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Elemental and Biomarker characteristics in a Pleistocene aquifer vulnerable to arsenic contamination in the Bengal Delta Plain, India

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Abstract

An elevated level of arsenic (As) in the Indo-Gangetic delta plain aquifers has been reported since the 1990s. Organic matter (OM) present in groundwater and aquifer sediments sustains the microbial communities in these aquifers. In this study, during installation of a drinking water well, 26 sediment intervals of 6 m each were retrieved up till 156 m from a Pleistocene brown sand aquifer (BSA). The aquifer sediments consist of medium to coarse sand except the surface sediments and those at bottom, which have high clay and slit content. Various geochemical analyses in the sediment samples include sequential extraction of trace metals and total extractable lipids. Arsenic (As) concentration in sediments range from 2 to 21 mg/kg and indicate a strong correlation with grain size. Arsenic is mostly associated with crystalline oxides and silicate-rich minerals. Arsenic shows significant correlation with Fe in different geochemical fractions and suggests presence of pyrite bound As-bearing minerals in these sediments. The sediments indicate diagnostic biomarkers such as *n*-alkanoic acids, *n*-alkanes, *n*-alkanols and sterols. The presence of these compounds is associated with terrigenous sources derived from vascular plants and microbial cell wall. The inference is supported by various diagnostic lipid ratios. The biomarkers are abundant in surface and deeper layers, which have high clay and silt content. The BSA sediments indicate preferential preservation of *n*-alkanes over other

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functionalized compounds, which are more reactive and subject to degradation. The thick clay lens at 132 to 156 m contains visible plant fragments, and OM in this layer indicates preferential preservation of the organic carbon perhaps due to the absence of microbial communities that degrade these compounds, and mobilize As into groundwater.

Key words: Arsenic, Pleistocene aquifers, Metals, Biomarkers, Microbes

1. Introduction

High arsenic (As) concentration in groundwater and its exposure to a large number of people in Southeast Asia has caused long term endemic health problems (Bhattacharya et al., 2002). This issue has received significant attention since the 1990s (Guha et al., 1988). The Bengal Delta Plain (BDP) in northeastern India and Bangladesh is one of these regions, which is most affected by the high As concentrations in drinking water. These deltaic plains are located in the river basins of Ganges, Brahmaputra and Meghna rivers, and extend over ca. $2 \times 10^6 \text{ km}^2$ (Curry and Moore, 1974).

Researchers have proposed many models for explaining the mechanism of As mobilization and explaining its source in these aquifers. The reductive dissolution of geogenic iron Fe(III) oxy-hydroxide minerals bearing As is widely accepted. This process underlines the complex biogeochemical processes which influence As mobilization in BDP aquifers (Bhattacharya et al., 1997; Nickson et al., 1998, 2000; Harvey et al., 2002; McArthur et al., 2004). The reductive dissolution Fe(III) oxy-hydroxide minerals is coupled with microbial utilization of organic matter (OM) as electron donor during respiration (Nickson et al., 2000; Berg et al., 2001; Harvey et al., 2002; Islam et al., 2004). Along with increase in As concentrations, the process results in increase of alkalinity and dissolved iron in groundwater (Nickson et al., 2000). Consistent with this, many studies have indicated that biological processes play a critical role by changing redox conditions or converting the As species (from As(V) to As(III)), and impacting its mobilization in surface and groundwater (Oremland and Stolz, 2003, 2005; Quemeneur et al., 2008).

Recent studies have demonstrated that sedimentary and dissolved organic matter (OM) in BDP aquifers utilized by *in situ* microbes are key factors controlling the release of As (Farooq et al., 2010; Postma et al., 2007; Rowland et al., 2006, 2007; Ghosh et al., *in review*). Various external and internal sources of OM have been proposed by researchers, which contribute to the OM pool in sedimentary aquifers. Anthropogenic sources of OM can be surface derived inputs (McArthur et al., 2001; Lawson et al., 2013), whereas autochthonous sources include relatively recalcitrant naturally derived OM, which are primarily found in organic rich clayey-peat lenses in the BDP aquifers (Umitsu, 1993; Goodbred and Kuehl, 2000; McArthur et al., 2004; Anwar et al., 2010). Earlier reports have also indicated the presence of natural gas and substantially rich petroleum reserves in BDP sediments (Alam, 1989; Ganguly, 1997; Milici et al., 2002). In addition, there are several studies where presence of thermally mature petroleum derived hydrocarbons are suggested as one of the key sources of *in situ* derived OM in the As contaminated Holocene grey sand aquifers in BDP (Rowland et al., 2006, 2007; Héry et al., 2010). Likewise, a similar situation is suggested for the deltaic sediments in Cambodia, Taiwan and Vietnam (Lawati et al., 2012a,b). Although, microbial degradation of petroleum derived hydrocarbons in shallow Fe(III) reducing aquifers is well established (Tuccillo et al., 1999; Chapelle et al., 2002), there are few instances where researches have investigated the complex interactions involving OM utilization by *in-situ* microorganisms, and high As levels in aquifers (Ghosh et al., 2014; Ghosh et al. *in review*). Organic matter in shallow Pleistocene aquifer from an As affected region in Vietnam has been characterized recently (Lawati et al., 2012a). OM characteristics in this aquifer however does not differ significantly with respect to the young Holocene aquifers in this region (Lawati et al., 2012a), which are more prone to elevated As levels. In light of the fact that Pleistocene aquifers are considered “As-safe” (Ravenscroft et al., 2001), it is important to investigate the biogeochemical characteristics in sediments, which impact As cycling. Therefore, the main objectives of this study are to: 1) characterize OM sources in the Pleistocene BSA sediments, and 2) establish the possible relationship between sedimentological characteristics vs. the distribution of trace metals and sedimentary OM distribution influencing As cycling. To the best of our knowledge this is the only study of OM characteristics in a Pleistocene BDP aquifer, and the data provides an opportunity to assess complex biogeochemical interactions in the sub-surface associated with As cycling and its mobility.

2. Geological settings

The shallow sedimentary aquifers in BDP have been divided into late Pleistocene and Holocene alluvial deposits dating back to ca. 18 ka BP (Acharyya et al., 2000). Collision of the Indian and Eurasian plates during Miocene formed the Himalayas ca. 50 Ma. Rapid physical and chemical weathering of the Himalayas and deposition of weathered sediments resulted in formation of the Bengal fan, which prograded as a clastic wedge in the Bengal Basin (Alam, 1989; Uddin and Lundberg, 1999). Further lowering of sea-level and erosion (Lindsay et al., 1991), and rapid deposition by rivers resulted in formation of the BDP alluvial deposits (Acharyya et al., 2000). The sedimentary Holocene deposits consist of grey micaceous sand with OM rich peat lenses. In contrast, iron-rich Pleistocene deposits have coarse to fine brown sand with intermediate clay and peat layers (Alam, 1989; Acharyya et al., 2000).

Nadia district in West Bengal, India has been widely tagged as an As “hot-spot” (Bhattacharya et al., 2002; Mukherjee et al., 2008; Biswas et al., 2012 (a)). Arsenic-rich groundwater in these aquifers exceeds beyond the 10 µg/l ‘safe’ drinking water limit proposed by the World Health Organization (WHO, 2011). The study area is located in Haringhata block (N 22°56.401’, E 88°32.389’) in the south eastern corner of Nadia district (Fig. 1). The aquifer is primarily recharged by rainfall during monsoon season. The annual rainfall in this district is ~1413 mm and the annual temperatures are 42 °C in summer and 9 °C in winter (Statistical Handbook, 2010). Recent investigations led by us indicate that arsenite oxidizing bacterial groups are absent in this well. However, these bacterial groups were detected in grey sand aquifers (GSA) in this region (Ghosh et al., 2014). The sediment profile in this aquifer contains fine to medium brown colored sand with clay lenses occurring in-between the layers. The top layer consists predominantly of clay; in addition, there is a thick clay lens at depth of 132-156 m. Arsenic concentration in the aquifer in 2011 was 22 µg/l.

3. Materials and Methods

3.1. Sediment collection

About 156 m of core segments were recovered by drilling a borehole using the conventional household technique, involving hand percussion and reversed circulation. This method allows continuous recovery of drilled sediments. Sediment samples from surface to every 6 m were

collected and labeled as samples 1 to 26. The samples were stored in individual separate zip lock polyethylene bags, and kept at 4 °C. All the samples were freeze-dried before further geochemical analysis.

3.2 Total organic carbon (TOC) analysis

To remove the carbonates, the samples were pre-treated with HCl (Brodie et al., 2011). The percent abundance of TOC in pre-treated sediment samples were determined by an infrared detector in a TOC analyzer (Elementar Analysensysteme GmbH) after combusting the samples at 1000 °C. The instrument was calibrated using the Canadian Stream Sediment (STSD-4 B-571).

3.3 Sediment grain size analysis

The sediments were wet sieved through a 250 µm and 63 µm sieve with 0.5 g/l of Na₃PO₄ solution. The suspension volume of this fine fraction was made upto 80 ml with the Na₃PO₄ solution and sonicated in a Vibracell CV334 (Sonics and Materials Inc.). The grain size distribution and % sand, silt, and clay in the sediment suspension was determined with a Sedigraph III coupled with an auto-sampler Mastertech 52 (Micromeritics).

3.4. Trace metal analysis

The BCR protocol of the Standards, Measurement and Testing Program (formerly the Community Bureau of Reference) of the European Commission (Quevallier et al., 1997; Rauret et al., 2000) was followed for sequential extraction of metals. The protocol involved three step-by-step extractions to separate the different mineral fractions (Table 1), and is further detailed in the original references (Quevallier et al., 1997; Rauret et al., 2000). The original BCR method requires 1 g of sample for extraction, however Routh and Hjelmquist (2011) have shown that half of the amount can also be used. Hence, we have used 0.5 g of freeze-dried sediment sample in this study. Each extraction step was followed by centrifugation at 3000 g for 20 min, and the supernatant was collected. After each step, a wash with 10 ml of deionized distilled water was done. The certified standard reference sediment CRM-601 was used for extraction to check the extraction efficiency. All the four fractions (named as fraction 1 to 3) were analyzed on a Perkin Elmer NexION 300D ICP-MS. The detection limits for the different trace metals detected in each step is detailed in the supplementary data table S1.

Total digestion of the sediments was carried out in accordance with the Swedish Standard Method (SIS, 1993) using same amount of sample (0.5 g) as in the BCR sequential extraction method. All the digestions were carried out by autoclaving the samples with 10 ml 7 M HNO₃ at 100 kPa and 121°C for 30 min. The digested solution was cooled down and centrifuged at 14,000 g for 15 min. The extracts were named as fraction 4. The reference standard CRM-601 was digested in the same way as the samples.

3.5. Extraction of lipid fractions

About 10 g of freeze-dried sample was used for extraction on a Dionex 300 automated solvent extractor (programmed for a 60 min extraction cycle at 1500 psi at 100 °C and another 60 min extraction cycle at 1500 psi at 140 °C) with a mixture of dichloromethane and methanol (9:1 v/v ratio) and recovery standard consisting of 50 mg/l deuterated hexatriacontane-d₅₀). Total volume of the lipid extract was reduced in a Büchi Syncore system. These total lipid extracts (TLE) were further separated into two different fractions using packed cartridges. The neutral and acid fractions were separated in 6 ml glass columns packed with 500 mg of Supelco Superclean LC-NH-2 (Kim and Salem, 1990); the sample was eluted with CH₂Cl₂/isopropanol (2:1, v/v; 15 ml; ‘neutral fraction’) and 2% acetic acid in diethyl ether (15 ml; ‘acid fraction’), respectively. The acid fraction was dissolved in 1 ml of bromo trifluoride (BF₃) in methanol and heated (70 °C for 120 min) to convert into their corresponding methyl esters. The derivatized products were further extracted with NaCl and hexane. The neutral fractions were separated into polar and non-polar fractions using Bond-Elut column cartridges (Agilent Bond Elut® AL-N 500 mg, 3 ml) with hexane (5 ml; non-polar fraction) and CH₂Cl₂/MeOH (1:1 v/v, 5 ml; polar fraction). The non-polar fractions were dried under a gentle flow of moisture and hydrocarbon free N₂ gas. The polar fractions were derivatized to their trimethylsilyl ethers using 100 µl bis (trimethylsilyl) trifluoroacetamide (BSTFA) and 100 µl of pyridine, and heated (70 °C for 120 min). To ensure no contamination was introduced during the extraction and separation procedure, blanks were prepared following the same protocol. All the lipid fractions were analyzed by gas chromatography-mass spectrometry (GC–MS) after adding specific internal standards. To

quantify the non-polar and polar fractions, androstane and deuterated tetracosane were used as internal standards, and for the acid fractions deuterated eicosinoic acid methyl ester was used.

3.6. Gas chromatography–mass spectrometry (GC–MS)

The lipid fractions were analyzed in an Agilent 6890 N gas chromatograph interfaced to an Agilent 5973 MSD mass spectrometer at 70 eV and scanning from m/z 40–600 at 2.62 scans/s. The non-polar and acid fractions were dissolved in hexane, and the polar fractions were dissolved in CH₂Cl₂:MeOH (2:1) before injection. The samples were injected in split-less mode (1 µl; inlet pressure of 10 psi with a flow rate 54.3 ml/min) and separated on a HP-5 MS capillary column (5% di phenyldimethyl polysiloxane; length 30 m, 250 µm, film thickness 0.25 µm). A constant flow (1.3 ml/min) of He was used as carrier gas. The interface was set at 300 °C, whereas the mass source was set at 230 °C and the MS quadrupole was maintained at 150 °C, respectively. The samples were injected at 35 °C and the oven was programmed to 130 °C at 20 °C/min and then at 6 °C/min to 320 °C where it was maintained isothermally for 15 min. Based on the retention time and mass spectra of different lipids, the compounds were identified by comparing our results with published literature (Philip, 1985; Peters et al., 2005) and online libraries (NIST library and Lipid library, 2011). The fractions were quantified with respect to the response of their internal standards.

3.7. Statistical analyses

To determine the correlation between As and other trace elements, a bivariate correlation test was done. Arsenic concentration in each fraction was correlated with the concentration of each element studied in that fraction, and a correlation matrix was plotted. A principal component analysis was carried out using the total concentration of different organic fraction (*n*-alkane, *n*-alkanoic acid and *n*-alkanol), and the inorganic elemental concentrations (in total digested fraction) with respect to sediment grain size. Furthermore to predict the prime variables, which have the highest influence on the studied niche, ten organic and inorganic variables were selected to make a predictor plot. All these analyses were done using IBM® SPSS® Version 22.

4. Results

4.1. Sediment grain size and organic carbon content

The sediments ranged from grayish brown to reddish brown and deep brown in color. The sediments predominantly consisted of brown sand; the top three samples were clayey. Samples at a depth of 66-72 m consisted of a clay layer. Another clay layer occurred at 132 m and persisted upto the bottom (156 m) of the well; this unit also contained plant fragments which could be identified visibly (Fig. 2). The clay rich samples had slightly higher TOC content (0.2 to 0.67 %) than sand (0.13 to 0.17 %; Fig. 2).

4.2. Distribution of trace metals

The reliability of BCR method for sequential extraction of metals was verified using the CRM-601 standard along with the sediment samples. After 84 m, alternate samples were selected for sequential extraction. In CRM-601, 6 trace elements were assessed in each extraction step and also for the pseudo-total elemental concentration (López-Sánchez et al., 1998). Since 7 M HNO₃ was used to extract pseudo total elemental concentration, it is not strictly comparable to the reported values in CRM-601.

Trace element concentrations of different extraction steps for CRM-601 standard were compared to their 95% confidence interval (López-Sánchez et al., 1998; Table 2). The concentrations for Cu, Ni and Zn in fraction 1 of this study were not within the confidence limit. In fractions 2, concentrations of Cr, Cu, Ni and Zn were not within the confidence limit, and in fraction 3 trace metal concentrations of Ni, Pb and Zn were not within the confidence limit. Zn was not measured within confidence in any of the extraction steps. From these results, it was concluded that all steps in the BCR extraction scheme in this study were only partially reliable, and was similar to our experience of using this protocol for sequential extraction in another study on BDP sediments (Routh and Hjelmquist, 2011).

Samples from top layers and the clay lenses had the highest concentrations for most elements. The total As concentration in these sediments varied between 1.73 and 21.2 mg/kg (Fig. 3). The highest As concentration occurred in samples 1, 2, 7, 8 and 24. Arsenic was mainly associated with the residual fraction. However, in other readily available forms (e.g., As complexes with organic matter and Fe–Mn oxy-hydroxides) extracted in fractions 1–3 ranged between 0.01 to

20.0 mg/kg. Arsenic associated with the reducible fraction 2 occurred in samples 1, 2, 7, 8, 9, 24 and 26, and ranged from 0.23 to 4.65 mg/kg (Fig. 3).

The total Fe concentration in sediments varied between 854 and 10,661 mg/kg (Fig. 3); the highest concentration occurred in fraction 4 followed by fractions 3 and 2. The total Mn content varied between 12.6 and 253 mg/kg. Mn primarily occurred in fractions 2 and 3 (Fig. 3). The total P concentration in sediments varied between 252 to 2530 mg/kg (Fig. 3). Phosphorus mainly occurred in fractions 2 and 4.

4.3. Distribution of sedimentary lipid biomarkers

n-Alkanes

The total *n*-alkane concentration in sediment samples varied significantly between the different depth intervals (Supplementary data, Table S1), and ranged from 0.05 to 25.0 ng/mg. The *n*-alkane concentrations did not show a steady decline with depth for the first 54 m, however after this depth interval, decline in *n*-alkane concentration was more rapid. The clay lens present at depth 138-154 m with visible trace of plant material showed high *n*-alkane concentration (Supplementary data, Table S1). Unimodal distribution of *n*-alkanes was observed with the predominance of high molecular weight (HMW) *n*-alkanes ($>n\text{-C}_{20}$) in samples with high (27 to 70 %) silt and clay content (1, 2, 3, 11, 24, 25). A predominance of HMW odd *n*-alkanes *n*-C₂₇, *n*-C₂₉, *n*-C₃₁ was observed in all these samples, except in sample 25 (144-150 m), where equal predominance of odd and even HMW *n*-alkanes was observed (Fig. 4). Overall the total HMW *n*-alkanes concentration ranged between 0.006 to 0.55 ng/mg, and the total low molecular weight (LMW) *n*-alkane concentration ranged between 0.03 to 2.49 ng/mg. The distribution of these biomarkers was used to assess the source(s) of these compounds based on diagnostic ratios as discussed below.

1) Carbon preference index (CPI) including *n*-C₂₃ to *n*-C₃₂ *n*-alkanes (Allan and Douglas, 1977) was modified based on the abundance and distribution of different alkane monomers in our samples. We separated them as CPI_{TOT} (*n*-C₁₃ to *n*-C₃₅), CPI_{LMW} (*n*-C₁₃ to *n*-C₂₁) and CPI_{HMW} (*n*-C₂₃ to *n*-C₃₅).

$$CPI_{TOT} = \frac{\Sigma(C_{13} \text{ to } C_{33})_{odd} + \Sigma(C_{15} \text{ to } C_{35})_{odd}}{2\Sigma(C_{14} \text{ to } C_{34})_{even}} \quad (1)$$

$$CPI_{LMW} = \frac{\Sigma(C_{13} \text{ to } C_{19})_{odd} + \Sigma(C_{15} \text{ to } C_{21})_{odd}}{2\Sigma(C_{14} \text{ to } C_{20})_{even}} \quad (2)$$

$$CPI_{HMW} = \frac{\Sigma(C_{23} \text{ to } C_{33})_{odd} + \Sigma(C_{25} \text{ to } C_{35})_{odd}}{2\Sigma(C_{24} \text{ to } C_{34})_{even}} \quad (3)$$

The CPI_{TOT} ranged from 0.22 to 6.03, whereas the CPI_{LMW} ranged between 0.12 to 1.41 and CPI_{HMW} ranged from 0.38 to 25.6 (Fig. 4).

2) Terrigenous/aquatic ratio (TAR) was proposed by Bourbonniere and Meyers (1996) to estimate the terrigenous or aquatic derived OM input to sediments. TAR was modified to accommodate the distribution of *n*-alkanes, and the value ranged from 0.60 to 42.7, however the TAR value in sample 25 was extremely high (Fig. 4).

$$TAR = \frac{\Sigma(C_{27} \text{ to } C_{31})_{odd}}{\Sigma(C_{15} \text{ to } C_{19})_{odd}} \quad (4)$$

3) Average Chain Length (ACL) proposed by Cranwell et al. (1987) to describe the type of vegetation ranged from 29 to 31 (Fig. 4) in this study.

$$ACL = \frac{\Sigma(nC_n \text{ to } mC_m)_{odd}}{\Sigma(C_n \text{ to } C_m)_{odd}} \quad (5)$$

where $n = 25$ and $m = 33$

***n*-Alkanoic acids**

The average total *n*-alkanoic acid concentration is greater than 99% of the total lipid extract (Σn -alkane + Σn -alkanoic acid + Σn -alkanol + Σ sterol). The total *n*-alkanoic acid concentration ranged from 307 to 6031 ng/mg in sediments (Supplementary data Table S2). The sediment samples from 0 to 36 m had higher concentration of total *n*-alkanoic acids, which decreased steeply until 138 m (Fig. 4). The LMW *n*-alkanoic acids *n*-C_{16:0} and *n*-C_{18:0} were most abundant followed by HMW *n*-alkanoic acids (*n*-C_{24:0}, *n*-C_{26:0}, *n*-C_{28:0} and *n*-C_{30:0}). Among odd *n*-

alkanoic acids, there was dominance of n -C_{25:0} followed by n -C_{23:0} and n -C_{17:0} monomers. Many unsaturated n -alkanoic acids were detected amongst which unsaturated C₁₈ alkanolic acids (18:1 ω 13 and 18:2 ω 6,9) were dominant followed by 15:1 ω 13. Various ratios were calculated based on the distribution of different n -alkanoic acids.

1) The Carbon Preference Index (CPI of fatty acids) was modified by (Matsuda and Koyama, 1977) by dividing it into low molecular weight CPI_L (C₁₂ to C₁₈) and high molecular weight CPI_H (C₂₂ to C₃₂) for determining the source of n -alkanoic acids.

$$CPI_L = \frac{\Sigma(C_{12} \text{ to } C_{16})_{\text{even}} + \Sigma(C_{14} \text{ to } C_{18})_{\text{even}}}{2\Sigma(C_{13} \text{ to } C_{17})_{\text{odd}}} \quad (6)$$

The CPI_L was high (ranged from 5.34 to 40.3) compared to CPI_H which ranged from 1.39 to 4.31 (Fig. 4).

2) The terrigenous:aquatic fatty acid ratio (TAR_{FA}; proposed by Meyers (1997) was used to predict the source of fatty acids in sediments.

$$TAR_{FA} = \frac{\Sigma(C_{24} \text{ to } C_{28})_{\text{even}}}{\Sigma(C_{12} \text{ to } C_{16})_{\text{even}}}$$

The TAR_{FA} value ranged from 0.03 to 0.89; sample number 26 had the highest TAR_{FA} value (Fig. 4).

n -Alkanol

The n -alkanol concentration within the sediment profile varied from 0.02 to 7.17 ng/mg, n -C₂₂ alkanol was the most predominant monomer. In order to assess the preferential preservation of different organic compounds, the higher plant alkane index (HPA; Westerhausen et al., 1993) was used.

$$HPA = \frac{(C_{24} + C_{26} + C_{28})_{\text{alkanol}}}{(C_{25} + C_{27} + C_{29})_{\text{alkane}} + (C_{24} + C_{26} + C_{28})_{\text{alkanol}}} \quad (8)$$

The HPA values ranged from 0.02 to 0.96 (Fig. 4).

Other than these compounds, sterols and stanols were also detected in a few samples. The top 0-6 core contains coprostane. The thick clay-rich layer at the bottom of the core (132 to 156 m) contained higher stanols and sterols including: brassicasterol, 5 α -brassicastanol, 5 α -stigmastanol and situstanol.

5. Discussion

5.1. Inorganic characteristics

Riverine deposits with sand fining upward into silt with clay bands is typical of BDP sediments (Tucker et al., 1991; Umitsu, 1993). These stacked packages of sediments were probably deposited in channels and the fining-upward sequences have formed during channel migration (Umitsu, 1993; Goodbred and Kuehl, 2000; McArthur et al., 2004). Because of the inherent dynamism associated with these depositional environments there is strong variability/heterogeneity in sediment characteristics observed in BDP. The predominantly brown colored iron stained sediment with low TOC content in the study area has previously been described as early to mid-Pleistocene deposits in age (Umitsu, 1993; Alam, 1989). The aquifer has orange to brown colored clayey sediments on top, with brown colored sands in deeper layers. The brown color is due to the high iron content in these sediments, and suggests oxidation of bound Fe-(hydr)oxides minerals (Biswas et al., 2012b).

The total As content (1.73 and 21.2 mg/kg) in these samples is above the world average value reported by Smedley and Kinniburgh (2002) for unconsolidated sediments. A bivariate correlation of As with other metals in each fraction from all the sediment samples (Table 3), indicate significant correlation of As with Fe in all fractions [fraction 1 ($r = 0.59$; $p = 0.007$), fraction 3 ($r = 0.76$; $p = 0.00$) and fraction 4 ($r = 0.79$; $p = 0.00$)].

In fraction 1 which contains carbonates, the samples have high concentrations of Ca, Mn and Na. The high Ca content in this fraction occurs in most samples (945 to 30,652 mg/kg; Fig. 3), and shows a strong correlation with Mn ($r = 0.97$; $p = 0.00$) indicating the abundance of carbonate minerals in BSA sediments. This is consistent with the high inorganic carbon content in groundwater (104 mg/l) and low DOC level (7.8 mg/l; Ghosh et al., *in review*). Fraction 1 also contains relatively higher Mn concentration than Fe and As (Fig. 3), suggesting incorporation of

Mn into carbonate minerals like rhodochrosite (MnCO_3) and $\text{CaCO}_3\text{-MnCO}_3$ (Stollenwerk et al., 2007; von Brömssen et al., 2008).

Fraction 2 containing (hydr)oxides have high concentration of many elements (Cd, Fe, Pb, V and Zn). Iron is more abundant in fraction 2 compared to its presence in the other fractions (678 to 6286 mg/kg); this is in agreement with other studies on BDP sediments from this region (Routh and Hjelmquist, 2011; Biswas et al., 2012b). However, there is absence of significant correlation of Fe with As in fraction 2 (Table 3), which is perhaps due to variation in redox potential along vertical or horizontal zones in BDP aquifers (Nickson et al., 2000; BGS and DPHE, 2001; McArthur et al., 2004).

Fraction 3 containing elements bound to sulfides and OM has high concentration of Mg and Mn. Arsenic has significant correlation with sulfidic metals (e.g., Ni, Cu, Pb, Cd and Cr) in fraction 3 (Table 3). Although the concentration of Fe in this fraction is lower (72 to 2058 mg/kg) compared to fraction 2, but significant correlation between As and Fe occurs in this fraction ($r = 0.76$; $p = 0.00$) thereby suggesting presence of As bearing sulfidic minerals such as pyrite. However, this needs to be confirmed with x-ray diffraction analysis. This trend provides evidence supporting the earlier studies, where presence of sulfidic minerals of Fe like pyrite was detected along with Fe (hydr)oxides such as ferric hydroxide [$\text{Fe}(\text{OH})_3$], goethite (FeOOH), hematite (Fe_2O_3), and magnetite (Fe_3O_4) (Das et al., 1996; Chakraborti et al., 2001; Biswas et al., 2012b). The overall low concentration of all elements in this fraction is also in agreement with the low TOC content in BSA sediments.

Fraction 4 containing metals bound to crystalline and silicate forms have high As, Fe and Mn concentrations. There is a significant correlation of As with many transition metals in fraction 4 (e.g., Ni, Cu, Zn, Pd, Cd, Mo, Cr and V; Table 3). This suggests strong association of most of these elements within the crystalline silicate lattice, which protects them from chemical/physical weathering processes (Hjorth, 2004; Routh and Hjelmquist, 2011).

Overall, there is an absence of significant correlation between As and Mn in all fractions, except fraction 4. Likewise, Mn occurs in low concentration in Haringhata groundwater (Ghosh et al., *in*

review), which contrasts with other studies in BSAs (Biswas et al., 2012b). The results indicate that BSAs are mainly a Fe dominated system (see supplementary data Fig. S1). Moreover, the trend suggests that complexation of As with Fe-bound minerals is preferred over Mn-bound minerals. This contrasts findings of other studies (Nickson et al., 2000; BGS and DPHE, 2001; McArthur et al., 2004; Routh and Hjelmquist, 2011). However, these studies were essentially conducted in the grey sand aquifers (GSA) in BDP.

5.2. Organic matter sources and their distribution

The overall low TOC content in these sediments (even in the clay-rich intervals) is typical of alluvial settings and has been reported from other sites in BDP aquifers (McArthur et al., 2004; Rowland et al., 2006; Routh and Hjelmquist, 2011). Alluvial sediments in general consist of degraded and reworked OM, and only the peat-rich intervals in BDP sediments have high TOC content (McArthur et al., 2004; Rowland et al., 2006). Moreover, BDP sediments have been subjected to microbial degradation of OM, which results in low TOC and C/N ratio as a result of early diagenetic changes (Routh and Hjelmquist, 2005). Previous studies suggest that OM degradation during microbial respiration in the BDP sediments drives these aquifers to anoxia, and impacts the release of As in groundwater (McArthur et al., 2004; Islam et al., 2004; Routh and Hjelmquist, 2005). Thus OM characteristics and its inherent quality play an important role in driving microbial processes associated with As cycling. While OM quality particularly its reactivity is an important aspect, nevertheless it is the presence of specific enzymes in the bacterial community, which determine if the OM can be used as a substrate for metabolic processes in the sub-surface.

The distribution and suite of different biomarkers identified in the BSA sediments is similar to those in groundwater analyzed from this aquifer (Ghosh et al., *in review*). Sediment and water samples from this well indicate unimodal distribution and predominance of HMW *n*-alkanes indicating a dominant input of terrestrial OM. The total *n*-alkane concentrations (except the clay lens at 138 m) are similar to the total *n*-alkane concentrations reported by Rowland et al. (2006) in GSAs. The steady decline in total *n*-alkane concentrations with depth as reported by Rowland et al. (2006) is however absent in this sediment core. The total LMW *n*-alkane concentration is high in the clay and silt rich layer, but is notably much lower than the HMW *n*-alkane

concentrations. This trend suggests low abundance of microbial derived inputs produced from degradation of sedimentary OM. The odd over even predominance of *n*-alkane monomers calculated using CPI_{TOT} shows there is higher input of plant derived OM in the clay and silt rich layers in the core. In particular, the bottom layers which indicate presence of plant fragments shows high CPI_{HMW} values confirming the presence of un-degraded and immature sedimentary OM in this interval (Peters et al., 2006). However within this clay interval (138-156 m), sample collected at depth of 144 to 150 m, which indicates plant fragments does not show an odd over even predominance of *n*-alkane distribution. Although the sample is lean in LMW *n*-alkanes and is instead enriched in HMW *n*-alkanes (*n*-C₂₃ to *n*-C₃₅), this sample interval lacks the dominance of odd over even monomers (Fig. 4). Such a distribution pattern of hydrocarbons in sediments suggests presence of OM derived from anthropogenic sources like bitumen and oils and/ OM derived from carbonate and/ rock evaporites (Peters et al., 2005; Burgman and Ali, 2009). The high dissolved inorganic carbon content in groundwater (104 mg/l; Ghosh et al., in review), abundance of Ca in the carbonate fraction in this sample (fraction 1; Fig. 3) and high loss-of-ignition value at 950 °C (D. Ghosh, unpublished study), supports the presence of carbonates in deeper layers.

TAR value corresponds well with the Sedigraph profile, and indicates increased input of higher plant matter in samples rich in clay and silt content. TAR increases rapidly in the bottom clay interval and further confirms the high terrigenous input in this sample. The ACL value ranges from 29 to 31 and confirms the overall predominance of terrestrial OM (Cranwell et al., 1987; Duan and He, 2011) in BSA sediments.

The high abundance of monounsaturated *n*-alkanoic acids C₁₆ and C₁₈ is unspecific because it can be derived from multiple sources e.g., marine/terrigenous bacteria, animals or plants. However, high abundance of long chain *n*-alkanoic acid (C₂₄ to C₃₀) is suggested to indicate epicuticular leaf waxes of vascular plants (Bianchi and Canuel, 2011). The predominance of C₁₈ derived unsaturated alkanoic acids suggest algae as well as higher plant inputs into these sediments. Notably, the even-over-odd predominance among LMW *n*-alkanoic acids indicate microbial reworking in the sediments (Zou et al., 2004; Bianchi and Canuel, 2011 and references

therein). The higher plant derived input in the deepest samples is also supported by their high TAR_{FA} values.

Amongst the *n*-alkanols, abundance of C_{22} -OH indicates presence of suberin derived from plant wax (Bull et al., 2000), or other sources such as eustigmatophytes (Volkman et al., 1999), phototrophic marine and fresh water microalgae (Jaffé et al., 2001). However, in these samples alcohols are mostly likely derived from suberin because significant input from other sources in sub-surface sediments is unlikely. Also, the HPA index is found to be >1 in the clay rich samples indicating preferential preservation of *n*-alkanes over *n*-alkanols. Bacterial reworking of more functionalized organic compound as alcohols is more likely than saturated *n*-alkanes (Routh et al., 2013). Along with *n*-alkanols, few sterol and stanol monomers have been detected in some samples associated with the polar fractions. For example, presence of coprostane in the top layer signifies anthropogenic inputs from sewage (Brown and Wade, 1984). The trend suggests that contamination from such anthropogenic inputs into the sub-surface is restricted to surficial layers and there is perhaps less mixing. The HMW sterols and stanols like brassicasterol, 5α -brassicastanol, 5α -stigmastanol and situstanol are diagnostic markers for plant derived OM sources (Volkman, 1986). Stanols are preferentially preserved over sterols (Routh et al., 2013), and presence of these functional compounds in clay rich sediments implies that despite better preservation, degradation of functional components still occurs in these sediment intervals.

High plant derived OM inputs occur in the clay rich intervals, where low CPI_{HMW} value indicates no apparent predominance of odd over even HMW *n*-alkanes and preferential preservation of *n*-alkanes over alkanols as denoted by the HPA ratio. In fact presence of LMW *n*-alkanes and *n*-alkanoic acids, which are derived from microbial cells suggest microbial degradation of OM (Cranwell et al., 1987; Meyers and Ishiwatari, 1993; Holtvoeth et al., 2010). Based on the lipid distribution profile, it can be said that OM in sand appears to undergo higher biodegradation in comparison to the clay lenses above or below it. Similar distribution of biomarkers with high terrigenous input has also been observed in GSA sediments in BDP (Rowland et al., 2006). Notably, these studies have shown the presence of petroleum derived thermally ‘mature’ hydrocarbons in GSAs, which are suggested to be the key source of OM sustaining the microbes participating in reductive dissolution of As in these aquifers (Rowland et al., 2006; Héry et al.,

2010). Interestingly, there is no indication of petroleum derived hydrocarbons in the Pleistocene BSA sediments, which instead indicate predominance of complex and young immature OM that has undergone limited post-diagenetic changes. Perhaps lack of some of these OM sources play a crucial role in driving As concentration and cycling in the BDP aquifers since this is one of the major differences we find in OM quality between the BSA and GSA sediments.

5.3. Correlation between organic and inorganic components

A principal component analysis was carried out to determine if the distribution of different lipid biomarkers correlated with elemental concentration grain size (Fig. 5). The correlation between the different components is described by the factor score, whereby higher values represent greater significance. The lowest level for significant factor score is chosen as 0.5. The three principal components (PC) represent 77.8% of variability in geochemical data (Table 4). The factor scores obtained for each component are a type of correlation coefficient, and higher values are associated with greater significance. PC1 describes 40.5% of the total variance with high loadings of metal forming As complexes within the silt and clay rich fraction, Fe and Mn. PC2 is described only by lipid biomarkers, and does not correlate with sediment type or metal complexes. The third component PC3 indicates correlation between fine sand and P content in the sample. Thus, from factor analysis it is clear that the inorganic and organic components in the aquifer sediment are naturally exclusive, and they are not interdependent.

A built model is created based on predictor space, where nearest neighbor analysis is done for the 20 samples used for biomarker characterization. Out of 20 samples, 15 are considered as training i.e., they describe 75% of the model, and remaining 5 samples are holdouts. A lower-dimensional projection of the predictor space (Fig. 6), contains a total of 10 different organic and inorganic predictors (As, Fe, Mn, P, and S concentrations, fine sand, silt and clay composition, Σn -alkane, Σn -alkanol, and Σn -alkanoic acid concentration) is plotted. The plot (Fig. 6) indicates that As, Fe and fatty acid content in the sediment samples are the key predictors of the model, which are probably driving the biogeochemical reactions of the aquifer system. This gives a clear indication that both organic and inorganic components co-influence the distribution and fluxes of different elements in the system.

6. Conclusions

This is the first study of BSA sediments in BDP aquifers from West Bengal, where we characterize different lipid fractions in the SOM pool in addition to measuring the distribution of metals in different fractions using the BCR sequential extraction protocol. Although microbial As(III) oxidation occurs in GSA aquifers in this region, this process is absent in the BSA investigated earlier (Ghosh et al., 2014). This is perhaps due to the absence of specific reactive organic and/or inorganic compounds, which impact the As oxidizing bacterial communities in these sediments. There are very few differences found amongst the BSA and GSA aquifers in BDP. In GSAs, As forms complexes with Mn-bound minerals. In contrast, in BSAs, As is mainly related to Fe-bound minerals and the overall concentration of Mn is low in these sediments. Distribution of lipids in BSA sediments shows the presence and better preservation of terrigenous OM along with less microbial degradation. However, such hydro-geochemical differences can potentially influence the indigenous microbial flora. Low microbial metabolism keeps the BSAs As-safe (Dhar et al., 2011) which perhaps results from better preservation of OM due to its complex structure. Moreover the microbial communities in the sub-surface are probably different, which impedes As mobilization and cycling in BSAs. However, in terms of the microbial communities in BSA and GSA groundwaters, we did not see major differences in the bacterial communities, and they are represented by common soil bacteria (Ghosh et al., 2014). Notably earlier studies have shown that imperfect sealing between the shallow GSAs and deep BSAs may transfer the arsenite reducing bacteria in groundwater from GSAs into BSAs (Dhar et al., 2011), which may eventually affect the ‘As-safe’ tag for Pleistocene aquifers in BDP. Thus, key differences between potentially vulnerable ‘As safe’ BSA and As contaminated GSA behooves further detailed studies including *in situ* microbial processes to understand the long term safety viability and characteristics in these aquifers.

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Table and figure captions

Table 1. Scheme of BCR sequential extraction protocol of elements from sediment

Table 2. Comparison of trace element concentrations in the CRM-601 standard obtained in this study with the original certified values reported by López-Sánchez et al. (1998)

Table 3. Correlation of As with other elements in the four different sediment geochemical fractions in Haringhata, West Bengal.

Table 4. Rotated Component Matrix for organic and inorganic variables distributed along the vertical sedimentary profile

Fig. 1. Location of the BSA well investigated in Haringhata, Nadia district, West Bengal, India.

Fig. 2. Downcore distribution of total organic carbon with respect to sediment grain size in Haringhata BSA well.

Fig. 3. Distribution of elements in different geochemical fractions in BSA sediments in Haringhata, West Bengal. Metal concentrations are plotted versus depth (m).

Fig. 4. Distribution of various lipid fractions and their ratios in BSA sediments in Haringhata.

Fig. 5. Component plot in rotated space, circles showing the aggregated loadings defined by each principal component.

Fig. 6. Predictor space plot showing As, Fe and fatty acid as selected predictors.

Supplementary Data

Table S1 Detection limits of different elements in matrix in four different fractions (in µg/l)

Table S2. Distribution of different lipid fractions along the vertical sedimentary profile in Haringhata BSA.

Fig. S1. Relative abundance of As, Fe and Mn in the four fractions extracted from BSA sediments with BCR protocol.

859 Table 1
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Fractions	Extracted with	Target phases ^a
1	20 ml, 0.11 M Acetic acid, shaken for 16 h	Carbonates (pH sensitive), including elements participating in sorption and desorption ion exchange processes in soluble phase.
2	20 ml, 0.5 M Hydroxylamine hydrochloride, pH 1.5, shaken for 16 h	Oxides sensitive to change in pE.
3	5 ml, 8.8 M Hydrogen peroxide, RT, 1 h. At 85°C, 1 h. 5 ml, 8.8 M Hydrogen peroxide, 85°C, 1 h. 25 ml, 1.0 M Ammonium acetate, pH 2.0, shaken for 16 hours.	Sulfides and organic matters
4	10 ml, 7 M Nitric acid, at 121°C, 100 kPa for 30 mins ^b	Pseudo total content including silicate and crystalline residuals

861 a Quevallier et al. (1997) and Rauret et al. (2000).

862 b SIS (1993).

863 RT is room temperature
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866 Table 2

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Elements	Step 1					Step 2					Step 3				
	BCR (2000)*	Present study*	SD values	95% confidence	Within confidence	BCR (2000)*	Present study*	SD values	95% confidence	Within Confidence	BCR (2000)*	Present study*	SD values	95% confidence	Within Confidence
Cd	4.46	4.04	0.63	1.23	YES	3.05	3.65	0.53	1.03	YES	1.80	1.53	0.17	0.33	YES
Cr	0.37	0.22	0.08	0.16	YES	1.42	5.31	0.90	1.76	NO	19.4	19.9	0.91	1.78	YES
Cu	10.4	14.7	0.43	0.84	NO	6.37	51.4	4.90	9.60	NO	116	120	9.00	17.6	YES
Ni	8.22	4.99	0.83	1.62	NO	5.55	8.73	1.20	2.35	YES	6.75	10.3	0.86	1.68	NO
Pb	2.07	1.25	0.49	0.96	YES	37.3	142	11.0	21.5	NO	108	58.7	19.0	37.2	NO
Zn	259	206	13.0	25.4	NO	175	214	17.0	33.3	NO	124	177	17.0	33.3	NO

869 *Trace metal concentration in mg/kg

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881 Table 3

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		Fe	Mn	P	Ni	Cu	Zn	Pb	Cd	Na	Mo	Cr	V	Ca	Mg
Fraction 1	Pearson Correlation	0.586**	-0.125	0.948**	0.734**	0.548*	0.562**	-0.274	0.052	-0.001	0.774**	0.770**	0.706**	-0.321	-0.154
	Sig. (2-tailed)	0.007	0.598	0.000	0.000	0.012	0.010	0.242	0.829	0.996	0.000	0.000	0.001	0.168	0.518
Fraction 2	Pearson Correlation	0.090	-0.034	0.460*	-0.064	0.074	0.017	0.035	0.219	0.006	-0.113	-0.084	0.179	-0.331	-0.413
	Sig. (2-tailed)	0.705	0.887	0.041	0.789	0.758	0.942	0.882	0.355	0.980	0.635	0.725	0.451	0.154	0.070
Fraction 3	Pearson Correlation	0.759**	0.152	0.428	0.640**	0.750**	0.280	0.804**	0.775**	0.773**	0.154	0.756**	0.290	0.271	0.295
	Sig. (2-tailed)	0.000	0.522	0.060	0.002	0.000	0.233	0.000	0.000	0.000	0.516	0.000	0.215	0.247	0.206
Fraction 4	Pearson Correlation	0.790**	0.587**	0.204	0.763**	0.846**	0.809**	0.877**	0.800**	0.438	0.849**	0.728**	0.685**	-0.288	0.690**
	Sig. (2-tailed)	0.000	0.007	0.388	0.000	0.000	0.000	0.000	0.000	0.054	0.000	0.000	0.001	0.218	0.001

883 *Correlation is significant at the 0.05 level (2-tailed).

884 **Correlation is significant at the 0.01 level (2-tailed).

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887 Table 4
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	Components		
	1	2	3
Eigen value	4.05	2.25	1.48
% total variance	40.5	22.5	14.8
Arsenic	0.70	0.11	-0.15
Iron	0.98	-0.01	-0.05
Manganese	0.92	-0.05	0.14
Phosphorus	0.31	-0.18	0.75
Fine Sand	-0.38	0.14	0.85
Silt and Clay	0.93	-0.17	-0.17
Alkane	0.06	0.80	-0.15
Sterol	0.04	0.86	0.04
Fatty Acids	-0.08	0.87	0.07

889 Extraction Method: Principal Component Analysis.
890 Rotation Method: Varimax with Kaiser Normalization.
891 Loadings >0.5 are in bold.
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Supplementary Table

Table S1

Metals	Step-1	Step-2	Step-3	HNO ₃	TD
Arsenic	0.10	0.10	0.10	0.10	0.20
Iron	10.0	10.0	10.0	300	300
Manganese	0.20	0.20	0.20	0.20	0.50
Phosphorus	10.0	10.0	30.0	30.0	30.0
Cadmium	0.10	0.10	0.10	0.10	0.20
Chromium	0.10	0.10	0.10	0.20	0.20
Nickel	0.10	0.10	0.10	0.30	0.30
Lead	0.10	0.10	0.10	0.20	0.20
Zinc	1.00	5.00	5.00	5.00	5.00

Table S2

Sample depth in meters	Concentration (ng/g) of different lipid fractions		
	<i>n</i> -alkane	<i>n</i> -alkanoic acid	<i>n</i> -alkanol
0-6	0.53	1071	0.11
6-12	0.44	201	4.47
12-18	0.69	1960	1.15
18-24	0.63	2220	5.31
24-30	1.16	5194	5.67
30-36	0.78	6031	6.20
36-42	0.96	1246	9.41
42-48	0.66	1088	0.05
48-54	0.89	930	0.27
54-60	0.05	885	1.10
60-66	0.09	1409	1.04
66-72	0.12	839	1.38
72-78	0.19	504	0.99
78-84	0.12	600	0.02
84-90	0.06	494	0.34
90-96	0.13	441	0.94
96-102	0.12	390	0.07
102-108	0.17	372	0.93
108-112	0.14	365	0.62
112-118	0.09	549	1.17
118-124	0.09	768	0.68
124-130	0.04	307	1.09
130-136	0.20	3794	1.64
136-142	0.54	3409	3.85
142-148	25.1	1674	3.40
148-154	0.38	4932	7.17

901
902 Fig. 1.

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906 Fig. 2.

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Fig. 3.

The dots on the curve represent the absolute concentration of biomarkers.

Fig. 4.

Fig. 5.

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933 Fig. 6.

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Fig. S1.