WaterJPI project

SMARTControl

Smart framework for real-time monitoring and control of subsurface processes in managed aquifer recharge (MAR) applications

Deliverable D5.2

Real-time observation platform at MAR scheme in Berlin-Spandau, Germany

Online estimation of groundwater hydraulic residence time and advanced microbial monitoring using flow-through cytometry

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Short summary

The present report describes the field works conducted at the Berlin-Spandau case study for the characterisation of uncertainities regarding the microbial safety. The activities included an upgrade of the monitoring system to allow the online estimation of groundwater hydraulic residence time using SMART-Control web-based tools, and advancing the microbial risk assessment by flow-through cytometry, DNA sequencing and microbial cultivation along the MAR scheme.

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ABSTRACT

This report summarizes the work for monitoring of hydraulic residence time (HRT) carried out at the Managed Aquifer Recharge (MAR) site Berlin-Spandau waterworks. The newly installed monitoring system consists of real-time online sensor data and evaluation algorithms implemented as a web-based software tool. The combination of online data with processing tools allows time-efficient HRT evaluation. Apart from HRT estimations, the monitoring also included measurements by flow-through cytometry (FCM), meta-genomic DNA sequencing and classical microbial cultivation-based analysis. FCM cell counting allows to quantitatively detect microbial cells after staining with a DNA-binding fluorescent dye. The aim of FCM measurements was to gain insights on microbial dynamics along the flow path from the infiltration basin to the abstraction well. The FCM device was installed to measure in the infiltration basin, groundwater observation well and abstraction well in a continuously flowing sampling line that allowed for automatic and continuous monitoring in water. Microbial indicators of viruses, bacteria and protozoa were sampled and analysed by classical cultivation-based methods in parallel to the FCM measurements. The combination of FCM with cultivation-based methods aimed to establish an indicative reference cell count representing a hygienically safe water. The high-frequency flow cytometry data revealed decreasing order of total cell counts from surface water in the infiltration basin water to groundwater in the abstraction well. The fairly constant measurements in the abstraction well may allow to use FCM fingerprinting as a fast monitoring tool in combination with cultivation based methods. However, long-term measurements of FCM for at least 6 months are recommended to assess seasonal fluctuation in both source water and groundwater. Water samples were in addition characterised by DNA sequencing enabling a complete "meta genomic" analysis and taxonomic profiling including bacterial, archaea, viral, eukaryotic DNA. The DNA sequencing in combination with FCM measurements showed that total cell counts decreased along the flow path while the biodiversity increased.

CONTENT

FIGURES

TABLES

1. INTRODUCTION

Managed aquifer recharge (MAR) is a widely accepted technique for augmenting water supplies for potable and non-potable uses. The success of a MAR system largely depends on substantial removal of chemical and biological contaminants during subsurface passage. Removal processes are often time dependent (e.g. biodegradation, inactivation), and the understanding of the hydraulic residence times (HRT) from the source, e.g. infiltration basin or river, to the point of abstraction is a key element in the management of aquifer recharge. During an initial risk assessment potential hazards that may pose risks to human health and environment were identified at all project MAR sites (Sprenger et al., 2020). At Berlin-Spandau the maximal risk assessment identified uncertainties regarding site-specific HRT and hence microbial safety. It was concluded that hydraulic monitoring of individual abstraction wells with critical residence times should be taken into account. This report summarizes the field work for improved monitoring of HRT carried out at the Berlin-Spandau waterworks and the HRT estimation using the SMART-Control web-based software tool T1 “Initial risk assessment. Part A. Estimation of groundwater hydraulic residence time” (Stefan et al., 2021). The newly installed monitoring system relies on real-time online sensor data and evaluation algorithms implemented in the SMART-Control platform. The use of online field data with evaluation software in a web-based environment simplifies the overall assessment procedure and offers advantages such as:

- easy access to information;
- automation of evaluation procedures;
- improved communication and dissemination.

During the evaluation procedure the raw sensor data are interpreted by evaluation algorithms to meaningful information on HRT. The information can be shared easily and the interpretation process is easy to understand. Although at the moment not fully automatic, the implemented routines hold potential to reduce human interventions. Another potential benefit is that it facilitates the communication with internal and external stakeholders and the further use of information, e.g. as part of a Quantitative Microbial Risk Assessment (QMRA).

The monitoring also included measurements by flow-through cytometry (FCM) along the MAR scheme. The FCM device was rented for two months and operated in automated offline mode. The aim of FCM measurements was to gain insights on microbial dynamics at the recharge site along the flow path of the aquifer recharge and recovery system. FCM cell counting allows to easily, quickly (15-30 minutes, depending on the ambient temperature) and quantitatively detect microbial cells after staining with a DNA-binding fluorescent dye. The parameters Total Cell Count (TCC), Intact Cell Count (ICC) and the proportion of cells with a low or high content of nucleic acids (LNA or HNA proportion) determined by FCM are microbiological sum parameters for the general microbiological state. Total cell count has no legally established value but typically 20.000-200.000 cells/mL are measured in hygienically safe drinking water. However, FCM cell count measurements have no hygienic implications since cell counting do not distinguish pathogenic and non-pathogenic microbes. Since FCM do not allow for health related microbial risk assessment directly, at least one representative for each group of pathogen (i.e. virus, bacteria and protozoa) was sampled and analysed by classical cultivation-based methods in parallel to the FCM sampling. The combination of FCM and classical cultivation-based methods aimed to establish an indicative reference cell count representing a hygienically safe water. FCM measurements holds potential to detect e.g. possible operational disturbance by comparing the actual detection to an previously defined reference cell count. In addition, water samples were characterised by DNA sequencing enabling a complete "meta genomic" analysis and taxonomic profiling including bacterial, archaea, viral, eukaryotic DNA. The DNA sequencing alone would result in the relative abundance of detected DNA, but the combined use of cell counts together with DNA sequencing data provides sample-specific absolute taxon abundances and holds the potential to get further insights into subsurface processes from community shifts along the flow path.

2. SITE DESCRIPTION

The study site is situated in the north-western part of Berlin (Germany) at the waterworks Berlin-Spandau. The waterworks Spandau abstracts groundwater in the range of about 25-30 Mm^3/year and recharges 15-20 Mm^3/year through constructed infiltration basins and near-natural lakes and ditches (Figure 1). Groundwater augmentation is necessary because natural groundwater recharge does not cover the actual water demand. The source water for groundwater augmentation is abstracted from the Havel River and pre-treated before recharge. Surface water is treated by flocculation and rapid sand filtration before recharge. The technical pre-treatment aims at removing suspended solids and nutrients. Site-specific conditions have been described during maximal risk assessment and can be found in the related deliverable D2.1 (Sprenger et al., 2020).

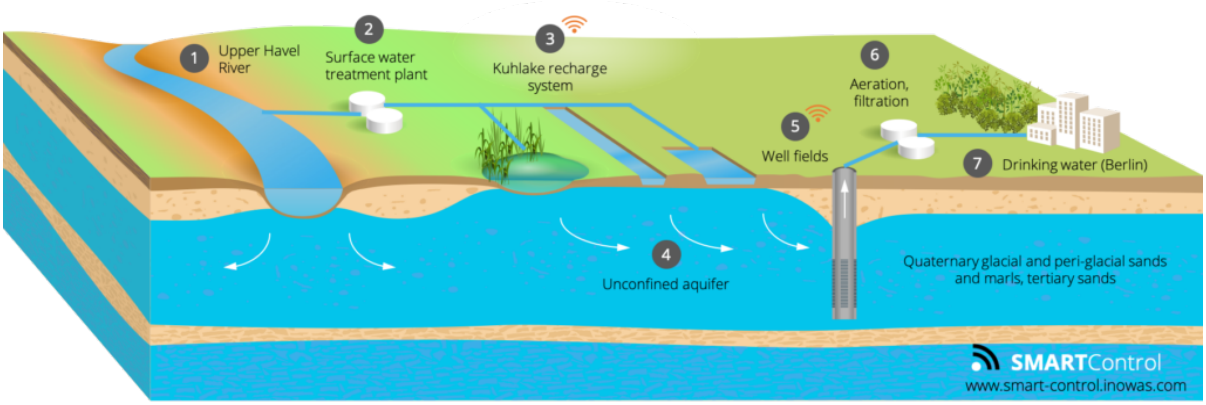

Figure 1. **Schematic representation with main components of the MAR facility at Berlin-Spandau waterworks** (source: www.smart-control.inowas.com)

At Berlin-Spandau, the project approach focuses on the well-field Spandau-Nord and consists in the assessment of HRT at its central abstraction well #5 situated between the rectangular infiltration basin 1+2 on the eastern side and the elongated basin 3 on the western side, both in about 90 m distance from the wells (Figure 2).

The well field Spandau-Nord consists of eight recovery wells with an average abstracted volume of 6.8 Mm^3/year. The total depth of the wells is 47-52 meter below ground level, with filter screen lenghts between 18 to 19 m. A hydrogeological cross-sectional view (true to scale) based on water level measurements from 2014-2016 (Sprenger, 2018) and geological explorations (Bruehl and Limberg, 1985) is depicted in Figure 3.

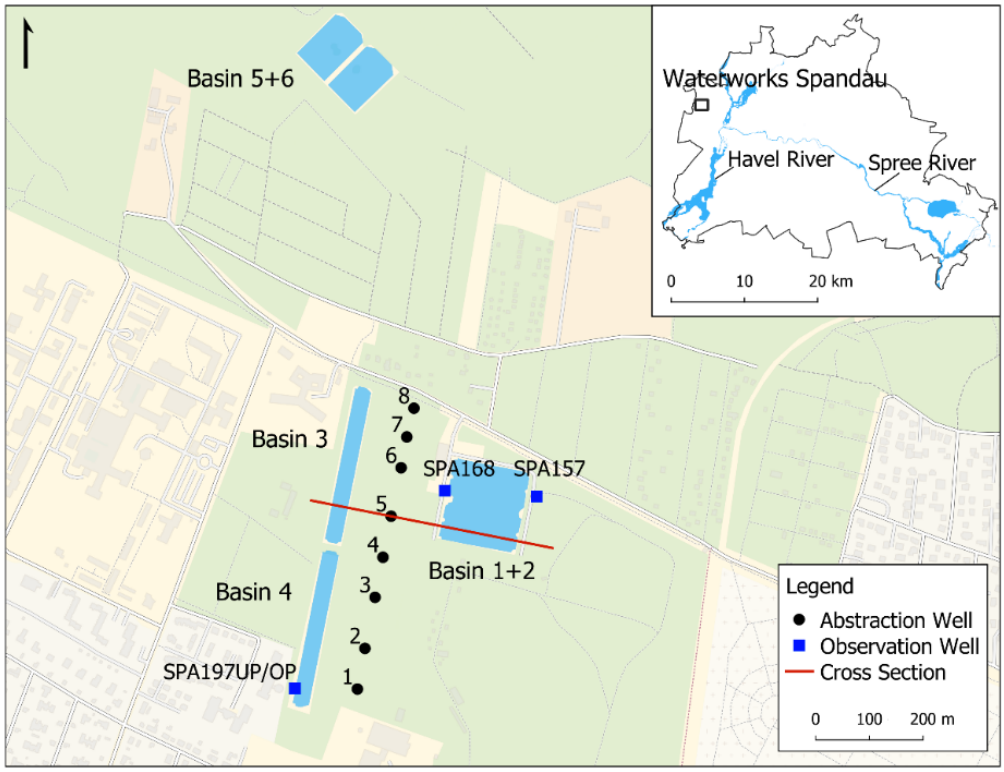

Figure 2. Overview of infiltration basins and abstraction wells at WW Berlin-Spandau

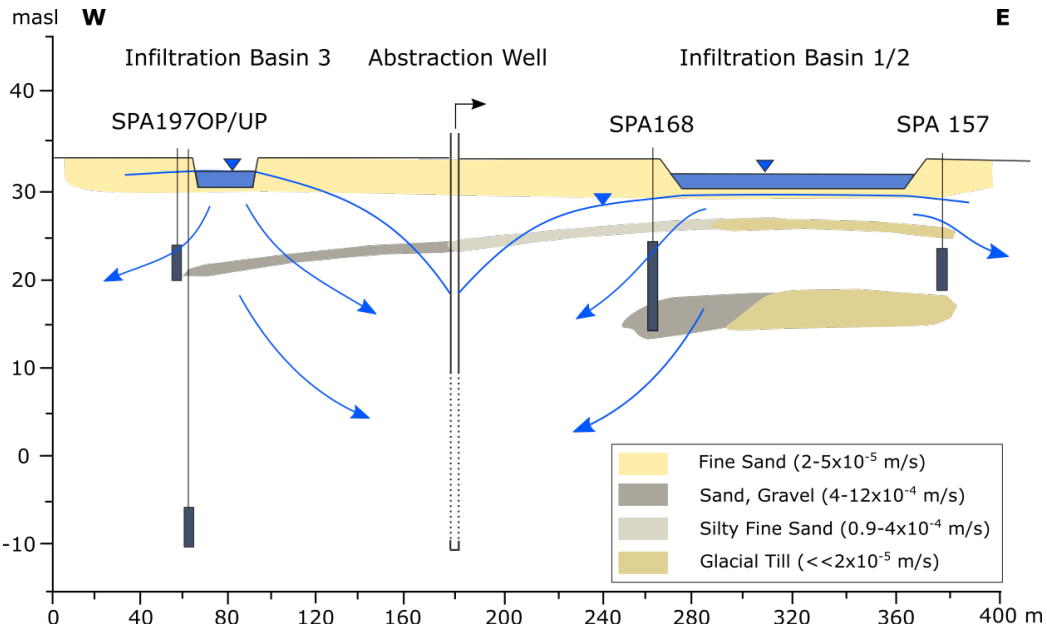

Figure 3. Cross-section of the groundwater recharge site at waterworks Spandau (vertical exaggeration 1:5)

The geology of the study site consists of quaternary sediments forming an aquifer complex that is characterized by various heterogeneous layers of glacial, peri-glacial and fluvial deposits. This dynamic sedimentary environment results in large small-scale hydraulic differences in the sedimentary sequence, that influences the infiltration and flow pattern to a large extend. The upper layer is composed of fine sands. Below basin 3 this low permeable layer ($2\text{-}5\times10^{-5}$ m/s) is max 0.5 m, while below basin 1/2 it is around 1 m in thickness (Bruehl and Limberg, 1985). Almost

the entire area of infiltration basin 1/2 is underlaid by this low permeable layer, which explains the relative low infiltration rates (Table 1) and the presence of an unsaturated zone of about 0.5 to 1 m thickness even at maximum basin filling level (Figure 3). At basin 3 no unsaturated zone was observed. The glacial till below is composed of extremely unsorted glacial deposit in the form of a ground moraine, deposited as a band that evolves to more coarse grained sediments from east to west (Bruehl and Limberg, 1985). The glacial till is spatially highly variable in thickness and extend and forms due to its very low permeability major flow barriers. However, the hydrogeological observations strongly suggest that the observation well SPA168 is on the groundwater flow path from the infiltration basin to the abstraction well.

Table 1. Geometry and hydraulic parameters of the infiltration basins 1/2, 3 and 4 (Sprenger, 2018)

Basin ID	Area (m^2)	Volume infiltrated 1995-2015 (Mill. m^3/a)	Hydraulic loading (m/d)	Infiltration rate 2015-2016 (m/d)
Basin 1/2	21,900	2.44	0.31	0 – 0.5
Basin 3	8,082	2.42	0.82	0 – 3.3
Basin 4	7,498	2.69	0.98	0 – 3.4

3. APPLICATION OF SMART-CONTROL WEB-BASED TOOLS AT THE BERLIN-SPANDAU CASE STUDY

3.1 DATA ACQUISITION

The MAR scheme at Berlin-Spandau is a full-scale facility equipped with advanced monitoring and control system. For testing and validation of the SMART-Control aproach, additional sensor devices were installed at three monitoring stations. The scope of these devices was to collect the data necessary for HRT calculation of well #5. The well itself was not equipped with new sensors because it was already fully equipped.

Data from the well (e.g. water temperature) were uploaded to the INOWAS platform and used as offline data during the evaluation procedure. Calculation of HRT is done using tool SMART-Control web-based tool T1_A (Stefan et al., 2021) following an established method based on seasonal heat transport as described by Sprenger et al. (2017). The installed sensor devices (WR-GPRS compact, Umwelt- und Ingenieurtechnik GmbH Dresden) are measuring water level and water temperature. Measurements are transferred via the General Packet Radio Service (GPRS) technology to UIT's SENSOweb server and from there to the INOWAS platform. For more details about data transfer see Deliverable D3.1 (Junghanns and Glass, 2020).

The online devices were installed at two infiltration basins (basin 1+2, basin 3) and one groundwater observation well (SPA168). At the infiltration basin 1+2 an abondoned slotted pipe was used as fixation system for sensor installation. At the infiltration basin 3 a steel construction at the basin served as a fixation point for the sensor device (Figure 4).

Figure 4. Manual water level meter (upper left) and installation of the online sensor devices at infiltration basin 1+2 (lower left) and basin 3 (right)

At the observation well (SPA168) the sensor was installed at the depth of 10 m below ground surface. Measurements were calibrated by manual depth-to-water measurements. Monitoring started on 13.11.2019 with a measurement interval of one hour and a daily transmission rate.
This was later reduced to weekly transmission and two hourly measurements. At the observation well previously measured data were uploaded manually (from 24.09.2019). All installed loggers automatically compensate for atmospheric pressure changes.

3.2 DATA INTEGRATION

The INOWAS platform provides the software infrastructure developed in the SMART-Control project to enable the import of third-party data and to perform evaluation processes. Details on the developed software infrastructure (tool T2) is given in the SMART-Control Deliverable 4.2 (Junghanns and Glass, 2020).

For the assessment of hydraulic residence times for the Berlin-Spandau case study online data from the three monitoring stations can be combined with offline data from abstraction well (Well#5) (Figure 5).

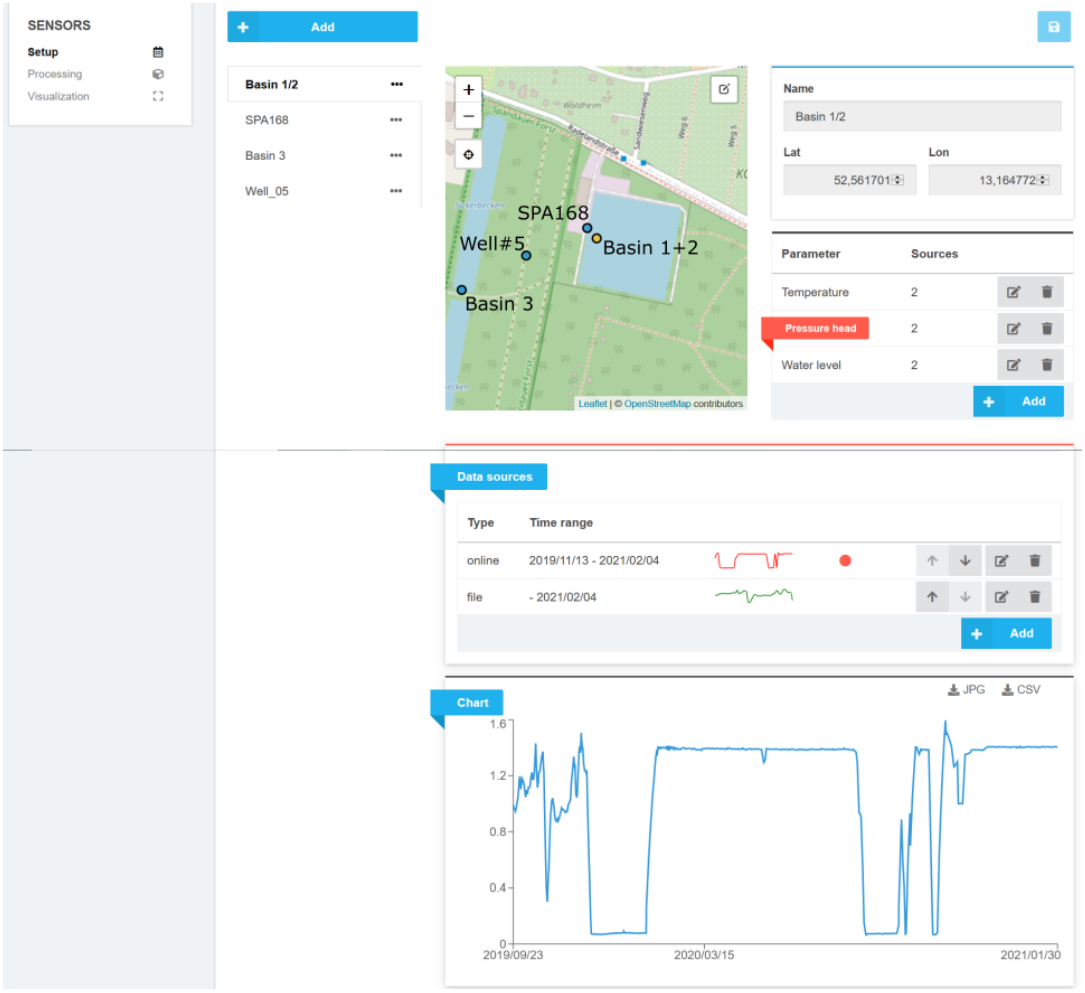

Figure 5. Screenshot of real-time sensor as data source on the web-based SMART-Control platform

Water pressure data from the two infiltration basins can be displayed in water level expressed as meter above sea level (masl) and water level expressed as meter above basin bed (mabb). Infiltration rates in the basins are controlled by the water level above basin bed. The operational practice is to maintain a certain water level in the basins by adjusting the inflow rate to the actual infiltration rate. Water pressure data from the observation well (SPA168) is shown as water level expressed as meter above sea level (masl) and pressure head expressed as meter above sensor. At the abstraction well water temperature measurements and the pumping rate can be displayed. Both data sets are obtained from the site operator and uploaded manually via csv files.

3.3 DATA PROCESSING AND RESULTS

The SMART-Control tool T1 (Part A) has been used to calculate the groundwater HRT at Berlin-Spandau site. For testing and validation purposes, temperature measurements collected from other locations at WW Berlin-Spandau were imported to the INOWAS platform by following the steps described in the previous section. To obtain plausible results it is necessary to select a full sine curve at both the inflow (surface water) and the outflow (groundwater). The time series have thus been cropped to a time range of approximately one year (for surface water between 23.04.2016 and 30.05.2017 and for groundwater between 19.06.2016 and 29.06.2017). The start and end date of selected time series are displayed and subsequently used for sine fitting calculation.

After pre-processing, the time series were loaded to the HRT tool as shown in Figure 6.

Figure 6. Screenshot of the Heat Transport Tool for hydraulic residence time assessment. Selection and display of input data

In the tool, the user input is further required to specify the thermal retardation factor. By default a value of 1.8 is set, representing sandy aquifers. After pressing the “run calculation and save” button, results from model fitting are displayed for surface water and groundwater (Figure 7).

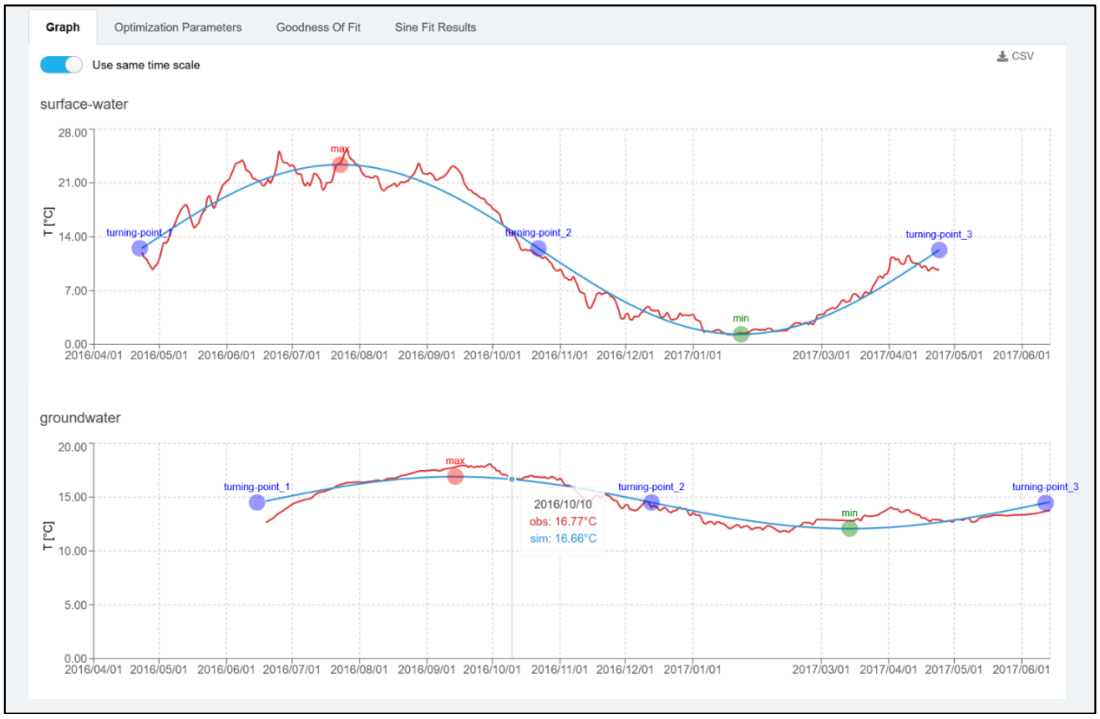

Figure 7. Screenshot of the Heat Transport Tool for hydraulic residence time assessment. Display of calculated min/max and turning points

The tool is thus offering the possiblity to inspect the

- "Optimization parameter" showing the sinusoidal parameter (period length, amplitude etc.)
- "Goodness of fit" showing deviation of the estimates from the observed values expressed by the root mean square error (RMSE) and r^2, and
- sine fit results

The tool allows to export the model results and calculated lag times as csv files for further processing and reporting. Sine fit results for the example calculated at Berlin-Spandau are shown in Table 2.

Table 2. Sine fit result table

	Surface Water (IN)	Groundwater (OUT)	Residence Time [d]	
			Thermal	Hydraulic
turning-point_1	2016-04-22	2016-06-15	54	30
Max	2016-07-23	2016-09-14	53	29
turning-point_2	2016-10-22	2016-12-13	52	29
Min	2017-01-23	2017-03-14	50	28
turning-point_3	2017-04-24	2017-06-12	49	27

4. ADVANCING MICROBIAL CHARACTERISATION: FLOW-THROUGH CYTOMETRY, DNA SEQUENCING, MICROBIAL CULTIVATION

4.1 FIELD SAMPLING

The field study was conducted over eight weeks (02.10.2019 to 26.11.2019) with a BactoSense flow cytometer that allows for automatic and continuous monitoring in water in 30 min (or longer) intervals (Sigrist-Datasheet). The BactoSens is currently the only instrument on the market that allows for automated sample preparation within the same instrument. From 02.10. to 31.10.2019 the FCM device was installed to measure in parallel at the infiltration basin 1+2 and the observation well (SPA168). In order to take samples from the two different sources a continuously flowing sampling line was installed. A peristaltic pump (GEMKE, 2020) was discharging water from the infiltration basin and a submersible pump (MP1 Grundfos) was pumping groundwater from the observation well (Figure 8, left).Time controlled magnetic valves were used to allow flow to the FCM device either from the basin or from the groundwater. The valves were switched every 6 h resulting in two measurements per day for each sampling location. The peristaltic pump was operated two times a day for 30 min before FCM sampling, while the submersible pump was operated continuosly. The flow cytometer was programmed to sample automatically either from the basin or from the groundwater. The outflow was discharged to the basin.

The peristaltic pump was operated with santoprene or silicone tubes with a diameter of 10 mm. The submersible pump was operating with a 13mm PVC tube and a permanent pumping rate of ~1000 mL per min. The flow rate to the device was adjusted manually to the required flow rate of 200 to 400 mL/min by a valve. The flow rate was measured and adjusted manually prior to each restart of the automatic sampling mode (see appendix 7. Annex Table 7). Prior to the installation of the submersible pump the pumping equipment including the pipes were disinfected and cleaned with ethanol and purified water. This was done to avoid possible microbiological contamination of the observation well through the pumping equipment. After cleaning the submersible pump was left in the obersvation well until the end of the sampling period. The peristaltic pump and sampling line were installed and stored in a box placed close to the basin and the observation well (Figure 8, right).

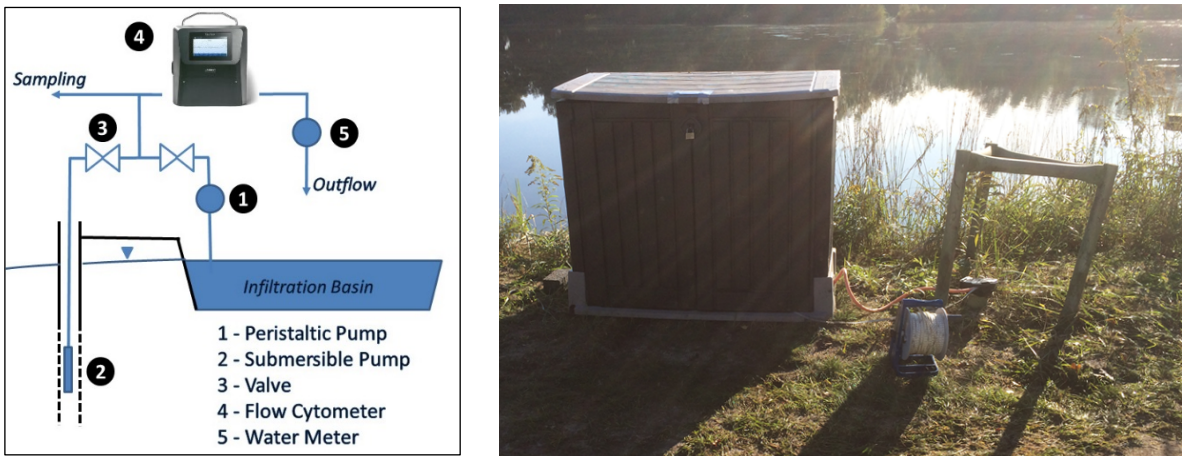

Figure 8. Sketch of sampling line for flow-through cytometry (left). Field box with sampling line next to the observation well SPA168 and infiltration basin 1+2 (right)

After four weeks, the equipment was moved from the basin and observation well to the abstraction well. At the abstraction well#5, the FCM was operated from 31.10. to 26.11.2019. The FCM was installed at the sampling bypass of the rising pipe inside the well chamber (Figure 9). Flow was provided by a time controlled magnetic valve for 30 min prior to the sampling. The outflowing water from FCM device was collected in a reservoir inside the well chamber and pumped out periodically.

Figure 9. Flow cytometer installed in well chamber (left); flow cytometer maintenance and manual measurements (right)

The FCM device was operated in automatic mode at all three sampling sites. Sample volumes of 260 µL were taken and mixed with fluorescent stains SYBR®Green and Propidiumiodide (Sigrist-Datasheet). SYBR®Green penetrates all cells and binds to double-stranded DNA (Hammes et al., 2008). Propidiumiodide (PI) binds to DNA, but only penetrates cells with defect cell membrane (Berney et al., 2007). Cells with an intact membrane fluoresce green, while cells with a damaged membrane are permeable to the red pigment PI. After staining with the the two fluorescent dyes, the cells are individually channeled through a glass capillary and detected by a laser light. Every particle encountered by the laser beam causes the light to be scattered, and any fluorescent dyes is stimulated to emit light. The resulting scattered light and the fluorescence signal is transmitted to detectors via filters and mirrors and recorded (Kötzsch et al., 2012). FCM allows to obtain information on a large number of particles, including bacteria, protozoa and viruses.

After incubation (~10-20 min, 37 °C), samples were analysed (FL1 channel at 525 nm, FL2 channel at 715 nm) using fixed gates to separate cells and background signals. The detection limit of the FCM device is given with 1×10^2 cells/mL, nominal range of 1×10^3 - 1 x 10^6 cells/mL (Sigrist-Datasheet), and the limit of quantification is 1000 cells per mL (SLMB).

During site visits manual measurement with the FCM device were performed in parallel. Manual measurements sometimes produced failures due to occurring bubbles (error message: bubbles detected). The measured Total Cell Count (TCC) increased to implausible high numbers. The underlying cause could not finally be identified. It is assumed that degassing of groundwater may be responsible, but sometimes also basin water was affected. Aerating and degassing the samples by opening the sample bottles for at least 30 min and in some cases up to several hours prior to the manual measurement avoided this failure in most cases. Another failure that caused implausible readings during automatic measurement was the error message: “enclosure too cold” that occurred during night. Low ambient temperatures of <5°C during night may have disturbed the incubation period and triggered the error message. All measurements with error and warning messages were not considered for further analysis and interpretation.

Cultivation based methods

During the sampling period from 10.10.2019 to 14.11.2019 samples for E.Coli, clostridium perfringens, somatic coliphages, and enterococci were taken twice a week, resulting in n=11 for each sampling station (SPA168, well#5 and basin1+2). Before sampling at well#5, the sampling tap was desinfected by flame sterilization followed by a 2-3 min flushing interval. Samples were taken while wearing sterile gloves. Samples were collected into 200 mL sterile sample bottles and transported to the laboratory within 6 h at the same day for analysis. Basin samples

were taken directly from the edge of the infiltration basin with sterile gloves. Samples from the observation well were taken from the at the beginning of the sampling period disinfected sampling line. Field sampling and sample transport was carried out by KWB. Laboratory measurements were carried out by BWB according to standardised methods as decribed in the following. Parameters were selected to have at least one representative for each group of pathogen (i.e. virus, bacteria and protozoa).

DNA sequencing

In total three samples were chosen for additional meta-genomic sequencing with the aim to identify and compare microbiological communities. The infiltration basin and the observation well (SPA168) was sampled on 17.10.2019 while the well#5 was sampled on 12.11.2019. The different sampling dates were chosen to account for the subsurface travel time from the basin to the recovery well. Samples were taken in 1 l sterile bottles, filled up to 80% with the sample and then filled up with ethanol to avoid bacterial growth during transport. Field sampling and sample transport was carried out by KWB. Metagenomic sequencing was performed by BlueBiolabs (see below).

In-situ physico-chemical measurements

On-site parameters were measured in an Eijkelkamp mini-flow cell using the Eijkelkamp Multimeter 18.50.01 and associated standard probes until stable values were reached. The pH and conductivity probes were calibrated against Eijkelkamp standard calibration solutions (pH 4.0 and 7.0 and 1,413 µS/cm) before sampling. A SmarTroll multimeter device was used to measure dissolved oxygen at the infiltration basin and the observation well on 29th November 2019. In-situ measurements are shown in the appendix Table 9 and summarized in Table 6.

4.2 LAB PROCEDURES

Cultivation based methods

E. coli is the most common indicator of faecal contamination in water treatment. *E. coli* is the first organism of choice in monitoring programmes including surveillance of drinking-water quality (WHO, 2011) and used in German drinking water guidelines (TrinkwV, 2011). *E. coli* is considered an appropriate indicator for the presence/absence of Campylobacter in drinking water systems (WHO, 2016). Laboratory measurements were following DIN-EN-ISO9308-2 (2014). *Clostridium perfringens* are spores or spore-forming sulphate-reducing bacilli and intestinal bacteria of humans and other vertebrates. *C. perfringens* has been proposed as an indicator of protozoa in treated drinking-water supplies (JRC, 2016; WHO, 2011). The applied DIN at the lab measures spores (DIN-EN-ISO14189, 2016) and is therefore indicative for protozoa. The presence of somatic coliphages in drinking water provides an indicator of faecal pollution and the potential presence of enteric viruses and other pathogens (WHO, 2011). Coliphages are not included in German drinking water guidelines (TrinkwV, 2011). Laboratory measurements according to (DIN-EN-ISO10705-2, 2002). *Enterococci* tend to survive longer in water environments than *E. coli* (or thermotolerant coliforms), are more resistant to drying and chlorination (WHO, 2011). Laboratory measurements as described in TrinkwV §15.

DNA sequencing

DNA sequencing was carried out by the company Blue Biolabs. The company provided a sampling kit, carried out sample preparation, sequence analysis and evaluation in its “Blue Biome” service. The sequence analysis is based on shotgun metagenomics, which represents a broadly applicable approach to assessing the microbial composition, diversity and metabolic potential of environmental samples. This method is also known as next-generation sequencing, in which thousands of microbial genomes are sequenced at the same time without the need for prior cultivation. The depth of coverage is determined by the project goals and the expected complexity of the samples. With the help of database comparisons, it is possible to assign the identified DNA to specific organisms.

The samples taken with the „Blue Biome"-kit were initially prepared by filtration through a 0.2 µm polycarbonate membrane in the laboratory. The DNA contained in the filter cake was then extracted using the Roboklon Soil DNA kit and prepared for sequencing. The sequencing was carried out on a NovaSeq 6000 system (2 x 150 bp paired-end sequencing mode). The subsequent bioinformatics evaluation included an assessment of the sequence quality before the annotation of the metagenome, as well as the removal of bases, adapters and primers of poor quality. In addition, with the help of comparison algorithms (Langmead et al., 2009) DNA fragments of human origin were excluded that may have contaminated the sample during sampling. The remaining sequence data were then subjected to a taxonomic identification algorithm. The phylogenetic classification of the sequences was carried out with the help of Kraken database (Wood and Salzberg, 2014) and the mini-octopus reference database. The sequence data determined are compared with those of already known organisms and a similarity assessment is carried out.

4.3 DATA PROCESSING

Flow Cytometry

Data from the BactoSens device were transferred using the data format "flow cytometry standard" (FCS). FCS files were transferred from the BactoSens device and processed with R-studio (R-CoreTeam, 2013). After data import with the R-package FlowCore (Ellis et al., 2019), cell counting and graphs were made with self-developed R scripts. Only about 90% of the measured data are stored in the FCS file. Therefore, cell counting conversion of 1000/90 (~11.1) is applied (e-mail communication with SIGRIST staff). Measurements are plotted using a 2D density estimation function from the MASS R package to color points by density with ggplot2. This helps to indicate regions with overplotting points (Figure 10, right). FL1 and FL2 are representing the fluorescence channels at wave lengths of 525 nm and 715 nm, respectively (Manickum, 2020). Additionally, fluorescence is measured at 488 nm for the Side Scatter (SSC). The two forward scatter signals (FL1, FL2) are related to particle size, while SSC signals are related to particle complexity and granularity (Manickum, 2020). The green fluorescence (FL1) versus red fluorescence (FL2) scatter plot help to distinguish background from intact and damaged cells.

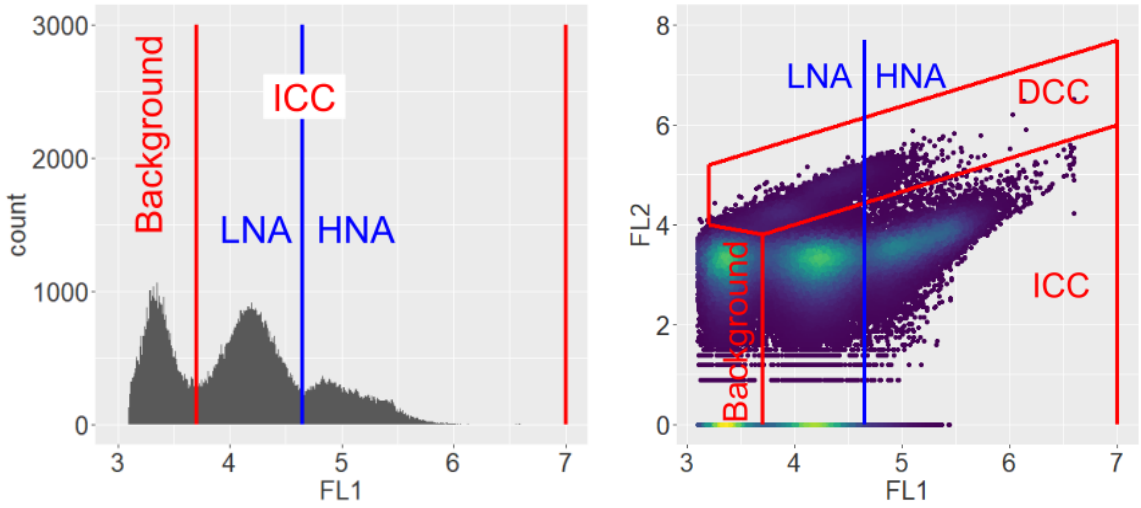

Figure 10. Gating based on single parameter histogram of FL1 (left) and FL1 against FL2 (right) to discriminate LNA/HNA (blue line), ICC and DCC (red lines) (sample: SPA168-20191105-103657UTC; all data points shown)

FCM data is typically analyzed through a processing called "gating". Gates are closed polygons in which cell clusters are isolated. The gate boundaries are defined on the basis of one- or two-dimensional representations of FCM parameters. Kötzsch et al. (2012) states that gates need to be corrected only in rare cases, since cell clusters do not change their position with optimally selected settings, but only vary in shape and size within the gates.
Hence, fixed gates settings were used for all samples to separate cell counts and background noise (abiotic particles and background signals), to define ICC and DCC cells, and to distinguish between high (HNA) and low

(LNA) nucleic acid content bacteria. Hammes and Egli (2010) state that in the current literature little consensus consists amongst researchers about which parameters exactly define LNA and HNA cells. The gates applied are often based on a subjective assessment that may limit the comparability of results from different studies (BfNT, 2018).

Adomat et al. (2020) monitored various treatment trains involving riverbank filtrated water by flow through cytometry using fixed gates. The LNA/HNA parting line was set to FL1 = ~4.8 and the TCC range from FL1=3.75 to 6.5 (derived from visual inspection of diagrams). In this study, gates were defined based a single-parameter histogram data of FL1 and the scatter diagram of FL1 vs. FL2 (Figure 10). Based on the histogram of FL1 the LNA/HNA parting line is set to 4.65. Cell counts above 4.65 are HNA, and LNA counts are below 4.65. The ICC gate limits to 3.7 and 7 and on the FL2 axis to 3.8 and 6. The DCC gate is set to 3.2 on the FL1 axis and 4, 5.2 and 7.7 and the FL2 axis. Please note that the ICC gate also covers data points that showed no fluorenscence in the red spectrum (FL2), i.e. FL2 = 0. All points outside of these gates are considered as background noise and not further respected.

DNA sequencing

The combined use of FCM total cell counts together with DNA sequencing data provides sample-specific estimated absolute taxon abundances (EAA). FCM total cell count from flow cytometric data were multiplied by the relative abundances of taxa to reveal EAA as suggested by Props et al. (2017).

4.4 RESULTS AND DISCUSSION

Microbial cultivation based methods

Laboratory results of cultivation based microbial measurements are listed in the appendix Table 8. At well#5 all measurements of coliform bacteria, E. coli, somat. Coliphages, clostridium perfringens, and enterococci were below detection limit. Results indicate that the well#5 abstracts hygienically safe water according to legally established analytical methods. However, from a microbial risk perspective the absence of microbial indicators do not provide sufficient guarantee for microbial safety.

One could now calculate the Log Removal Value (LRV) by simply taken the mean conentration values and calculate:

$$LRV = log_{10}(C_o) - log_{10}(C_x)$$

Where C_o is the input (e.g. infiltration basin) concentration and C_x (e.g. observation well) the concentration at the output. Log removal is used to express the relative number of microbes that are reduced along the flow path.

Conservative assumptions were applied for measurements with zero counts. For a given microbial parameter below limit of detection (LOD) were replaced by the LOD (1 organism/100 mL). Measurements given with “greater as sign” are treated as absolute numbers. Best case or maximal LRV are calculated by max C_o– min C_x of log-concentrations. Worst case or minimal LRV are calculated by C_{min} inflow - C_{max} outflow of log-concentrations. Mean LRV are calculated by using the arithmetic mean of log-concentrations. Log removal values are calculated for the subsurface flow paths between the infiltration basin and observation well and between the observation and the abstraction well#5 (

Table 3). Due to the specified assumptions, the given LRV indicate the minimal microbial removal. The actual microbial removal of the subsurface is likely to be higher.

Table 3. Calculated Log Removal Values (LRV) along the flow path

Flow path		Coliform Bacteria	*E. coli*	Somatic Coliphages	*Clostridium perfringens*	*Enterococci*
Basin 1+2 to SPA168	Max	>2.3	>1.4	>2.2	>0.8	>2.3
	Mean	**>1.9**	**>0.9**	**>1.5**	**>0.2**	**>1.5**
	Min	0.8	NA	NA	NA	0.3
SPA168 to well#5	Max	>1.1	NA	NA	NA	NA
	Mean	**>0.4**	NA	NA	NA	NA
	Min	NA	NA	NA	NA	NA

NA= not applicable; each sampling station n=11 samples; sampling period 4 weeks

Based on the above described assumptions the mean LRV for *E.Coli* is 0.9 log unit. The mean LRV can be considerd as conservative since observed microbial detection in observation well are lower and hence the actual LRV is higher. Since almost all measurements in groundwater are below detection limit, the observed microbial concentrations do not allow for a sound evaluation. It is therefore recommended to apply sampling and analytical procedures with detection limits that account for low microbial concentration in groundwater. Bacterial accumulation by means of filtration modules, e.g. hemodialysis filters (Mull and Hill, 2012; Smith and Hill, 2009) are recommended to be conducted in future.

Flow through cytometer

Over the full sampling period, a total of 180 samples were measured at the three sampling stations. The median cell counts of TCC, ICC, DCC and HNA show a decreasing order from the basin water to the observation well and further to the well#5 (Table 4).

Table 4. Summary of TCC, ICC, DCC, LNA and HNA

Sampling station		TCC ($\times10^6$ cells/mL)	ICC ($\times10^6$ cells/mL)	DCC ($\times10^4$ cells/mL)	LNA ($\times10^6$ cells/mL)	HNA ($\times10^6$ cells/mL)	HNA (%)
Basin 1+2 (n=50)	max	3.65	3.35	4.07	0.71	2.75	82
	med	**2.57**	**2.36**	**1.69**	**0.55**	**1.80**	**76**
	min	1.45	1.26	0.77	0.35	0.84	67
SPA168 (n=39)	max	1.21	1.15	0.98	0.84	0.44	38
	med	**0.94**	**0.91**	**0.24**	**0.72**	**0.18**	**21**
	min	0.40	0.39	0.08	0.32	0.07	18
Well#5 (n=91)	max	0.39	0.38	1.91	0.34	0.083	23
	med	**0.32**	**0.32**	**0.66**	**0.28**	**0.039**	**12**
	min	0.30	0.29	0.56	0.23	0.034	11

TCC = total cell count; DCC = defect cell count; ICC = intact cell count; HNA = high nucleid acid; LNA = low nucleid acid

Compared to other studies with similar conditions, the measured median TCC in the abstraction well are in the same order of magnitude, e.g. Besmer et al. (2016) measured TCC in groundwater abstracted by a well influenced by bank filtrate between 0.12 – 0.28×10^6 cells/mL. In Austria, bank filtration wells showed median concentration of 0.32×10^6 cells/mL with maximum concentration of up to ~0.38×10^6 cells/mL (BfNT, 2018). Therefore, the measured TCC in the abstraction well is within a plausible range and similar to other studies. The measurements in the basin water are also in the same order of magnitude as compared to other studies, e.g. Adomat et al. (2020) measured TCC in Elbe River at Dresden with an average of 9.4×10^6 cells/mL (fluctuation from 4.4×10^7 cells/mL to 1.9×10^6 cells/mL) and Kötzsch et al. (2012) measured lakewater at Zürichsee with an average TCC of 1.2 ×10^6 cells/mL.

The time series of FCM measurements are presented and discussed below. While in the infiltration basin and the observation well a decreasing trend in TCC, HNA and LNA can be observed, the measurements in the abstraction

well are relatively constant (Figure 11). A gradual decrease of total cell counts in the infiltration basin from >3×10^6 cells/mL in October to <2×10^6 cells/mL in November and from approx. 1×10^6 cells/mL to 0.4×10^6 cells/mL in the observation well was obeserved. It can be argued that this gradual decrease is related to the seasonal decrease in temperature. During the sampling period the local average air temperature dropped from about 14°C to 2°C. Higher water temperatures promote bacterial growth and TCC values were found to be higher at elevated temperatures, e.g. in drinking water distibution systems as shown by Liu et al. (2013). However, whether this decrease is actually due to an atmospheric temperature decrease, cannot be clarified finally. It would be interesting to see how long term measurements would look like.

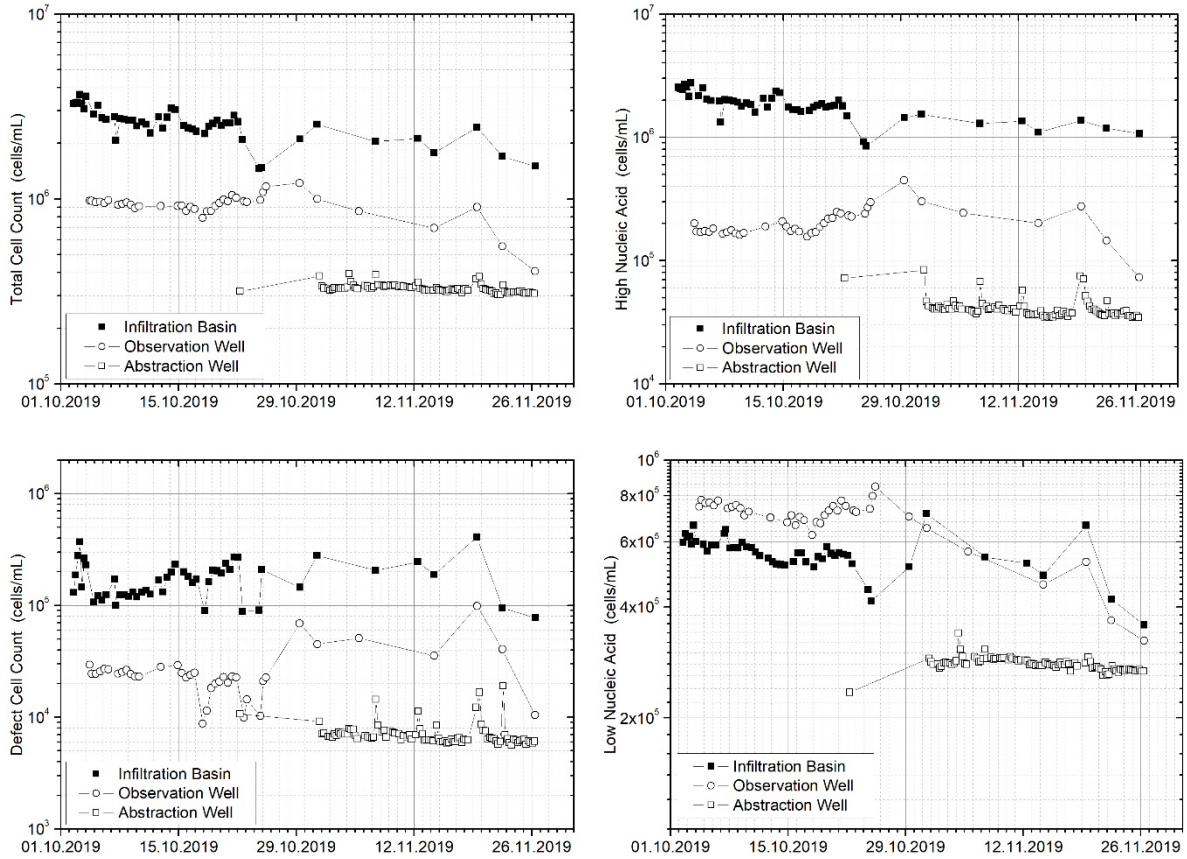

Figure 11. Time series of Total Cell Count (TCC), High Nucelic Acid (HNA), Defect Cell Count (DCC) and Low Nucleic Acid (LNA) at infiltration basin, observation well and abstraction well

The time series of DCC shows relatively strong fluctuations but appears to be stable over the period of observation (Figure 11). DCC at the sampling stations is two orders of magnitude lower than TCC. ICC are in the same range as TCC in terms of absolute numbers and show a similar temporal course at each sampling stations and is therfore not shown in Figure 11.

The comparison of scatter plots indicate the general decrease of cell counts from basin to observation well to the abstraction well (Figure 12). The shift of point cluster of HNA in the basin to LNA cluster in groundwater is visualised by the coloured density in Figure 12. This shift is important because high HNA counts are associated to pathogenic contamination (Servais et al., 2003; Sharuddin et al., 2018). Therefore, the absence of pathogens and indicators in the abstraction well as measured by cultivation based methods is confirmed by FCM measurements.

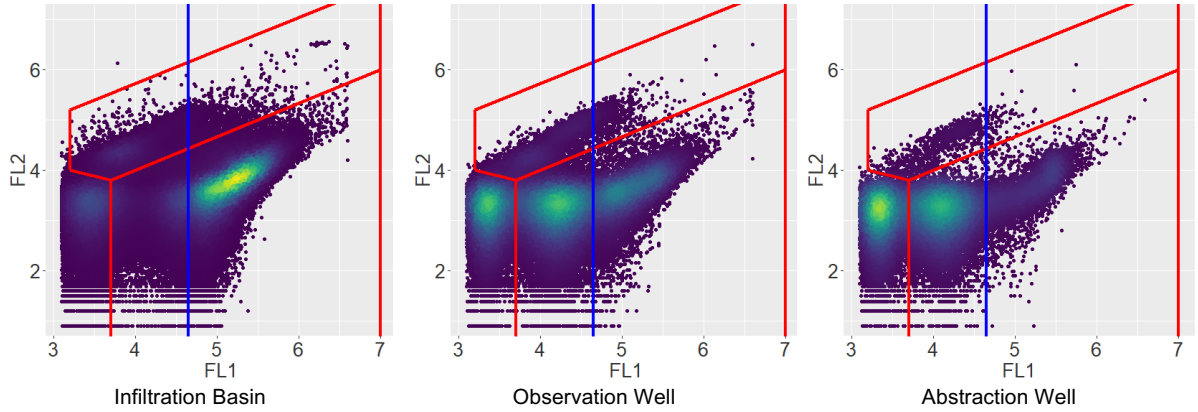

Figure 12. Scatter diagrams of infiltration basin 1/2, observation well and abstraction well

FCM measurements holds potential to detect e.g. possible operational disturbance by comparing the actual detection to an previously defined reference cell count. Major aim of FCM measurements in this study was the definition of an indicative reference cell count that is considered to be hygienically safe. Hygienically safety is ensured by cultivation based methods as described above. Based on the absence of bacterial, viral and protozoan indicators in abstraction well samples FCM measurements may be selected as reference cell counts. An exemplary scatter diagram of well#5 with parallel cultivation based measurements is shown in Figure 13.

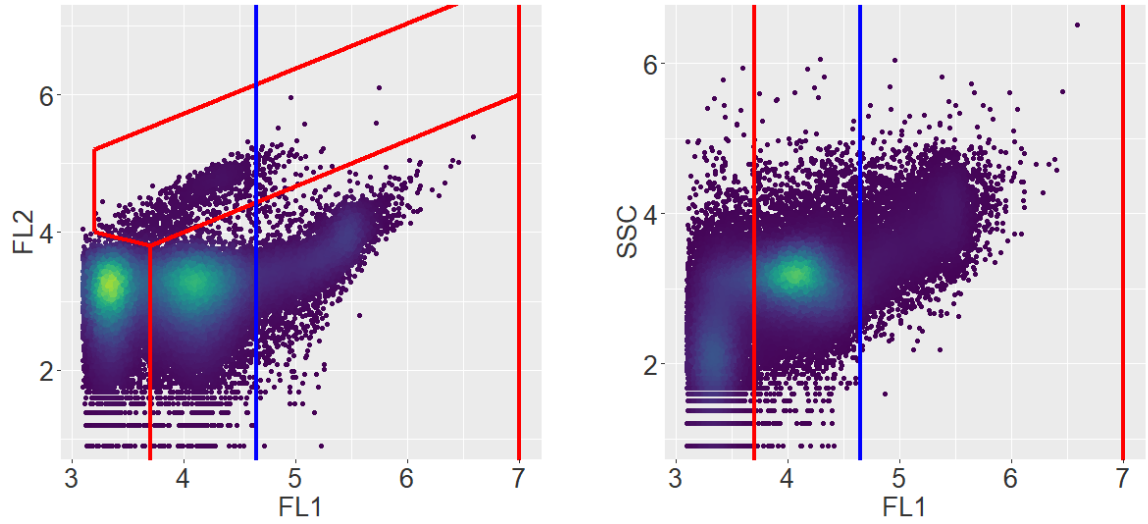

Figure 13. Flow cytometric analysis of indicative reference composition of well#5 (sample from 12.11.2019, measured at 12 am; green vs red fluorescence left; green vs side scatter (SSC) right)

The temporal variation over the sampling period was found to be rather stable in well#5 (see Figure 11 and Table 4). This finding may allow the establishment of an reference cell count. It can be argued that breakthrough of pathogens will alter the cell cluster and cell counts, since pathogens do not occur as single organisms but are acompained by bacterial communities. This cell counting reference concept can be included in monitoring procedures. In case of indicator detection on the water works level, FCM measurements allow fast screening of suspicous wells. Compared to conventional cultivation-based methods, FCM results are readily and much faster available.

DNA sequencing

DNA sequencing is revealing the bulk microbial taxonomic domains present in the samples. DNA counts were grouped in four superkingdoms: bacteria, viruses, eukaryota and archaea (Table 5).

Table 5. Distribution of taxa

Sampling station	Bacteria	Viruses	Eukaryota	Archaea
Basin 1 + 2 (n=1)	96 %	2.9 %	0.9 %	0.02 %
SPA168 (n=1)	82 %	0.05 %	14 %	3.4 %
Well#5 (n=1)	82 %	0.2 %	16 %	1.7 %

Bacteria is the most dominant taxonomic superkingdom at all sampling stations, showing a percental decrease from basin to groundwater samples. Viruses are found in the basin sample in the highest relative abundance. Eukaryota show an increase along the flow path. Organisms that have eukaryotic cells include protozoa and fungi. Archaea are single-celled microorganisms with structure similar to bacteria. These microorganisms lack cell nuclei and are therefore prokaryotes. Archaea are obligate anaerobes, living in environments low in oxygen and are found in the observation well and the abstraction well sample in elevated abundances.

The redox state along the flow path is oxic in the infiltration basin and SPA168 (Table 6). Unfortunately, oxygen measurements at abstraction well#5 are not available, but it is likely that redox state at the abstraction well is characterised by Fe/Mn-reducing conditions. Dissolved iron (Fe^{2+}) was measured in previous studies in concentrations of 1-2 mg/L at the well field Spandau-Nord.

Table 6. Physico-chemical water quality parameter (min-max)

Sampling station	Dissolved oxygen (mg/L)	Temperature (°C)	pH (-)	Electrical conductivity (µS/cm)
Infiltration basin (n = 10)	~11.1*	6 - 15	7.5 – 8.6	435 - 600
SPA168 (n = 10)	~4.6*	7 - 17	7.2 – 7.5	440 – 535
Well#5 (n = 9)	NA	13 - 14	7.3 – 7.5	520 – 621

NA = not available; *measured during single campaign with SmartTroll multimeter

The relative abundances of all examined samples on the phylum level is shown in Figure 14. The taxonomic composition of the microbial community is derived from the DNA sequencing results. Species that make up less than 0.1% of the total reads are shown as NA´s.

The abundances of bacterial phyla shows an increase in the microbial diversity from the basin to groundwater stations. In the basin only 5 different bacterial phyla were identified, of which the most abundant phyla are Actinobacteria (87%) and Proteobacteria (7.8%). In the observation well 23 different bacterial phyla and in the abstraction well 27 phyla were identified with more than 0.1% of the total reads. Relative abundances show that in the observation well about 65% and in the abstraction well about 49% of all species make up more than 0.1% of the total reads.

A very large proportion of Actinobacteria was detected in the basin sample. Actinobacteria are the most abundant microbes in freshwater systems (Neuenschwander et al., 2018). Actinobacteria are characterized by their ability to decompose organic matter in water. Species of the phylum Actinobacteria are omnipresent in the Basin sample (87 %), in the observation well (38%) and only 0.7% in the well sample. About 70% of the Actinobacteria in basin sample is classified as Candidatus Nanopelagicus. Metabolic reconstructions for Candidatus Nanopelagicus suggested an aerobic chemoheterotrophic lifestyle (Neuenschwander et al., 2018).

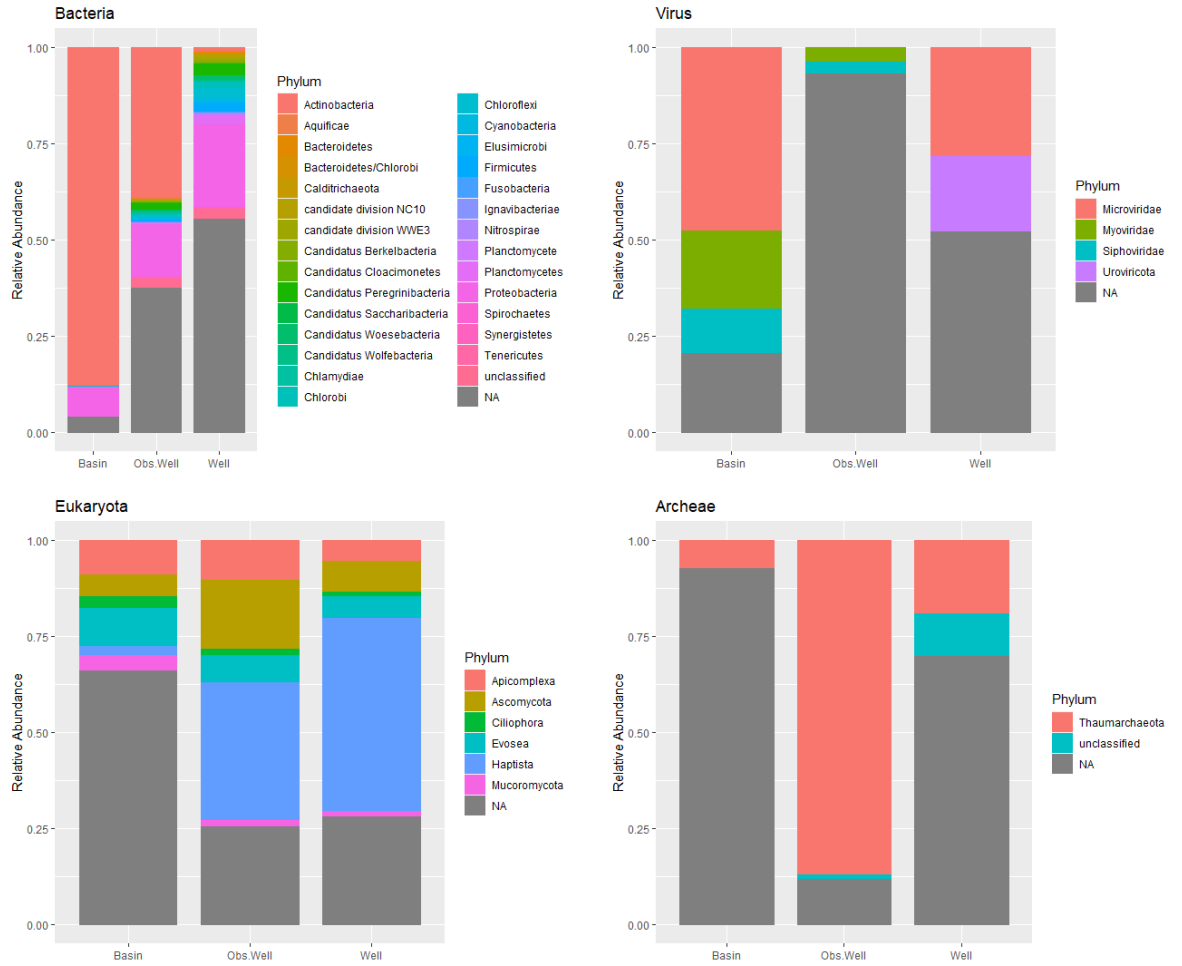

Figure 14. Relative abundances of phyla (NA = cut-off value 0.1%)

The Proteobacteria represents the only phylum that remains rather stable between sample groups in terms of relative abundance (Basin = 8%; Obs.Well = 11%; Well=17%). Proteobacteria contains most of the "commonly known" species, such as *E.Coli*. *E.Coli* was detected by cultivation in relative high abundances in all basin water samples (ranging from 1 to 23.8 cfu/100 mL, see Table 8 appendix). DNA sequence reads related to that species (e.g. *Escherichia coli, Escherichia albertii*) confirm the cultivation based analysis.

To explain the depth of analysis in DNA sequencing, it is useful to consider that only a total of approximately 0.5 million individual DNA sequences were measured in the infiltration basin sample. The total cell count in the basin sample from FCM is about 2.5 million cells/mL (see Table 4), or about 2.5×10^9 cells per litre. Given a sampling volume of approximately 1 litre of water (actually 800 ml) and that all cells from this volume that were retained on the filter are extracted, with a number of 2.5 billion cells, only 0.02 % of the DNA is actually measured. In the groundwater samples it may be assumed that the proportion of measured DNA is similar. In SPA168 a total of approx. 57,000 individual DNA sequences were measured. With a total cell count of approx. 0.9 million cells/mL (see Table 4), the proportion of measured DNA is then ca. 0.006%. A total of approx. 30,000 individual DNA sequences were measured in the well#5. With a total cell count of approx. 0.3 million cells/mL (see Table 4), the proportion of measured DNA is then about 0.01%. These estimations make clear that only a fraction of the total DNA was actually sequenced and it becomes clear that some species were not detected at all. General taxonomic profiling of all organisms present in a given sample is possible. However, it only gives a first overview of genera in complex metagenomes and individual species that occur in very low numbers will be difficult to detect.

The most abundant species in the well sample is the Eukaryota Emiliania huxleyi (8.1 %). Emiliania huxleyi is a species of coccolithophore found in almost all ocean ecosystems from the equator to sub-polar regions. The second abundant species found in the well sample is the Proteobacteria Sulfuricella denitrificans (7.6 %). Sulfuricella is a genus of facultatively anaerobic sulfur oxidizing bacteria often found in freshwater lakes. They are rod-shaped, mobile and gram-negative. They are able to oxidize elemental sulfur and thiosulfate to sulfate as the only energy source. The bacterium is microaerobic and could also use nitrate as electron acceptor and reduce it to nitrogen. The third most abundant species makes at least 2.4 % of the bacterium Candidatus Peribacter riflensis found in the well sample. The bacteria was found in groundwater in Colorado (USA) (Anantharaman et al., 2016a), but not much is known about their metabolism. The bacteria Candidatus Kuenenia stuttgartiensis was found with 1.8% in the well sample. This organism is capable of anammox reaction. This is an ammonium oxidation using nitrite as a reducing agent with the final product molecular nitrogen. After that Chlorobium phaeobacteroides occurs with 1.2 %. Chlorobium (also known as Chlorochromatium) is a genus of green sulfur bacteria. They are photolithotrophic sulfur oxidizers. The next abundant species is Dehalococcoides mccartyi that make up 1.0% of total count. Dehalococcoides mccartyi are anaerobic specialist restricted to reductive dehalogenation activity with its use of chlorinated and brominated compounds as their terminal electron acceptor (Löffler et al., 2013). Further, about 43 different species of clostridia have been found in the well sample. Clostridia are gram-positive, obligate anaerobic, spore-forming bacteria of the family Clostridiaceae. The endospores are heat resistant and can survive for many hours in boiling water, some of them for about one hour at 110 °C. Clostridia, with the exception of C. perfringens, can actively move with peritrichous flagella. The bacteria occur everywhere (ubiquitously), especially in soils and in the digestive tract of higher organisms. Through dust and soil particles they also get into food, where they can cause serious problems. The genus Clostridia contains both pathogens (pathogenic germs) and apathogenic species, some of which are used in biotechnology. Among the pathogenic species, Clostridium botulinum (causes botulism) and Clostridium tetani (causes tetanus) are the most important. The pathogens Clostridium botulinum were found with 0.2 % and Clostridium tetani with 0.003 % of total reads.

The most abundant viruses in the well were Gokushovirinae GNX3R (Phylum: Microviridae) and Lactobacillus phage Lrm1 (Phylum: Uroviricota) that make up 47% of the total viral DNA. Rotavirus A is the only DNA sequence of human pathogenic virus that was detected in well#5. In this context, it should be emphasized that the identification of DNA is based on the closest relative. It does not necessarily mean that the exact species with the exact characteristics is present in the sample. All other viral DNA are bacteriophages and not human pathogenic. In the obervation well no microviridae or uroviricota were found, instead siphoviridae and mayviridae..

The Bacteroidetes are widely distributed in the environment, including in soil, and sediments, as well as in the guts and on the skin of animals. Bacteroidetes are mainly particle-associated and strongly dependent on the availability of organic matter (Newton et al., 2011).

FCM total cell counts together with DNA sequencing data provides sample-specific estimated absolute taxon abundances (EAA). FCM total cell counts measured by flow cytometric data were multiplied by the relative abundances of taxa to reveal EAA as suggested by Props et al. (2017). EAA of detected taxa is shown in Figure 15.

As discussed before, the microbial diversity increases from the basin to the observation well and the abstraction well (Figure 15). In basin water only 7 phylums with species >0.1% constitute 93% of all reads, while in observation well 27, and in abstraction well 33 different phylums were measured. In the surface water Actinobacteria and Proteobacteria are dominimant. The groundwater at the abstraction well is characterised by Actinobacteria (31 %), Proteobacteria (9.9 %), Haptista (5.0 %), Thaumarchaeota (3.0 %), Ascomycota (2.3 %) and Apicomplexa (1.3 %). At the abstraction well the microbial diversity is even more pronounced.The Proteobacteria is here the most dominant phylum (16 %), followed by Haptista (8.1 %), Firmicutes (2.2 %), Chloroflexi (2.05 %), Planctomycetes (1.9 %), Chlorobi (1.3 %), Ascomycota (1.1 %), and Cyanobacteria (1.0 %).

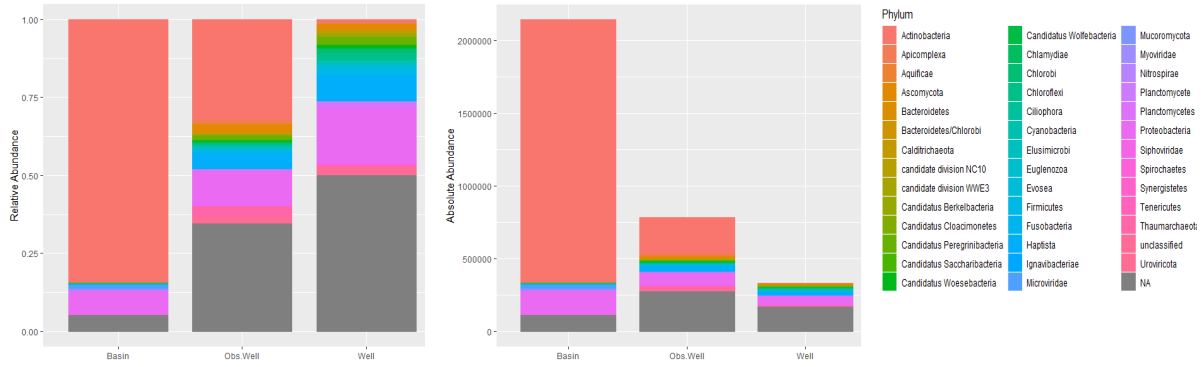

Figure 15. Relative and absolute abundances of total phyla (NA = cut-off value 0.1%)

Other studies show that metabolic plasticity involving the use of multiple electron donors and acceptors appears to be extremely common in microorganisms in subsurface environments (Anantharaman et al., 2016b). Therefore, multiple metabolic functions must be associated to a single organism. The interpretation of metabolic functions is discussed here only to a certain extend and many aspects remain open. Moreover, DNA sequencing was carried out for a single set of sample due to budget constraints. Results must be therefore considered preliminary.

5. SUMMARY AND OUTLOOK

The INOWAS platform provided the software infrastructure to perform evaluation processes for hydraulic residence time (HRT) calculation. The existing online monitoring system at the water works Berlin-Spandau was expanded by installation of additional sensors. The combination of online data with processing tools allows time-efficient HRT evaluation.

Automatic measurements by flow-through cytometry (FCM) were carried out under field conditions taking samples from surface water (infiltration basin) and groundwater (observation well, abstraction well) in parallel. The high-frequency flow cytometry data revealed decreasing order in total cell counts (TCC) from 2.5 $\times 10^6$ cells/mL in basin water, over 0.9 $\times 10^6$ cells/mL in the observation well to 0.3 $\times 10^6$ cells/mL in the abstraction well. The high-nucleic acid (HNA) counts in basin water are in absolute abundance and percentage higher than in the measured groundwater samples, revealing a microbial community shift towards higher percentage of LNA content bacteria in groundwater. The low-nucleic acid (LNA) and high-nucleic acid (HNA) content bacteria largely corroborated the changes in TCC fluctuations.

The observed constant measurements in the abstraction well may allow to use FCM fingerprinting as a fast monitoring tool in combination with cultivation based methods. However, long-term measurements of FCM for at least 6 months are recommended to assess seasonal fluctuation in both source water and groundwater. No measurement system has yet been able to detect fecal indicators such as *E.Coli* or coliform bacteria in the required concentration range for drinking water systems. On the other hand the total biological activity could be measured continuously. Other studies have shown that there was a correlation between the total biological activity and the total cell count (Brand and Wülser, 2018).

The DNA sequencing showed the presence of a diverse bacterial community and the distribution of various phylogenetic groups in surface- and groundwater. It was demonstrated to genomically describe thousands of microorganisms from an groundwater augmentation site. Total cell counts from flow cytometric data were multiplied by the relative abundances of taxa to reveal sample-specific estimated absolute taxon abundances. The DNA sequencing in combination with FCM measurements showed that total cell counts decreased along the flow path while the biodiversity increased. In future studies, the genome-specific metabolic potential should be determined by profiling all the genes against specific databases to reveal metabolic functional groups.

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7. ANNEX

Table 7. Flow rate control of FCM device

Location	Date Time	Flow rate (mL/min)
Basin1+2	02.10.2019 14:00	380
SPA168	02.10.2019 14:00	400
Basin1+2	04.10.2019 12:00	350
SPA168	04.10.2019 12:00	370
Basin1+2	07.10.2019 15:30	300
SPA168	07.10.2019 15:30	400
Basin1+2	10.10.2019 15:30	350
SPA168	10.10.2019 15:30	400
Basin1+2	15.10.2019 11:45	380
SPA168	15.10.2019 11:45	410
Basin1+2	17.10.2019 14:30	300
SPA168	17.10.2019 14:30	310
Basin1+2	22.10.2019 11:00	350
SPA168	22.10.2019 11:00	280
Basin1+2	24.10.2019 09:30	350
SPA168	24.10.2019 09:30	380
SPANord5	31.10.2019 14:00	270
SPANord5	05.11.2019 12:00	260
SPANord5	07.11.2019 12:00	300
SPANord5	12.11.2019 13:00	280
SPANord5	19.11.2019 13:00	270
SPANord5	22.11.2019 09:00	280

Table 8. Microbial cultivation based results

Sampling site	Sampling date	Coliform Bacteria (cfu/100 mL)	Escherichia coli (cfu/100 mL)	Somat. Coliphages (pfu/100 mL)	Clostridium perfringens (cfu/100 mL)	Enterococci (cfu/100 mL)
Basin 1+2	10.10.2019	>200.5	1	0	0	5.3
	15.10.2019	>200.5	23.8	30	3	88.5
	17.10.2019	>200.5	20.7	93	0	12.4
	22.10.2019	>200.5	7.5	162	0	8.7
	24.10.2019	>200.5	7.5	62	0	>200.5
	29.10.2019	>200.5	13.7	4	0	19.2
	31.10.2019	>200.5	6.4	4	0	22.2
	05.11.2019	>200.5	9.9	8	6	22.2
	07.11.2019	>200.5	4.2	14	0	5.3
	12.11.2019	129.8	1	0	0	2
	14.11.2019	88.5	1	8	0	2
SPA168	10.10.2019	1	0	0	0	0
	15.10.2019	1	0	0	0	0
	17.10.2019	1	1	0	0	0
	22.10.2019	0	0	0	0	0
	24.10.2019	0	0	0	0	0
	29.10.2019	13.7	0	0	0	0
	31.10.2019	0	0	0	0	0
	05.11.2019	2	0	0	0	0
	07.11.2019	0	0	0	0	0
	12.11.2019	1	0	0	0	0
	14.11.2019	2	0	0	1	0
Well#5	10.10.2019	0	0	0	0	0
	15.10.2019	0	0	0	0	0
	17.10.2019	0	0	0	0	0
	22.10.2019	0	0	0	0	0
	24.10.2019	0	0	0	0	0
	29.10.2019	0	0	0	0	0
	31.10.2019	0	0	0	0	0
	05.11.2019	0	0	0	0	0
	07.11.2019	0	0	0	0	0
	12.11.2019	0	0	0	0	0
	14.11.2019	0	0	0	0	0

Table 9. In-situ measurements with Eijkelkamp Multimeter 18.50.01

Sampling station	Sampling Date	pH	el. Cond (µS/cm)	Water temp. °C
Well#5	07.10.2019	7.49	559.0	13.2
	10.10.2019	7.42	520.8	13.7
	15.10.2019	7.44	527.3	14.3
	17.10.2019	7.42	571.7	14.0
	22.10.2019	7.49	620.8	13.9
	24.10.2019	7.43	567.3	14.0
	29.10.2019	7.34	573.5	12.5
	05.11.2019	7.50	523.0	13.8
	12.11.2019	7.49	520.0	13.7
Basin 1+2	07.10.2019	7.72	440.3	11.6
	10.10.2019	7.56	445.0	13.1
	15.10.2019	7.53	443.1	15.3
	17.10.2019	7.48	535.2	13.9
	22.10.2019	7.57	502.6	14.1
	24.10.2019	8.59	435.0	14.2
	29.10.2019	7.78	456.0	11.6
SPA168	07.10.2019	7.22	440.3	17.0
	10.10.2019	7.24	494.4	16.2
	15.10.2019	7.25	535.1	15.7
	17.10.2019	7.40	452.9	14.9
	22.10.2019	7.34	455.8	15.4
	24.10.2019	7.30	516.9	14.8
	29.10.2019	7.25	452.0	14.1
	05.11.2019	7.38	458.0	13.0
	12.11.2019	7.24	467.0	11.8
	29.11.2019	7.54	534.0	7.10