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Mineralogical and geomicrobiological investigations on groundwater arsenic enrichment in Bangladesh

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Abstract

Sources of As in the Ganges sediments and microbial mechanisms of its release in groundwater were examined in the present study, where the authors have systematically examined the pertinent mineral species present in the sediments using XRD, TEM-EDS and EPMA techniques. The results show several As-bearing minerals in the Ganges sediments, in western Bangladesh. Iron-sulfide minerals consist of near-amorphous and/or crystalline precursors of framboidal pyrite and pyrite both of which contain As. Several types of Fe oxyhydroxides (oxides), which contain variable amounts of As were also found in muddy sediments. The content of As increases from Fe-oxides to the precursors of framboidal pyrite and pyrite. Four different chemical forms of As from the core sediments were determined. The sequentially extracted chemical forms are as follows: (1) acid soluble form (As mainly fixed in carbonates), (2) reducible form (As fixed in Fe- and/or Mn-oxides), (3) organic form, (4) insoluble form (As fixed mainly in sulfide and rarely in silicate minerals). Arsenic is dominantly sorbed on to Fe- and/or Mn-oxides, organic forms and sulfide minerals in most samples, although their relative abundances differ in different samples. Geomicrobial culture experiments were carried out to test the hypothesis that microbial processes play a key role in the release of As in groundwater. Batch culture and circulating water system experiments were designed using the sediments from Bangladesh. In the batch experiments, As was released at low Eh values a few days after adding nutrients containing glucose, polypepton and yeast extract, urea and fertilizer under a dominantly N_2 atmosphere. This contrasts with the control experiments without nutrients. Circulating water experiments with sand layer in a N_2 atmosphere showed similar results. These results support the hypothesis that microbial processes mediate the release of As into groundwater under reducing conditions. Glucose and polypepton used in the experiments may correspond to C and N sources, respectively. Younger sediments contain abundant organic matter, which is easily used by bacteria. So, the enhanced bacterial activity may correspond to simulation of accelerated natural diagenetic process using organic matter, or some fertilizer/wastewater effects.

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1. Introduction

Groundwater As enrichment in the Ganges delta area has been recognized as a serious health problem to thousands of people in the region (Welch and Stollenwerk, 2003). It is estimated that about 60–70 million people are exposed to the potential risk of As toxicity (Rahman, 1997; Chowdhury et al., 2000). High concentrations of As are mainly restricted to sedimentary aquifers of Holocene age (Bhattacharya et al., 1997; Ishiga et al., 2000; Anawar et al., 2002, 2003). Two important questions currently asked about As enrichment in groundwater focus on (i) sources of As and (ii) mechanisms that release As into groundwater. It is likely that the constituent minerals in these alluvial deposits are the most probable sources of As-enriched groundwater, similar to other highly As-enriched groundwater (e.g. Masuda et al., 1999). One potential source of As to groundwater is the oxidation of As-bearing sulfides (Chakraborti, 1995). Alternatively, As can be released by reductive dissolution of Fe(III) oxyhydroxides (Bhattacharya et al., 1997; Nickson et al., 1998). However, mineralogical data are insufficient to support these possibilities. For this reason the mineral species in alluvial deposits of the Ganges Delta, which may contribute to the release of As were investigated. In this study the As contents and their various chemical forms were determined through sequential extraction of the sediments.

A review of the literature suggests limited focus on the role of microbes on the As release mechanism into groundwater. In the present paper the role of bacterial activity and redox environments in natural sediments on the mobilization of As into groundwater is studied. Recent researches have focused on microbial activities in natural sediments, and implications for the evolution of water chemistry in both surface and subsurface environments. Microorganisms present in shallow aquifers catalyze most of the oxidation-reduction reactions (McNabb and Dunlap, 1975; Chapelle, 2001) through enzymatic processes and control redox speciation of metals in natural environments. Alternatively, As minerals can be directly oxidized by bacteria (Ehrlich, 1964; Wakao et al., 1988; Sehlinin and Lindstrom, 1992) and arsenate [As(V)] is also directly reduced by bacteria (Newman et al., 1997; Zobrist et al., 2000; Ahmann et al., 1994; Langner and Inskeep, 2000). Some organisms show resistance to As toxicity (Silver, 1996). The bacterial effects on As behavior in anoxic sediments have also been reported (Dowdle et al., 1996; Ahmann et al., 1997).

Here, the authors propose that microbial processes promote As release into groundwater. Bacteria in the early diagenetic processes use organic matter in the sediments. In addition a great amount of fertilizer is used in Bangladesh and this may or may not infiltrate into the ground, while disposal of private wastewaters near the tube wells are also problematic. Experiments were performed on sediments from the Ganges Delta to test this hypothesis, the preliminary results being presented in Akai et al. (2000).

2. Sampling and methodology

2.1. Sampling

Sediment samples were collected from a borehole drilled at Samta village in Jessore district of Bangladesh within the framework of a joint project of the Research Group of Applied Geology, Japan (RGAG), Asian Arsenic Network (AAN) and Miyazaki University Research Group (MURG). General geological details, sediment and related groundwater characteristics have been described in detail previously (RGAG-MURG, 2000). Samples (51) obtained from one drilling core of 61 m in 1998 indicated mostly sandy sediments. However, muddy sediments were encountered at depths of 3–9 m and locally at depths of 33–46 m. All the samples were preserved in 50 cc plastic bottles. Concentrations of As ranged between 7 and 16 mg/kg in mud layers and 2 and 5 mg/kg in sandy layers (Rahman and Ishiga, 1999). In addition, 10 core samples were also collected from the upper muddy layer from Samta Village during 1999. The lithological characteristics of these sediments were described earlier by Yamazaki et al. (2000). Ten samples were preserved in a bag with an O_2 absorbent. Typical sediment samples were selected and examined for mineralogical details with a binocular microscope, polarizing microscope, scanning electron microscope (SEM), transmission electron microscope (TEM) and Electron Probe X-ray Microanalyzer (EPMA). Some samples collected during 1999 were used also for culture experiments.

2.2. Mineralogical experiments

The distributions of elements were mapped by Electron Probe X-ray Microanalyzer (EPMA), JXA 8800EPMA (JEOL), operating at 15 kV and 2×10^{-8} A beam current. For TEM observations, the samples were suspended in water and put onto a C-coated microgrid. The TEM used is a JEM 200CX (JEOL) and JEM 2010 (JEOL) operating at 200 kV. Qualitative and quantitative EDS analyses were carried out using a Voyager IV, Noran Instruments. Co. Ltd. For X-ray Diffractometry (XRD) determination of mineral species, a Geiger Flex Rad-X (Rigaku Denki KK) was used, operating at 35 kV and 20 mA with Cu target.

Sequential extraction of the sediments was carried out for determining the association of As with different mineral phases. Procedures for extractions were basically those described by Ito (2001), which were modified from Thomas et al. (1994). The extractions for chemical forms and leaching agents are as follows: (i) acid soluble form (As mainly fixed in carbonates), which was leached using 0.1 M acetic acid; (ii) reducible form (As fixed in Fe- and/or Mn-oxides), which was leached using 0.1 M hydroxylamine hydrochloride solution, adjusting pH to 2 using HNO_3; (iii) organic form, which was first decomposed by 0.1 M sodium pyrophosphate solution heated on a hotplate and dissolved into 1 M ammonium acetate solution; and (iv) insoluble form (As fixed mainly in sulfide and rarely in silicate minerals), which was dissolved by a mixture of concentrated HNO_3 and $HClO_4$. Some amorphous Fe(III)-oxides may also dissolve in acetic acid. For some samples, residues after these sequential extractions of As were fused using alkali reagents to dissolve residual minerals. Arsenic concentrations in the solution were determined by a Seiko (SAS7500) atomic absorption spectrophotometer (AAS) equipped with a hydride generator (THG-1200). Accuracy and reproducibility of the total As concentrations were checked using the standard sedimentary rock sample distributed by the Geological Survey of Japan, and are within 5%. The differences between the total As contents determined for whole sediment digestions and for the sum of chemical extracts are within ±5%.

2.3. *Culture experiments*

2.3.1. *Electron microscopic (EM) observation of bacteria and precipitates*

Bacteria and their biomineralization products were observed by transmission electron microscopy (TEM) and optical microscopy. The samples for TEM observations were prepared by putting a drop of well water onto a C-coated microgrid and air-drying.

Sediment samples collected from Samta Village, in May 1999 were used as starting materials in culture experiments. Two different culture experiments were carried out: (1) batch culture, and (2) circulating water flow experiments. Algal growth was not observed during the experiments despite being carried out under lighted laboratory conditions rather than in a dark room.

2.3.2. *Batch culture experiments*

Experiments were conducted under anaerobic conditions, in a N_2 gas environment. Control experiments were carried out, using distilled and autoclaved nutrient-rich water with sediments.

2.3.2.1. Culture with Sakurai Medium. This experiment was carried out using Sakurai Medium (0.075 g of glucose, 0.3 g of polypepton and 0.15 g of yeast extract and 150 ml distilled water). Ten grams of mud from core specimens (sp No. B7-20/22) were put in flasks with the Sakurai Medium. All materials were autoclaved-except the mud. Flasks were placed in N_2 filled bags in an incubator at 30 °C. Nitrogen gas was added twice for a week to expell any O_2. Distilled water was added during the experiments as needed to replace water lost due to evaporation.

2.3.2.2. Enriched culture experiments. The enriched culture experiments were carried out using beakers with and without addition of different nutrients in a N_2-gas environment. The enriched nutrients used were glucose, polypepton, urea and fertilizer. Control experiments were carried out using distilled water with mud and using autoclaved nutrients and water.

Twenty grams of mud (sp No. B7-20/22) were placed in a beaker with 300 cc of distilled water and 0.15 g of glucose and/or 0.6 g of polypepton. The ratio of polypepton and/or glucose:water:mud was kept constant in all the experiments. The beakers were placed into glove bag filled with N_2 gas and placed in an incubator at 30 °C. Samples were taken daily from the beakers followed by stirring the water and adding N_2 . Urea containing 46% N and commercial fertilizer containing 7% N as urea, 14% P and 8% K were used and experiments were carried out at room temperature (20–25 °C).

2.3.3. *Circulating water experiments with flow-through columns (strata model)*

The sediment samples were inner parts of drilled core that were refrigerated. The circulating water experiments with flow-through columns were designed to observe interaction between water and sediments. The reaction chamber of the strata model is 4×4×10 cm in size (Fig. 1). Bangladesh sand samples (sp. No. B7-45) were spread in the lower part of the box. Bangladesh mud samples (sp.No.B7-20/22) were layered on the sand (upper part of the box). The box cover was tightly closed. Distilled water, ca. 400 cc, was added to the system and was circulated by a peristaltic pump with the flow speed of 1 cc/min. The experiments were carried out in the N_2 filled box at room temperature (20–25 °C) and N_2 was supplied continuously.

2.3.4. *Analysis of arsenic by the modified Gutzeit method*

During the culture experiments, pH and Eh were measured using a POT-101M pH-ORP meter (Shibata Sci. Co. Ltd.), and EC was measured using the EC meter (TDScan-20, Iuchi Seieido Co. Ltd.). Ten milliliters of water samples were collected for As analysis at appropriate intervals.

Dissolved As was quantitatively analyzed by the modified Gutzeit method (Hironaka-method; Hironaka, 2000), which is a conventional technique useful in the field and its sensitivity corresponds to the atomic absorption method (Tanabe, 1998). 0.1 g of KI, 0.1 g of $ZnCl_2$ and 0.3 g of powdered Zn are added into a 10 ml

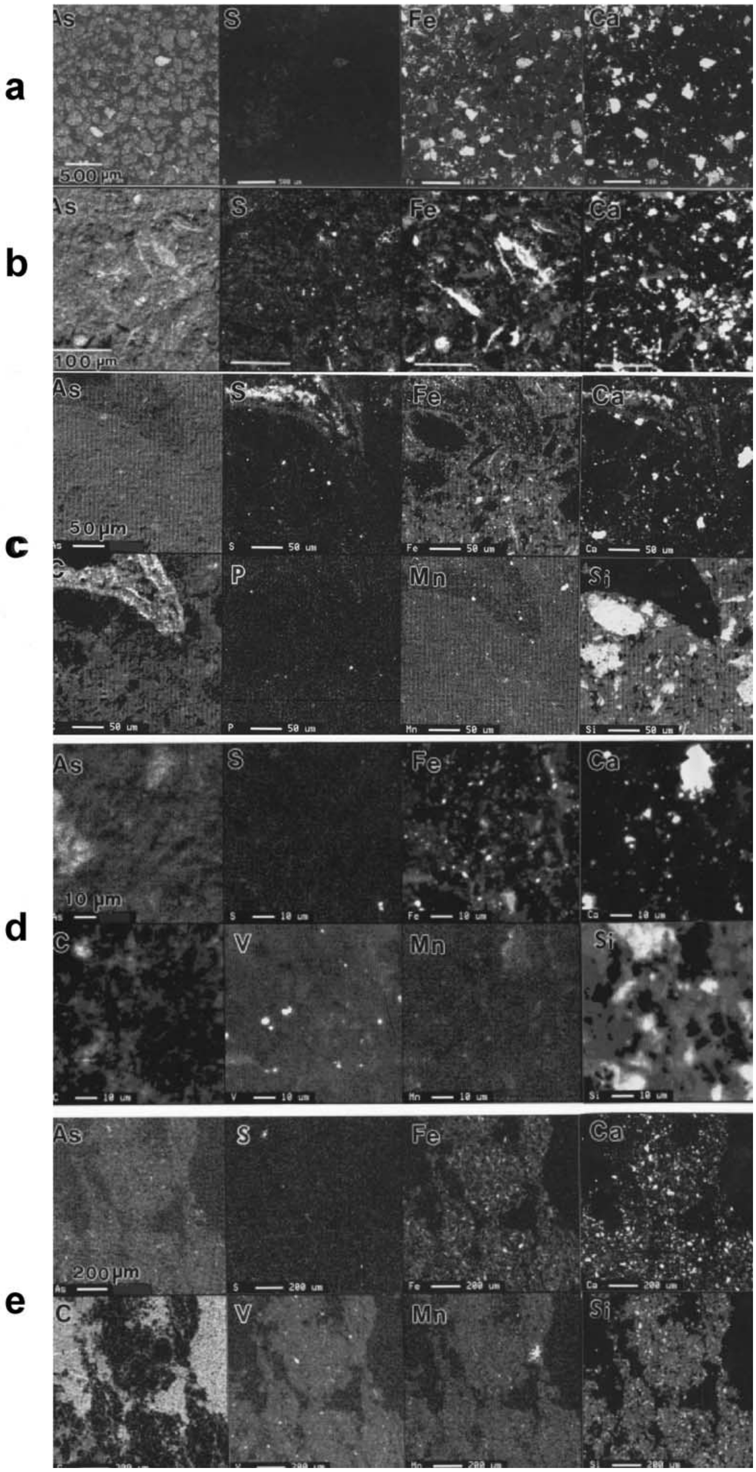

Fig. 1. Area analysis of element distribution (As, S, Fe, Ca, C, P, Mn, V and Si) by EPMA. (a) Sand specimen at 53.1 m depth (sp. No.B-177ft); (b) mud specimen (sp. No.B-30 ft); (c) Mud specimen (sp. No.B4-23 ft); (d) and (e) mud specimen (sp. No.B4-18ft). See text for details.

sample solution. Then, 2 ml of 6 N HCl is added. By this treatment, AsH_3 will be produced and will react with $HgBr_2$, forming a concentration-dependent color, from yellow to brown. Arsenic concentration can then be quantified by comparing colors derived from standard solutions. The detection limit is 0.01 mg/l and the most reliable working range using this method ranges between 0.01 and 0.4 mg/l (Hironaka, 2000).

3. Results

3.1. Mineral species and their relative abundance based on XRD studies

Results of XRD showed that the principal minerals present in the sediments are quartz, micas, feldspars, chlorite, amphibole and clay minerals (ARGNU, 2000). The sandy sediments comprise major quartz > > plagioclase > mica and minor K-feldspar > chlorite, and amphiboles. Although clay minerals are not abundant in the sediment, relatively clay rich sediments tend to contain more As than sandy sediments. Small amounts of smectite and kaolin minerals as well as mica and chlorite were identified by XRD of the bulk samples. Based on the optical microscope observations and EDS (Energy Dispersive Spectrometer) analyses, accessory minerals include pink garnet (almandine), calcite, dolomite, clinopyroxene (augite, diopside), orthopyroxene, magnetite, ilmenite, topaz, zircon, tourmaline, pyrite, Ti-oxide, gypsum etc. Lesser amounts of lithic fragments (mainly sedimentary rocks) and wood flakes also were identified. Gypsum was rarely found using the TEM. However, it is not clear whether the gypsum was in the sediments or is a decomposition product formed after drilling.

3.2. EPMA mapping of chemical composition

Major elemental distribution patterns in sandy and muddy sediments were analyzed by EPMA (Fig. 1a–e). Although patterns of elemental distribution were complicated in mud specimens, it was still possible to recognize a positive relationship between: (i) As and Fe (as well as Mn; Fig. 1b), (ii) As and Mn (Fig. 1c), (iii) As and Ca, S and Fe (Fig. 1d), and (iv) As and Si, Mn, V, Fe and Ca (Fig. 1e). In the sandy sediments at a depth of 53 m, strong correlation between As and Ca was found (Fig. 1a), along with weak but persistent correlation amongst As, Fe and S, is found in a grain (Fig. 1a). This suggests that As in this grain is in Fe-sulfides within calcite. In the shallow muddy sediments collected from 5.5–9 m depth, As correlates with Fe and Mn, indicating its association with the Fe- and Mn-mineral phases. However, no correlation between As and P was apparent in any of the specimens. An inverse correlation was observed between C and As. Complex heterogeneous distribution patterns in EPMA mapping are characteristic for these sediments. EPMA studies on these sediments indicate complex and heterogeneous patterns in the distribution of As as well as its correlation with other elements.

3.3. TEM observations

3.3.1. Pyrite

Pyrite was found in the upper muddy layer of ca. 3–9 m and also in the lower muddy layer at a depth of 43–44 m (Fig. 2a). Pyrite also occurs in the sandy sediments and is occasionally associated with wood flakes and always contains 1–0.3 wt.% As_2O_3. Chakraborti (1995) previously reported pyrite in sediments of the Ganges delta. This study provides more details on the characteristics of the pyrite. Most pyrite grains are framboidal, although some of them are rectangular in shape. Icosahedral structures in framboidal pyrite, which was recently described by Ohfuji and Akai (2002) have not been found in this area until now.

3.3.2. Precursor minerals of framboidal pyrite

Examination of some sandy sediments (e.g., 48.6 m deep) revealed the presence of precursor minerals of framboidal pyrite (Fig. 3a). They are often spheroidal aggregates and are amorphous or crystalline. The ED pattern obtained from one of them (Fig. 3b) suggests greigite (Fe_3S_4). The Fe/S mole ratio is close to 1:1. Precursor minerals of framboidal pyrite may be amorphous 'FeS', greigite or mackinawite (Sweeney and Kaplan, 1973; Roberts and Turner, 1993; Wilkin and Barnes, 1997). Small spheroidal and amorphous sulfides associated with carbonate in sandy sediments at 49 m depth (Fig. 3b) had mottled internal structures and contained less As (0.1–0.4% As as As_2O_3). These grains may represent embyronic crystals of framboidal pyrite or decomposition products of precursor pyrite grains.

3.3.3. Varieties of Fe-oxyhydrocides (oxides)

Fe-oxides occur in different forms in these sediments. A typical grain of ferrihydrite in muddy sediments is shown in Fig. 4a. This grain is characterized by a distinct As peak corresponding to 0.3 wt.% as As_2O_3 in the EDS spectrum. Small spheres of Fe-oxyhydroxide (ferrihydrite) are present in the sandy sediments at a depth of 49 m. Small grains of geothite, which show a very small peak of As in the EDS spectra, were found in the muddy sediments (Fig. 4b). The As-bearing Fe-oxides are not uniformly distributed, even in the shallow muddy sediments. Fig. 4c shows hematite grains coating quartz grains in the sandy sediments. Arsenic was rarely detected by EDS analysis of the hematite.

3.3.4. Characteristics and some textures of wood flakes

Based on the EDS analysis, wood characteristically contains Ca and very small amounts of Fe and S

(Fig. 5) and EPMA mapping analysis showed no direct correlation between As and C. In Fig. 5, a complex pit-like texture was observed in the wood flakes. The shape and sizes of the pits are similar to those found in pyrite that are interpreted to be bacterial leaching patterns (Rodrigue-Levia and Tributsch, 1988).

3.4. Sequential extraction analysis of arsenic

Mineral analyses by EDS provides important information, but the detection limit by EDS analyses is fairly high (ca. 0.1%) and busk As concentrations in the sediments cannot be provided by this technique. In order to estimate the As contents quantitatively, 4 different chemical forms of As were chemically extracted from the core samples as previously described. Table 1 shows the results of the extractions. Dominant As forms in the most samples are Fe- and/or Mn-oxides, organic matter and sulfides, although relative abundances differ among the samples. Little As is adsorbed to minerals and fixed in carbonates.

3.5. Batch culture experiments using Sakurai Medium

Culture experiments using Sakurai Medium (glucose, polypepton and yeast extract) and sediments (sp. No. B20-9) were carried out for a week at 30 °C. Arsenic (0.01 mg/l) and Fe (5.7 mg/l) was released from sediments into water in the presence of bacteria after 7 days

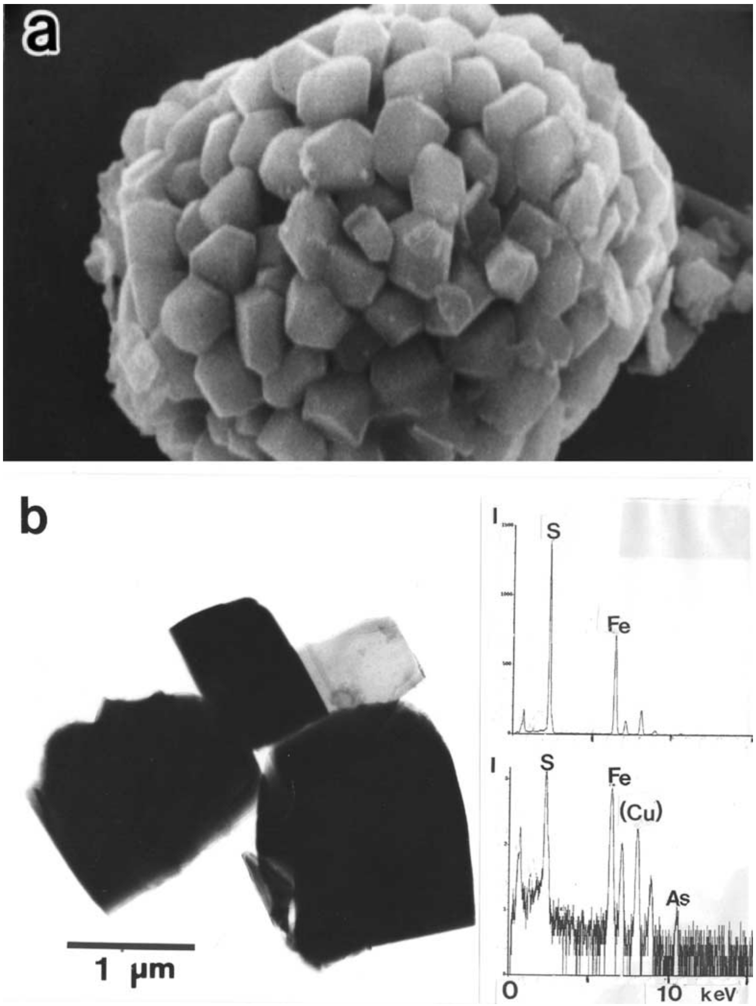

Fig. 2. Pyrite grains. (a) SEM photograph of framboidal pyrite on wood flakes in representative mud at 43.5 m (sp. No.B-145 ft); (b) TEM image of pyrite occurring in the upper muddy layer (sp. No.B-30ft). EDS spectra of the grains are inserted. Vertical scales in EDS spectra represent characteristic X-ray intensity (I) at normal (above) and log (below) scales. Label of (Cu) represents peak due to specimen supporting grids and only main peaks are labelled (also in the following figures). Arsenic content is 0.6 wt.% as As_2O_3.

without stirring the water (final Eh value was 45 mV). Arsenic was below the detection limit in the control experiments where distilled water was added to sediments (final Eh value was 352 mV), and iron was not detected in water of the control experiment. Fig. 6 shows a TEM image of bacteria found in the culture products using Sakurai medium. The bacteria were sometimes associated with amorphous phases containing Ca, P and Fe. Phosphorus would be from the bacteria. The P released after bacterial death might have reacted with Ca in the sediment to form such materials.

3.6. Batch culture experiments with enrichment of nutrients

Batch culture experiments were systematically carried out using several types of nutrients including glucose, polypepton, urea and fertilizer. Fig. 7 shows pH–As diagrams. The pH increased slightly for all experiments, but is not obviously related to As content. In contrast, the Eh–As diagram clearly shows changes due to microbial activity (Fig. 8). The Eh decreased a few days after adding some nutrients. Arsenic was released only when Eh values decreased to below 0 mV, suggesting

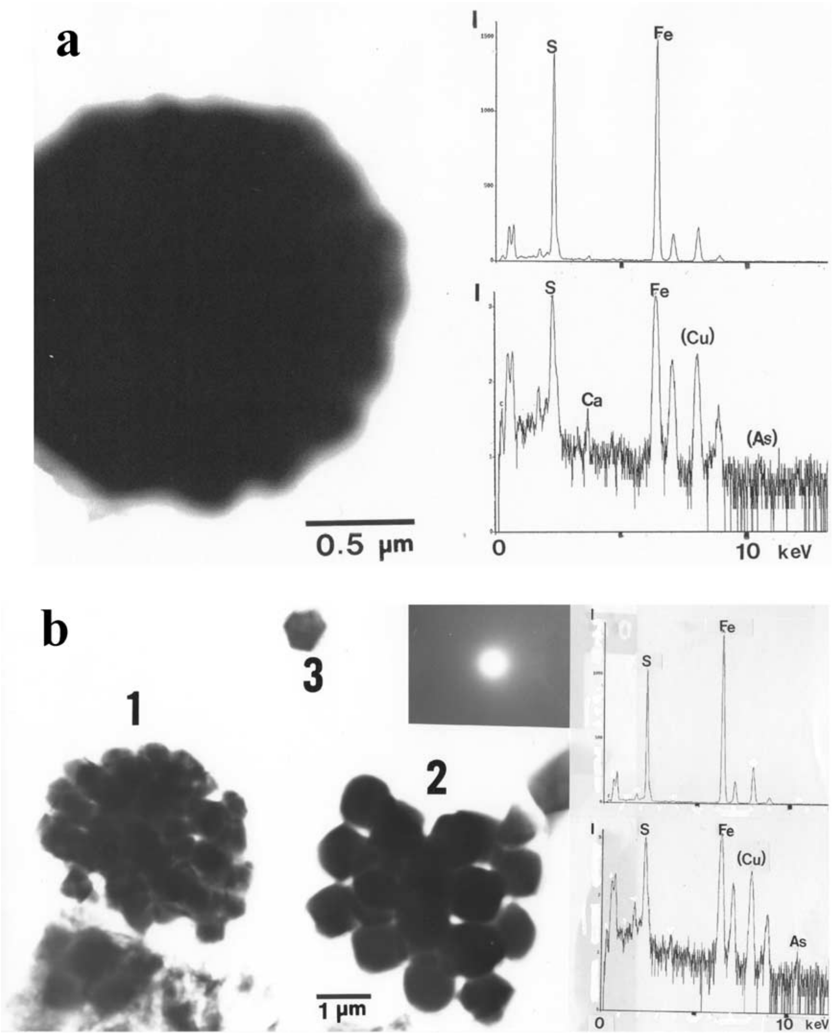

Fig. 3. Precursor minerals of framboidal pyrite. (a) TEM image of the precursor minerals of framboidal pyrite in 48.6 m sandy specimens (sp. No.B-162ft). EDS spectra indicates Fe:S at 1:1 ratio, (b) TEM image of a small spherical precursor mineral associated with carbonate in the same sample. Grains 1 and 2 are precursor minerals, while grain 3 is a crystalline pyrite. ED pattern (see inset b) for grain 1, suggests the presence of the amorphous mineral, greigite. Arsenic content is 0.3 wt.% as As_2O_3. Label of (As) in the spectra shows the position of the As peak, even though it is not seen clearly for all the grains.

that As was dissolved when reductive conditions were produced by microbial activity. Arsenic was not observed in experiments involving distilled water and mud with autoclaved nutrients, although some Eh decreasing tendency was observed. The decreasing Eh may have occurred only in the first few days of the experiment; a preliminary one year experiment also showed no low Eh values (ca. 300 mV) and no As release. For As release, factors other than incubation with distilled water appear to be required. The results demonstrate a lag in the release of As even after the lowest Eh value is attained. This lag may be caused by chemical reactions such as oxyhydroxides (and oxides) dissolution of Fe-oxides. Complete consumption of nutrients may raise Eh conditions again. Changes in EC values are shown in Fig. 9. Concentration of Fe in the water 3 days after the addition of nutrients was 5.74 mg/L (for glucose and polypepton), 0.36 mg/L (for polypepton) and 7.57 mg/L (for glucose). Concentrations of As correlate well with Fe, and EC values correlate with these As and Fe concentrations. The results suggest that dissolved As is originally adsorbed to Fe(III)-hydroxides. In these experiments, at least 3 types of dominant bacteria were found: one is a rod shaped bacterium, the second one is rod shaped one with a bubble like texture inside and the third one with granules (Fig. 10). Fig. 10 indicates EDS

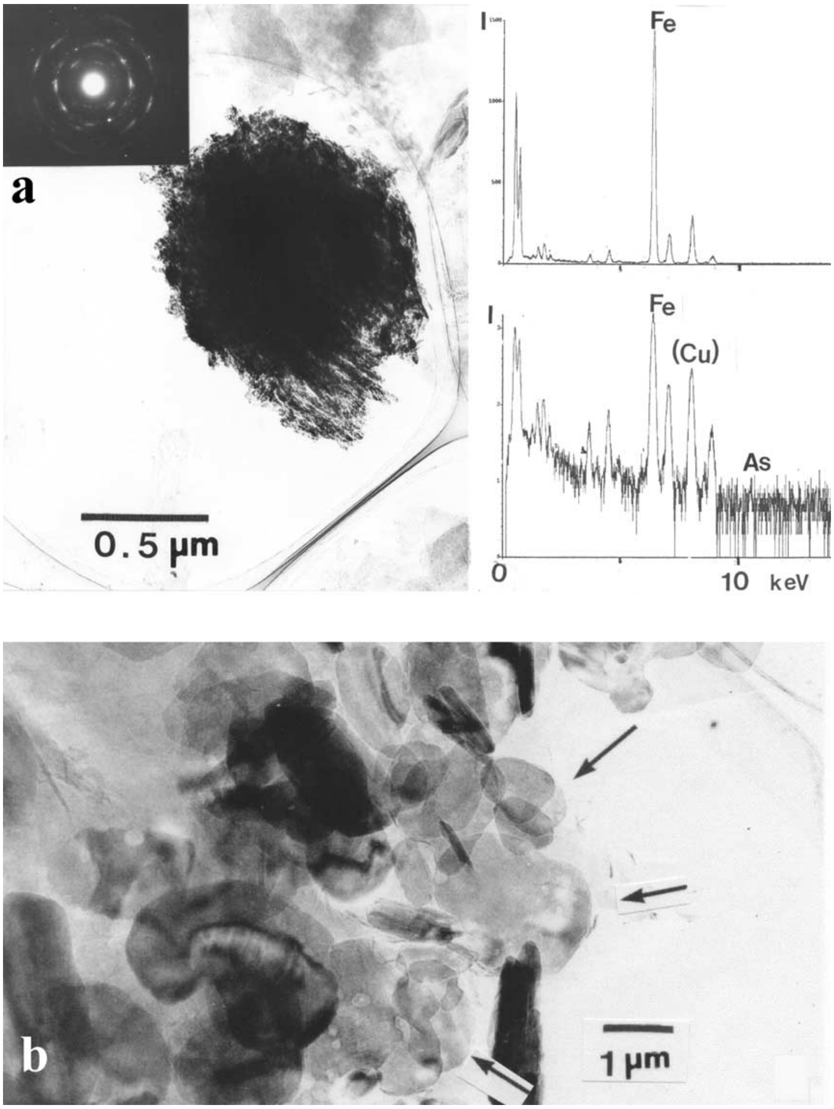

Fig. 4. TEM image of Fe-oxyhydroxide mineral. (a) Fe oxyhydroxide (goethite) grain found in upper muddy layer at 9 m (sp.No.B-30ft). ED pattern and EDS spectra are presented as insets. Vertical scale of the lower EDS spectrum is log scale; (b) TEM image of hematite grains (arrows in the figure) coating on quartz grain in sands (sp. No.B-162ft).

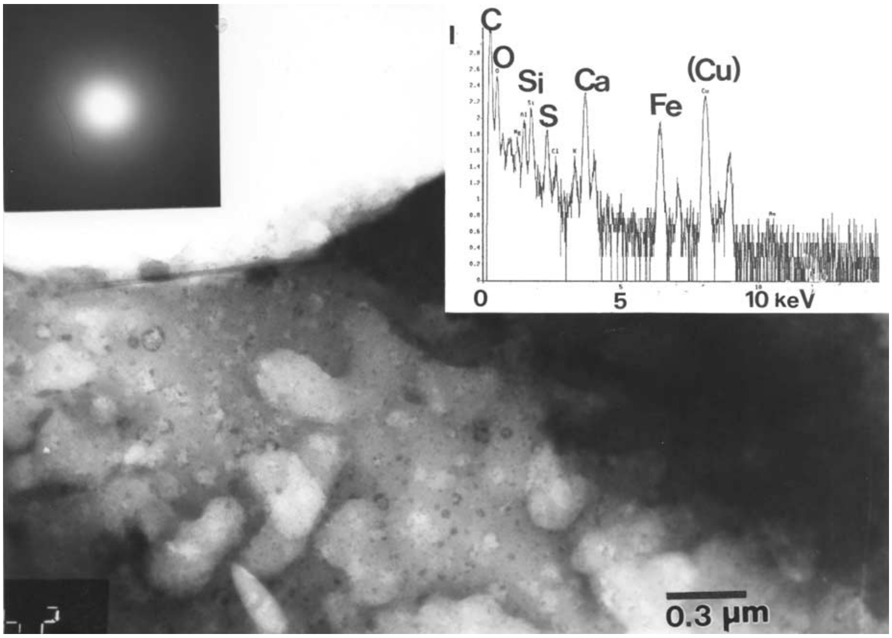

Fig. 5. TEM image and EDS spectrum of wood flakes in muddy sediments at 43.5 m (sp. No.B-145ft). ED pattern (inset) indicates its amorphous nature.

Table 1
Results of sequential extraction analysis of As (in ppm) from Bangladesh sediment samples

Sample	Acid soluble form	Reducible form	Organic form	Insoluble form	Total As
B30 Mud	0.1	4.8	2.5	5.6	15.4
B177 Fsd	0.0	0.1	0.2	0.4	0.6
B7-20/22-Iz. mud	1.7	10.4	6.2	13.2	36.8
B20-9 mud	0.1	2.7	2.9	2.1	7.9
B4-23 mud	0.2	2.0	4.7	4.4	11.2
B7-45A sd (without org.m.)	0.03	0.8	0.7	0.2	1.7
B7-45B sd (bulk)	0.2	1.5	2.8	0.9	5.2

mapping results also. Phosphorus rich portions indicated by the arrows in Fig. 10 may be polyphosphate granules (Friedberg and Avigad, 1968; Rashid et al., 2000).

3.7. *Culture experiments of circulating water system experiments through experimental columns (strata model)*

Experiments using the water flow system reaction vessel were conducted (Fig. 11). Fig. 12 summarizes the changes of pH, Eh, EC and As content. After distilled water flow under N_2 gas for a month, nutrients were added. In the presence of only distilled water, As concentration was less than the detection limit with a nearly constant Eh (ca. 300 mV). However, pH values became slightly elevated (ca. 8.0), probably due to carbonate dissolution, because calcite grains were included in the sediment samples

Addition of 1 g polypepton (marked as P in Fig. 12) in the same system in a N_2 environment, caused the As concentration to increase with lowering of Eh. Subsequently, the Eh value generally increased and the As-decreased. Six days later, 1 g of glucose was added to the system (marked as G in Fig. 12). Four days after the addition of glucose the aqueous Fe concentration was 3.97 mg/L and 12 days after the glucose addition the Fe concentration was 1.93 mg/L. The TEM image of a rod-shaped bacteria is shown in Fig. 13. Other bacteria show globular structures under TEM and are polyphosphatic in composition. Most bacteria cultured after addition of nutrients may be heterotrophic, although no detailed

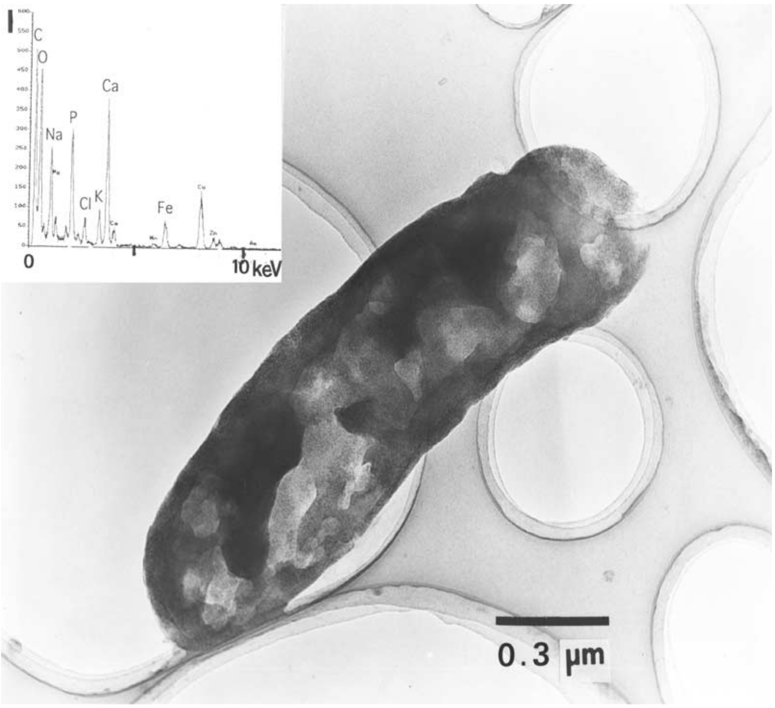

Fig. 6. TEM image of bacteria in the Sakurai culture media after a week. The bacterium is coated with material containing mainly Ca and P. Fe and As content is low in the coating.

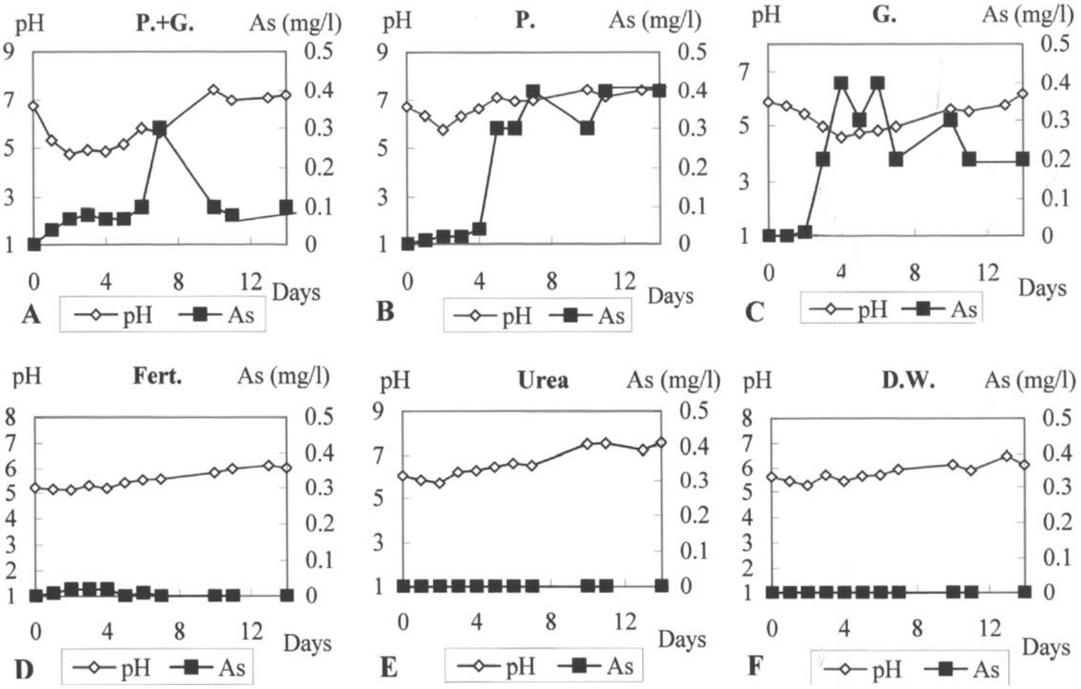

Fig. 7. Variation of pH and As release with time during the enriched culture experiments using muddy sediments (sp. No.B7-20/22) with addition of nutrients. (A) polypepton + glucose (P. + G.); (B) polypepton (P.); (C)glucose (G.); (D) commercial fertilizer containing K, P and N; (E) urea (Urea); (F) distilled water (D.W.).

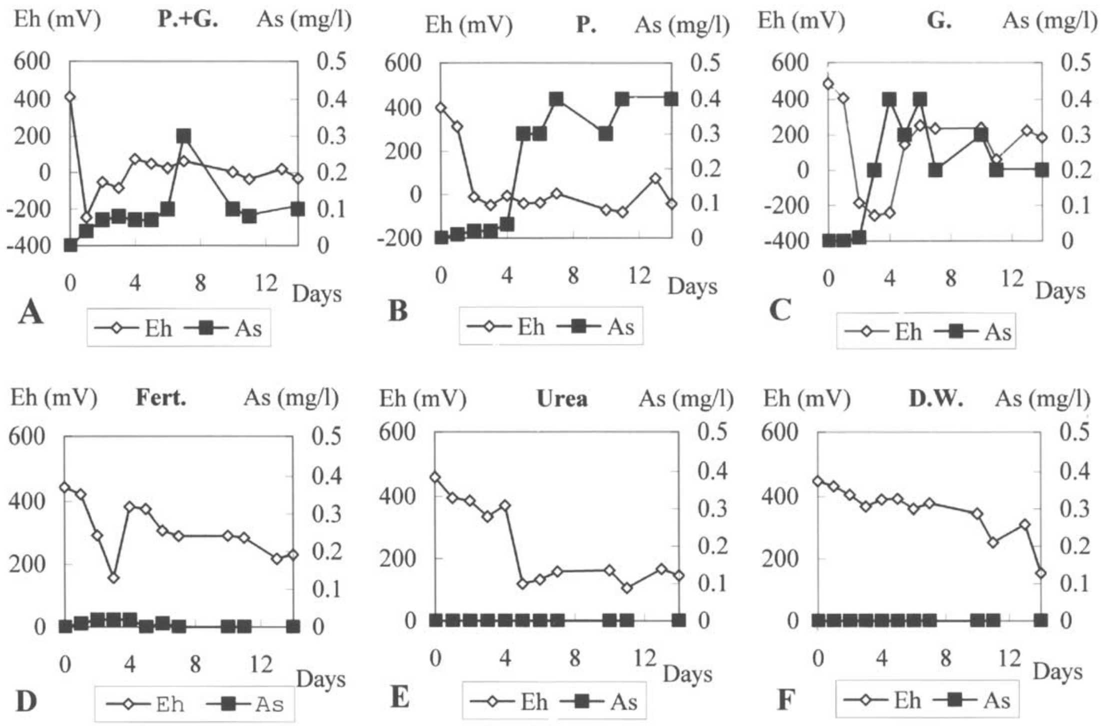

Fig. 8. Variation of Eh and As release with time during the enriched culture experiments using muddy sediments (sp. No.B7-20/22) with addition of nutrients. For other explanations, refer to Fig. 7.

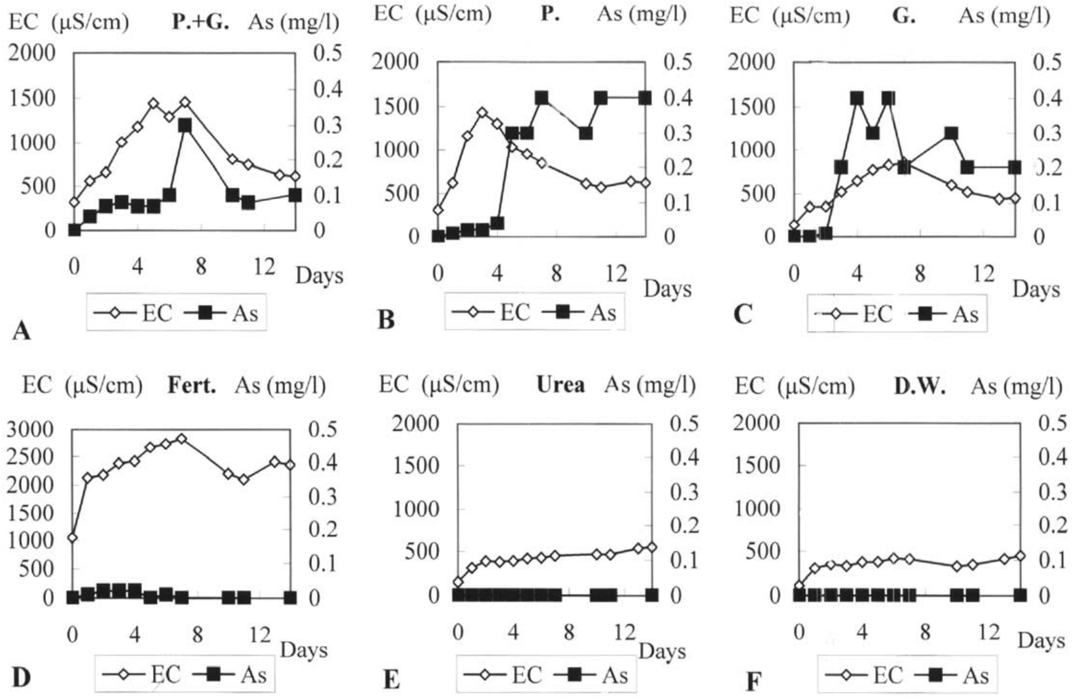

Fig. 9. Variation of EC and As release with time during the enriched culture experiments using muddy sediments (sp. No.B7-20/22) with addition of nutrients. For other explanations, refer to Fig. 7.

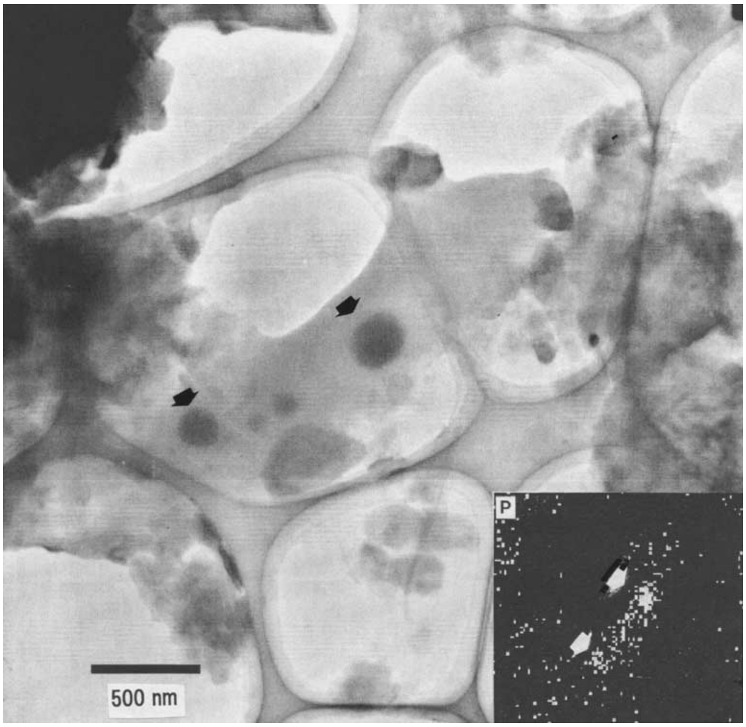

Fig. 10. TEM image of bacteria found in the cultured specimen with enrichment nutrients (3 days after addition of glucose). Inset shows the EDS pattern of P enrichment at the granules (indicated by arrows).

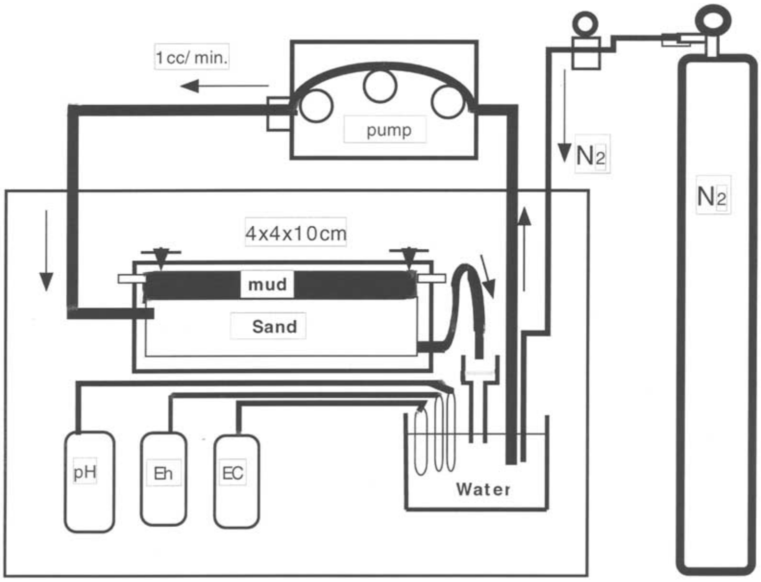

Fig. 11. Experimental design of the circulating water system with strata model.

micorobiological examinations were carried out. Genetic identification of the bacteria is currently in progress.

4. Discussion

4.1. Minerals associated with arsenic and their origin

Arsenic in the sediments is clearly associated with pyrite (especially, framboidal pyrite). Precursor minerals of pyrite also contained As. The Fe-oxyhydroxides (and/or oxides) contain low concentrations of As which is often solid less than EDS detection limit. Arsenic was not detected in the solid organic matter by EDS but may be contained in minerals around wood fragments. EPMA results are consistent with this observation.

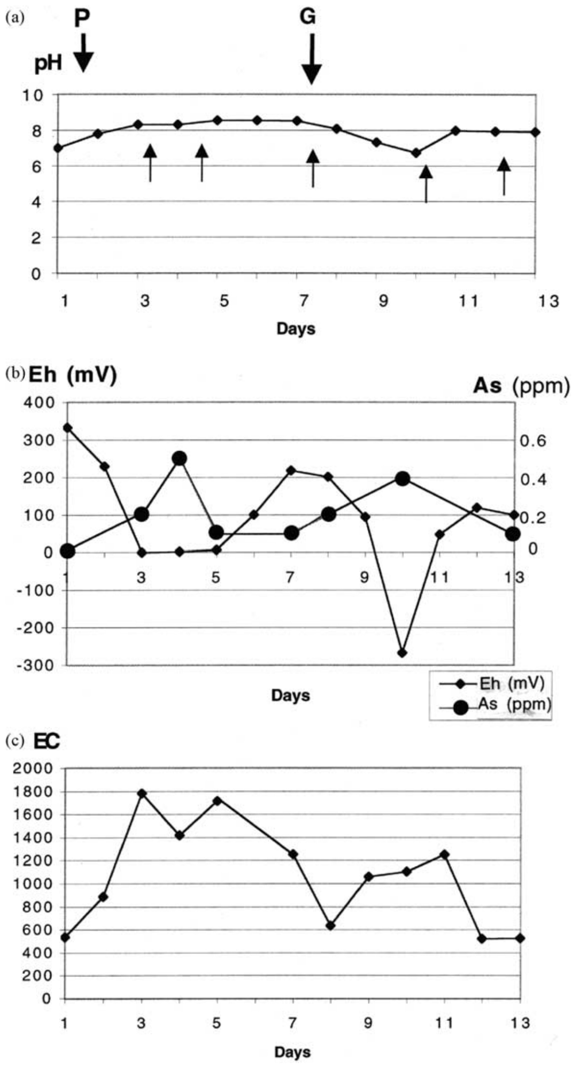

Fig. 12. Arsenic release in circulating water flow experiments using Bangladesh sediments when polypepton and glucose are added. (A) pH changes with additions of glucose and polypepton as shown by arrows G and P respectively. The other short arrows indicate additions of distilled water when water quantities became less; (B) Change in the Eh and As concentrations; and (C) Changes in the EC.

EPMA reveals a complex As distribution in the sediments. These complex variations may represent a heterogeneous As distribution and the binding of As to different minerals. The local chemical distributions of As in the sediments may be due to the local biogeochemical reactions, such as oxidation of Fe and Mn due to bacterial activities coupled with inorganic reactions. The As distribution pattern suggests that the finer particles concentrate and/or the less permeable sediments retain more As. This pattern may be related to adsorption of As to Fe- and Mn-oxides and probably aluminosilicates in the clay and silty sand sediments.

Sequential extraction shows that the dominant As hosts are Fe- and/or Mn-oxides, organic matter and sulfide minerals in most samples. The data showed that adsorbed As on mineral surfaces is not abundant and carbonates contain little As. Nearly equal quantities of As are contained in oxides, in organic matter and in sulfide minerals, although relative abundances differ in each sample. However, As in organic matter was not detected using EPMA. The As concentration in organic matter may be less than the detection limit but the total quantity may be large. The behaviour of As-containing minerals may be as follows: if sufficient reduced S is present in the anoxic environments of the buried sediments, As-bearing pyrite may form. The As content in each grain increased in the order Fe-oxyhydroxides (oxides), mineral precursors to pyrite, and pyrite.

Higher concentrations of As are characteristic of reductive conditions in groundwater. So, under strongly reducing conditions, the mobility of As is coupled with dissolution of Fe- and Mn-oxides. Although the main source for As in such reducing systems are oxides of Fe and Mn, the present study reveals that it is contained in several other chemical forms. It is pertinent to mention that natural processes may be complex, particularly due to oscillation of oxidation and reduction fronts during the sediment-water interactions. In such instances, some pyrite could be partially oxidized and re-precipitated as Fe-hydroxide along with As.

4.2. Implications of culture experiments

During the laboratory experiments, As elution from sediments apparently occurred following rapid decrease in Eh, caused by the bacterial activity. The Eh decreased markedly in the presence of organic matter, polypepton and/or glucose as compared to the control experiments. Bacterial growth was slow without addition of the nutrients, and after depletion of readily degradable organic matter in the sediments.

Considering the rapid response pattern of Eh values after addition of nutrients (polypepton and/or glucose), the bacteria are probably predominantly anaerobic. It is likely that Fe(III)-reducing bacteria accelerate dissolution of Fe-oxyhydroxide (Lovley, 1997; Lovley and Chapelle, 1995; Lovley et al., 1995,1996). In most of the analyzed sediments, As combined with organic matter, sulfides and oxides are abundant among the 4 sequentially extracted forms. The acetic acid soluble form contains less As. Rapid As release under reducing condition suggests that reducible materials such as Fe-oxyhydroxides and Mn oxides are the main sources of As released to groundwater.

Addition of nutrient may accelerate the diagenetic changes of organic matter. In the sedimentary environment, organic matter may decompose by natural biogeochemical processes, which lead to reducing conditions in the aquifers. Bacteria may more easily use organic matter in younger sediments, compared to organic matter in older sediments. Additionally, infiltration of nutrients due to the application of fertilizer during agricultural practices may play a role in the release of As (Ueno, 2000).

4.3. Groundwater chemistry and related environments

Chemical characteristics of groundwater in Bangladesh have been studied in great detail during recent years (Mashiko et al., 1999; Bhattacharya et al., 2002; Bhattacharya, 2002; Anawar et al., 2003). Arsenic concentrations range from 0.01 to 0.3 mg/L with predominance of higher values. pH ranges are generally 7.2–7.6. Eh values are generally around +100 to −150 mV. EC values are 600–1000 μS cm^{-1}. Moreover, these groundwaters are characterized by low NO_3^- (0–1.4 mg/L), while the concentrations of Fe^{2+} (2–8 mg/L), NH_4^+ (3–6 mg/L) and PO_4^{3-} are high. Occasionally, very high levels of NH_4^+ (10–20 mg/L) are reported in groundwaters elsewhere in Bangladesh (Mashiko et al., 1999; Bhattacharya, 2002; Anawar et al., 2003). The groundwater tables are deeper in the dry season and agricultural irrigation effects are also added. Due to the lowering of the water table, aerobic environments appear in the deeper sediments. In such conditions with stratified sediments, organic matter can decompose. These changes create favourable conditions for some aerobic bacteria to grow causing significant changes in the aquifer environments. In general, knowledge of soil science shows that organic matter in once dried soil is well decomposed and much bacteria will grow after it is refilled by water also because of their starvation rates. These conditions do not correspond exactly to Bangladesh case, but a very similar mechanism is strongly inferred.

5. Summary

The present study on the mineralogy of the sediments from Bangladesh indicates various forms of Fe- and Mn-oxides (or oxyhydroxides), and Fe-sulfides as the most important source minerals for As. Iron-oxides and hydroxides are hematite, goethite, ferrihydrite, and near amorphous phases, while Fe-sulfide phases include pyrite, greigite, a near amorphous phase. The As contents of each mineral decrease from pyrite to Fe-oxides through a variety of intermediate phases, e.g., precursor minerals of framboidal pyrite.

Four different chemical forms of As were extracted from the core samples. The extracted chemical forms are

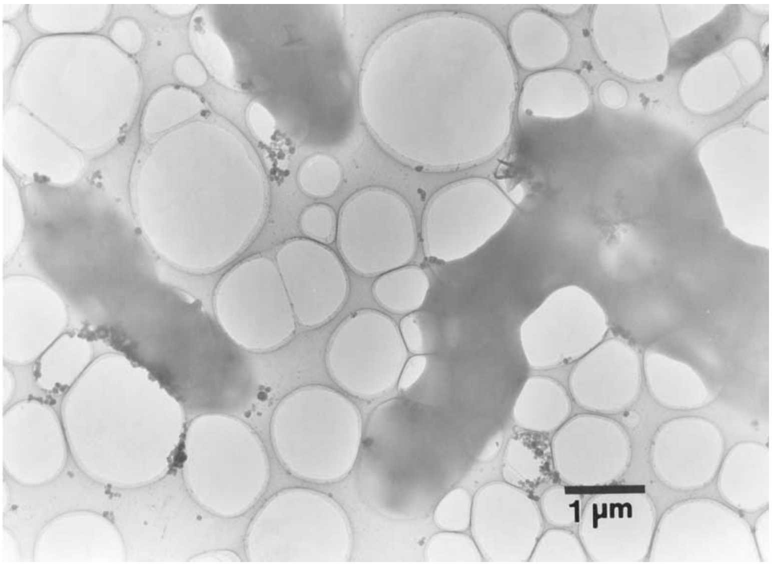

Fig. 13. Bacteria grown in the circulating water flow experiments (5 days after addition of glucose).

as follows: (1) acid soluble form (As mainly fixed in carbonates): (2) reducible form (As fixed in Fe-and/or Mn-oxides): (3) organic form: (4) insoluble form (As fixed mainly in sulfides and rarely in silicates). Arsenic occurs predominantly in the reducible form, organic form and insoluble form.

In order to test the bacterial role in dissolution, two types of culture experiments were designed: a batch culture experiment and a circulating water system experiment using Bangladesh sediments. In the batch culture experiments, addition of glucose and/or polypepton leads to the release of As following the lowering of Eh values. Control experiments without nutrients revealed contrasting results. In the circulating water flow system experiments with strata model; As was released when polypepton or glucose was added. These results support the hypothesis that microbial processes mediate the release of As release into water. Release of As into groundwater may be a reductive processes as experimentally confirmed in this study.

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