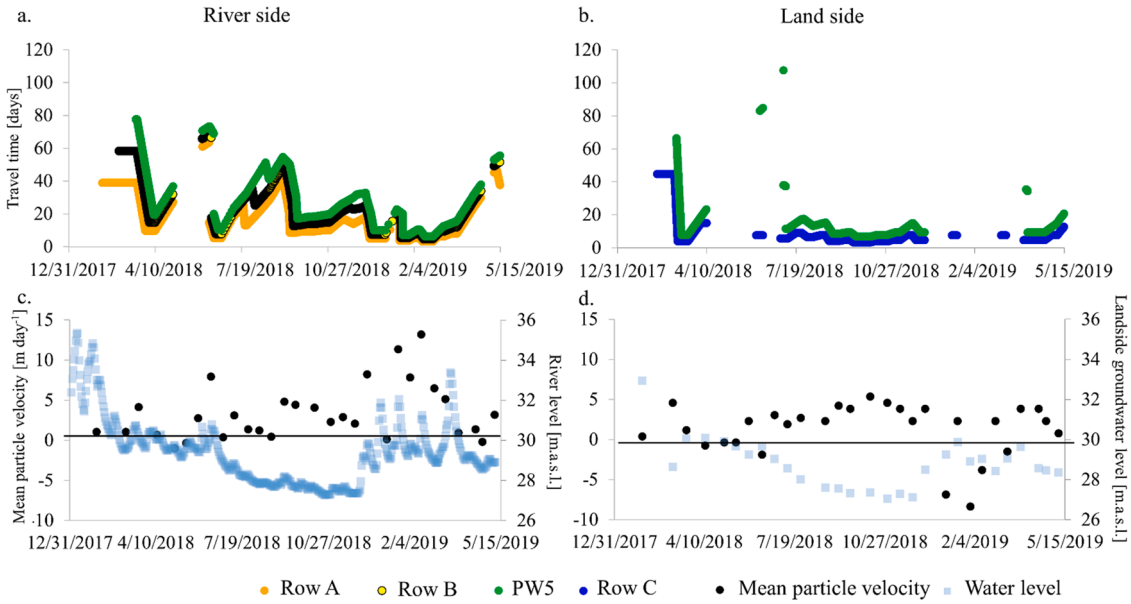

Fig. 3. Mean travel time from river and from land side (a and b), respectively, as well as mean particle velocity of bank filtrate at individual observation wells (c and d).

study, but a clear gradient between the river (8.1 ± 0.2) and water in OO8 (7.3 ± 0.2). The mean pH value at PW5 was similar to that at OO8 with 7.3 ± 0.1. The DO concentration in the water samples exhibited a clear seasonality decreasing with increasing water temperature (Fig. 2). Compared with the land side groundwater, high DO concentrations were observed in river water ranging from 7.3 to 13.5 mg L^{-1} with a mean of 10.4 ± 1.8 mg L^{-1}. The concentration of DO in groundwater at OO8 and PW5 ranged from below detection to 6.9 mg L^{-1}, with a mean of 2.2 ± 1.7 mg L^{-1} and 3.5 ± 2.3 mg L^{-1}, respectively. DO decreased as river water passed through the riverbank (Fig. 4f). The decline in DO was also mirrored to a lesser degree in a decrease in DOC (Figure S3). Further distant from the river, the DOC concentration remained constant. Noticeably, the DO concentration in the riverbank filtrate dropped to zero several times during summer. However, in connection to the local disappearance of DO, no obvious change in nitrate concentrations was observed (Figure S3).

3.5. Dynamics of bacterial indicators

The total number of TCC in the river ranged from 3.0×10^8 to 2.8×10^9 cells L^{-1} positively correlated with the water temperature (Fig. 5). As water flows through the riverbank, TCC readily declined 16-fold on average between the river and wells in row A. Although the annual mean values of TCC in water from the different observation wells were in the same order of magnitude, there was a significant difference between different locations along the transect to the production well, PW5 (*Table S1*). The lowest TCC values were observed in groundwater from row C. A similar pattern was found with microbial activity (ATP) (Fig. 6a). The highest ATP values were found in the river water, on average $6.4 \times 10^{-10} \pm 5.2 \times 10^{-10}$ M. ATP decreased not only with distance to the river but also with distance to well OO8; hence, the lowest ATP was found in water in PW5 ($2.4 \times 10^{-12} \pm 8.1 \times 10^{-12}$ M).

The fecal indicators, *E. coli* and coliforms, were frequently detected in river water, but only occasionally in water from the observation wells (Fig. 5). There was no obvious trend in the dynamics of *E. coli* or coliforms in the river, depending on the season. The mean *E. coli* concentration in river water was 803 MPN/100 mL with a large variance (±1148 MPN/100 mL). Only 12 of 283 water samples from the observation wells were positive for *E. coli*, and *E. coli* was never found in any of the land side groundwater samples (Fig. 5). The concentration of coliforms in the river was approximately 5 times higher than *E. coli*, with an average of 4884 ± 6027 MPN/100 mL. Coliforms were observed more frequently in water from the observation wells (in 83 of 283 samples). Considering hydrological situations and travel time, coliforms were detected in water in PW5 at low river level (Figure S5).

The occurrence of *C. perfringens* in river water did not show a seasonal trend, similar to other bacterial indicators. Compared with *E. coli* and coliforms, the average concentration of *C. perfringens* was lower, with 197 ± 163 CFU/100 mL. *C. perfringens* was also detected in the monitoring wells of row A, even when the river was at medium levels. In total, 38 of 268 water samples from the observation wells were *C. perfringens* positive.

3.6. Viral indicators

A mean of $3.2 \times 10^{10} \pm 1.2 \times 10^{10}$ virus particles L^{-1} was detected in the river, which was on average is 23 times higher than the TCC of the same samples and 38 times higher than TVPC in row A. The lowest TVPC was found in water samples from row C ($5.7 \times 10^8 \pm 3.7 \times 10^8$ virus particles L^{-1}).

Human enteric adenoviruses were found in the Rhine River during the entire sampling period. The mean concentration of adenovirus in surface water was $1.2 \times 10^4 \pm 3.0 \times 10^4$ particles L^{-1}, whereas the virus concentration in well A2 was already two to three orders of magnitude lower with $5.8 \times 10^1 \pm 3.6 \times 10^1$ particles L^{-1}. No adenovirus was above the limit of detection (LOD) in the selected samples of well B1.

The occurrence of *F*+ coliphages and somatic coliphages in the river differed. *F*+ coliphage concentration was the highest in January 2018 with 12 PFU/100 mL overall with a mean concentration of 2.6 ± 3.8 PFU/100 mL. Effective attenuation of *F*+ coliphages during riverbank filtration was observed between the river and rows A and B. Only a few samples were detected as positive in wells A2 and B1 in spring 2018 (Fig. 7). No *F*+ coliphages were found in groundwater from other wells. Only 5 of 74 water samples from the observation wells contained active *F*+ coliphages above the LOD. The highest concentration of somatic coliphages detected in the river was 116 PFU/100 mL in February 2019, and other samples exceeding a concentration of 40 PFU/100 mL were detected only in January and December 2018. The mean concentration of active somatic coliphages in river water was 24.1 ± 28.0 PFU/100

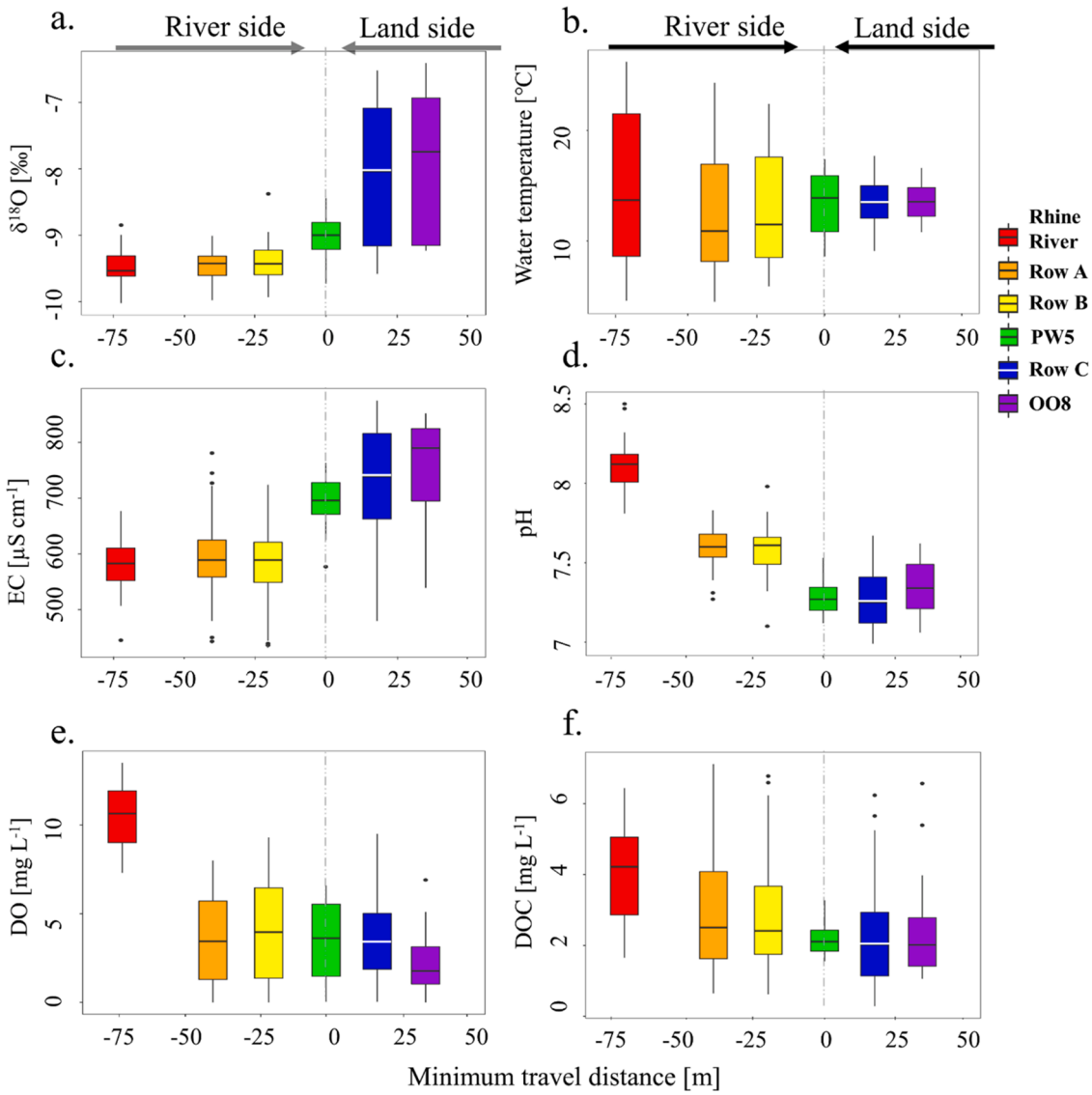

Fig. 4. Gradients of selected physico-chemical variables monitored in water from the Rhine River and from selected groundwater observation wells.

mL, 9-fold higher than *F*+ coliphages. Only 7 of 74 water samples from the observation wells were positive for somatic coliphages. However, somatic coliphages were occasionally detected in groundwater at C1 in spring 2018.

3.7. River water, bank filtrate, and groundwater in comparison

Spearman rank correlation analysis revealed that all biological variables (including adenovirus) recorded for the riverbank filtration transect are significantly correlated (Figure S6). Noticeably, all biological indicators positively correlated with pH and negatively correlated with the travel time. Furthermore, all biological indicators negatively correlated with $\delta^{18}O$ and EC, except adenovirus. The DO concentration positively correlates with viral indicators, *E. coli* and *C. perfringens*, rather than coliforms and TCC. The river and groundwater level co-varied with three bacterial indicators, i.e., *E. coli*, coliforms, and *C. perfringens*. A covariation was also discovered between TCC, TVPC, coliforms, and temperature.

The correlation analysis exhibited different patterns when data from surface water and observation wells were analyzed separately. For the river samples, fewer correlations were obtained although all biological indicators were detected all year round. For bacterial indicators, the concentration of coliforms correlated with both *C. perfringens* and *E. coli*. A positive correlation occurred between the viral indicators *F*+ coliphages and somatic coliphages, as well as for TVPC and *F*+ coliphages. However, none of the biological variables correlated with the abundance of adenovirus. The relationships between the biological indicators in the riverbank filtrate and groundwater were different. *C. perfringens* correlated with most of the biological indicators, including *F*+ coliphages, coliforms, *E. coli*, TCC, TVPC, and ATP. Additionally, the number of coliforms correlated with *E. coli* and TCC. The two groups of coliphages did not correlate with each other; nonetheless, somatic coliphages correlated positively with ATP, whereas *F*+ coliphages correlated with *E. coli* and *C. perfringens*. Once more, no correlation between adenovirus and other biological indicators was found.

Taking a specific look at the correlations between adenovirus and individual physico-chemical parameters at different hydrological situations, pH was the only variable correlating independent of the hydrological condition. The abundance of adenovirus during bank filtration correlated positively with pH. Elevated DOC concentrations found at high river levels correlated positively with an increased abundance of adenovirus. At medium river levels several factors (i.e., travel time, temperature, pH, DO) were found to correlate with adenovirus. At low river levels it was only pH and travel time that revealed a statistical

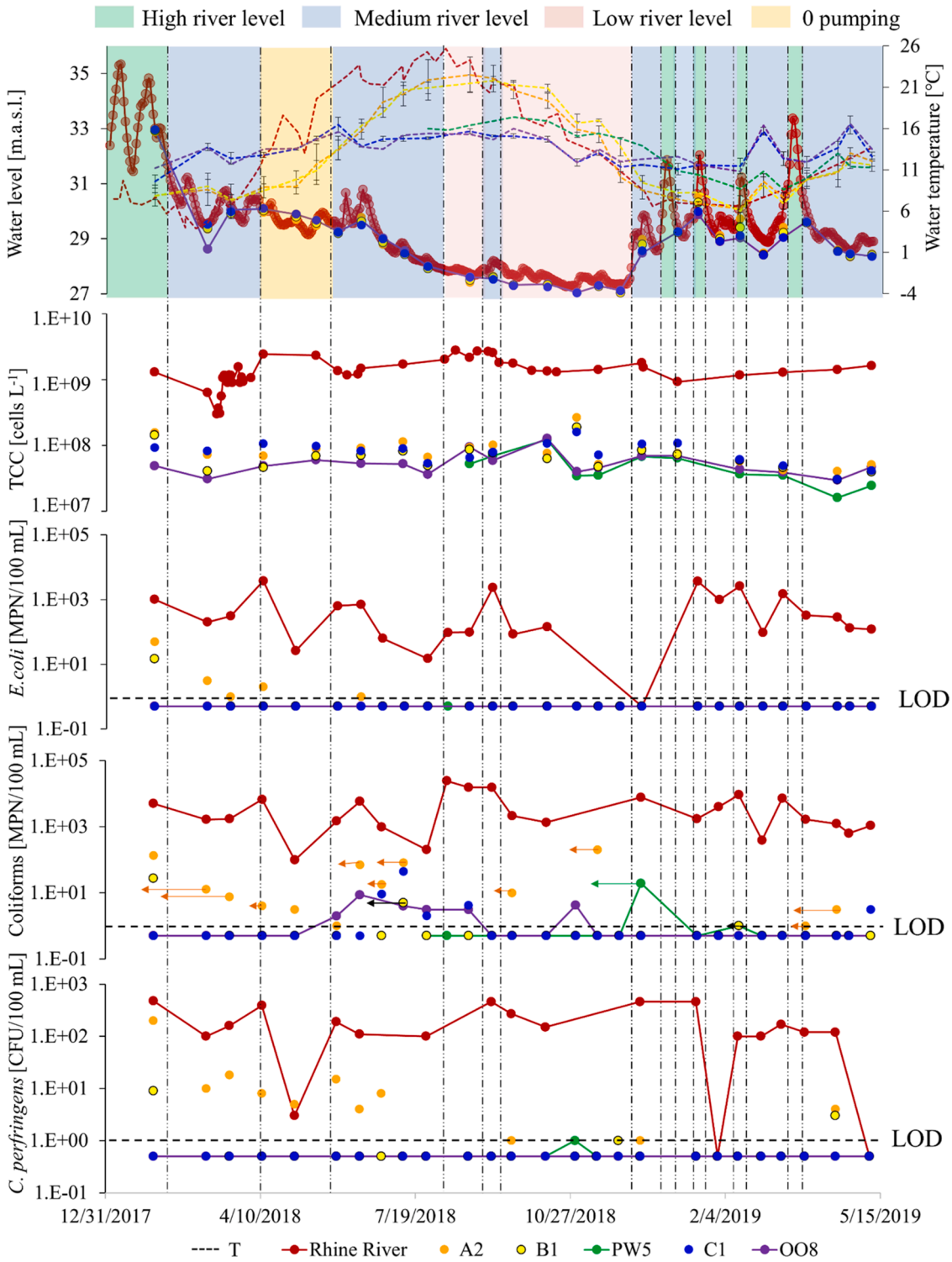

Fig. 5. Seasonal dynamics of selected bacterial indicators, as monitored in the Rhine River and water from the different groundwater observation wells. T = water temperature; LOD = limit of detection. The arrows with the coliform data exemplarily show the affiliation to specific hydrological situations when accounting for the time delay caused by the travel time to wells A2, B1, and PW5. Data from selected sampling sites (river, PW5, and OO8) are connected with a line.

significant correlation to adenovirus. With respect to other biological indicators, adenoviruses correlated with *E. coli*, somatic coliphages, TCC, and TVPC during high river levels, with coliforms, somatic coliphages, ATP at medium river levels, and with coliforms, TVPC, and ATP at low river levels, respectively.

Principal component analyses (PCA) (Fig. 8) depict the spatial and temporal differences within water samples collected from the river and observation wells with respect to the selected physico-chemical and biological variables. The difference between river water and water from the observation wells was explained by principal components with 47.1% of the total variance and PC2 explained another 17.4% (Fig. 8a). Although river water had significantly higher concentrations of coliforms, *E. coli, C. perfringens*, ATP, TCC, TVPC, and DOC, the riverbank filtrate and groundwater samples were closely clustered in the PCA plot

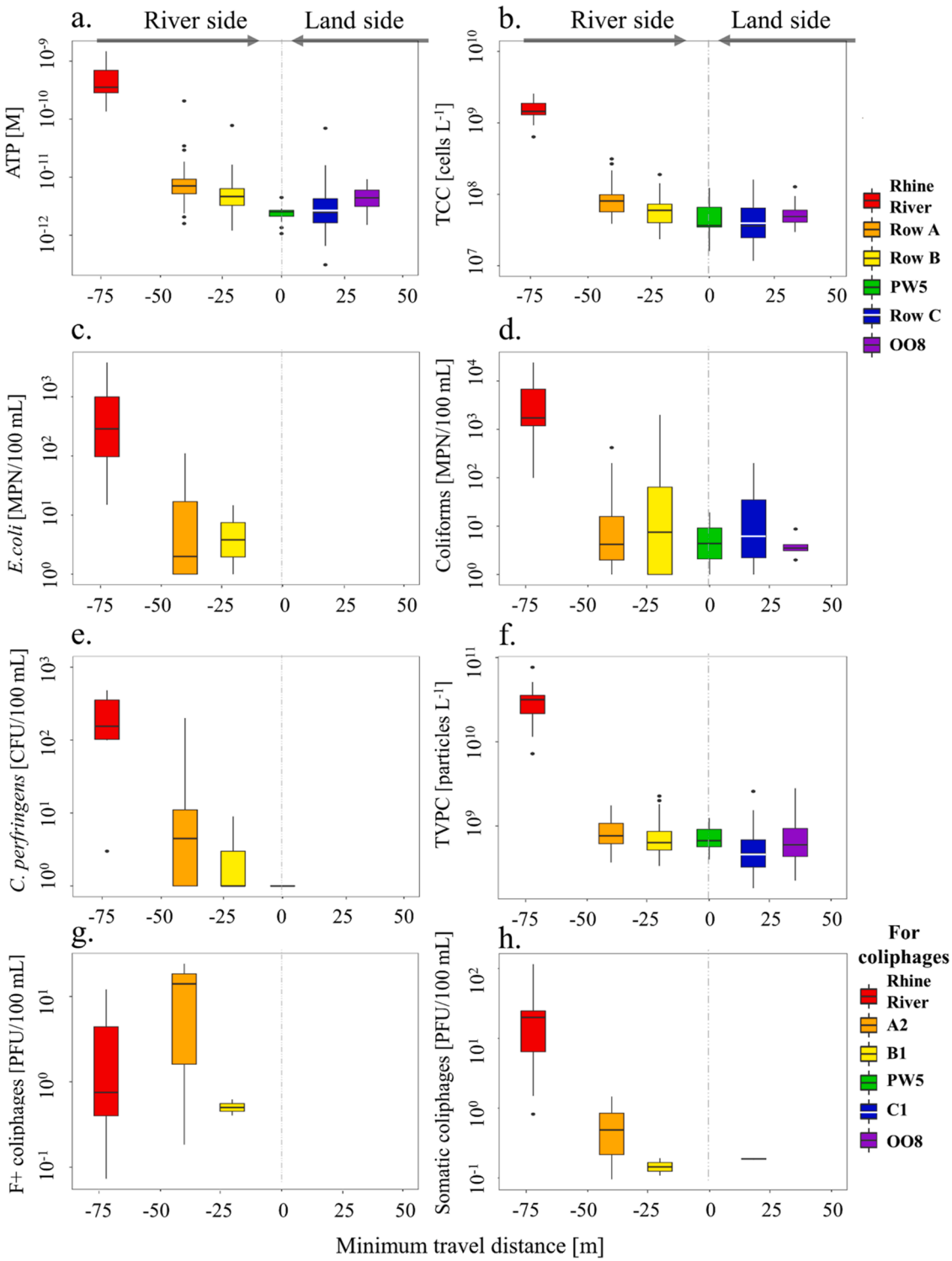

Fig. 6. Gradients of selected bacterial and viral indicators monitored in water from the Rhine River and from selected groundwater observation wells.

(Fig. 8a). Excluding river samples from the analysis, the trend in water samples could be explained by PC1 with 29.5% and PC2 with 19.4% (Fig. 8b). PC1 is mainly represented by the distance from the river, whereas seasonality (represented by temperature and DO) is the dominant factor for the variance in PC2.

3.8. *Efficiency of riverbank filtration in removing pathogens and fecal indicators*

We observed that in surface water concentrations of several bacterial and viral indicators, i.e., *E. coli, C. perfringens*, adenovirus, *F+* coliphages, and somatic coliphages, were higher during high river levels (flood situation) than during low and medium river levels (Fig. 9).

The absolute removal efficiency during riverbank filtration, in terms of $\log_{10}$ reduction, was higher during high river levels than in other

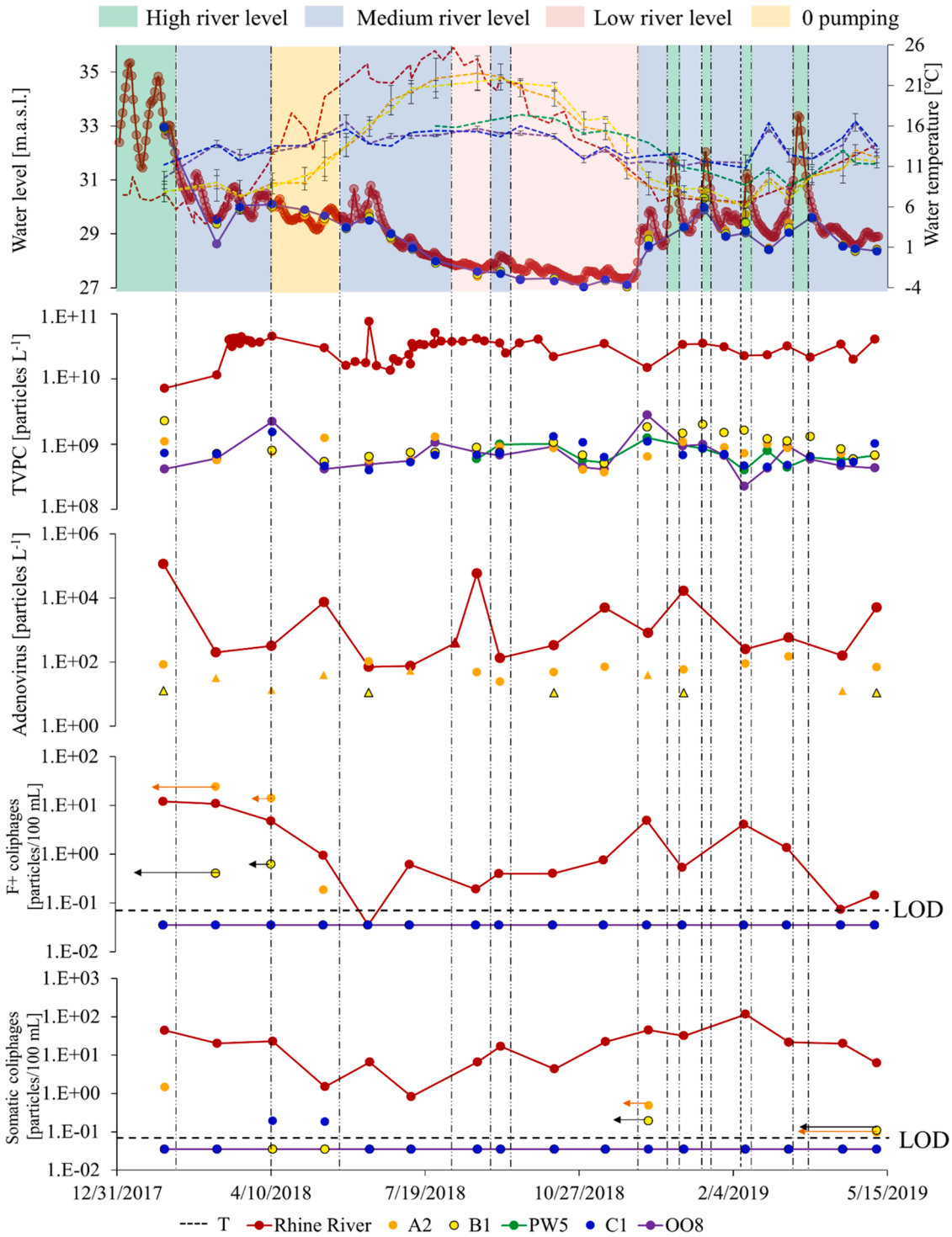

Fig. 7. Seasonal dynamics of selected viral indicators, as monitored in the Rhine River and water from different groundwater observation wells. T = water temperature; 'LOD' and triangles with adenovirus data exemplary show the limit of detection. The arrows with F^+ and somatic coliphages data exemplarily show the affiliation to specific hydrological situations when accounting for time delay caused by the travel time to wells A2, B1, and PW5. Data from selected sampling sites (river, PW5, and OO8) are connected with a line.

hydrological phases for most biological indicators (Fig. 9). However, most of the biological indicators could not be detected in the production well PW5, regardless of the hydrological conditions. The highest $\log_{10}$ reduction during passage through the riverbank was observed for coliforms (>3.7 $\log_{10}$), followed by *E. coli* (>3.4 $\log_{10}$), somatic coliphages (>3.1 $\log_{10}$), *C. perfringens* (>2.5 $\log_{10}$), and *F*+ coliphages (>2.1 $\log_{10}$) at high river levels. The concentration of adenovirus decreased by 3.1 log units between the river and observation well row A.

It is obvious from our data that the most pronounced removal of viruses and bacteria takes place in the early sediment passage from the river channel to well row A (Table 1). For this first stretch of sediment passage, low river levels had a beneficial effect on the removal of *C. perfringens*, TVPC, and *F*+ coliphages, with a reduction of >2.3 $\log_{10}$, 1.8 $\log_{10}$, and >0.8 $\log_{10}$, respectively. TCC, *E. coli*, and somatic

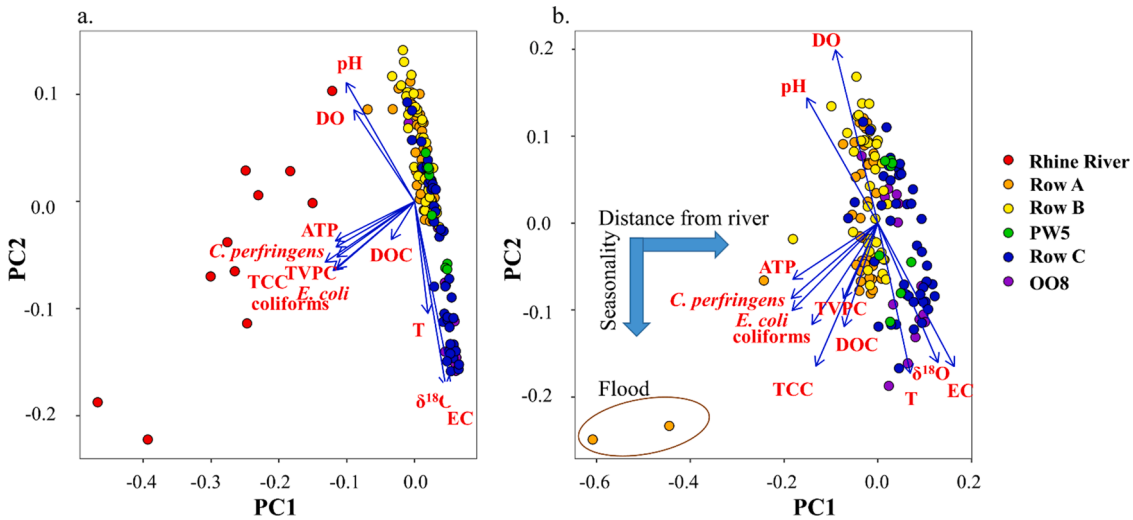

Fig. 8. PCA of all water samples and sampling sites (a) and of samples from the observation wells only (b).

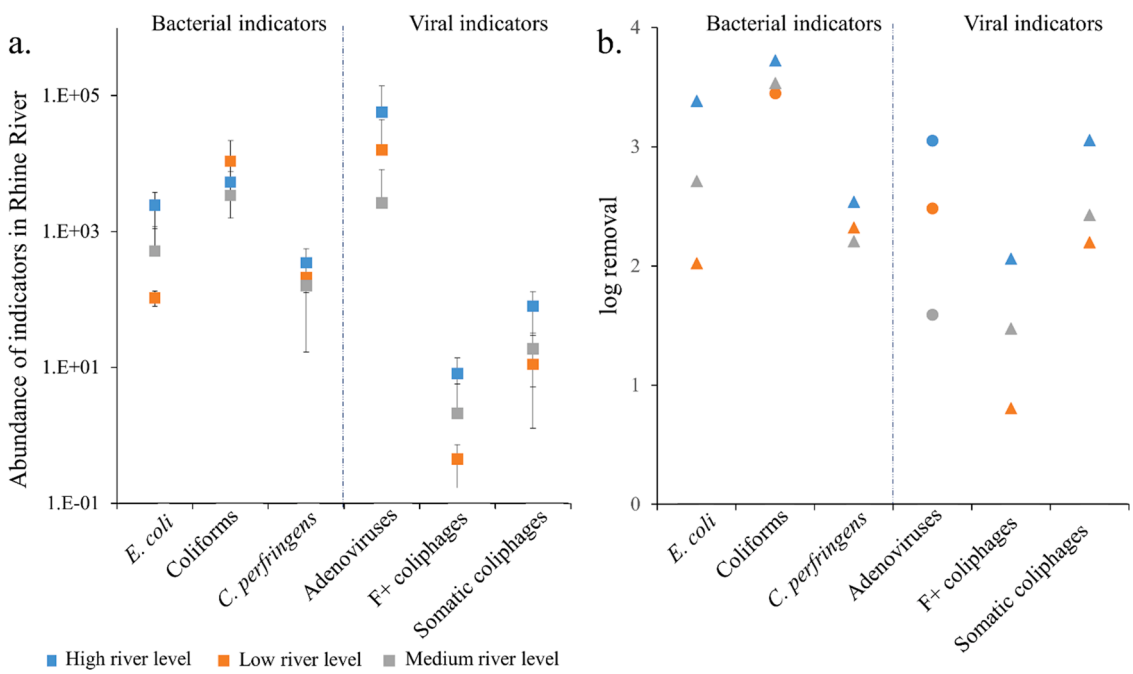

Fig. 9. Abundance of bacterial and viral indicators in the Rhine River (a), and its removal efficiencies by riverbank filtration (b). The abundances of *E. coli*, coliforms, *C. perfringens*, adenovirus, F^+, and somatic coliphages are in MPN/100 mL, MPN/100 mL, CFU/100 mL, particles L^{-1}, PFU/100 mL, and PFU/100 mL, respectively. Triangles in chart (b) refer to the minimum log removal. Calculation of the log removal in adenovirus was limited to the reduction between the river and well A2.

coliphages were reduced most efficiently at medium water levels by 1.3 $\log_{10}$, >2.7 $\log_{10}$, >2.4 $\log_{10}$. Adenovirus was the only agent that exhibited the highest removal efficiency (3.1 $\log_{10}$) between the river to well row A at high river levels (Table 1).

4. Discussions

4.1. Attenuation of adenovirus and other viral and bacterial indicators during riverbank filtration

In our study, we investigated the fate of adenovirus compared with several viral and bacterial fecal indicators during riverbank filtration across a transect of >72 m from the Rhine River to the production well of a waterwork facility (Fig. 1). Using ddPCR, we detected adenovirus all year round in Rhine River water at concentrations of 7.1×10^1 to 1.2×10^5 particles L^{-1}. Although adenovirus could occasionally still be detected in the riverbank filtrate water after >32 m of sediment passage at A2 (Fig. 7), all samples analyzed from the observation well B1 (>52 m travel distance from the river) were adenovirus negative, given the LOD of approximately 10 particles L^{-1}. Consequently, we assume the risk of adenovirus contamination at concentrations >1 virus particle L^{-1} in water at the production well (PW5) to be very low; however, an occasional occurrence at lower concentrations cannot be excluded. In agreement with this observation, none of the other tested viral indicators, such as coliphages, were detected in water from the production well and individual bacterial fecal indicators, such as *C. perfringens* and coliforms, were detected only rarely.

Besides the risk assessment of adenovirus contamination in raw water at the waterworks in Duesseldorf, Germany, a major focus of our study was on the suitability of individual viral and bacterial indicators.

Table 1
Mean log removal of selected bacterial and viral indicators with riverbank filtration during different hydrological conditions (n/a: the value is not available).

	Location	High river level	Low river level	Medium river level
TCC	RR-A2	1.2	1.1	1.3
	A2-B1	0.08	0.1	0.09
	B1-PW5	0.3	0.2	0.1
E. coli	RR-A2	2.6	>2.0	>2.7
	A2-B1	0.5	n/a	n/a
	B1-PW5	>0.3	n/a	n/a
Coliforms	RR-A2	2.4	2.4	2.4
	A2-B1	0.7	>1.6	>1.2
	B1-PW5	>0.6	n/a	n/a
C. perfringens	RR-A2	1.0	>2.3	1.9
	A2-B1	1.2	n/a	>0.3
	B1-PW5	>0.3	n/a	n/a
TVPC	RR-A2	1.4	1.8	1.6
	A2-B1	n/a	n/a	n/a
	B1-PW5	0.2	0.08	0.2
Adenovirus	RR-A2	3.1	2.5	1.6
	A2-B1	>0.7	>0.7	>0.8
F+ coliphages	RR-A2	0.2	>0.8	0.2
	A2-B1	1.8	n/a	1.4
	B1-PW5	>0.06	n/a	>0.1
Somatic coliphages	RR-A2	2.4	1.8	>2.4
	A2-B1	>0.7	>0.4	n/a
	B1-PW5	n/a	n/a	n/a

Expectedly, viral and bacterial indicators when transported through aquatic sediments differ in terms of attenuation including processes such as straining, adsorption, persistence, and decay (Schijven and Hassanizadeh, 2000). Although straining is considered one of the most important processes for attenuating bacteria, it has been shown that it mainly occurs when the ratio of the particles of concern (viruses 0.02–0.08 μm; bacteria 0.2–2 μm) and the medium grain size (this study 0.5 to 10 mm) is >0.5% (Bradford et al., 2004). As such, straining is considered to be negligible for our setting. Previous studies revealed that total adenovirus particles had the highest persistence (decay rate of 0.008–0.027 $\log_{10}$ day^{-1}), followed by other selected indicators, i.e., *C. perfringens* (inactivation rate of 0.027 $\log_{10}$ day^{-1}), infectious coliphages (inactivation rate of 0.03–1.0 $\log_{10}$ day^{-1}), *E. coli* and coliforms (inactivation rate of 0.02–1.5 $\log_{10}$ day^{-1}), when incubated at 15 °C in groundwater or autoclaved surface water (Medema et al., 1997; John and Rose, 2005; Ogorzaly et al., 2010). Moreover, the persistence of selected indicators decreases to varying degrees at elevated temperatures, the presence of active autochthonous microbes, and unsaturated conditions (Feichtmayer et al., 2017; Schijven and Hassanizadeh, 2000). The adsorption of viruses and bacteria is particle-specific (i.e., surface charge and hydrophobicity), mineral surfaces-specific (i.e., surface charge heterogeneity) and depends on water chemistry (i.e., pH, ionic strength, cations, and organic matter) as summarized in the DLVO (Derjaguin–Landau–Verwey–Overbeek) theory (Schijven and Hassanizadeh, 2000). Therefore, even among viruses of a similar size, different transport and survival behavior have to be expected and documented (A. Bosch et al., 2006). Nevertheless, numerous studies claim that individual fecal indicators, such as *E. coli*, can adequately account for bacterial and viral contamination (Donn et al., 2020; Love et al., 2014). Meanwhile, at first glance, the different indicators tested in our study provided similar information. However, at a closer look, numerous differences are found. From the perspective of removal efficiency during the sediment passage from the river to the production well, the routinely used indicators coliforms and *E. coli* experienced a stronger reduction, i.e., 3.6 $\log_{10}$, and >2.9 $\log_{10}$, respectively, than viral indicators; i.e., somatic coliphages (>2.5 $\log_{10}$), *F*+ coliphages (>1.6 $\log_{10}$), and total viral particles (TVPC, 1.6 $\log_{10}$). *Clostridium perfringens* performed worse than somatic coliphages (>2.3 $\log_{10}$), and the total number of TCC revealed the least reduction (1.4 $\log_{10}$) of all indicators tested. The main reduction in viral and bacterial indicators generally takes place in the early sediment passage (Schijven and Hassanizadeh, 2000). This is consistent with our findings. Focusing only on the transect between the Rhine River and row A and ignoring various hydrological situations, the mean reduction of adenovirus was 2.3 log units, which is comparable with the removal of *E. coli* (2.6 $\log_{10}$), coliforms (2.4 $\log_{10}$), and somatic coliphages (2.3 $\log_{10}$), and higher than that of other selected microorganisms. A higher spatial resolution would have been advantageous to explore the attenuation pattern, i.e., exponential vs. linear reduction, in more detail.

A large range in the reduction of adenovirus from approximately 1 to 5 log units is reported for riverbank filtration of comparable distances (31–92 m) from previous studies (Betancourt et al., 2014; Verbyla et al., 2016; Sprenger et al., 2014). Removal efficiencies are very site-specific (Partinoudi and Collins 2007; Rohns et al., 2006; Weiss et al., 2003, 2005). Variations in reduction are mainly caused by the different adenovirus source concentrations, sediment properties, water residence times, and the LOD of quantification methods used. The same is true for other indicators. A reduction of coliphages by >1.9 to >4.4 log units after a sediment infiltration distance of 27–177 m has been documented (Verbyla et al., 2016; Weiss et al., 2003, 2005; Rohns et al., 2006). Note that an approximate 5-$\log_{10}$ reduction of somatic coliphages after only 3.8 m of riverbank passage was reported by Sprenger et al. (2014) for site water infiltration is not forced by pumping, implying the water residence time to be a key factor (Sprenger et al., 2011). The removal efficiency of bacterial fecal indicators (i.e., coliforms, *E. coli*, and *C. perfringens*) during riverbank filtration has been investigated in several studies. A reduction of 2–4 log units has frequently been observed for sediment travel distances of 12–55 m and source concentrations $\leq 10^4$ PFU/100 mL (Partinoudi and Collins, 2007; Ren et al., 2019; Weiss et al., 2003). Although studies that directly compared the removal efficiency for viral and bacterial pathogens and/or indicators are rare, bacterial indicators showed a higher reduction level than viral indicators same as found in our study (Weiss et al., 2003, 2005).

Besides specific bacterial and viral indicators, we monitored the behavior of TVPC and TCC during riverbank filtration, which are variables that have rarely been considered in previous studies, especially for the underground passage at field scale. Compared with adenovirus and other indicators, TVPC and TCC exhibited the lowest log removal during the passage of river water to the production well. Similar to the other indicators, most of the removal occurred in the sediment passage between river and well row A. A low removal of TVPC and TCC, comparable with that in the present study, ranging between 1 and 1.7 $\log_{10}$ units after 1.1 m of vertical infiltration and 70 m of horizontal infiltration through aquatic sediments were observed by Mindl et al. (2015) and Fillinger et al. (2020). Contrary to other more specific indicators, TVPC and TCC may contain offspring from microbes and viruses, which can replicate in the bank sediment. Thus, they may not be suitable indicators for monitoring attenuation efficiency for pathogens during riverbank filtration.

WHO (2017) regarded 10^{-5} pathogenic viruses L^{-1} as a safe threshold concentration for drinking water, which is six orders of magnitude below our LOD for adenovirus (<10 particles L^{-1}). There is no routine technique to concentrate viruses from water by greater than or equal to six to seven orders of magnitude (Pei et al., 2012; Seidel et al., 2016). Hence, this target quality of riverbank filtration can be evaluated considering the viral load in river water and predicted reduction during riverbank filtration. The removal of adenovirus and the viral indicators tested meet the minimum removal for viruses of >2.1 $\log_{10}$ expected in riverbank filtration, as stated by WHO (2017). Additionally, the reduction of most other targeted bacterial fecal indicators exceeded the minimum removal of 2 $\log_{10}$ for bacteria with the sediment passage from the river to the production well.

4.2. Correlations between biological indicators and physico-chemical variables

As human pathogenic viruses cannot propagate in an aquatic environment, the abundance of adenovirus in river likely correlates with the fraction of wastewater from the runoff of sewage treatment plants, which may be increased during and after heavy rain events (Passerat et al., 2011) and low river level periods (Karakurt et al., 2019). Concretely, our study revealed increased numbers of coliphages in river water at high levels when compared with other hydrological conditions. Note that there was a strong negative correlation between the number of cultivable coliphages and river water temperature, suggesting that low temperatures experienced during high river levels may have favored virus survival, as viruses generally experience thermal destabilization and gradation of the viral capsid when exposed to higher temperatures (John and Rose, 2005). Additionally, low temperatures reduce the activity of antagonistic microbes and biological mechanisms contributing to virus survival (Gordon and Toze, 2003; Yates et al., 1990). TCC and TVPC, mainly reflecting the autochthonous microbes and viruses, exhibited a higher density at high temperatures, resulting in a high concentration in river water during low river levels. TCC was also positively correlated with temperature, as reported in a previous study by Liu et al. (2013). Furthermore, we observed a negative correlation between *C. perfringens* and EC in river water. Heavy rainfall accompanied by high river levels with low EC in river water due to dilution effects has the potential to mobilize adsorbed microorganisms from river bed sediments and soils of the catchment (Landry et al., 1979). Moreover, albeit a high concentration of coliforms in river water was observed at low river levels, no correlation with any drought-specific physico-chemical parameter was found. Noticeably, the occurrence and abundance of adenovirus in the river water and bank filtrate did not correlate with any of the bacterial and viral indicators monitored nor with any of the physico-chemical parameters tested. Thus, based on the fate of fecal bacterial indicators (*E. coli*, coliforms), viral surrogates (coliphages), TCC, and TVPC, it would not have been possible to predict the occurrence and abundance of pathogenic viruses in either river water or bank filtrate.

4.3. Impact of hydrological extremes on riverbank filtration efficiency

Another focus of our study was the effect of different river levels on the transport and reduction of adenovirus and viral and bacterial fecal indicators during riverbank filtration. The water travel time through bank sediments is a complex function of parameters, including hydraulic conductivity of subsurface sediments, pumping rate at the production well, land side groundwater levels, and river stages. Expectedly, with longer travel times, all selected biological indicators were reduced. This correlation supports the typical hypothesis that a decrease in travel time caused by short–termed increases of the river stage increases the risk of viruses reaching the production well. *Vice versa*, at slow changing and generally low river levels, reduced water flow velocity and increased water residence time can be assumed, improving removal efficiency for pathogenic agents.

High river levels are believed to impair the natural water purification efficiency in riverbank filtration mainly due to potentially elevated concentrations of viruses and bacteria in surface water, a reduced travel time and less dilution with groundwater. In our study, concretely, most of the targeted viral and microbial indicators either showed the highest concentrations in surface water during flood events or concentrations were in the same range in all hydrological situations. We found travel times from the Rhine River to the production well of less than 10 days at high river levels, compared to maximum travel times of up to 78 days. Mean travel time was 31 days. Previous studies and well-established raw water protection schemes recommend a mean water travel time of 10–60 days to be sufficient for efficiently removing pathogenic viruses and bacteria. Worth mentioning, based on our $\delta^{18}O$ data, we can show that water from the production well entirely originated from the river at high water levels, while the river water share was 84% and 77% during medium and low water levels, respectively. Furthermore, at times of high river levels, a full sediment passage from the river to the production well was required to efficiently remove the tested biological indicators, whereas, under other hydrological situations, particularly during low river levels, a sediment passage from the river to the nearby observation wells at row A was already sufficient to reduce most targeted indicators to below the LOD. Another aspect related to high river level situations was raised by Irmscher and Teermann (2002), who claimed that low activity filter media do not remove pathogens efficiently, and, as the river level rises, the infiltrating surface water reaches unsaturated subsoil zones unconditioned for effective natural attenuation. Additionally, factors, including low ionic strength, elevated DOC, and high flow velocities, caused by floods may not only lower the adsorption of microorganisms and viruses to the sediment matrix, significantly decreasing the attenuation capacity of riverbank filtration, but also trigger a remobilization of previously adsorbed bacteria and viruses, promoting their long-distance underground transport (Sprenger et al., 2011; Maliva, 2019).

The highest removal efficiency for adenovirus and most viral and bacterial indicators tested in our study was observed during high river levels. Following the above-mentioned findings and assumptions, this is surprising. However, the data obtained are worth a second look. The higher removal efficiency for pathogens and indicators by riverbank filtration during high river levels are related to higher absolute numbers of respective agents in river water during flood situations (Fig. 9). In fact, high coliform and coliphage numbers in combination with high water levels, as reported by Rohns et al. (2006), could indeed pose a serious risk to water quality at the production well, exceeding the capacity of riverbank filtration.

Taking a specific look to the different hydrological situations, the abundance of adenovirus during bank filtration correlated positively with pH, supporting the idea that virus particles attach to a lesser extent at a higher pH, due to increased electrostatic repulsion according to the DVLO theory (Schijven and Hassanizadeh, 2000). Elevated DOC concentrations found at high river levels correlated positively with an increased abundance of adenovirus. In fact, dissolved organic matter was shown to compete with viruses for sorption sites on sediment surfaces (Gerba, 1984). At low river levels it was only pH and travel time that revealed a statistical significant correlation to adenovirus. No common biological indicators were found to correlate with the abundance of adenovirus during sediment passage at different river levels. While at high river level, adenoviruses correlated with *E. coli*, somatic coliphages, TCC, and TVPC, a correlation with coliforms, TVPC, and ATP was revealed at low river levels. In consequence, for the site investigated, a combination of *E. coli*, coliforms and coliphages seems safe to monitor raw water quality.

The only contamination of raw water at the production well was the occasional occurrence of coliforms and *C. perfringens* during the long period of extremely low river levels in the summer of 2018. Low river levels entail the risk of river bed sediment clogging. Here, clogging includes excessive biomass accumulation, sedimentation of accumulated suspended particles, and precipitation of Mn^{4+} or Fe^{3+} after consumption of DO and denitrification (Sprenger et al., 2011; Engesgaard et al., 2006; Diem et al., 2013). Clogging may not only reduce the bank filtrate quantity but may also lead to preferential flow paths and impaired removal efficiency of pathogens and microbiological indicators (Jaramillo, 2011). Conversely, an increased number of fine particles and iron oxide minerals may constitute an effective barrier leading to an increased adsorption of viruses (Schijven and Hassanizadeh, 2000). Additionally, viruses and bacteria have been demonstrated to be less inactivated and removed under anoxic conditions, which we have observed during the long-term drought, when compared with oxic conditions (Klitzke et al., 2015; Frohnert et al., 2014).

Thus, we summarize that both high river levels with reduced

sediment travel times for water carrying a huge particle load and low water levels with increased water temperature and the risk of bank sediment clogging may pose a risk to raw water quality at the production well. This conclusion is supported by earlier findings of Rohns et al. (2006), who investigated the same riverbank filtration site.

5. Conclusion

In this study, we compared the performance of a riverbank filtration site for the removal of adenovirus and several commonly used bacterial and viral water quality indicators during different hydrological situations. Surprisingly, high river levels were characterized not only by an increased number of pathogens and indicators but also by the highest removal efficiency with riverbank filtration. During drought and low river levels, coliforms and *C. perfringens* were occasionally present in raw water at the production well of the waterworks. Adenovirus, quantified via droplet digital PCR, correlated with *E. coli*, somatic coliphages, TCC, TVPC, pH, and DOC at high river levels. At low river levels, adenoviruses correlated with coliforms, TVPC, pH, and water travel time. For the site investigated, a combination of *E. coli,* coliforms and coliphages for assessing raw water quality was proved safe. For adenovirus, and probably other human pathogenic viruses, viral fecal indicators such as coliphages may occasionally fail predictability, as shown for low river levels, which is an issue that awaits a more detailed exploration. Extreme hydrological events and their influence on the performance of riverbank filtration should be studied on an event-to-event basis at a significantly higher spatial and temporal resolution. This is a difficult task because flood and drought periods cannot be precisely forecasted when planning a field study. Finally, there is an urgent need for lower LODs of pathogenic viruses in natural waters. Since molecular tools such as the ddPCR cannot be significantly improved, the pre-concentration of viral particles from larger water volumes (>100 L) is the way to achieve higher sensitivity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2021.117961.

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