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Arsenic removal from drinking water by a household sand filter in Vietnam — Effect of filter usage practices on arsenic removal efficiency and microbiological water quality

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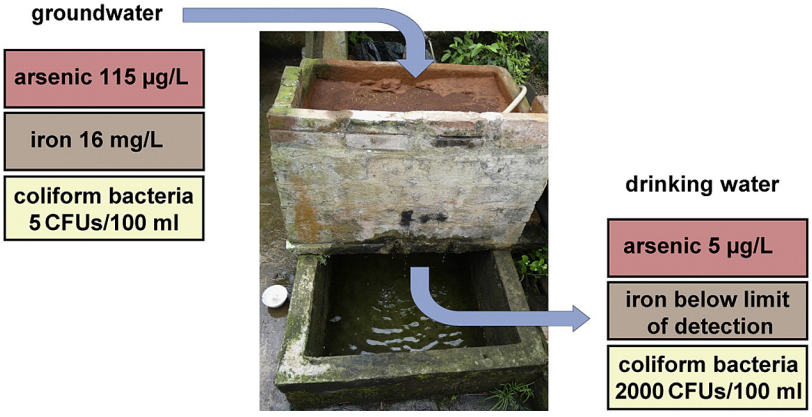
HIGHLIGHTS

- Efficiency of As removal from As(III)-/Fe(II)-rich water by sand filter was studied.
- Fe(II) in groundwater is oxidized and resulting Fe(III) minerals bind As.
- Periods of intense daily filter use or non-use did not affect As removal efficiency.
- Filter efficiency was maintained even directly after sand replacement.
- CFUs of coliform bacteria increased during filtration causing potential health risk.

GRAPHICAL ABSTRACT



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ABSTRACT

Household sand filters are applied to treat arsenic- and iron-containing anoxic groundwater that is used as drinking water in rural areas of North Vietnam. These filters immobilize poisonous arsenic (As) via co-oxidation with Fe(II) and sorption to or co-precipitation with the formed Fe(III) (oxyhydr)oxides. However, information is lacking regarding the effect of the frequency and duration of filter use as well as of filter sand replacement on the residual As concentrations in the filtered water and on the presence of potentially pathogenic bacteria in the filtered and stored water. We therefore scrutinized a household sand filter with respect to As removal efficiency and the presence of fecal indicator bacteria in treated water as a function of filter operation before and after sand

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replacement. Quantification of As in the filtered water showed that periods of intense daily use followed by periods of non-use and even sand replacement did not significantly ($p < 0.05$) affect As removal efficiency. The As concentration was reduced during filtration from 115.1 ± 3.4 μg L^{-1} in the groundwater to 5.3 ± 0.7 μg L^{-1} in the filtered water (95% removal). The first flush of water from the filter contained As concentrations below the drinking water limit and suggests that this water can be used without risk for human health. Colony forming units (CFUs) of coliform bacteria increased during filtration and storage from 5 ± 4 per 100 mL in the groundwater to 5.1 ± 1.5 × 10^3 and 15 ± 1.4 × 10^3 per 100 mL in the filtered water and in the water from the storage tank, respectively. After filter sand replacement, CFUs of *Escherichia coli* of <100 per 100 mL were quantified. None of the samples contained CFUs of *Enterococcus* spp. No critical enrichment of fecal indicator bacteria belonging to *E. coli* or *Enterococcus* spp. was observed in the treated drinking water by qPCR targeting the 23S rRNA gene. The results demonstrate the efficient and reliable performance of household sand filters regarding As removal, but indicate a potential risk for human health arising from the enrichment of coliform bacteria during filtration and from *E. coli* cells that are introduced by sand replacement.

1. Introduction

South and South-East Asian countries, including Vietnam, are affected by microbially contaminated surface waters and As-contaminated groundwater (Smedley and Kinniburgh, 2002; van Geen et al., 2003). This causes a dilemma for the supply of safe, consumable drinking water. Pathogenic microorganisms in the drinking water can cause diarrheal diseases or other severe health problems with potentially lethal consequences (UNICEF and WHO, 2009; WHO, 2011). Continuous consumption of drinking water exceeding the World Health Organization (WHO) guideline level of 10 μg L^{-1} As may lead to chronic health impairments such as skin lesions, various forms of skin and internal cancer, peripheral vascular disease, neurological effects, hypertension, and cardiovascular disease (Naujokas et al., 2013; Muehe and Kappler, 2014).

Until the mid-1990s, surface waters and shallow dug wells were the main sources of drinking water for the rural inhabitants of the Red River Delta in North Vietnam (Berg et al., 2001). Inadequate sanitary standards frequently resulted in the introduction of fecal bacteria into surface water bodies and dug wells. Therefore, the rural drinking water supply was shifted, in addition to using rainwater, to presumably pathogenic-free groundwater by installing tens of thousands of household tube wells (~10–100 m deep) (Berg et al., 2006). Unfortunately, at that time, quality control of the groundwater according to international guidelines did not include As. In the mid-2000s, the inhabitants of the Red River Delta began to show the first symptoms indicative of chronic As poisoning (Tobias and Berg, 2011) stemming from As that is present in the aquifers together with high concentrations of dissolved Fe(II) (Winkel et al., 2011). High amounts of aqueous Fe(II) impair the taste and color of drinking water (Berg et al., 2006) and therefore, in the mid-1990s, people in the rural areas of North Vietnam installed household sand filters to remove Fe(II) from groundwater (Fig. 1) (Berg et al., 2006). These simple sand filters are mainly applied for purification of smaller amounts of water for drinking, cooking, and washing for individual households and not for purification of larger amounts of water, e.g. for irrigation purposes.

During water filtration, dissolved Fe(II) is oxidized to Fe(III) (oxyhydr)oxide precipitates (Voegelin et al., 2014). Later was it found that As is simultaneously removed from the contaminated groundwater by arsenite oxidation and either sorption to or co-precipitation with the newly formed Fe(III) phases (Berg et al., 2006). This study showed that over 90% of the investigated 43 sand filters reduced As concentrations to levels below the previous national limit of 50 μg L^{-1} and 40% of the filters to levels below the WHO guideline value of 10 μg L^{-1}. Additionally, these authors quantified As concentrations in the outflow water of four sand filters in 3-min intervals for 10 min and observed no variations of As concentrations between the different time points. The efficiency of the sand filters was shown to depend on the Fe:As ratio in the groundwater, which should be ≥50 for As removal to ensure a reduction of dissolved As to concentrations below 50 μg L^{-1}. Since this condition is mostly fulfilled in As-contaminated groundwaters in North Vietnam

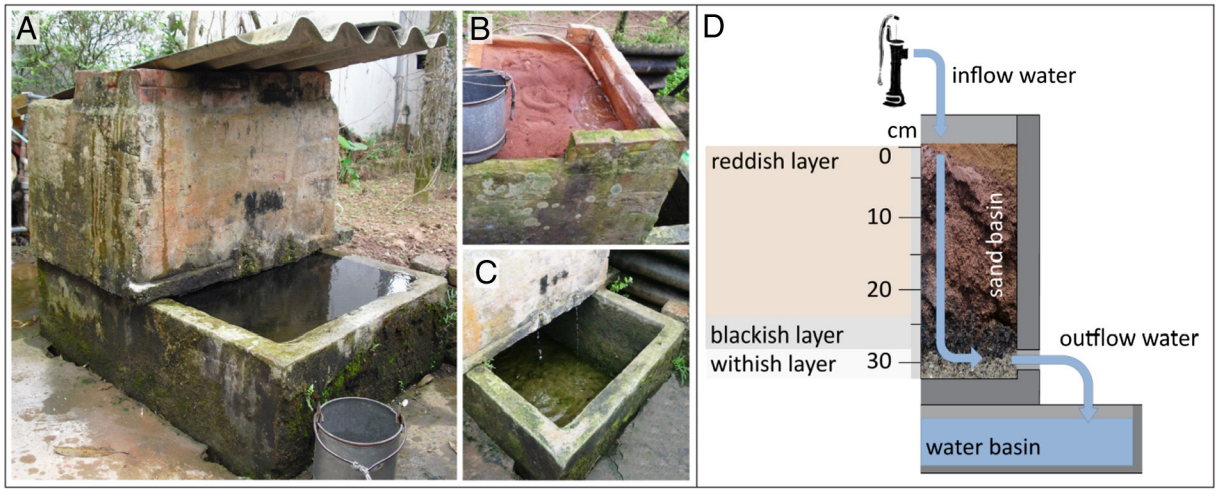

Fig. 1. Example of a household sand filter, commonly used in the rural areas of the Red River Delta, North Vietnam, to reduce As and Fe concentrations in drinking water. (A) Filters consist of two basins stacked on top of each other and covered by a corrugated metal sheet. (B) The upper basin is filled with locally available sand (e.g., from the river banks of the Red River) and groundwater is pumped from a tube well onto the upper basin, trickles through the sand and leaves through holes at the bottom. (C) The water is collected in the lower basin, which serves as a water storage tank. (D) Schematic cross section of a household sand filter depicting the vertical profile of the sand material, which is differently colored according to the precipitation of orange/brown iron minerals and black manganese minerals. The whitish layer at the bottom shows the original color of the sand that was used.

(Berg et al., 2008), these sand filters are a widely used water treatment method, in comparison e.g. to Bangladesh, where this criteria is often not fulfilled (Hug et al., 2008). Sand filters are therefore the preferred treatment method in rural areas in North Vietnam in comparison to membrane-based technologies, photochemical/photocatalytical oxidation, and application of ion-exchangers, adsorbents, and coagulant-flocculants (Berg et al., 2006; Mondal et al., 2013). The advantages of sand filters are that they can be built from easily accessible materials by the affected communities, their operation procedures are simple, installation and operation costs are low, and an amount of groundwater sufficient for one household can be treated within just a few minutes (Tobias and Berg, 2011). Potential disadvantages of such filters are clogging over time and the formation of preferential flow paths within the filter, which may lower the contact time of water with the sand material, the lack of quality control and the lack of incentives that lead to changes in filter usage behavior.

Although household sand filters in North Vietnam have been shown to be an effective tool for mitigating As exposure, the efficiency, i.e. the ability of the filter to remove As to concentrations $<10\ \mu g\ L^{-1}$ has not been investigated systematically with respect to varying filter usage practices. Potential changes in As removal efficiency have not been examined to date with respect to intermittent filter use or different usage durations, or after several days of not operating the filter. Variations in As removal efficiency might occur after several hours/days of filter disuse. Waterlogging and the presence of organics in the filter could stimulate Fe(III)-reducing bacteria and the reductive dissolution of the As-loaded Fe-minerals in the sand filter (McArthur et al., 2001; Islam et al., 2004; Weber et al., 2009). Additionally, As(V)-reducing bacteria could reduce As(V) to As(III) (Zobrist et al., 2000; Tufano et al., 2008). Arsenite is expected to sorb to a lesser extent to Fe(III) (oxyhydr)oxides than arsenate at low As concentrations and at pH values of ≤ 7 (Dixit and Hering, 2003; Stachowicz et al., 2008; Huang et al., 2011) and hence could be released into the water. Furthermore, the effects of sand replacement on As removal are not known; i.e., whether an equally effective As removal occurs immediately after sand exchange. Filter sand replacement could reduce the As removal efficiency, because the new sand does not initially contain sufficient Fe(III) (oxyhydr)oxides and such minerals have yet to be formed on the new sand. Fe(III) (oxyhydr)oxide precipitates, however, are essential for a fast heterogeneous Fe(II) oxidation, which is suggested to be the prominent Fe(II) oxidation and Fe(III) precipitation mechanism in such sand filter systems (van Beek et al., 2012; Voegelin et al., 2014), leading to efficient sorption and co-precipitation of the As. In addition to insufficient As removal in the presence of fresh sand, the introduction of microorganisms from the sand into the water as well as the presence and growth of harmful microorganisms in the open water storage tank after filtration may pose a risk for the health of the consumers.

The main goal of the present study was therefore to determine whether prolonged or discontinuous use of a sand filter as well as sand replacement influences the efficiency of As removal from the water. To this end, we quantified Fe and As concentrations in the inflow and outflow water during different filter operation scenarios. We also quantified fecal indicator bacteria in the groundwater, in the filtered water, and in the water storage tank. The overall aim of this study was to gather critical knowledge on how filter operation affects filter performance parameters with respect to As removal and pathogen load.

2. Material and methods

2.1. Sampling site

The study was conducted with a representative household sand filter (Fig. 1) in the rural village of Van Phuc, 10 km Southeast of Hanoi City, Vietnam (20°55′08″ N, 105°54′08″ E). In this area, the aquifer groundwater is contaminated by As (average 121 $\mu g\ L^{-1}$ and up to 340 $\mu g\ L^{-1}$) (Berg et al., 2008) and in most cases the groundwater contains at least 50 times more Fe than As (concentration ratio) and low phosphate concentrations ($<1\ mg\ L^{-1}$), which is a prerequisite for the ability of the sand filter to lower the As concentration in the water to values below $50\ \mu g\ L^{-1}$ (Berg et al., 2006). The sand filter is privately owned and regularly used by one family. The sand filter studied here is common and representative for many other filters in terms of its shape, size and materials (M. Berg, personal communication). The filter bed is composed of fine gravel to very fine sand and usually originates from the Red River banks. The groundwater is obtained via a 45 m deep tube well and contained ~115 $\mu g\ L^{-1}$ of As (see Results) and is therefore also representative for this area. Detailed information regarding filter dimensions and filter bed properties are summarized in Voegelin et al., (2014). For the present study, the sand filter was sampled twice, in March 2012 and in March 2013, to verify the sand filter performance over a larger time frame. The sampling schemes for both campaigns are shown in Tables SI 1 and SI 2.

2.2. Sampling and treatment of sand filter water

The inflow water (freshly pumped groundwater before it entered the sand filter) and the outflow water (water that had passed through the sand filter) were sampled three times per day (at 10 am, 1 pm, and 4 pm). The sampling schedule simulated typical daily filter use of a Vietnamese family in rural areas with a water demand in the morning, during lunchtime and in the afternoon. The outflow water samples were taken immediately with the first drops of filtered water. For the 10 am sampling session, additional outflow water samples were taken at 10 and 30 min after the first water flush, to check the filters for short-term and long-term As-leaching. The sampling included outflow water samples during days of intensive filter use, after hours to days of non-use, and after filter sand replacement. Samples were taken for total As, Fe, Mn, arsenite and PO_4^{3-} for lab analyses and for total Fe and Fe(II) for field analyses and were preserved for analysis as described in the SI. Dissolved organic carbon (DOC) and inorganic ions (Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}, F^-, Cl^-, NO_3^-, SO_4^{2-}) were determined in the daily 1 pm samples and preserved for analysis as described in the SI.

2.3. Sand filter solid phase characterization

For sampling of the sand filter material, a vertical profile was dug into the sand filter material. Bulk samples were collected from 6 different depths by pushing 50 mL plastic tubes horizontally into the sand filter material. Samples were stored anoxically in Anaerocult® bags (Merck) and cooled in the dark until further processing. The analyses of the filter sand by X-ray fluorescence (XRF), micro X-ray diffraction (μXRD), Mössbauer spectroscopy, and sequential extractions of Fe after Roden and Zachara (1996) modified by Amstaetter et al. (2012) and of As after Huang and Kretzschmar (2010) are described in the SI.

2.4. Analytical methods

Flow cell electrodes for dissolved O_2 (FDO 925, WTW), pH (Sentix 940, WTW), and electrical conductivity (TetraCon 925, WTW) analyses were connected to a multi-parameter analyzer (Multi 3430, WTW). Redox potential was measured with a combination electrode (SensoLyt WQL-Pt, WTW) with a pH/mV device (pH 340i, WTW).

Total Fe and Fe(II) of water samples were quantified in triplicates in the field using the ferrozine assay (Stookey, 1970) and a portable spectrophotometer (DR/2010, HACH). The inflow and outflow water samples were tested at ratios of sample to ferrozine solution of 1:5 and 1:2 (v/v), respectively. Total elemental concentrations (Fe, Mn, As) in water samples were quantified in duplicates by ICP-MS (ICP-MS, Agilent 7500ce). Arsenic was separated in the field into total As and arsenite by filtration through a disposable anion exchange cartridge (MetalSoft Center, Piscataway, USA) (for details see the manufacturer's manual) and quantified in duplicates by ICP-MS. Inorganic ions (Na^+,

NH_4^+, K^+, Mg^{2+}, Ca^{2+}, F^-, Cl^-, NO_3^-, SO_4^{2-}) were determined in duplicates by ion chromatography (DionexDX120 equipped with an AS9HC column and an AG9HC precolumn). DOC was quantified in triplicates with a total organic carbon analyzer (High TOC II, Elementar). Phosphate was analyzed colorimetrically in duplicates by the molybdenum blue method using a Varian Cary 100 photometer. According to Boltz and Mellon (1947) no interference of As at present As and phosphate concentrations are expected with this assay.

2.5. Quantification of fecal indicator bacteria

The presence of fecal indicator bacteria in the inflow, outflow, and storage water was investigated with cultivation-dependent and cultivation-independent techniques during the sampling campaign in 2013 (10 am sampling session over several days, as shown in Table SI 1). For culture-dependent experiments, water was collected in sterile 1 L glass bottles which were cooled on ice during transport and analyzed in the laboratory at Hanoi University. For culture-independent quantification of fecal indicator bacteria, 100 mL of water were filtered on site through membrane filters to concentrate biomass for nucleic acid extraction (0.22 μm Express® Plus Membrane, Millipore). Filters were cooled on ice, transported to the laboratory, and stored at −20 °C until further use. Total cell numbers and fecal indicator bacteria were quantified by plate counts in duplicates or triplicates according to German drinking water regulations (1990) and guidelines of the USEPA (EPA Methods 1603 and 1600). 16S/23S rRNA gene targeted quantitative PCR (qPCR) was carried out in duplicates as described in detail in the SI. Coliform bacteria, *Escherichia coli*, and *Enterococcus* spp., were quantified by plate counts while *E. coli* and *Enterococcus* spp. were also quantified by 16S/23S rRNA gene based qPCR.

The plate count method is a standardized procedure described in national and international water quality assessment guidelines and it is frequently used to quantify microorganisms and to monitor the bacterial contamination of water resources. However, this method is limited to

Table 1
Geochemical parameters of the inflow and outflow water of the household sand filter in Van Phuc, Hanoi, Vietnam. Shown are mean values and standard deviations and the number of samples n in brackets for all parameters of the sampling campaigns in 2012 and 2013, respectively.

	2012		2013	
	Inflow water	Outflow water	Inflow water	Outflow water
pH	6.89 ± 0.04 (n = 4)	7.06 ± 0.03 (n = 4)	6.88 ± 0.01 (n = 28)	7.08 ± 0.10 (n = 28)
O2 [mg L^{-1}]	<0.05 (n = 6)	5.42 ± 0.36 (n = 6)	<0.05 (n = 28)	5.16 ± 0.66 (n = 28)
Eh [mV]	n.d.	n.d.	−166 ± 11 (n = 7)	+171 ± 20 (n = 10)
EC [μS cm^{-1}]	1353 ± 1 (n = 6)	1233 ± 11 (n = 6)	1318 ± 5 (n = 28)	1196 ± 140 (n = 28)
Fe species [mg L^{-1}]				
Fe (ICP-MS)	15.7 ± 2.0 (n = 9)	<0.01 (n = 14)	16.3 ± 0.5 (n = 16)	<0.01 (n = 20)
Fe (total) (ferrozine assay)	19.7 ± 1.8 (n = 19)	<0.05 (n = 32)	18.1 ± 0.8 (n = 28)	<0.05 (n = 122)
Fe(II) (ferrozine assay)	19.2 ± 1.8 (n = 19)	<0.05 (n = 32)	17.3 ± 0.6 (n = 28)	<0.05 (n = 122)
As species [μg L^{-1}]				
As (total)*	117.8 ± 5.0 (n = 9)	4.5 ± 1.0 (n = 14)	115.1 ± 3.4 (n = 16)	5.3 ± 0.7 (n = 20)
Arsenite	107.9 ± 6.0 (n = 9)	<0.1 (n = 14)	110.8 ± 5.0 (n = 16)	<0.1 (n = 20)
Mn [μg L^{-1}]*	1228.00 ± 139.6 (n = 9)	169.4 ± 155.3 (n = 14)	1187.8 ± 31.2 (n = 16)	4.2 ± 7.4 (n = 22)
Cations/anions [mg L^{-1}]				
Na^{+}**	28.5 ± 3.0 (n = 4)	27.9 ± 6.1 (n = 4)	36.0 ± 0.7 (n = 10)	36.0 ± 1.0 (n = 10)
$NH4^{+}$*	n.d.	2.1 ± 1.0 (n = 4)	6.4 ± 0.3 (n = 10)	1.6 ± 0.8 (n = 10)
K^{+}**	3.5 ± 0.7 (n = 4)	2.8 ± 0.8 (n = 4)	2.7 ± 0.1 (n = 10)	2.7 ± 0.2 (n = 10)
Mg^{2+}**	31.2 ± 3.9 (n = 4)	28.2 ± 4.7 (n = 4)	42.5 ± 2.0 (n = 10)	44.3 ± 1.8 (n = 10)
Ca^{2+}**	130.6 ± 15.3 (n = 4)	104.6 ± 24.6 (n = 4)	196.0 ± 10.7 (n = 10)	167.8 ± 19.8 (n = 10)
F^{-}*	0.2 ± 0.1 (n = 4)	0.1 ± 0.0 (n = 4)	0.3 ± 0.0 (n = 10)	0.1 ± 0.0 (n = 10)
Cl^{-}**	69.3 ± 2.9 (n = 4)	67.9 ± 5.4 (n = 4)	64.6 ± 0.6 (n = 10)	64.0 ± 0.8 (n = 10)
$NO3^{-}$*	1.3 ± 1.0 (n = 3)	26.3 ± 8.4 (n = 4)	0.1 ± 0.0 (n = 9)	19.0 ± 7.0 (n = 10)
$SO4^{2-}$*	16.4 ± 2.2 (n = 4)	10.2 ± 2 (n = 4)	8.0 ± 0.3 (n = 9)	3.8 ± 0.4 (n = 10)
$PO4^{3-}$-P*	0.5 ± 0.7 (n = 6)	0.1 ± 0.0 (n = 6)	0.5 ± 0.0 (n = 10)	0.1 ± 0.0 (n = 10)
DOC [mg L^{-1}]**	2.9 ± 0.2 (n = 6)	2.5 ± 0.5 (n = 6)	2.8 ± 0.2 (n = 10)	2.7 ± 0.4 (n = 10)

Eh, redox potential; O2, dissolved oxygen; EC, electric conductivity; n.d., not determined.
* Extremely significant ($p < 0.001$) difference between inflow and outflow water.
** No significant ($p > 0.05$) difference between inflow and outflow water.

the detection of microorganisms that are able to grow under laboratory conditions on the selected media, which is suggested to be less than 1% of the total microbial community (Zengler, 2009; Puspita et al., 2012). Moreover, the method is based on the assumption that one CFU descends from only one cell; hence, cell aggregation or clumping of cells underestimates the real number of living cells. In contrast, the detection of gene copy numbers by qPCR avoids the disadvantages of the cultivation bias of the plate count methods; however, one has to take into account that qPCR quantifies gene copy numbers regardless of whether the amplified gene originates from living cells, dead cells, or free DNA. Furthermore, qPCR based 16S/23S rRNA gene counts are usually higher than direct cell counts because many microorganisms contain more than one ribosomal RNA operon. Thus, plate counts generally underestimate and qPCR generally overestimates total or group-specific cell numbers. According to this method-inherent cell number trend, we quantified approx. 1000-fold higher cell numbers by qPCR compared to plate counts.

3. Results

3.1. Geochemical analyses (including pH, E_h, and dissolved oxygen) and quantification of major ions in inflow and outflow water

Results of the geochemical analyses of water samples collected during both sampling campaigns are summarized in Table 1. The average pH of the inflow and outflow water was near neutral and constant in both sampling years 2013 and 2012, with values of 6.88 ± 0.01 and 6.89 ± 0.04 in the inflow water and 7.08 ± 0.10 and 7.06 ± 0.03 in

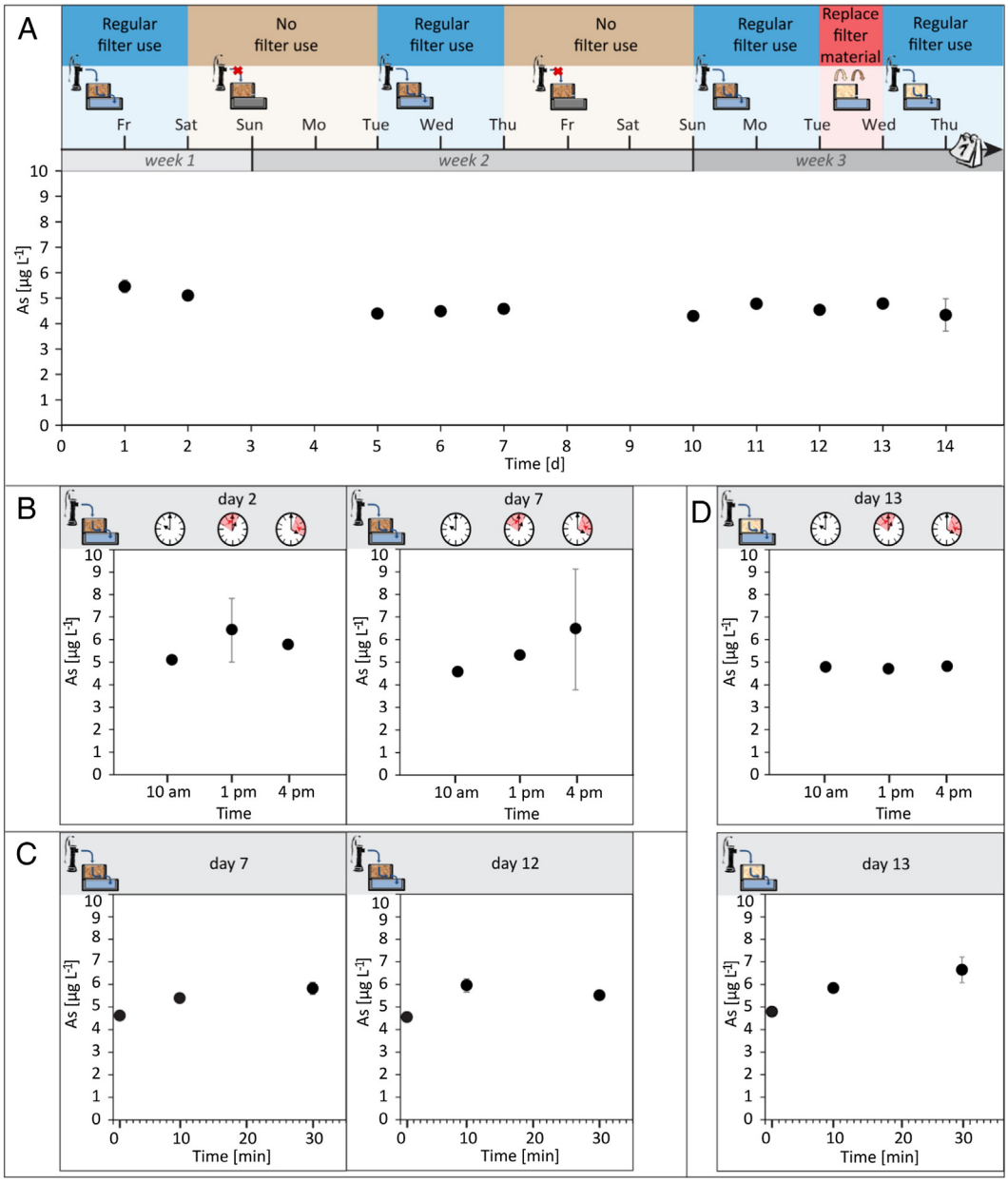

Fig. 2. Arsenic concentrations in the water after filtration through the sand filter (outflow water) during the sampling campaign in 2013. (A) Concentration of As in the initial first milliliters of the filtered outflow water, quantified over a period of 14 days during periods of regular daily use followed by periods of non-use, and after sand material replacement. (B) Concentration of As in the initial first milliliters of the filtered outflow water at two different days (days 2 and 7) during regular filter use in the morning (10 am), at midday (1 pm), and in the afternoon (4 pm). (C) Concentration of As in the filtered water collected after 1, 10, and 30 min of continuous filter operation on days 7 and 12. (D) Concentration of As in the filtered outflow water after the filter sand material was replaced. The data quantified at different times during day 13 (upper panel) and after 1, 10, and 30 min of continuous filter operation (lower panel) are shown. Mean values and range of duplicate field samples are shown. If no error bars are visible they were smaller than the symbols.

the outflow water, respectively. The average concentrations of dissolved oxygen were <0.05 mg L^{-1} in the inflow water (2013 and 2012) and 5.16 ± 0.66 mg L^{-1} (2013) and 5.42 ± 0.36 mg L^{-1} (2012) in the outflow water. The redox potential of the inflow water was −166 ± 11 mV and indicated reducing conditions, while suboxic conditions were present in the outflow water, which had a redox potential of +171 ± 20 mV measured in 2013.

The inflow water during the sampling campaign in 2013 contained, on average, 115.1 ± 3.4 μg L^{-1} total As, of which ~96% was present as arsenite. The inflow water contained 16.3 ± 0.5 mg L^{-1} total Fe (ICP-MS analysis) while the spectrophotometric ferrozine assay yielded a slightly higher value of 18.1 ± 0.8 mg L^{-1} total Fe with ~96% Fe(II). No significant variations in As and Fe concentrations were observed in the inflow water during the entire several-week sampling periods of both campaigns (Figures SI 1 and SI 2). The outflow water contained, on average, 5.3 ± 0.7 μg L^{-1} of total As in 2013. In the outflow water, the arsenite and total Fe were below the detection limit (0.1 μg L^{-1} for As (ICP-MS), 10 μg L^{-1} for Fe (ICP-MS) and 50 μg L^{-1} for Fe (ferrozine assay)) for most of the samples. Fe and As concentrations measured in the inflow and outflow water during the sampling campaign in 2013 were comparable to the data collected in 2012 (Table 1). Mn concentrations of >1000 μg L^{-1} were quantified in the inflow water in both sampling years. Mn concentrations in the outflow water showed strong differences between as well as during the two sampling campaigns. Concentrations as low as 0.4 ± 0.3 μg L^{-1} (2013) and 20.0 ± 4.2 μg L^{-1} (2012) and maximum concentrations of 17.8 ± 20.5 μg L^{-1} (2013) and 470.5 ± 108.2 μg L^{-1} (2012) have been quantified (Table 1).

The DOC concentrations of the inflow water of 2.8 ± 0.2 mg L^{-1} (2013) and 2.9 ± 0.5 mg L^{-1} (2012) did not differ significantly to the concentrations in the outflow water of 2.7 ± 0.4 mg L^{-1} (2013) and 2.5 ± 0.5 mg L^{-1} (2012) (Table 1). The concentrations of PO_4^{3-}, SO_4^{2-}, NH_4^+, and F^- were lower and NO_3^- was higher in the outflow compared to the inflow water in both sampling years (Table 1). In particular, NH_4^+ decreased from 6.4 ± 0.3 mg L^{-1} (inflow) to 1.6 ± 0.8 mg L^{-1} (outflow) while NO_3^- increased from 0.1 ± 0.0 mg L^{-1} (inflow) to 19.0 ± 7.0 mg L^{-1} (outflow). The concentrations of other ions including K^+, Mg^{2+}, Ca^{2+}, and Cl^- did not change between the inflow and the outflow water (Table 1).

3.2. Sand filter performance

The results of the sand filter performance experiments during the sampling campaign in 2013 are summarized in Fig. 2 and for the year 2012 in Figure SI 3. Total As concentrations in 2013 remained constant in the outflow water over a period of 14 days during regular daily use, after periods of non-use, and after sand replacement (Fig. 2A). On days where the filter was used three times (at 10 am, 1 pm, and at 4 pm), total As concentrations did not change in the outflow water (Fig. 2B). A slight but significant ($p < 0.05$) increase in total As concentration was quantified in the outflow water between 1, 10 and 30 min of continued sand filter use (Fig. 2C) while a similar increase was not quantified in 2012 (Fig. SI 3 C and D). Variation in total As in the outflow was low within one day and within 30 min of filter usage or after sand material was replaced (Fig. 2D). The data collected in 2012 showed similar results (Fig. SI 3). Overall, more than 95% of total As and 100% of total Fe were removed from the inflow water during filtration by the sand filter during both sampling campaigns. Concentrations of As were reduced below the WHO recommended value of 10 μg L^{-1}.

3.3. Sand filter solid phase characterization

The filter sand was characterized by a reddish colored layer down to ~23 cm depth, followed by a blackish layer with a thickness of ~5 cm, and a bottom layer of whitish sand of ~5 cm in 2012 (Fig. 1). In 2013, the reddish layer was ~17 cm thick, the blackish layer was more pronounced with 16 cm thickness, and the whitish layer was still ~5 cm thick.

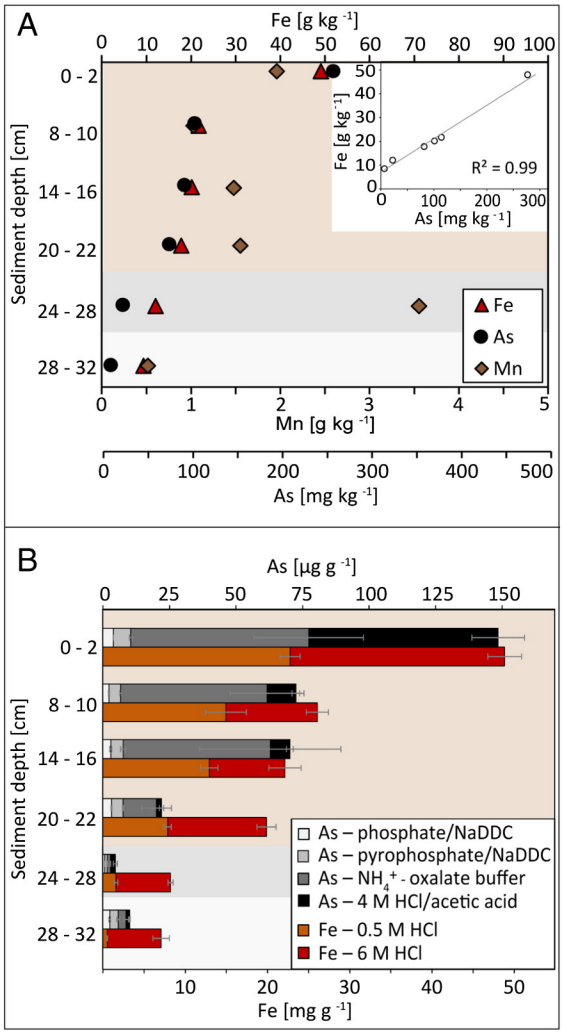

Fig. 3. Depth profiles of: (A) total Fe [g kg^{-1}], total As [mg kg^{-1}], and total Mn [g kg^{-1}] in the sand material present in the upper filter basin as determined by XRF. The insert shows the linear correlation of Fe and As. (B) As and Fe concentrations in different fractions of the sand filter material as quantified by sequential extractions. As extracted with a phosphate/NaDDC mixture (representing soluble and exchangeable As (Huang and Kretzschmar, 2010)), As extracted with a pyrophosphate/NaDDC mixture (representing either As bound to organic matter (Huang and Kretzschmar, 2010) or As in colloidal particles), As extracted with NH_4^- oxalate buffer (representing As bound to poorly crystalline Fe (oxyhydr)oxides (Huang and Kretzschmar, 2010)), As extracted with 4 M HCl/acetic acid mixture (representing As bound to crystalline Fe (oxyhydr)oxides (Huang and Kretzschmar, 2010)). Fe extracted with 0.5 M HCl (representing poorly crystalline Fe minerals (Amstaetter et al., 2012)) and Fe extracted with 6 M HCl (representing crystalline Fe minerals (Amstaetter et al., 2012)). Mean values and standard deviations of triplicate extractions are plotted. The reddish zone (depth of 0–23 cm) indicates orange/brown iron phases, while the blackish layer (23–28 cm) indicates black manganese precipitates, and the whitish layer (28–32 cm) represents the original colored sand. Data obtained during the sampling campaign in 2012.

Total Fe, As, and Mn concentrations were quantified in the filter material by XRF in 2012 (Fig. 3). The highest concentrations of Fe (49 g kg^{-1}) and As (260 mg kg^{-1}) were quantified in the top 2 cm of the red colored filter material. Fe and As concentrations decreased significantly with filter material depth. In the bottom whitish layer, concentrations of Fe and As were similar to the concentrations in the unused sand material (data not shown). Mn concentrations were highest (1135 mg kg^{-1}) in the blackish layer (24–28 cm depth). A strong correlation (coefficient of determination of 0.99) was observed between Fe

and As in the filter sand material (Fig. 3). Similar values were observed in 2013 (Fig. SI 4).

Sequential extraction of Fe from the sand filter material in 2012 revealed that 0.5 M HCl- and 6 M HCl-extractable Fe make up similarly sized fractions in the reddish layers (0–23 cm), while more Fe was extractable with 6 M HCl than with 0.5 M HCl in the blackish and whitish layers (80% and 92%, respectively) (Fig. 3). The data from 2013 showed the same trends (Fig. SI 4). The use of 0.5 M HCl and 6 M HCl to estimate the fraction of Fe associated with poorly crystalline and crystalline Fe minerals was based on a protocol described by Amstaetter et al. (2012).

Major amounts of As were extracted from the red filter layers (0–23 cm) by ammonium oxalate buffer (45–78% of total amount of As extracted by sequential extraction) and 4 M HCl/acetic acid mixture (9–48% of total extracted As), which have been suggested to reflect As bound to poorly crystalline and crystalline Fe(III) (oxyhydr)oxides, respectively (Huang and Kretzschmar, 2010). Minor amounts of As in the red layers were extracted by a phosphate/sodium diethyldithiocarbamate trihydrate (NaDDC) mixture (3–15% of total extracted As) and by pyrophosphate/NaDDC (4–20% of total extracted As), which were assigned to reflect soluble/exchangeable As and either As bound to organic matter (Huang and Kretzschmar, 2010) or As mobilized with colloidal Fe in the pyrophosphate extract (Regelink et al., 2013). The lowest amount of As in all fractions was extracted from the blackish layer (24–28 cm). Only little As (0.4–3.1%) was extracted from all sand filter layers by $NH_2OH \cdot HCl$ (suggested to reflect As bound to manganese (Mn) oxides (Huang and Kretzschmar, 2010) (data not plotted in Fig. 3)).

μXRD mineral analyses of the filter sand before its use confirmed that the sand is dominated by quartz. We also detected traces of calcite but observed no reflections corresponding to Fe(III) (oxyhydr)oxide minerals (e.g., magnetite, hematite, ferrihydrite, goethite), probably due to their low concentration and low crystallinity compared to the dominant quartz background. Analysis of the original sand by Mössbauer spectroscopy, a technique that is specific for Fe analysis, provided evidence for the presence of poorly crystalline Fe(III) oxyhydroxides and the absence of more crystalline Fe(III) minerals, as well as for the presence of a minor fraction of Fe(II) most likely contained in primary phyllosilicates (see details in the SI; Fig. SI 5 and SI 6; Table SI 4).

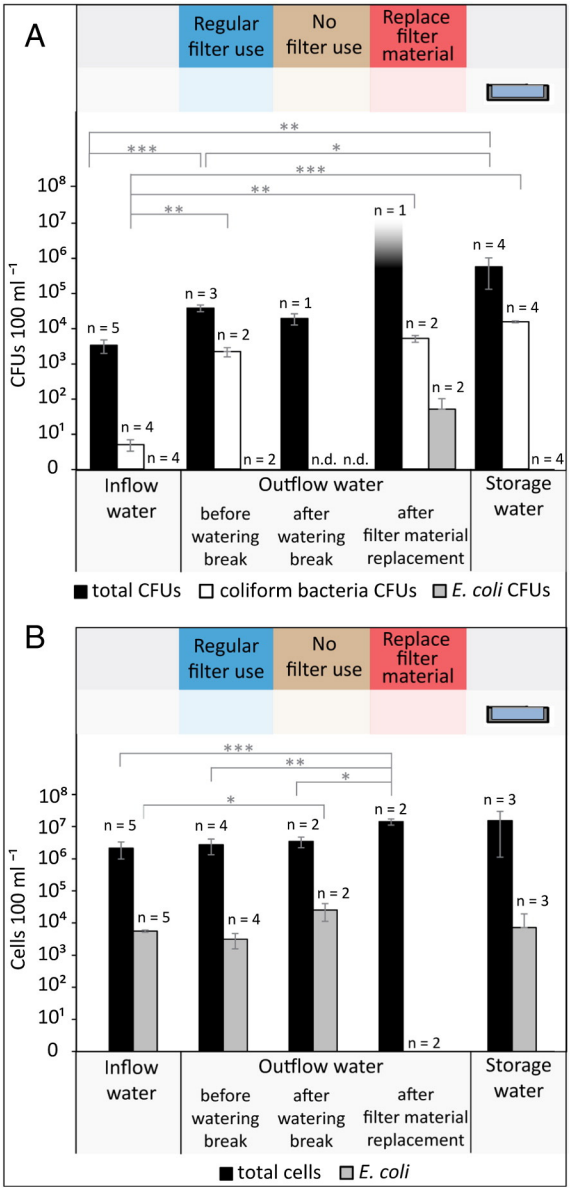

Fig. 4. Quantification of fecal indicator bacteria and total cell numbers in the inflow, outflow, and storage tank water. (A) Colony forming units (CFUs) as quantified by plate counts. The black to white color gradient indicates numbers above the upper limit of detection. (B) Total cells (*Bacteria* and *Archaea*) and *E. coli* cell numbers as quantified by 16/23S rRNA gene copy number based qPCR. Mean values and standard deviation of n independent samples are shown. Asterisks indicate significant differences between samples using the unpaired t-test at a 95% confidence interval (* for $p < 0.05$, ** for $p < 0.01$ and *** for $p < 0.001$). No Asterisk indicates nonsignificant differences between samples ($p > 0.05$).

3.4. Quantification of fecal indicator bacteria

We quantified total colony forming units (CFUs) and CFUs of coliform microorganisms, including *E. coli* and *Enterococcus* spp., and found $3.3 \pm 2.0 \times 10^3$ CFUs per 100 mL and 5.0 ± 4.0 per 100 mL in the inflow water, respectively (Fig. 4A). Significantly higher numbers of total CFUs and coliform CFUs were found in the outflow water. Before a watering break (i.e., before several days of non-use of the filter) total CFUs and coliform CFUs in the outflow water yielded $37.6 \pm 1.2 \times 10^3$ and $2.2 \pm 1.1 \times 10^3$ CFUs per 100 mL, respectively. After a watering break, the number of total CFUs was not significantly different from the value before the break ($19.0 \pm 6.6 \times 10^3$ CFUs per 100 mL). The number of CFUs for coliforms in the outflow water was not determined after the watering break. After sand replacement, the number of total CFUs increased by several orders of magnitude in the outflow water and was even above the upper limit of quantification of the method (i.e. 1×10^6 CFUs per 100 mL), while the number of CFUs for coliform microorganisms showed no significant differences in the outflow water compared to the values before sand replacement ($5.1 \pm 1.5 \times 10^3$ CFUs per 100 mL). In the storage water, total CFUs and coliform CFUs were $5.6 \pm 3.1 \times 10^5$ CFUs per 100 mL and $15.3 \pm 1.4 \times 10^3$ CFUs per 100 mL, respectively, and thus higher than the numbers in the inflow and the outflow water (except for the total number of CFUs after filter material replacement). With the exception of one sample in which one *E. coli* colony grew on one out of three plates, no *E. coli* and *Enterococcus* spp. were detected by plate counts (<1 CFU per 100 mL).

In addition to plate counts, we used qPCR to estimate the total cell number (calculated from 16S rRNA gene copy numbers of *Bacteria* and *Archaea*) as well as the number of *E. coli* and *Enterococcus* spp. (based on 23S rRNA gene copy numbers). All gene copy numbers were normalized to cell numbers based on average rRNA operon numbers per cell as derived from the ribosomal RNA operon database (Klappenbach et al., 2001; Lee et al., 2009).

In the inflow water, we found $2.1 \pm 1.2 \times 10^6$ total cells and $55.6 \pm 4.1 \times 10^2$ *E. coli* cells per 100 mL. Higher numbers of total cells and in many cases also of *E. coli* were quantified in the outflow water compared to the inflow water as follows (see Table 4B): Before the watering break, the number of total cells and of *E. coli* in the outflow water yielded $2.7 \pm 1.4 \times 10^6$ and $3.3 \pm 1.6 \times 10^3$ cells per 100 mL^{-1}, respectively. After the watering break, the number of total cells

($3.5 \pm 1.2 \times 10^6$ per 100 mL) was not significantly different compared to the value before the break but the number of *E. coli* cells ($2.6 \pm 1.4 \times 10^4$ per 100 mL) increased by one order of magnitude. The *E. coli* cells were significantly enriched in the outflow water compared to the inflow water after the watering break. After sand replacement, the total cell number ($14.3 \pm 3.2 \times 10^6$ per 100 mL) was significantly higher compared to the values before sand replacement. No *E. coli* cells were detected by qPCR in the outflow water after sand replacement. Quantification of total cells and *E. coli* in the storage water yielded cell numbers of $1.6 \pm 1.4 \times 10^7$ and $7.2 \times 10^3 \pm 1.2 \times 10^4$ cells per 100 mL^{-1}, respectively. In all samples, total cell numbers were dominated by *Bacteria* (>92%) as determined by individual qPCRs for Bacteria and Archaea. In all samples, the cell numbers of *E. coli* were <1% of total cell numbers. *Enterococcus* spp. was not detected by qPCR.

4. Discussion

4.1. Biogeochemical transformation of Fe, As and Mn

Understanding the functioning of the sand filter first requires analysis of the biogeochemical transformation of Fe, As and Mn. We determined the spatial distributions of these elements as well as the mineralogy of the sand filter material.

4.1.1. Fe(II) oxidation, Fe(III) precipitation, and As binding in household sand filters

Dissolved Fe in the pumped groundwater is present as Fe(II) and starts to oxidize immediately when the water is exposed to atmospheric O_2 (Davison and Seed, 1983). The possibility of biotic Fe(II) oxidation by microaerophilic bacteria in suboxic microsites or by denitrifying microorganisms in anoxic microsites in the sand filter is also conceivable (Emerson and Moyer, 1997; Klueglein et al., 2014). The fact that nitrate concentrations are increasing from the inflow to the outflow water suggests nitrification rather than denitrification as an important process. And indeed, a metagenome study with the filter material (K. S. Nitzsche, unpublished data) provided evidence for the presence of ammonium- and nitrite-oxidizing microorganisms.

Virtually all Fe(II) was removed from the water during filtration and red-brownish precipitates were formed that were identified as 0.5 M- and 6 M-HCl-extractable Fe-fractions (Figs. 3 and SI 4). Based on Mössbauer spectroscopy (Fig. SI 5 and 6, Table SI 4), these corresponded to a poorly crystalline Fe(III) oxyhydroxide mineral phase which was previously identified as ferrihydrite-like precipitate by X-ray absorption spectroscopy (XAS) (Voegelin et al., 2014). Ferrihydrite has a high specific surface area and is well known to strongly bind As via relatively strong inner-sphere complexes (Raven et al., 1998; Sherman and Randall, 2003; Ona-Nguema et al., 2005; Hohmann et al., 2011). Indeed, based on sequential As-extraction, we found a dominating As-fraction associated with this mineral phase and only a minor portion of As to be soluble and exchangeable (Fig. 3), suggesting a strong binding of As to the Fe(III)-precipitate and thus a low probability of As remobilization from the sand filter material. This is supported by the Fe and As content in the sand filter material, which showed a strong positive correlation ($R^2 = 0.99$), as also observed by Voegelin et al. (2014). This indicates that co-precipitation and adsorption of As onto the ferrihydrite-like precipitate are likely the dominant processes of As removal from the pumped groundwater during the downwards migration of Fe- and As-containing water through the sand filter material. Aging of the ferrihydrite precipitates resulting in an increase of crystallinity could lead to a reduction in As adsorption capacity over time and thus potentially to a release of As. However, ferrihydrite was still present as main Fe phase in the sand filter that was in use for eight years, as shown by Voegelin et al. (in press).

4.1.2. Precipitation of Mn oxide minerals and their role as oxidants for Fe(II) and arsenite

The dissolved Mn present in the groundwater was mostly retained in the sand filter as precipitate of Mn(IV) oxides. In line with μ-XRF and SEM-EDX data (Voegelin et al.,2014), sequential extractions suggest that only a minor fraction (0.4–3.1%) of the total extractable As was associated with Mn oxides. Element distribution profile (Fig. 1) revealed that the blackish layer just beneath the Fe- and As-rich red-brownish layers (at a filter depth of 24–28 cm) contained up to ~2.7 times higher Mn concentrations and the lowest extractable As fraction of all layers. A low amount of As bound to Mn oxides is expected since the negatively charged arsenate has only a low tendency to bind to these Mn oxides due to the negative surface charge of the Mn oxides (point of zero charge of birnessite, i.e. MnO_2, is ~2 (Tan et al., 2008)).

Mn(IV) oxides are oxidants for Fe(II) (Postma, 1985; Villinski et al., 2001) and As(III) (Tournassat et al., 2002; Ying et al., 2012). It can be expected that during water infiltration through the filter initially Fe(II), As(III) and Mn(II) are oxidized abiotically or biotically but due to the excess of As(III) and Fe(II) compared to the Mn(II), the initially formed Mn(IV) oxides function as chemical oxidant for Fe(II) and As(III) leading to Fe(III) oxyhydroxide precipitation and arsenate immobilization. As a consequence, the Mn would be remobilized as Mn(II) as long as Fe(II) and As(III) are present in the water and the black Mn-rich layer thus indicates the filter depth at which no As(III) and Fe(II) are present anymore.

Interestingly, occasionally we observed a breakthrough of Mn and the presence of measurable Mn concentrations in the outflow water. This might be explained by the fact that Mn(II) oxidation by O_2 is slower than Fe(II) oxidation and needs microbial catalysis to be effective since Mn(II) oxidation by O_2 is rather slow (Farnsworth et al., 2011). Microbial enrichment studies indeed provided evidence for the presence of aerobic Mn(II)-oxidizing bacteria throughout the filter with the by far highest numbers of cells found in the black Mn-rich layer (K. S. Nitzsche, unpublished data). Thus, fluctuations in O_2 supply in the deep filter layers causing lower microbial activity would lead to varying efficiencies of Mn(II) oxidation and thus in some cases to incomplete Mn(II) oxidation and a Mn release into the outflow water. Additionally, limiting O_2 supply in combination with the presence of organic matter could allow microbial Mn(IV) reduction to take place releasing Mn(II) (Voegelin et al., 2014). Although it is known that Mn ingestion can cause neurological deficiencies and intellectual impairments (Bouchard et al., 2011), it is currently unknown whether the occasional release of Mn from the filter represents a health risk.

4.2. Efficiency of As removal depending on sand filter usage and recommendations for water use

The investigated sand filter removed 95% of As from the treated water and lowered As to levels below the WHO recommended value of 10 μg L^{-1}. This result is in agreement with previous reports that demonstrated efficient As removal by such filters (Berg et al., 2006; Hug et al., 2008). Other technologies perform similarly or even better regarding their As removal efficiencies, e.g., chemical coagulation or electrocoagulation (depending on As species up to 93–99% As removal efficiency (Ratna Kumar et al., 2004)) or pressure-driven membrane-based methods such as nanofiltration or reverse osmosis (both up to 99% arsenate removal efficiency (Mondal et al., 2013)). However, these techniques are much more expensive than the simple sand filter systems and have high operation and maintenance costs (Mondal et al., 2013). Some methods, such as oxidation treatments by ozone or coagulation–flocculation, require the addition of chemicals that produce toxic or carcinogenic by-products (Mondal et al., 2013). Filters based on activated carbon are not as efficient regarding As adsorption, having an As removal efficiency of only up to 60% (Mondal et al., 2013). Most of these technologies can be applied for As removal in large and medium scale treatment plants for centralized services but are not suitable for

rural areas where only untrained personnel are available to install and maintain these technologies for daily domestic use. Decentralized biosand filter systems (3-Kolshi, SONO or Kanchan™ filters) have been applied in rural areas in Nepal, Bangladesh and Cambodia, but differ with respect to design and build-up compared to the sand filters used in North Vietnam. Since groundwater in the other regions tend to have lower Fe concentrations, and hence, form less Fe(III) oxyhydroxide precipitate required for As removal, these filters contain several layers made of a zero-valent Fe and are thus adapted to low groundwater Fe:As ratios as well as to high phosphate:Fe ratios (Hug et al., 2008; Noubactep et al., 2009). Several studies showed that these filter systems remove As between <40–99%, depending on groundwater composition (Ngai et al., 2007; Chiew et al., 2009; Neumann et al., 2013). Therefore, as already concluded by Berg et al. (2006), if the Fe:As ratio is >50 and the groundwater concentration of competing phosphate is <2.5 mg L^{-1}, simple sand filters are excellent alternatives to these elaborate techniques and advanced sand filter systems in rural areas.

Our simulation of different scenarios of usage, such as repeated water filtration several times per day with differing lengths of continuous water filtration, or several days of filter non-use, consistently demonstrated efficient removal of As. This shows, in contrast to our initial hypothesis, that waterlogging and the possible establishment of anoxic microsites leading to Fe(III) mineral reduction and thus a potential release of As are either not occurring on the filter or play only minor roles at least in the sand filter studied here. In addition, the As removal efficiency after sand replacement showed no differences compared to the As removal before exchange of the filter material. The fact that the original sand that was initially free of Fe(III) oxyhydroxide minerals removed the As as efficient as the old and Fe(III) precipitate-enriched sand is probably due to the high Fe:As ratio in the groundwater, leading to instant precipitation of sufficient Fe(III) oxyhydroxides and thus sufficient binding sites for As.

Overall, As concentrations flushed through the sand filter are reduced as recommended below 10 μg L^{-1} irrespective of the filter use practices studied here. Initial flushing of the filter (e.g., to avoid potentially higher As concentrations in the initial volumes of filtered water) or additional water treatment are not required to lower the As content in the outflow water.

4.3. Evidence for nitrification occurring in the drinking water filter

In both sampling campaigns (2012 and 2013) we observed that concentrations of NH_4^+ decreased and NO_3^- increased significantly comparing the inflow to the outflow water (Table 1). In combination with the observed presence of microorganisms known for nitrification in a metagenomic microbial community analysis of the filter (K.S. Nitzsche, unpublished data) this suggests nitrification in the sand filter. NH_4^+ oxidation and simultaneous NO_2^- or NO_3^- formation during water filtration have been observed in previous studies of biosand filters in field and lab experiments (Chiew et al., 2009; Murphy et al., 2010; Mangoua-Allali et al., 2012; Baig et al., 2013). Although the NO_3^- concentrations in the outflow water of the investigated sand filter were below the WHO guideline value of 50 mg L^{-1}, our study suggests putting more attention on this geochemical parameter in future studies since elevated NO_3^- concentrations in drinking water might harm infants, causing methemoglobinemia (Fan and Steinberg, 1996).

4.4. Fecal indicator bacteria in sand filters and recommendations for water use

In the inflow water, we quantified ~10^3 total CFUs per 100 mL water using cultivation-based plate counts and estimated ~10^6 total cells per 100 mL based on bacterial and archaeal 16S rRNA gene targeted qPCR. These numbers are within the same order of magnitude as previously published for groundwater (Hazen et al., 1991; Lleo et al., 2005; Kozuskanich et al., 2011). With more specific plate growth assays we found 5 CFUs of coliform bacteria per 100 mL groundwater, a common finding in groundwater (Leber et al., 2011), but no CFUs for *E. coli* and *Enterococcus* spp. In contrast to the CFU analysis, *E. coli* could be quantified by qPCR in the inflow water with rRNA gene copy numbers corresponding to ca. 10^3 cells per 100 mL. Similar gene copy numbers have been determined by qPCR in groundwater samples of a highly populated rural area in Bangladesh (Ferguson et al., 2012).

In the outflow water, the total number of cells did not increase significantly (CFU plate counts and qPCR) after several days of not using the filter, but the number of *E. coli* cells was higher (qPCR). After filter sand replacement, the total number of cells increased (CFUs and qPCR), while the number of coliform bacteria (CFUs) and the number of *E. coli* cells (qPCR) remained at the same level. However, CFU analysis showed that *E. coli* cells were present in the outflow water after the sand replacement posing a potential risk for human health (WHO, 1997). This suggests that the replacement of the filter material and thus the source of the new sand, but not the filter operation practice is a critical parameter for microbial contamination of the filtered drinking water.

Compared to the inflow and outflow water, the water in the storage tank contained higher numbers of total cells (based on qPCR and plate counts) and coliform bacteria (based on plate counts) due to growth of the microorganisms in the storage tank. It is therefore recommended to preferentially use the freshly filtered water rather than the water from the storage tank.

Enterococcus spp. were not detected by plate counts or by qPCR. However, the numbers for coliform bacteria clearly exceed the recommended value of zero CFUs 100 mL^{-1} and their presence generally poses a potential risk for human health, although only a small group of these fecal bacteria are human pathogens. This result (CFUs) confirms our initial hypothesis that potentially harmful microorganisms are enriched in the sand and are flushed out with the outflow water. It has been shown for biosand filter systems in rural areas in Nepal, Bangladesh, Cambodia and other developing countries, that general fecal indicator bacteria such as coliform bacteria and *E. coli* can be removed from the water and hence diarrhea diseases are limited when a layer of small-grained sand (<1 mm) is used (Ngai et al., 2007; Chiew et al., 2009; Stauber et al., 2009; Jenkins et al., 2011). This material has been suggested to retain fecal indicator bacteria by physical straining (Noubactep et al., 2009; Jenkins et al., 2011). Since the sand filter investigated here showed a larger and more heterogeneous grain size distribution (one third of the particles were >1 mm and two thirds <1 mm) our filter might not be able to mechanically remove microbial cells as efficiently as other systems.

Seasonal variation of fecal contamination was reported for shallow (<37 m deep) and deep (~100 m deep) tube wells in rural areas of Bangladesh where the quantities of fecal indicator bacteria in the water were higher during the wet compared to the dry season (Howard et al., 2006; Ferguson et al., 2012; van Geen et al., 2011). Similar variations might be present in some shallow groundwater wells in Vietnam, but our sampling scheme did not account for the analysis of seasonal variations.

Based on the results from the present study and in order to prevent diarrheal diseases, we recommend avoiding direct consumption of outflow and storage tank water before it is sterilized by boiling (thermal disinfection), solar or chemical disinfection, or sterile filtration. Boiling of the water before consumption as drinking water is a simple and efficient solution. Furthermore, livestock and other potential sources of fecal contamination must be kept at a distance from the sand filter. The container with the sand material and the water storage tank should be covered at all times to limit phototrophic growth of bacteria and external contamination. Following a discontinuation of the filter use for several hours/days or after the sand filter material has been replaced, the sand filter should be flushed several times in order to decrease the cell numbers in the filtered water. Alternatively, the new sand that is typically taken from the Red River bank should be washed, boiled and dried before replacement of the used sand. Additionally, the use of

sieved sand <1 mm in size might limit the number of microorganisms in the outflow water by physical straining (Noubactep et al., 2009; Jenkins et al., 2011).

4.5. Implications and transferability to other household sand filters used in Vietnam

It has been demonstrated (Berg et al. (2006)) that sand filters are effective to remove As over a wide range of groundwater compositions. Changes in groundwater conditions (e.g. the concentrations of As/Fe, concentrations of competing substances such as bicarbonate, organic matter or silicate, or the redox potential and ionic strength) can influence the adsorption of As onto the Fe(III) (oxyhydr)oxide coatings on the sand filter and hence affect the effectiveness of As removal. Groundwater composition and aquifer structures are often very heterogeneous throughout the As-affected regions around the globe, with significant differences observed over distances of few tens of meters (van Geen et al., 2003; Fendorf et al., 2010; Guo et al., 2010; Michael, 2013). This has to be taken into account when drawing general conclusions from the sand filter scrutinized in this study. Besides the variations in As and Fe, it has been shown that the presence of fecal indicator bacteria in the groundwater depends on geological conditions (Leber et al., 2011) and indeed variable geological conditions are present in North Vietnam (Berg et al., 2008; Winkel et al., 2011; van Geen et al., 2013). It was also observed that with well depth, the number of *E. coli* in groundwater is varying (Leber et al., 2011). Consequently, depending on present geological conditions, well depth, and groundwater quality, the bacterial load in the inflow water will likely vary from well to well, leading to a varying enrichment of fecal indicator bacteria in the sand filter and outflow water.

Our study showed that the investigated sand filter removes As efficiently from drinking water irrespectively of usage practices and sand usage duration. The findings regarding As removal efficiency and microbiological water quality of a sand filter are probably transferable to other sand filters if similar groundwater compositions, geological settings, well and sand filter properties exist.

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Appendix A. Supplementary data

Supporting information includes Table SI 1 and 2, which show the sampling schedule for 2012 and 2013, a detailed method describing the sampling including the sample treatment of sand filter water, the description of the solid phase characterization (including Mössbauer-, XRD- and μXRF analyses), as well as sequential Fe and As extraction, the procedure for quantification of fecal indicator bacteria (with the qPCR primers used shown in Table SI 3). Results including Figure SI 1 showing concentrations of As and Fe in the inflow water over several days in sampling campaigns in 2013 and 2012, Fig. SI 2 showing Fe(tot) and Fe(II) concentrations of inflow water over several days in sampling campaigns in 2013 and 2012, Figure SI 3 showing sand filter performance regarding As concentrations in sampling campaign 2012, Fig. SI 4 including depth profiles of total As, Fe, Mn in the sand material from 2013, and a depth profile of different Fe fractions in the sand material from 2013. A description of Mössbauer results, including room temperature Mössbauer spectra and fitted parameters, is included in Fig. SI 5 and Table SI 4, as well as low temperature Mössbauer spectra in Fig. SI 6. This material is available free of charge via the Internet at http://dx.doi.org/10.1016/j.scitotenv.2014.09.055.

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