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Characterisation of organic matter and microbial communities in contrasting arsenic-rich Holocene and arsenic-poor Pleistocene aquifers, Red River Delta, Vietnam

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Abstract

High concentrations of geogenic As in the groundwaters of south and SE Asia, which are used as drinking waters, are causing severe health impacts to the exposed human populations. It is widely accepted that As mobilization from sediments into these shallow reducing groundwaters requires active metal-reducing microbes and electron donors such as organic matter (OM). Although OM in such Holocene aquifers has been characterised, there is a dearth of data on Pleistocene aquifers from the same areas. Reported here are preliminary studies of OM and microbial communities present in two aquifers, one of Pleistocene and one of Holocene age, with contrasting concentrations of As (viz. Pleistocene: low As <10 µg/L; Holocene: high As up to 600 µg/L) from Van Phuc village in the Red River Delta, Vietnam. Results revealed OM inputs from multiple sources, including potential contributions from naturally occurring petroleum seeping into the shallow aquifer sediments from deeper thermally mature source rocks. Although concentrations vary, no noticeable systematic differences in biomarker distribution patterns within the OM were observed between the two sites. Microbial analyses did not show a presence of microbial communities previously associated with As mobilization. All clone libraries were dominated by α -, β -, and γ -*Proteobacteria* not known to be able to reduce Fe(III) or sorbed As(V). Furthermore, representatives of the Fe(III)-reducing genus *Geobacter* could only be detected at very low abundance by PCR, using highly selective 16S rRNA gene primers, supporting the hypothesis that metal reduction is not a dominant *in situ* process in these sediments. No correlation between As concentration in groundwater and OM composition nor microbial community in the host sediments was found. This suggests that either (i) As is not being significantly mobilized *in situ* in these sediments, instead As appears to be mobilized elsewhere and transported by groundwater flow to the sites or (ii) sorption/desorption processes, as implicated by geochemical data from the cores, play a critical role in controlling As concentrations at these sites.

1. Introduction

Over a hundred million people across the globe are chronically exposed to high concentrations (>10 µg/L) of the geogenic poison As, in shallow reducing groundwaters utilised as drinking water (Ravenscroft et al., 2009). Several possible mechanisms have been postulated to lead to As release from the hosting sediments into the groundwaters, including: (i) the oxidation of As-bearing sulfides (Ravenscroft et al., 2009), (ii) reduction of As(V) (Zobrist et al., 2000), (iii) reductive dissolution of Fe(III) oxyhydroxides (Bhattacharya et al., 1997; Nickson et al., 1998), (iv) alkali desorption (Bottomley, 1984; Boyle et al., 1998; Ayotte et al., 2003; Bexfield and Plummer, 2003) and (v) geothermal release of As (Ballantyne and Moore, 1988). However, the most widely

accepted mechanism of As release into the aquifers, being under Fe(III)-reducing conditions (Zheng et al., 2004), in the As hot spot sites of West Bengal, Bangladesh (Nickson et al., 2000; Ravenscroft et al., 2001; Smedley and Kinniburgh, 2002; Islam et al., 2004; McArthur et al., 2004; Zheng et al., 2004), Cambodia (Polya et al., 2003, 2005; Berg et al., 2007; Buschmann et al., 2007; Pederick et al., 2007; Rowland et al., 2007) and Vietnam (Berg et al., 2001, 2008; Postma et al., 2007; Eiche et al., 2008; Winkel et al., 2011) is believed to be the microbially mediated reductive dissolution of As-bearing Fe oxyhydroxides. The role of degradable organic C and the availability of specialized microbial communities in this process has been recognized extensively (Charlet and Polya, 2006; Rowland et al., 2006, 2007, 2009; Postma et al., 2007; van Dongen et al., 2008; Neuman et al. 2010; Mladenov et al. 2010).

Results of organic geochemical and radiocarbon analyses of sediments from high As aquifers in southern and south eastern Asia (e.g., West Bengal and Cambodia), to date, show that organic matter (OM) is likely to have derived from multiple sources including (i) OM laid down at the time of deposition, (ii) reworked older sedimentary OM eroded from older unconsolidated sediments upstream of the sampling location, (iii) groundwater-transported surface derived modern OM and (iv) naturally occurring thermally mature petroleum-derived hydrocarbons seeping into shallow sediments from deeper source rocks (van Dongen et al., 2008). The presence of bio-available petroleum derived sources of OM is of potential importance, since recent microcosm experiments provide direct evidence that this material is oxidised during *in situ* As mobilization (Rowland et al., 2009). Electron donors such as OM are required to drive this process, yet As release rates are often poorly correlated to total OM (Gault et al., 2005) – this may in part reflect the varying bioavailabilities of different types of OM (van Dongen et al., 2008; Rowland et al., 2009), including biologically degradable and recalcitrant organic C (Neumann et al. 2010), and humics (Mladenov et al. 2010), or the potential involvement of other electron donors, as suggested recently from the observation of As mobilization from organic-lean sediments (Héry et al., 2010).

The role and nature of OM in As mobilization, therefore, remains rather ambiguous, perhaps in part due to the limited number of studies to date. An improvement in understanding this link is required to better understand long-term anthropogenically-driven secular increases in groundwater As in reducing aquifers (Harvey et al., 2002; Polya and Charlet, 2009). In addition, it is presently unclear whether the presence of specific electron donors, such as petroleum derived hydrocarbons, in high As aquifers is a coincidence or whether they are widely spread, playing a significant role in the mobilization of As in contaminated aquifers.

In addition to the role of OM, several studies have shown that the indigenous anaerobic metal(loid)-reducing microorganisms can play a vital role in mediating As mobilization into groundwater aquifers (Islam et al., 2004; Lloyd and Oremland, 2006; Lear et al., 2007). However, the identity of these microorganisms has been studied in only a few examples (reviewed in Héry et al., 2008), where Fe(III)- and As(V)-respiring *Geobacter* and *Sulfurospirillum* have been implicated in playing a role in As mobilization.

Taken together, there is an urgent need for a better understanding of the electron donors and their interactions with microbial consortia present in other As hot spot areas outside those studied so far (i.e., West Bengal, Bangladesh and Cambodia). Interestingly, previous studies have focussed on characterisation of OM in Holocene but not Pleistocene aquifers. Since previous analyses (Zheng et al., 2004; Ravenscroft et al., 2005; Sutton et al., 2009) indicated that high levels (>10 µg/L) of As were typically associated with Holocene aquifers and not with Pleistocene aquifers there is a need to analyse OM in Pleistocene aquifers as well. In this study, the areas studied has been extended to

include the Red River Delta (RRD), Vietnam (Berg et al., 2007, 2008), where OM and microbial communities in two contrasting (site H, Holocene, high As groundwater vs site L, Pleistocene, low As groundwater) aquifers from Van Phuc village in the RRD (Eiche et al., 2008; Weinman et al., 2007, 2008; Weinman, 2010) are characterized and groundwaters analysed for the first time. The focus of the organic analyses was on the extractable (lipid) fraction of the OM. This is to test if the sedimentary OM has contributions from sources comparable to those observed in other aquifers suggested to be potentially involved in the microbial As mobilization (Rowland et al., 2006, 2009; van Dongen et al., 2008).

Using these data and previous geological and hydrogeochemical data (Berg et al., 2001, 2008; Eiche et al., 2008), the relationship between OM bioavailability, microbial communities, sediment lithology, hydrochemistry and redox status and the concentrations of As in the pore water are discussed. The findings provide, for the first time, a detailed comparison of OM between Holocene and Pleistocene aquifer sediments, as well as one of the few studies of microbial communities in Pleistocene aquifers (cf. Sutton et al., 2009). This study also increases the number of locations where thermally mature OM has been reported in association with As-rich shallow aquifers.

2. Materials and Methods

2.1. Study area

The Van Phuc village is located in the RRD in northern Vietnam (Fig. 1), about 10 km SE of Hanoi and is known for the variability of groundwater As concentrations in the aquifers (Berg et al., 2007, 2008). In the larger Hanoi area, both Holocene and Pleistocene sediments are present (Trafford et al., 1996; Winkel et al., 2011). The Holocene and Pleistocene aquifers along the Red River are mainly recharged by river water (Berg et al., 2007, 2008) with a minor fraction in the Holocene aquifer being derived from precipitation. The study area, including the lithology of the RRD sediments, has been described previously (Berg et al., 2001, 2008; Eiche et al., 2008; Postma et al., 2007; Winkel et al., 2011). Recently, core sediments and groundwater samples were collected from two sites in the RRD area, only 700 m apart but with contrasting environments (site L and H; Fig. 1; Eiche et al., 2008). For instance, the As concentrations in the groundwater (20-50 m depth) of these two sites are very different, e.g. being typically <10 µg/L for site L and between 170 and 600 µg/L for site H (Eiche et al., 2008; Fig. 1 and Table 1). Some of the key chemical characteristics of the groundwater and sediments, such as dissolved As, DOC, HCO₃⁻ and solid phase Fe and As, of both sites are shown as depth profiles (Fig. 3; data obtained from Eiche et al., 2008). In addition, the aquifer at site L is about 15 ± 2 ka (Pleistocene) whereas the aquifer at site H is about 0.8 ± 0.1 ka (Holocene; Weinman et al., 2007, 2008; Weinman, 2010). In the present study, 6 samples from distinctively different horizons (based on the TOC and the lithology; Fig. 2) from each site were chosen for organic and/or microbial geochemical analyses.

2.2. Sample collection

In April 2006, about 55 m of sediment cores were recovered by rotary drilling from sites L and H (Fig. 1). In summary, sediments (about 100 g) were placed in polypropylene bags and then flushed with N₂, in order to minimize oxidation between sampling and analysis. The samples were packed into Mylar bags and again flushed with N₂ before transportation. These were sent to Karlsruhe, Germany and kept frozen until analysis. Subsamples were sent to the United Kingdom for organic and microbial analysis. Upon arrival in Manchester, they were stored anaerobically at -20°C until analysis.

2.3. Extraction and fractionation

Twelve subsamples (ca. 30-50 g each) from both cores and from different depths were weighed accurately, freeze-dried, crushed and extracted using a Soxhlet apparatus with a dichloromethane/methanol (DCM/MeOH, 2:1 v/v) mixture for 24 h. The total lipid extracts (TLEs) obtained were concentrated using rotary evaporation, where aliquots were taken and two internal standards (540 ng of tetracosane-d₅₀; and 5400 ng of 2-hexadecanol) were added. Subsequently, the aliquots were separated into two fractions using Bond-Elut® column chromatography (Strata NH₂; 500 mg, 6mL, glass; e.g., Kim and Salem, 1990), eluting with DCM/isopropanol (2:1, v/v; 12mL; „neutral lipid fraction“) and 2% acetic acid solution in diethyl ether (12mL; „acid fraction“). The neutral lipid fractions were further separated into two fractions using a column packed with (activated) Al₂O₃ by elution with hexane/DCM (9:1 v/v; 3 mL; „apolar fraction“) and DCM/MeOH (1:1 v/v; 3 mL; „polar fraction“). Polar fractions were dissolved in bis(trimethylsilyl) trifluoroacetamide (BSTFA), and heated (70 °C; 60 min) to convert alkanols into their trimethylsilyl ethers. The acid fractions were derivatized, after addition of 540 ng of a tetracosane-d₅₀ standard, with bromotrifluoride (BF₃) in MeOH and heated (70 °C; 60 min) to convert the acids into their corresponding methyl esters (Rowland et al., 2007). Blanks were run to ensure that no contamination was introduced during the extraction and separation procedures and were found to be less than 1:100 for all target analytes. All fractions were analysed using gas chromatography mass spectrometry (GC-MS).

2.4. Gas chromatography-mass spectrometry (GC-MS)

GC-MS was performed using an Agilent 789A GC interfaced to an Agilent 5975C MSD mass spectrometer operating with electron ionization at 40 eV and scanning from m/z 50-600 at 2.7scans/sec. The GC was equipped with an Agilent 7683B auto sampler and a programmable temperature variation (PTV) inlet. The samples were dissolved in hexane prior to injection, injected using pulsed split-less injection (1 µL; inlet pressure of 25 psi for 0.25 min) and separated on an HP-5 capillary column (J & W scientific column 5% diphenyldimethyl polysiloxane; length 30 m, I.D. 250 µm, film thickness 0.25 µm). The samples were run at a constant flow (1 mL/min) with He as a carrier gas. The heated interface temperature was set to 280°C, with the mass source temperature at 230°C and the MS quadrupole at a temperature of 150°C. The samples were injected at 70°C and the oven was programmed to 130°C at 20°C /min and then at 4°C /min to 300°C at which it was held isothermally for 25 min. Compounds were identified by comparison of retention times of mass spectra with those presented in the literature (Petrov et al., 1993; Peters et al., 2005). The quantitative data were obtained by comparing the individual peak areas with a known concentration of the internal standard used.

2.5. Microbial community analyses

DNA was isolated from approximately 0.25 g of a range of high-As and low-As sediments using the PowerSoil™ DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA), following the manufacturer's instructions. PCR was performed in all samples using the universal bacterial 16S rRNA gene primers 8F (Edwards et al., 1989) and 1492R (Lane, 1991). Clone libraries of the whole bacterial community were then prepared from sediment samples L2870, H0970, H1860 and H3055 (from 36.5 m, 10.9 m, 20.8 m and 35 m depth, respectively) as described previously (Héry et al., 2010). PCR products were purified using a Qiagen PCR purification kit (Qiagen, Inc., Valencia, CA, USA) and then ligated into the PGEM-T easy vector system (Promega, Madison, WI, USA)

and transformed into One Shot® TOP10 chemically competent *Escherichia coli* cells (Invitrogen, Inc., Carlsbad, CA, USA). Positive clones (96 per library) were screened by PCR using SP6 and T7 primers, and sequenced using the ABI Prism® BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Life Technologies Corporation, USA). Clone libraries from samples at 36.5, 20.8 and 35 m depth were sequenced using forward primer 530F (Lane, 1991) and the clone library from sample at depth of 10.9 m was sequenced using primer 8F (Edwards et al., 1989).

In addition, PCR using *Geobacteraceae* specific 16S rRNA gene primers GEO564F and GEO840R (Cummings et al., 2003) was performed on all sediment samples and one clone library was prepared for the 10.9 m depth sample. The purified PCR gene fragments were ligated directly into a cloning vector containing topoisomerase I-charged vector arms (Agilent Technologies, Wokingham, UK) prior to transformation into *E. coli* competent cells expressing Cre recombinase (Agilent Technologies, Wokingham, UK). Positive clones were screened by PCR using primers complementary to the flanking regions of the PCR insertion site of the cloning vector, and sequenced using the ABI Prism® BigDye™ Terminator v3.1 Cycle Sequencing Kit and forward primer 564F (Cummings et al., 2003).

The 16S rRNA gene sequences that were obtained were checked for chimera formation using Mallard (Ashelford et al., 2006). The sequences of this study were aligned by Mothur v.1.6.0 (Schloss et al., 2009) with the needleman alignment method using the greengenes alignment with 7,682 positions. Then, only the overlapping sequences for each clone library were kept using the filter.seqs command in Mothur. A DNA similarity matrix was produced with dnadist.exe in Phylip (Felsenstein, 1989) using the Jukes-Cantor model (Jukes and Cantor, 1969). The sequences were finally clustered into OTUs (Operational Taxonomic Units) at a level of similarity of 97% using the average neighbour algorithm in Mothur. The closest relatives for all OTUs/sequences of this study were identified by nucleotide Blastn nucleotide search, using the default settings. In addition, the most closely phylogenetic association was found using the RDP classifier of the Ribosomal Database Project (Cole et al., 2009). All partial 16S rRNA gene sequences from this study were deposited in GenBank under the accession numbers JN014470 - JN014831.

3. Results

3.1. Extractable organic matter

3.1.1. Saturated hydrocarbon fraction

The saturated hydrocarbon fractions of all sediments were dominated by a homologous series of C₂₀ to C₃₄ *n*-alkanes with summed concentrations of the high molecular weight (HMW; >C₂₀) *n*-alkanes ranging from 44 to 200 ng/g sediment at site H and from 23 to 520 ng/g sediment at site L. The sediments in the top sections of both cores (above approximately 15 m depth) clearly indicated a predominance of the odd-numbered homologues as illustrated by CPIs between 2.3 and 3.6 (Table 2 and Fig. 3). In contrast, sediments below approximately 15 m depth were not dominated by the odd-numbered homologues as illustrated by the low CPI values. In addition, the most abundant homologue (C_{max}) and average chain length (ACL) of the HMW *n*-alkanes shifted between the top and bottom core sections. The top sections were dominated by either the C₂₉ or C₃₁ *n*-alkane with an ACL between 26.4 and 29.4 while the deeper cores were dominated by the C₂₀ or C₂₁ *n*-alkane with an ACL between 22.7 and 24.3 (Table 2 and Fig. 3).

Considerable amounts of hopanes and hopenes were also present in all sediments. At both sites, their distribution patterns shifted from being dominated by hopanes predominantly in the 17βH, 21βH-configuration in sediments of the top sections to being dominated by the αβ-configurations in

deeper sediment cores as illustrated by the ratio of C₃₀ ββ-hopane to the sum of the C₃₀ ββ and αβ hopane (Fig. 3). Besides the *n*-alkanes and hopanes, trace amounts of steranes could be detected in all sediments. Furthermore, unresolved complex mixtures (UCMs) were only detectable in the deeper sediments of both cores (Table 2).

3.1.2. Acid fraction

All acid fractions contained a homologous series of C₁₄-C₃₄ *n*-alkanoic acids, dominated by the C₂₆, C₂₈ or C₃₀ homologues, except for the sediment at 27.6 m depth in core L, which was dominated by the C₂₄ homologue, and a predominance of the even numbered homologues (Table 3). The CPI ratios of the HMW *n*-alkanoic acids ranged from 2.8 to 6.1 and 5.1 to 7.9 for site H and L, respectively. The *n*-alkanoic acids ACLs varied from 22.7 to 28.3 (Table 3). In addition to the *n*-alkanoic acids, homologous series of C₁₉-C₃₁ ω-hydroxy alkanoic acids were also abundantly present in the sediments of the upper core section, dominated by the even numbered homologues with C₂₄ the most abundant homologue at site H and C₂₈ or C₂₂ at site L. The HMW ω-hydroxy alkanoic acids CPIs for the top section ranged from 2.3 to 2.7 and 3.4 to 18.9 for site H and L, respectively (Table 3). In contrast, HMW ω-hydroxy alkanoic acids were only detectable in trace amounts in the shallower sediments (e.g., to about 20 m depth in both cores).

3.1.3. Polar fraction

All polar fractions contained a homologous series of C₁₆-C₃₁ *n*-alkanols, dominated by the C₂₄, C₂₆ or C₂₈ homologues and a predominance of the even numbered homologues (Table 3). The CPI ratios of the HMW *n*-alkanols ranged from 6.8 to 47.5 and 3.8 to 63, for sites H and L, respectively. The *n*-alkanol ACLs varied from 23.4 to 27.4 (Table 3). In addition, minor amounts of sterols, predominantly cholesterol and/or campostanol and sitosterol were present in all samples with various concentrations (ranging from 12.2 to 107 ng/g sediment at site H and from 15.9 to 239 ng/g sediment at site L) throughout both cores (Table 3). Trace amounts of hopanols could also be detected.

3.2. Microbial community diversity

PCR DNA amplification using universal bacterial 16S rRNA gene primers was performed in all sediment samples and then clone libraries prepared from samples L2870, H0970, H1860 and H3055 (from 36.5 m, 10.9 m, 20.8 m and 35 m depth, respectively). Subsequent phylogenetic analyses showed that the microbial communities in all samples were dominated by α-, β-, and γ-*Proteobacteria* (Fig. 4). OTU-based statistical analysis by Mothur (Schloss et al., 2009) indicated that the microbial communities were fairly diverse, as 36, 23, 36, and 34 OTUs/species were identified (at sequence similarity level of 97%) out of 64, 62, 77, 73 sequences within the sediments at 36.5 m, 10.9 m, 20.8 m and 35 m depth clone libraries respectively. The statistical analysis also showed that there was limited but not significant overlap between samples H1860 at 20.8 m and H3055 at 35 m, which both came from the borehole with the high As content (Table 1; Fig. 3; Fig. 4).

When *Geobacteraceae* specific 16S rRNA gene primers were used to detect *Geobacter* species indicative of Fe(III)- and potentially As(V)-reducing bacteria (Héry et al., 2008; Lear et al., 2010), PCR products were retrieved from only 30% (6 out of 20) of the sediment samples (Fig. 5). However the *Geobacteraceae* specific PCR products were in much lower concentrations in comparison to the PCR products of the universal 16S rRNA gene primers, as indicated by the weak

intensity of the bands in the agarose gel (Fig. 5). Quantitative PCR using the *Geobacter* primers was unsuccessful at such low template concentrations (data not shown). The specificity of the GEO564F and GEO840R primers (Cummings et al., 2003) was tested and confirmed after cloning and sequencing of *Geobacteraceae*-specific PCR products from sample H0970 taken from 10.9 m depth. The retrieved sequences were all members of the δ -*Proteobacteria*, with the majority of them closely related to members of the family *Geobacteraceae* and the *Geobacter* genus (data not shown).

4. Discussion

4.1. Sources of organic matter in Van Phuc aquifer sediments

Based on TOC and biomarker distribution patterns, particularly the relative abundances and distributions of HMW *n*-alkanes, *n*-akanoic acids, ω -hydroxy akanoic acids, *n*-alkanols, sterols and hopanes as well as the presence/absence of an UCM, it is possible to divide all sediments used for organic analyses into different clusters (e.g., cluster I, II and III; Fig. 3).

The first cluster, (I), consists of the two shallowest sediments from site L (L0580 at 5.6 m and L0990 at 12 m depth). These sediments are characterised by a relatively high TOC content (0.27 - 4.6 %; Table 2), compared to the deeper sediments in the core. In addition, these sediments are dominated by compounds such as C₂₀-C₃₄ *n*-alkanes (with odd-over-even predominance), C₁₄-C₃₄ *n*-alkanoic acids, C₂₀-C₃₁ ω -hydroxy alkanolic acids and C₁₆-C₃₁ *n*-alkanols (all with even-over-odd predominance) all characterized by a CPI generally > 3; typical for sediments with a relatively high input of higher plant derived OM (Eglinton and Hamilton, 1963, 1967). Also, plant derived sterols such as cholesterol, campostanol and sitosterol are abundantly present supporting a predominantly higher plant origin. The hopane distribution in these sediments are dominated by 17 β , 21 β -hopanes with the ratio of $\beta\beta/(\alpha\beta+\beta\beta) \geq 0.5$ (Fig. 3) and no clear UCM could be detected, indicating a predominance of OM of „immature“ origin. A dominance of „fresh“ plant derived OM in surface section sediments of Asian As rich aquifers has been observed before such as in Kandal province of Cambodia (Rowland et al., 2007; van Dongen et al., 2008).

The second cluster, (II), consists of all sediments from both sites taken below 20 m depth. In contrast to the sediments from the first cluster, these sediments are characterised by a relatively low TOC content (0.01-0.03%; Table 2) and a general dominance of compounds such as C₂₀-C₃₄ *n*-alkanes with no odd-over-even predominance (CPIs $\approx$ 1; Table 2 and Fig. 3). In addition, although present, the abundances of C₁₄-C₃₄ *n*-alkanoic acids and C₁₆-C₃₁ *n*-alkanols are generally low if compared to their concentrations in the first cluster and no ω -hydroxy alkanolic acids nor plant derived sterols could be detected. However, an UCM is clearly observed as well as abundant levels of hopanes, mostly in the $\alpha\beta$ configuration with the ratio of $\beta\beta/(\alpha\beta+\beta\beta) < 0.2$ (Fig. 3). Combined, these observations suggest a dominance of thermally mature derived OM in these sediments (Bray and Evans, 1961; Lichtfouse and Rullkotter, 1994; Peters et al., 2005). However, the presence of HMW *n*-alkanols and *n*-alkanoic acids, with CPIs > 3, still support a minor input of “fresh” higher plant derived OM. A dominance of thermally mature OM input was recently observed in other Asian As rich aquifer sediments from West Bengal (Rowland et al., 2006) and the Kandal province of Cambodia (Rowland et al., 2008; van Dongen et al., 2008). Although it remains unclear how exactly the thermally mature hydrocarbons have migrated into these aquifers, their presence is not surprising, given that the Song Hong Basin, offshore north eastern Vietnam, is known to have reserves of gas and oil (Petersen et al., 2004). In addition, hydrocarbons, comparable to those observed in the sediments have been observed in wells, in the Hanoi trough, the onshore extension

of this basin (Nielsen et al., 1999). It is speculated that, comparable to similar sediments in West Bengal and Cambodia, it is possible that the thermally mature derived OM in the Van Phuc area is migrating upwards from greater depths along fault planes or other zones of enhanced permeability and spreading laterally along porous, permeable sandy horizons. Reworking of sediments into which such OM has previously migrated cannot be discounted.

The third sediment cluster, (III), comprises the two shallowest sediments from site H (taken at 7.14 and 12.4 m depth) and one from site L (taken at 19.7 m depth), which do not fit completely into either of the previous two clusters. The TOC values in this cluster, for instance, ranging from 0.06 to 0.1% (Table 2), are between those values observed for sediments from the first and second cluster. These 3 sediments share some characteristics with the sediments of the first cluster. These include the presence of substantial amounts of C_{14} - C_{34} *n*-alkanoic acids, C_{20} - C_{31} ω -hydroxy alkanolic acids and C_{16} - C_{31} *n*-alkanols (all with even-over-odd predominance; CPIs >3) and plant derived sterols, all typical for an input of higher plant derived OM. Furthermore, no clear presence of an UCM could be detected in the two sediments from site H, whereas the sample from site L had a very minor UCM, in line with an immature origin of these sediments. However, the HMW *n*-alkanes in these sediments show a much lower abundance (with odd-over-even predominance; CPIs between 1.3 and 3.0; Table 2 and Fig. 3) compared to sediments from the first cluster. This indicates a greater predominance of mature OM, comparable to the second cluster sediments. This would also explain the abundant presence of $\alpha\beta$ hopanes as illustrated by values for the $\beta\beta/(\alpha\beta + \beta\beta)$ ratio ranging from 0.2 to 0.5 (Fig. 3). This suggests that these 3 sediments can be considered to be part of a transition/mixing zone with a substantial influx of OM dominating either sediments from the first cluster, (immature) terrestrial plant derived OM, or sediments from the second cluster, thermally mature (e.g. petroleum) OM.

Combined, the organic analyses indicate that both Holocene and Pleistocene cores are dominated by various sources of similar types of OM input, comparable to observations in other Asian As Holocene aquifer sediments (Rowland et al., 2006, 2008; van Dongen et al., 2008). In addition, focusing on the hydrocarbon fractions in both sites, a clear transition to completely mature OM dominated sediments can be observed in both cores, suggesting that the relative influx of mature OM becomes more important down core.

However, small differences between both cores can be observed. The top section of site L (above 15 m) is clearly dominated by higher plant derived OM whereas deeper down the core, the input from petroleum derived OM becomes more dominant and the relative abundance of the plant derived OM drops, with a sample from 19.7 m being part of the transition/mixing zone. In contrast, no clear dominance of higher plant derived OM could be observed in the top zone of site H (above 15 m), indicating that this section is already influenced by a substantial influx of mature OM. An absence of a predominantly higher plant dominated section was also observed in a core taken in West Bengal (Rowland et al., 2006) and it was suggested recently that this was due to the absence of a clay layer preventing petroleum migration upwards to very shallow depths (van Dongen et al., 2008). However, the lithology of core H (Fig. 2; Eiche et al., 2008) indicates that a clay horizon is present in the top 12 m of this core, suggesting that the absence/presence of such a clay horizon alone cannot explain the absence of a predominantly higher plant dominated section. The largest difference between the top sections of the cores is the total amount of OM present. The TOC in the top section of core H is substantially lower, around 0.06 %, if compared to the same depth interval in core L, values between 0.27 % and 4.6 % (Table 2). This suggests a substantially lower influx of higher plant input at site H if compared to site L. Taking into account the situation in the deeper

sections, one can assume that comparable amounts of mature OM are present in the surface sections of both sites but this is overwhelmed by the very large contribution of plant derived OM at site L.

To summarize, the organic geochemical data highlight that the OM in a given sedimentary horizon from the Van Phuc site likely derives from multiple sources, comparable to the analyses of Cambodian and West Bengal aquifer sediments (Rowland et al., 2006, 2008; van Dongen et al., 2008). Both Holocene and Pleistocene aquifer sediments show comparable origin/distribution of OM although with varying amounts of the relative contribution of higher plant and petroleum inputs. Furthermore, such OM as well as that from other labile sources (Neumann et al. 2010; Mladenov et al. 2010) not included in the present study, has the potential to fuel microbial *in situ* As mobilization (cf. Rowland et al., (2009) microcosm experiments).

4.2. Microbial analyses

The *in situ* microbial ecology of 4 Vietnamese sediment samples taken from site L at 36.5 m depth and from site H at 10.9 m, 20.8 m and 35 m depth, was assessed by 16S rRNA gene cloning and sequencing. The phylogenetic analyses indicated the presence of diverse microbial communities, with no significant similarity to each other. Sequences closely related to those of bacterial genera, such as *Acidovorax*, *Hydrogenophaga* and *Thiobacillus*, commonly found in pristine or contaminated aquifer sediments were identified. However, known arsenate-reducing genera such as *Sulfurospirillum* (Oremland and Stolz, 2003), *Geobacter* (Héry et al., 2008), *Shewanella* (Malasarn et al., 2004), *Desulfitobacterium* (Niggemyer et al., 2001), and *Chrysiogenes* species (Macy et al., 1996) were not detected in any of the clone libraries. The fact that *Geobacteraceae*-specific PCR products were amplified from 30% of the sediment samples of this study indicates that *Geobacter* species are present even in sediments sampled from the borehole with low groundwater As content. However, their absence in the clone libraries prepared with broad specificity primers indicates that they most probably constitute a minor portion of the overall microbial community.

Therefore, although PCR techniques suggest low abundance of organisms associated with metal-reducing and As-mobilizing subsurface environments, microbially-mediated metal reduction does not appear to be a dominant process in the Van Phuc sediments.

4.3. General discussion

The most widely accepted theory of As mobilization in reducing environments is by microbially mediated reductive transformations of As-containing Fe(III) (hydr)oxides (Harvey et al., 2002; Héry et al., 2010; Islam et al., 2004; McArthur et al., 2004; Postma et al., 2007). However, although the putative metal-reducing As mobilizers, such as *Geobacter* species (Héry et al., 2010), are present in these sediments, the results of molecular ecology analyses suggest that these processes do not appear to dominate *in situ* in either the Holocene or the Pleistocene aquifer sediments at the study sites (it is recognised, though, that the possibility of the existence of hitherto unknown As-mobilizing bacteria in the clone libraries cannot be eliminated). Previously, it was also suggested that low groundwater As in Pleistocene aquifers elsewhere might be attributable to the lack of electron donors, associated with lower DOC levels, or a lack of bioavailable solid-phase As or Fe(III) oxide electron acceptors (Polizzotto et al., 2006; Stollenwerk et al., 2007). However, mineralogical analyses (Eiche et al., 2008) of these sediments revealed the presence of sufficient amounts of As (1.0 – 30.3 mg/kg) and Fe (0.5 – 8.1 wt-% Fe₂O₃; Table 1; Eiche et al., 2008), indicating that the lack of As mobilization is not likely to be due to the deficiency of As or Fe in these sediments. Furthermore, the organic analyses clearly indicate that potential electron donors,

such as mature hydrocarbons, are present in abundance (Table 2 and Fig. 2). In addition, in contrast to a previous study in which DOC levels in Holocene and Pleistocene aquifers were compared and were clearly different (Sutton et al., 2009), in this study DOC levels in the Holocene and Pleistocene aquifers were very similar (Table 1). Combined, the results of the microbial, OM and mineral characterisation suggest that although the DNA signatures for known As-mobilizing metal reduction processes are weak in these sediments, there do seem to be sufficient electron donors, mineral surfaces or solid phase As in these sediments to support such activities.

Alternative explanations for the difference in groundwater As concentrations observed between the Holocene and Pleistocene aquifers studied here include: (i) a control by sorption/desorption processes within the aquifer (cf. Berg et al., 2008; McArthur et al., 2008; Polizzotto et al., 2006; Fig. 6); (ii) transport of As, mobilized upstream (with respect to groundwater flow) of the study sites, and within sediments with the redox-cline closer to the oxidised surface; or (iii) a combination of these processes. Eiche et al., (2008) observed contrasting reflectance (ΔR) values for both aquifer sediments. The Pleistocene aquifer at site L is characterised by relatively high ΔR values (up to 0.8) whilst all the Holocene aquifer sediments at site H are characterised by relatively low ΔR values (on average < 0.25 ; Table 1; Fig. 3). In the past, reflectance values have shown strong inverse correlations with the Fe(II)/ Total-Fe ratio in aquifer sediments (Horneman et al., 2004). This suggests that the general Fe(II)/ Total-Fe ratio in the Pleistocene aquifer at site L is, most likely, substantially lower (estimated between 0.2 and 0.4; based on the equation given in Horneman et al., 2004) if compared to the relatively high Fe(II)/ Total-Fe ratio (estimated between 0.6 and 0.7; based on the equation given in Horneman et al., 2004) in the Holocene aquifer at site H, consistent with sorption processes being important. However these processes alone cannot explain why the oxidation state of the two aquifers are so different; site L being predominantly brown and, though reducing, relatively oxidised, and site H being predominantly grey and highly reduced (Table 1). To be able to explain these differences, microbially mediated redox processes upstream (with respect to groundwater flow) of the study sites needs to be invoked, such as has been proposed by Polizzotto et al. (2008) in the Mekong River Basin and Postma et al. (2007) in the Red River Basin. However, it cannot be excluded that other microbial processes, which do not facilitate As mobilization, may also be taking place in the aquifers, creating the differences in the redox status observed. This is supported by the relatively high concentrations of HCO_3^- , $\text{P}_{(\text{tot})}$ and NH_4^+ (Table 1; Sutton et al., 2009), with NO_3^- being below detection limits, at site H compared to their much lower concentrations at site L (Table 1), indicating that the reducing conditions are at least partly due to other types of microbial activity. Lastly, it is noted that the redox behaviour of sulfur, particularly the formation of sulfide minerals which could act as sinks for As, could play a role in controlling As mobility (cf. Jessen et al., 2008); but further work beyond the scope of this study would be required to fully test this.

Berg et al. (2008) have already suggested that the groundwater As enrichment observed in these wells in the Van Phuc area was caused by the reductive mobilization of ionically bound and/or adsorbed As from the sediments. However, it is more likely that this mobilization takes place elsewhere than the study sites, after which the liberated As is transported through the aquifer sands, where sorption/desorption processes become increasingly important in controlling As concentrations in the groundwater. Since there are indications for a slow groundwater movement from site H towards site L based on i) measured hydraulic heads that diverge by some 0.5 m, and ii) differing $^3\text{H}/^3\text{He}$ groundwater ages (site L: <55 a, ^3H -absent; site H: 15-23 a; ^3H -present) (Frei,

2006), differences in the As concentrations observed at these sites is primarily associated with processes taking place upstream (with respect to groundwater flow paths).

Combined, these results show that although As mobilization is undoubtedly controlled by a complex range of processes including the interplay between microbiology, mineralogy and geochemistry at the nano-scale, larger-scale transport processes are also crucial in dictating the final distribution of the metalloid in at-risk aquifers.

5. Conclusions

Organic geochemical analyses on sediments, from two aquifers (one of Holocene age and with high groundwater As; one of Pleistocene age and with low groundwater As) in Van Phuc village in the Red River Delta of Vietnam, reveal lipid biomarkers of predominantly terrestrial origin with an influx of petroleum-derived OM source evidenced by the stereochemistry of the hopanes and the presence of an UCM. These petroleum-derived hydrocarbons have probably originated from a deeper, thermally mature, source. Despite the contrasting groundwater As associated with the different cores, no clear difference in sedimentary OM composition was observed between the Holocene and Pleistocene aquifer sediments.

Microbial community analyses indicate an absence of microbial communities previously associated with Fe(III) or As(V) reduction. In contrast to other As-rich aquifers, where the Fe(III)-reducing genus *Geobacter* is often abundant, *Geobacter* 16S rRNA gene sequences were detected at relatively low concentrations in the Van Phuc cores, suggesting that metal reduction is not a dominant process in these sediments.

These observations collectively suggest that (i) As concentrations observed in the groundwaters at both sites is most probably not caused by *in situ* microbially mediated reductive transformations of As-containing Fe(III) (hydr)oxides and (ii) these processes are not limited by an absence of electron donors, mineral surfaces or solid phase As in these sediments.

This and the difference in redox status between the two aquifers suggest that, most likely, microbially-mediated As mobilization takes place elsewhere, after which the liberated As is transported laterally or sub-vertically through the aquifer sands to the study sites. This implies that the differences in As concentrations observed at both sites is primarily associated with processes taking place at locations upstream along relevant groundwater flow paths, with modification of these concentrations through subsequent sorption/desorption processes as groundwater flows through aquifer sediments with varying sorption capacity.

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Figure Captions

Figure 1. Map showing the study sites in Van Phuc village situated about 10 km SE of Hanoi city centre (modified map after Berg et al., 2007). Arsenic shows an irregular distribution in the aquifers of this village. The investigated sediment cores are some 700 m apart and marked as follows: L (low) with low levels of As ($3 \pm 2 \mu\text{g/L}$) and H (high) with high levels of As ($400 \pm 135 \mu\text{g/L}$).

Figure 2. Lithological logs of the boreholes drilled in April 2006 at site L and site H. The labelling on the left side of the logs marks the samples taken for lipid extractions (all samples except H0970) and/or microbial analyses (samples L2870, H0970, H1860 and H3055). The scale on the right side indicates the depth in meters (modified after Eiche et al., 2008).

Figure 3. Concentrations of dissolved As, Fe, DOC, HCO_3^- , solid phase As, Fe, % TOC, ΔR (data from Eiche et al., 2008) as well as total concentration, Carbon Preference Index (CPI) and Average Chain Length (ACL) of the High Molecular Weight (HMW) *n*-alkanes ($\Sigma \text{C}_{20}\text{--}\text{C}_{34}$) and the ratio of $\text{C}_{30} \beta\beta$ hopane to the sum of $\text{C}_{30} 17\alpha\text{H}$, $21\beta\text{H}$ and $\text{C}_{30} 17\beta\text{H}$, $21\beta\text{H}$ hopane in sediments from Van Phuc, Vietnam. Roman numbers indicate the different clusters mentioned in the text.

Figure 4. The major bacterial phylogenetic groups that were identified in the 16S rRNA gene clone libraries of sediment samples L2870 (36.5 m), H0970 (10.9 m), H1860 (20.8 m) and H3055 (35 m).

Figure 5. PCR amplified products from Van Phuc Vietnam samples using universal or *Geobacteraceae*-specific 16S rRNA gene primers.

Figure 6. Schematic cross-section of the study area illustrating the core from site H (lithology taken from Fig. 2), idealized groundwater flow paths, and hypothetical zones upstream site H where microbial reduction and sorption/desorption processes may be controlling As mobility. The roman numbers refer to the organic geochemical clusters as described in the text, e.g. I = immature plant origin dominated OM, II = mature (petroleum) derived OM, and III = mixed contributions of immature and mature OM.

Table 1. Characteristics of groundwater and sediments from the study sites at Van Phuc Village, Vietnam (data from Eiche et al. 2008).

	Site L	Site H
Location	N 105.89208, E 20.92318	N 105.89758, E 20.91972
Age	Pleistocene	Holocene
Depth of cores (m)	54	57
Depth of monitoring wells (m)	24-54	17-57
<i>Groundwater characteristics</i>		
Dissolved As ($\mu\text{g/L}$)	0.9 - 7.8	7.0 - 600
Fe (mg/L)	0.72 - 2.7	2.4 - 20.4
pH	6.36 - 7.04	7.0 - 7.23
Dissolved SO_4^{2-} ($\mu\text{g/L}$)	<15 ^a - 11500	<15 ^a - 1050
NH_4^+ (mg N/L)	0.1 - 0.8	2.0 - 34
DOC ^b (mg/L)	0.9 - 2.8	1.7 - 6.1
HCO_3^- (mg/L)	140 - 370	420 - 620
P_{tot} ($\mu\text{g/L}$)	25 - 320	170 - 1330
NO_3^- (mg/L)	<0.25 ^a	<0.25 ^a
Eh	+60 - +150	-98 - -164
<i>Sediment characteristics</i>		
As (mg/kg)	2.1 - 28.7	1.0 - 30.3
Fe_2O_3 (%)	0.75 - 8.12	0.47 - 7.08
TOC (%)	0.01 - 4.6 ^c	0.01 - 0.8
Reflectance (ΔR) ^d	< 0.1 - > 0.7	< 0.25

^a below detection limit.^b DOC = dissolved organic carbon.^c Contains an organic rich peat layer with TOC of 4.6 % (see Fig. 2).^d Aquifer sediments only.

Table 2. Organic contents, abundance, and ratios of high molecular weight *n*-alkanes in sediments samples of sites L and H

Sample ID	Depth (m)	TOC ^a	Σ <i>n</i> -alkanes ^b	C _{max} ^c	CPI ₂₀₋₃₄ ^d	ACL ₂₀₋₃₄ ^e	UCM presence ^f
H0750	7.14	0.061	61	31	3.0	27.2	-
H1090	12.4	0.063	44	29	2.3	26.4	-
H1860	20.8	0.023	68	20	1.2	23.3	+
H2450	27.4	0.026	198	20	1.1	23.2	+
H3055	34.8	0.016	47	20	1.2	22.7	+
H3605	45	0.014	83	20	1.3	24.3	+
L0580	5.6	0.265	360	31	3.6	28.8	-
L0990	12	4.55	521	29	3.3	29.4	-
L14100	19.7	0.103	63	31	1.3	26.8	+/-
L2250	27.6	0.03	47	21	1.0	24.0	+
L2870	36.5	-	23	20	1.3	23.9	+
L3290	44.6	0.011	103	20	1.1	23.7	+

^a Total organic C (TOC) values in sediments (%); (Data obtained from Eiche et al., 2008).

^b The sum of concentrations of the HMW *n*-alkanes, Σ (C₂₀-C₃₄), ng/g sediment.

^c Chain length of the alkane with highest concentration.

^d CPI_{i-n} = carbon preference index over the range C_i-C_n. (calculated following the method of Schefuss et al., 2003).

^e ACL_{i-n} = Average chain length over the range C_i-C_n. (calculated following the method of Schefuss et al., 2003).

^f UCM = unresolved complex mixture (+ = present in high abundance, +/- = present in low abundance, and - = absent).

Table 3. Total high molecular weight *n*-alkanoic acids, *n*-alkanols, sterols and ω -hydroxy alkanolic acids abundance (in ng/g sediment) in sediments samples of sites L and H

Sample ID	Depth (m)	<i>n</i> -alkanoic acids				<i>n</i> -alkanols				Sterols	ω -hydroxy alkanolic acids ^a		
		Σ (C ₂₀ -C ₃₄)	CPI ₂₀₋₃₂ ^a	ACL ₂₀₋₃₂ ^b	C _{max} ^c	Σ (C ₂₀ -C ₃₁)	CPI ₂₀₋₃₀ ^a	ACL ₂₀₋₃₀ ^b	C _{max} ^c	Σ Sterols ^d	Σ (C ₂₀ -C ₃₁)	CPI ₂₀₋₃₀ ^a	C _{max} ^c
H0750	7.14	54	3.2	28.1	30	230	16.5	26.2	26	29.4	5.2	2.7	24
H1090	12.4	79	4.5	28.0	30	190	33.9	26.0	28	16.7	9.7	2.3	24
H1860	20.8	55	3.0	26.6	26	82	47.5	25.9	24	12.2	7.0	2.7	24
H2450	27.4	10	4.0	24.4	20	640	6.8	26.7	28	171	Bd ^e	Bd ^e	Bd ^e
H3055	34.8	8	6.1	22.7	20	98	20.4	23.4	24	30.2	Bd ^e	Bd ^e	Bd ^e
H3605	45	32	2.8	25.9	28	80	12.3	25.4	24	107	Bd ^e	Bd ^e	Bd ^e
L0580	5.6	260	5.8	28.3	28	2800	10.6	26.4	28	239	53	18.9	28
L0990	12	18000	7.6	27.5	26	11000	15.0	27.4	24	137	13000	6.9	28
L14100	19.7	18	5.1	26.0	26	350	16.8	25.5	26	62.8	4.1	3.4	22
L2250	27.6	28	6.0	26.8	24	31	3.8	25.6	28	40.8	Bd ^e	Bd ^e	Bd ^e
L2870	36.5	120	7.9	27.0	26	170	63.0	26.8	28	15.9	Bd ^e	Bd ^e	Bd ^e
L3290	44.6	39	6.3	26.7	26	170	14.5	25.9	28	26.9	Bd ^e	Bd ^e	Bd ^e

^a CPI_{i-n} = carbon preference index over the range C_i-C_n (calculated following the method of Schefuss et al., 2003).

^b ACL_{i-n} = Average chain length over the range C_i-C_n (calculated following the method of Schefuss et al., 2003).

^c Carbon number of the HMW homologue with the highest abundance.

^d Summed concentration of cholesterol, campesterol and sitosterol.

^e bd = below detection limit.

Figure1

Figure3

figure6

Highlights:

- First lipid analysis of Pleistocene aquifer sediments from a groundwater As-prone region
- No difference in lipids between (As-rich) Holocene and (As-poor) Pleistocene aquifers
- Lack of *in situ* microbially mediated As mobilization in any of the sediments.
- Groundwater As at depth controlled by As mobilization processes elsewhere.