Inorganic arsenic and iron(II) distributions in sediment porewaters investigated by a combined DGT - colourimetric DET technique

William Bennett  
*Griffith University*

Peter R. Teasdale  
*Griffith University*

David T. Welsh  
*Griffith University*

Jarad Panther  
*Griffith University*

Ryan R. Stewart  
*Griffith University*

See next page for additional authors

Follow this and additional works at: [https://ro.uow.edu.au/scipapers](https://ro.uow.edu.au/scipapers)

Part of the *Life Sciences Commons, Physical Sciences and Mathematics Commons, and the Social and Behavioral Sciences Commons*

**Recommended Citation**

Bennett, William; Teasdale, Peter R.; Welsh, David T.; Panther, Jarad; Stewart, Ryan R.; Price, Helen L.; and Jolley, Dianne F.: Inorganic arsenic and iron(II) distributions in sediment porewaters investigated by a combined DGT - colourimetric DET technique 2012, 31-40.  

Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: research-pubs@uow.edu.au
Inorganic arsenic and iron(II) distributions in sediment porewaters investigated by a combined DGT - colourimetric DET technique

**Keywords**
inorganic, technique, det, combined, investigated, porewaters, sediment, distributions, ii, iron, arsenic, colourimetric, dgt, CMMB

**Disciplines**
Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

**Publication Details**

**Authors**
William Bennett, Peter R. Teasdale, David T. Welsh, Jarad Panther, Ryan R. Stewart, Helen L. Price, and Dianne F. Jolley

This journal article is available at Research Online: [https://ro.uow.edu.au/scipapers/4262](https://ro.uow.edu.au/scipapers/4262)
Inorganic arsenic and iron(II) distributions in sediment porewaters investigated by a combined DGT – colourimetric DET technique

William W. Bennett\textsuperscript{a}, Peter R. Teasdale\textsuperscript{a}\textsuperscript{*}, David T. Welsh\textsuperscript{a}, Jared G. Panther\textsuperscript{a}, Ryan R. Stewart\textsuperscript{a}, Helen L. Price\textsuperscript{b} and Dianne F. Jolley\textsuperscript{b}

\textsuperscript{a}Environmental Futures Centre, Griffith University, Gold Coast campus, QLD 4222, Australia

\textsuperscript{b}School of Chemistry, University of Wollongong, Wollongong, NSW 2522, Australia

*Corresponding Author: p.teasdale@griffith.edu.au
Environmental Context

Contamination of aquatic ecosystems with inorganic arsenic species is a concern for both environmental and human health. Sediments provide an important sink for dissolved arsenic, but may also act as a source of arsenic due to human-induced changes in the aquatic systems. This paper describes a new approach for investigating the status of inorganic arsenic in sediments, based on recent developments in diffusion-based sediment sampling techniques.

Abstract

A new approach for investigating the biogeochemistry of inorganic arsenic and iron(II) in freshwater, estuarine and marine sediments is reported. The recently developed Metsorb DGT technique for the measurement of total inorganic arsenic and colourimetric DET technique for the measurement of iron(II), were utilised in combination to determine co-located depth profiles of both solutes in sediment porewaters. DGT-measured porewater arsenic concentrations were typically less than 40 nmol L\(^{-1}\), while iron(II) concentrations reached up to 704 µmol L\(^{-1}\). Statistically significant (p < 0.0002) correlations between porewater arsenic and iron(II) profiles were observed (r > 0.92) in mesocosms of each sediment type. This approach to investigating arsenic and iron geochemistry in sediments allows the in situ determination of arsenic and iron species at exactly the same location in the sediment at three-millimeter resolution for arsenic and one-millimeter resolution for iron(II). The technique was capable of detecting very low concentrations of arsenic, with a detection limit of 0.27 nmol L\(^{-1}\) (0.02 µg L\(^{-1}\)) for a 48 h deployment time. Porewater iron(II), which is often present over a wide range of concentrations, was detectable up to 2000 µmol L\(^{-1}\). This study shows the application of these recently developed DGT and DET techniques for the in situ investigation of inorganic arsenic and iron biogeochemistry in sediments. This approach has the potential to enable simple, yet highly representative assessment of the biogeochemical status of arsenic and iron in a variety of natural sediments, including groundwater sediments where mobilised arsenic is responsible for significant human health risks.
Introduction

Diffusive gradients in thin films (DGT) and the diffusive equilibration in thin films (DET) techniques allow the in situ study of solute concentration profiles and distributions in sediment porewaters at higher spatial resolution than is typically possible employing conventional techniques.\textsuperscript{[1-3]} High-resolution measurements, based on the diffusion of analytes into these passive samplers, allow a much smaller three-dimensional volume of sediment to be sampled compared to traditional core slicing techniques.\textsuperscript{[3]}

This results in far less averaging of fine-scale features, thus facilitating the investigation of mechanistic interactions between chemical species within the sediment.\textsuperscript{[3]} In addition, several models have been developed to assist with interpretation of the high resolution DET and DGT responses obtained.\textsuperscript{[2, 4-6]}

Furthermore, the in situ nature of the DGT and DET techniques limits inaccuracies caused by removal and processing of sediment, particularly when determining profiles of reduced species that may be rapidly oxidised in air.

Recently, a new DGT technique has been developed for the measurement of total inorganic arsenic. Bennett and co-workers\textsuperscript{[7]} described the use of a titanium dioxide-based (Metsorb) DGT technique for the measurement of total dissolved inorganic arsenic (As(III) and As(V)) in water. The technique accurately and predictably accumulated arsenic over pH and ionic strength ranges typical of both fresh and marine waters. Arsenic is ubiquitous in the environment and can be mobilised through a variety of natural pathways, however, anthropogenic influences have also contributed to increased arsenic contamination through activities such as mining, and fossil fuel processing and combustion.\textsuperscript{[8]}

Sediments provide an important sink for dissolved arsenic, but may also release bound arsenic depending upon the redox conditions of the aquatic system.\textsuperscript{[9]}

The mobilization/sequestration of arsenic in sediments is closely linked to the biogeochemistry of iron.\textsuperscript{[10, 11]} Arsenic readily adsorbs to insoluble iron(III) phases, typically amorphous iron oxide minerals such as hydrous ferric oxide (HFO) and more crystalline phases like goethite.\textsuperscript{[12]} Microbially-mediated
reduction of these iron oxides in sub-oxic and anoxic sediment zones results in the release of bound arsenic into the porewater.\textsuperscript{13, 14} Adsorption onto iron oxide minerals and subsequent release through reductive dissolution is the primary pathway of arsenic cycling in sediments and their overlying waters.\textsuperscript{9, 12} Therefore, investigation and understanding of the biogeochemical cycling of arsenic and iron is integral to the management of arsenic contamination, which has become a significant human health issue in certain parts of the world.\textsuperscript{15-17} Over 100 million people in South and Southeast Asia have been exposed to dangerous concentrations of arsenic via groundwater used for drinking.\textsuperscript{18} This mass poisoning is due to natural mobilisation of arsenic from groundwater sediments through the release of arsenic adsorbed to iron oxide minerals.\textsuperscript{18} A recent review by Fendorf and co-workers\textsuperscript{18} highlights the importance of understanding the current biogeochemical state of groundwater sediments as well as anthropogenic factors that contribute to arsenic mobilization in groundwater such as organic carbon input. They recommend increased testing and monitoring of drinking water wells in order to identify the sources of drinking water that minimize exposure to arsenic\textsuperscript{18} – a key component of which is the arsenic and iron status of the groundwater sediment.

A new DET technique has recently been described\textsuperscript{19, 20} that allows highly representative measurements of porewater iron(II) distributions within sediment and facilitates interpretation of iron biogeochemistry. Robertson and co-workers\textsuperscript{19} developed an improved DET technique based on colourimetry for the high-resolution (1 mm) two-dimensional measurement of iron(II) in sediments, utilizing an extremely sensitive and selective colourimetric reagent (Ferrozine). The arsenic DGT and iron(II) DET measurement techniques can be combined in a single sampling device, as the diffusive layer of the Metsorb DGT technique may act as the DET gel for colourimetric analysis of iron(II). This allows arsenic and iron(II) to be measured at the same location within the sediment, decreasing artifacts caused by sediment heterogeneity and assisting with interpretation of the processes leading to arsenic mobilization and/or sequestration. Furthermore, these techniques are simple to use and are not subject to the many interferences associated with traditional sediment sampling techniques.\textsuperscript{3, 19}
In this study, we used combined DET and DGT measurements to investigate the interaction between porewater arsenic and iron(II) in freshwater, estuarine and marine sediments at high resolution. To further decrease the role of sediment heterogeneity in obscuring overall trends, mesocosms of stabilised, homogenised sediment were employed, allowing deployment under controlled laboratory conditions. The replication of the combined DGT/DET measurements was improved as a consequence, allowing the differences in arsenic mobilization between the sediments to be clearly observed and interpreted. This study describes the first application of the Metsorb DGT technique to sediment porewater analysis of total inorganic arsenic and the first application of a combined DGT – colourimetric DET technique for the investigation of arsenic and iron sediment biogeochemistry at the same location in the sediment.

**Experimental**

**Reagents, Materials and Solutions.** Deionised water (Milli-Q Element) was used to prepare all solutions. Iron(II) stock solutions and bisacrylamide diffusive gels were prepared as described by Robertson and co-workers.\(^{19}\) Metsorb binding gels were prepared as described by Bennett and co-workers\(^{7}\) with the exception that bisacrylamide cross-linker was used, instead of agarose-based cross-linker, as the resultant physical properties allowed more precise slicing of the gels. DGT components (including materials used to prepare DGT gels) were acid-cleaned in 10% (v/v) HNO\(_3\) (AR grade, Merck) for at least 24 h and rinsed thoroughly with deionised water prior to use. All salts used to prepare solutions were AR grade or better.

**Preparation of Sediment Mesocosms.** Sediment was collected from three sites on the Gold Coast, Queensland, Australia. All sites were located on the Coomera River: the first site was freshwater (266 µS cm\(^{-1}\) conductivity, pH 8.30), the second site was in the upper estuarine zone (5.8 salinity, pH 8.14) and the third site was in the lower estuarine/marine zone (28 salinity, pH 8.20). Following collection, sediment and water from the sites was transported back to the laboratory where the sediment was sieved...
to < 2 mm, homogenised and transferred into 20 L polyvinylchloride (PVC) (240 mm Ø, 500 mm height) cylinders (containing approximately 15 L of sediment) that were placed in 70 L plastic containers with ~60 L of water collected from the same site. The mesocosms were incubated in the dark in a constant temperature room at 24 ± 1°C. The overlying water column was constantly mixed using an aquarium pump and sparged with air to ensure oxygen saturation. Sediments were allowed to equilibrate for two months prior to the deployment of samplers.

**Sediment Characterisation.** All sediment characterisation was performed after retrieval of the combined DGT/DET samplers. Sediment oxygen demand (SOD) was determined from the time dependent decrease in water column oxygen concentrations during closed incubations as described by Dunn and co-workers.\[^{21}\] Small cores of sediment (28 mm Ø, 200 mm depth) were hand collected from each mesocosm, the sediment was extruded, homogenised and split into six subsamples; three samples for porosity/grain size analysis and three samples for organic matter content analysis. Porosity (mL H\(_2\)O mL sediment\(^{-1}\)) was measured as loss of wet weight of a known volume of sediment upon drying at 105°C for 24 h.\[^{22}\] Particle size distribution was measured by dry sieving of previously dried sediment (105°C for 24 h) through 2000, 1000, 500, 250, 125 and 63 µm-mesh sieves.\[^{22}\] The silt fraction was defined as < 63 µm. Organic matter content was determined by loss on ignition (LOI) of previously dried and weighed sediments (105°C for 24 h) at 550°C for 4 h.\[^{23}\]

**Sampler Assembly.** Sediment DGT/DET sampling devices were supplied by DGT Research Ltd. A Metsorb binding gel (0.04 cm thickness) was placed in the sampler, followed by a polyacrylamide diffusive gel (0.08 cm thickness) and a 0.45 µm polysulfone filter membrane (Supor, Pall) of 0.01 cm thickness. Samplers had an exposure window of 18 mm wide by 150 mm long. The combined thickness of the diffusive gel and membrane filter (0.09 cm) was used for all DGT calculations. The diffusive gel of the DGT probes was used as the DET for colormetric iron(II) determination, allowing the measurement of arsenic and iron(II) at exactly the same location in the sediment.
**Deployment and Analysis.** Prior to deployment, the combined DGT/DET probes were deoxygenated overnight in 0.01 mol L\(^{-1}\) NaNO\(_3\) for freshwater and upper estuarine deployments and 0.7 mol L\(^{-1}\) NaCl for marine deployments, by sparging with high-purity nitrogen gas. This ensured the probes did not introduce oxygen into the anoxic zone of the sediment upon deployment, which could interact with reduced species. In each mesocosm, three probes were carefully inserted into the sediment, with \(\approx 10\) mm of the exposure window left above the sediment water interface (SWI). Probes were removed after 48 h and the gels cut from the sampler window with a razor blade. The diffusive gels were analysed for iron(II) within two minutes of retrieval using a slightly modified version of the colourimetric computer imaging densitometry (CID) method described by Robertson and co-workers.\(^{[19]}\) Modifications were the increase in Ferrozine concentration to 0.01 mol L\(^{-1}\) and the use of a different colour channel (red vs. green) to allow a larger range of iron(II) concentrations to be determined. The calibration curve based on these slight modifications fitted the data very well \((R^2 = 0.997)\) and allowed the measurement of 13.2 – 2000 \(\mu\)mol L\(^{-1}\) iron(II). This method relies on the staining of iron(II) within the diffusive gel by a colourimetric reagent (Ferrozine), which is then scanned and converted to grayscale. The grayscale intensities are then converted to iron(II) concentrations by way of the calibration curve. Distributions can be presented as two-dimensional contour plots, showing data at 1 mm by 1 mm resolution, or they can be horizontally averaged to provide traditional one-dimensional depth profiles. See Robertson *et al.* (2008)\(^{[19]}\) and Robertson *et al.* (2009)\(^{[20]}\) for further information and evaluation of this technique.

The Metsorb binding gels were washed in 50 mL of deionised water to remove excess unbound salts and then sliced horizontally at 3 mm intervals.\(^{[7]}\) Gel slices were eluted overnight in 0.2 mL of 1 mol L\(^{-1}\) NaOH, diluted 15-fold with 2% nitric acid (Baseline, Seastar) and analysed for total arsenic by ICP-MS (Agilent 7500a).\(^{[7]}\) ICP-MS limit of detection (LOD; 3\(\sigma\) of blank) for arsenic (m/z 75) was 0.19 nmol L\(^{-1}\) \((0.014 \, \mu\text{g} \, \text{L}^{-1})\). The limit of quantitation (LOQ; 10\(\sigma\) of blank) was calculated to be 0.61 nmol L\(^{-1}\) \((0.046 \, \mu\text{g} \, \text{L}^{-1})\) arsenic and all measured samples were above this value. Quality control standards were
analysed every 20-30 samples and showed low variation and quantitative recoveries of 105 ± 1.3%. The ArCl (m/z 75) interference of arsenic (m/z 75) was avoided, even in samplers deployed in marine sediments, due to the selective accumulation of arsenic in the presence of chloride by the DGT samplers, which is then eluted into a simple matrix prior to analysis. Yttrium (m/z 89) was spiked into every sample to a final concentration of 10 µg L⁻¹ as an internal standard for ICP-MS analysis to minimize the effect of instrument drift. Yttrium counts varied by no more than 3.8% for each analytical run, indicating minimal instrument drift and the absence of significant matrix effects. DGT concentrations of total inorganic arsenic were calculated at depth using the DGT equation as described previously. It is possible that organic species of arsenic, if present in the sediment, could bind to the Metsorb binding phase and contribute to the measured concentration. However, studies of arsenic speciation in marine, estuarine and freshwater sediments have reported inorganic arsenic as the predominant form, with organic species often undetectable or contributing to < 10% of the total arsenic measured. The method detection limit (MDL) for the Metsorb DGT was calculated, based on a deployment time of 48 h, to be 0.27 nmol L⁻¹ (0.02 µg L⁻¹) arsenic. Collectively, this data indicates that the features observed in the arsenic porewater concentration profiles are accurate representations of the porewater arsenic distributions and are not artifacts of the analysis.

Results and Discussion

Sediment Characterisation. There were differences between the mesocosm sediments depending on the source location, particularly for % silt, organic matter content (OM %) and SOD (Table 1). These results suggest that the freshwater sediment mesocosm was the most productive, with a higher SOD and organic matter content than the estuarine and marine sediment mesocosms. In a meta-analysis of bacterial production data from marine and freshwater sediments, Sander and Kalff found that, on average, bacterial production was higher in freshwater river sediments (1235 mg C m⁻² day⁻¹) compared to marine sediments (959.9 mg C m⁻² day⁻¹) and similarly, that the organic carbon content was higher in river sediments (9.4 g C m⁻²) than marine sediments (3.9 g C m⁻²). Furthermore, they reported a positive
correlation between bacterial production and organic carbon content in both freshwater ($r^2 = 0.58$) and marine sediments ($r^2 = 0.68$). This supports our finding of a higher SOD in the freshwater mesocosm, which is indicative of elevated bacterial production, associated with higher organic matter content, compared to lower values for the marine sediment mesocosm.

Table 1. Sediment characteristics: porosity, % silt fraction, organic matter content (OM % dry weight) and sediment oxygen demand (SOD)

<table>
<thead>
<tr>
<th>Mesocosm</th>
<th>Porosity</th>
<th>% silt</th>
<th>OM (%)</th>
<th>SOD (mmol O$_2$ m$^{-2}$ h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freshwater</td>
<td>0.356 ± 0.003</td>
<td>4.30</td>
<td>2.01 ± 0.04</td>
<td>1.02</td>
</tr>
<tr>
<td>Upper Estuarine</td>
<td>0.415 ± 0.006</td>
<td>3.98</td>
<td>1.40 ± 0.01</td>
<td>0.49</td>
</tr>
<tr>
<td>Marine</td>
<td>0.341 ± 0.006</td>
<td>1.38</td>
<td>0.46 ± 0.01</td>
<td>0.64</td>
</tr>
</tbody>
</table>

**Freshwater Mesocosm.** The results of combined DGT – colourimetric DET deployments in the freshwater sediment mesocosm show a clear relationship between the reductive dissolution of iron(III) to iron(II) and the mobilisation of arsenic into the porewater (Figure 1). The arsenic concentration profile is driven by the large increase in arsenic, from 5-10 mm depth, producing arsenic concentrations in the range 23-38 nmol L$^{-1}$. The concentration of iron(II) (maximum 220-700 μmol L$^{-1}$) also increases over the same 5-10 mm depth horizon. This is indicative of reductive dissolution of solid phase iron(III) oxyhydroxides, which results in release of iron(II) and adsorbed arsenic into the porewater. This mechanism of arsenic mobilisation has been observed in freshwater sediments and is accepted as the primary mechanism of sediment arsenic mobilisation. The zone of iron(III) reduction extends down to a depth range of 20-80 mm and the concentrations of both arsenic and iron(II) are quite stable in the deeper sediments until a sharp decrease is observed at depths from 90-110 mm. This decrease in both iron(II) and arsenic was determined to be due to a possible sampling artifact due to the entrapment
of methane bubbles on the probe surface (discussed in detail in the section: ‘Two-dimensional distributions of iron(II)’).

The steep concentration gradient that results due to the mobilisation of arsenic in the iron(III)-reduction (sub-oxic) zone will produce a diffusive flux of dissolved arsenic and iron(II) towards the sediment-water-interface. While the DGT data appears to indicate a concentration gradient between the water column and the arsenic minima at three to six millimeter depth, interpretation of this gradient is not straightforward. Since the measured gradient may, at least in part, be an artifact of the DGT measurement due to more efficient analyte resupply in the well-mixed water column compared to the static sediment porewater, it may or may not be indicative of an actual flux across the SWI.

At depths of 5-10 mm below the SWI, both the arsenic (mean 1.6 ± 0.5 nmol L⁻¹) and iron(II) concentrations reach minimum values. This is indicative of the interface between the suboxic and oxic zone where iron(II) is reoxidised by aerobic chemoautotrophic iron-oxidising bacteria and/or by direct reaction with dissolved oxygen diffusing across the SWI, to form insoluble iron(III) oxyhydroxides.²⁹ Both As(III) and As(V) would re-adsorb to these newly-formed mineral phases,¹² explaining the co-occurrence of the concentration minima observed in this zone. In natural sediments trace elements concentrations may increase at the sediment-water-interface due to their release during aerobic decomposition of organic detritus settling on the sediment surface. However, in the mesocosms employed in this study, this process was absent due to the closed nature of the system.
Figure 1. Replicate depth profiles of porewater inorganic arsenic and iron(II) concentrations as measured by DGT and colourimetric DET, respectively, in a freshwater sediment mesocosm. Negative depth values indicate measurements in the water column and positive values indicate measurements in the sediment.

**Upper Estuarine Mesocosm.** The results of combined DGT – colourimetric DET deployments in the upper estuarine sediment mesocosm (Figure 2) show very different concentration profiles of porewater arsenic and iron(II) compared to those recorded in the freshwater sediment (Figure 1). The reproducibility between samplers is in good agreement (see Table 2), with similar profile shapes observed for each replicate. As observed in the freshwater sediment mesocosm, the increase in porewater iron(II) concentrations at depths greater than 5-10 mm, due to reductive dissolution of iron(III) oxide minerals, is associated with a corresponding increase in porewater arsenic.
This process was much more gradual than in the freshwater sediment with iron(II) and arsenic concentrations increasing down to 120 mm and deeper. The iron(II) gradient in this sediment may reflect the lower overall demand for terminal electron acceptors as indicated by the lower sediment oxygen demand. Iron(II) (maximum 180–380 μmol L\(^{-1}\)) was present at lower overall concentrations than in the freshwater sediment, most likely due to some iron(II) being immobilised as FeS in the estuarine system where sulfate is available for sulfide production and subsequent precipitation with iron(II). The slight decrease apparent in the iron(II) concentrations in the lowest part of the profiles may reflect an actual maxima in iron(III)-reduction or may be due to the formation of sulfide by bacterial sulfate-reduction in the sediment at these depths (see the Marine Mesocosm section for further discussion).
Figure 2. Replicate depth profiles of porewater inorganic arsenic and iron(II) concentrations as measured by DGT and colourimetric DET, respectively, in an upper estuarine sediment mesocosm. Negative depth values indicate measurements in the water column and positive values indicate measurements in the sediment.

Marine Mesocosm. The combined DGT – colourimetric DET samplers deployed in the marine sediment mesocosm clearly show a strong interaction between porewater arsenic and iron(II) porewater concentrations in the upper part of the profiles (Figure 3). The agreement between replicates (see Table 2) suggests that these profiles are accurate indications of the relationship between arsenic and iron in the marine sediment mesocosms. The sub-oxic sediment zone is clearly defined by the increase in iron(II) between 10-40 mm depth, due to microbial reduction of iron(III) to iron(II) (maximum 150-300 μmol L⁻¹).
Within this iron(III)-reduction zone, porewater arsenic concentrations again increase proportionally (maximum 12-27 nmol L⁻¹) with iron(II) concentration and remain quite constant with increasing depth. These concentrations are more similar to those in the upper estuarine than in the freshwater sediment mesocosm. The iron(II) concentrations reached a peak between 30-45 mm depth, after which iron(II) concentrations gradually decrease to below 50 µmol L⁻¹. In marine sediment, a zone of sulfate-reduction in which sulfide is produced via microbial sulfate-reduction, occurs below the iron(III)-reduction zone. Sulfide forms insoluble FeS with iron(II) and would cause the iron(II) concentration to decrease as observed. This differs from the estuarine results, which display a gradual increase in iron(II) concentration with depth. This may be a result of the lower sulfate concentration in the estuarine system compared to the marine system, which would correspond to a lower sulfide concentration available to consume iron(II) in the sediment porewater. Future studies should utilise the sulfide DGT method to assess whether free sulfide is present in the porewater so that the iron(II) biogeochemistry can be further explained.

Insoluble arsenic-sulfide minerals such as realgar (AsS(s)) and orpiment (As₂S₃(s)) can form in anoxic sediments where the solubility of these compounds is exceeded. O’Day and co-workers modelled the interactions between iron, sulfide and arsenic in sediment porewater and determined that an arsenic concentration of between 10 to 100 µmol L⁻¹ is required to permit the formation of insoluble arsenic-sulfide minerals. The concentration of arsenic measured in the marine sediment mesocosm did not exceed 30 nmol L⁻¹, indicating that arsenic would not be removed from solution by precipitation as arsenic-sulfide minerals. This explanation is also supported by the solubility constants of realgar (K_{sp} = 1.5 × 10⁻⁶) and orpiment (K_{sp} = 8.4 × 10⁻¹⁶) being much higher than that of FeS (K_{sp} = 6.3 × 10⁻¹⁸), indicating that iron(II) would preferentially precipitate with sulfide before arsenic. Given the solubility constant of FeS and the fact that iron(II) is still measureable in the deepest zone of the sediment, it is...
also probable that only negligible concentrations of free sulfide were present in the sediment, as any microbially produced sulfide would be rapidly precipitated out of solution as FeS.

Figure 3. Replicate depth profiles of porewater inorganic arsenic and iron(II) concentrations as measured by DGT and colourimetric DET, respectively, in a marine sediment mesocosm. Negative depth values indicate measurements in the water column and positive values indicate measurements in the sediment.

It is important to note that DGT measurements provide a different measure of porewater solute concentration compared to traditional sampling techniques. DGT relies on the resupply of solute from the solid phase to the porewater to sustain the flux to the DGT sampler. This resupply may be one of three cases: fully sustained from the solid phase, where DGT represents the bulk porewater concentration; diffusion alone, where there is no resupply from the solid phase and DGT significantly
underestimates porewater concentrations; or partial resupply, where the solid phase releases some solute into the porewater but not at a rate capable of sustaining the flux to the DGT.\textsuperscript{[3, 35]} Arsenic has previously been measured by DGT in sediment porewater and was determined to be partially resupplied from the solid phase.\textsuperscript{[3]} However, as the biogeochemistry of arsenic is closely linked to that of iron, the resupply of arsenic from solid phase iron pools is likely to be affected by the oxidation and reduction reactions of iron in the sediment. The adsorption of arsenic onto iron oxide in the oxic zone would limit resupply and, conversely, the reductive dissolution of iron in the suboxic zone would at least partially resupply dissolved arsenic to the DGT sampler. Although DGT measurements made in sediment porewater may not accurately indicate true porewater concentrations, they do provide a useful tool for the interpretation of mechanistic interactions occurring in the sediment and avoid the interferences typical of traditional techniques (see “Evaluation of DGT/DET coupled with sediment mesocosms to investigate biogeochemistry” for further comparison of traditional and DGT techniques).

Correlation of porewater arsenic and iron(II). Statistical correlation analysis was performed on the porewater iron(II) and arsenic concentration profile data to determine the strength of the relationship between these chemical species (Table 2). The entire concentration profile for the upper estuarine data was included in the correlation analysis. For the marine sediment, however, due to the differential influence of sulfide on the porewater iron(II) and arsenic concentrations at depth, only the profiles between the sediment surface and the iron(II) maxima for each profile (30-45 mm depth) were included in the correlation analysis, as described by Stockdale and co-workers.\textsuperscript{[36]} This ensured that the correlation between the iron(II) and arsenic porewater concentrations was not confounded by the effect of sulfide on the porewater iron(II) concentrations. Similarly, the correlation analysis of the freshwater data was only performed for 0-80 mm depth, to eliminate the effect of the artifact observed below this zone (See section on two-dimensional distributions of iron(II)).
Table 2. Pearson $r$ values of correlations between DGT-measured arsenic and colourimetric DET-measured iron(II) in marine, upper estuarine and fresh water sediment mesocosms. R1, R2 and R3 are replicate probes from the same mesocosm. All correlations are significant ($p < 0.0002$).

<table>
<thead>
<tr>
<th>Sediment Type</th>
<th>Correlation Coefficient ($r$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R1</td>
</tr>
<tr>
<td>Marine</td>
<td>0.97</td>
</tr>
<tr>
<td>Upper Estuarine</td>
<td>0.94</td>
</tr>
<tr>
<td>Freshwater</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Statistically significant ($p < 0.0002$) correlations between porewater iron(II) and arsenic concentrations confirm a strong positive relationship in all three sediment mesocosms. This statistically confirms the inference that reductive dissolution of iron(III) minerals concomitantly releases iron(II) and adsorbed arsenic species into the porewater. All correlation co-efficients ($r$-values) are greater than 0.9 except for R1 in the freshwater sediment. As can be seen from the depth profiles in Figure 3, the arsenic concentration in R1 experiences a significant spike between 10-20 mm depth, probably contributing to the weaker $r$-value for this replicate.

Two-dimensional distributions of iron(II). The colourimetric DET technique allows the distributions of porewater iron(II) to be represented in two-dimensions for the entire area of the probe window. This has the benefit of allowing a more accurate and detailed interpretation of the iron biogeochemistry due to a better representation of analyte heterogeneity. This is evident in the two-dimensional distributions of iron(II) measured by the colourimetric DET technique in the freshwater mesocosms (Figure 4). Porewater iron(II) distributions, even in homogenised sediment, clearly show heterogeneity both within and between the replicates. The lateral variability exhibited in these two-dimensional distributions implies that porewater arsenic would also exhibit a similar degree of heterogeneity, especially
considering the strong correlation observed between porewater iron(II) and arsenic profiles. This finding is consistent with the work of Shuttleworth and co-workers\textsuperscript{37} who observed variation in both porewater iron and manganese on the horizontal and vertical scale by traditional DET measurements at three millimeter resolution, as well as localised zones of high concentration on the millimeter to sub-millimeter scale. This work supports the findings of other studies that emphasise the importance of measuring analytes in two-dimensions in order to make the most accurate quantitative and qualitative interpretations of sediment processes.\textsuperscript{37-40} Unfortunately, a comparably simple method for the two-dimensional analysis of porewater arsenic is not available, and so interpretations regarding its biogeochemistry must be based on one-dimensional measurements.
Figure 4. Two-dimensional distributions of porewater iron(II) in a freshwater sediment mesocosm as measured by colourimetric-DET. Contour plots were generated from grayscale intensities in Matlab R2010b. The approximate location of the SWI is indicated by a dashed white line.

These two-dimensional measurements indicate that the decrease in both iron(II) and arsenic observed in the bottom 40 mm of the profiles is most likely due to bubbles of methane gas, the product of methanogenesis, becoming trapped on the surface of the probe and preventing diffusion of both dissolved iron(II) and arsenic into the DGT/DET probe. The rounded shape of the areas of very low iron(II) concentration is indicative of gas bubbles and the observation of this effect in all probes supports the inference that methane bubbles were present on the surface of the probes during deployment. As probes were being inserted into the freshwater sediment, bubbles of gas were observed escaping from the sediment; a phenomenon not observed during the deployment of probes in the other two mesocosms, as the presence of sulfate from seawater would favour bacterial sulfate reduction over methanogenesis as a pathway for organic matter mineralization in these sediments, due to the higher energy yield of the former process.\textsuperscript{[41, 42]} To confirm the presence of methane in the sediment, an inverted glass funnel was submerged and placed over the sediment, stoppered and allowed to capture bubbles of gas during physical disturbance of the sediment. Flammability of the captured gas confirmed the presence of methane, and thus that methanogenesis was occurring in the freshwater sediment. This is not unexpected, as due to the very low abundance of sulfate, methanogenesis is the primary anoxic microbial metabolism in freshwater sediments.\textsuperscript{[41, 42]} This possible measurement artifact is something that DGT and DET users will need to be aware of for deployments in productive freshwater sediments.

\textbf{Evaluation of DGT/DET coupled with sediment mesocosms to investigate biogeochemistry.} Sediment heterogeneity can make the interpretation of mechanistic interactions between chemical species very challenging. Field deployments of DGT and DET techniques provide excellent resolution and data
quality, but many factors are uncontrolled and induce a higher degree of spatial heterogeneity leading to difficulties in the interpretation of the porewater profiles. The use of mesocosms can overcome these challenges by allowing sources of heterogeneity to be decreased or controlled. This is possible by sieving the sediment to remove large particulate organic matter and biota which induce heterogeneity, and by homogenizing the sediment to redistribute organic matter and chemical species so that new profiles of porewater solute concentrations are established. A recent study by Porter and co-workers investigated the effect of sediment manipulation on sediment biogeochemistry in laboratory-based systems. They found that although homogenisation of sediments can significantly influence solute and gas fluxes, the manipulated systems exhibited similar fluxes to intact, non-homogenised sediment after a stabilisation period of two to three weeks. The sediment in this study was allowed to age for a period of eight weeks following homogenisation, ensuring that dissolved nutrient and gas fluxes stabilised prior to sampling. It may take longer, however, for the re-establishment of concentration gradients of analytes such as iron(II) and arsenic in the porewaters as they are generated from solid mineral phases.

In natural sediments, organic matter and oxidized mineral phases such as iron(III) oxyhydroxides would be more abundant in the surface layers. Consequently, sediment homogenisation during mesocosm preparation could affect the shape of the measured profiles of arsenic and iron(II) in the porewater by providing iron(III) as a terminal respiratory electron acceptor and readily biodegradable organic matter that will stimulate microbial iron reduction and concomitant release of adsorbed arsenic deeper within the sediment. The results from mesocosm studies should therefore be interpreted carefully with respect to natural sediments as they may not accurately represent the complexity of biogeochemistry that occurs in sediment porewaters. Rather, their advantages lie in allowing investigation of mechanistic interactions that may otherwise be obscured by the heterogeneous nature of chemical distributions in sediment porewaters, such as the tight coupling between iron(II) and arsenic release observed in the sediment suboxic zone in this study.
Diffusion-based techniques like DGT and DET result in a very small volume of sediment porewater actually being sampled, further limiting the effect of heterogeneity and improving their ability to detect mechanistic interactions between analytes. Traditional core-slicing techniques rely on the extraction of porewater from much larger sediment volumes (typically in the milliliter range), resulting in the averaging of the chemical profile over the entire volume and, potentially, the reaction of chemical species with each other or with atmospheric oxygen. These reactions may result in inaccurate measurement of distributions of chemical species being obtained, and thus false interpretations of the interactions between chemical species. In terms of arsenic and iron(II) porewater analysis, the reaction of chemical species within a porewater extract could result in dramatic changes in the measured distributions. The oxidation of iron(II) by atmospheric oxygen could result in the formation of iron oxide minerals and the subsequent adsorption of dissolved arsenic. Whereas, the mixing of sediment containing solid phase iron and dissolved sulfide during processing, would result in the reductive dissolution of iron and the release of adsorbed arsenic. In situ sampling techniques such as DGT and DET avoid these problems, as well as providing the option of measuring concentration profiles at high spatial resolution. Recent developments in this field have expanded the number of DGT-measurable analytes to include selenium(IV) and dissolved reactive phosphorus, in addition to the existing techniques capable of measuring trace metals and dissolved sulfide.

Application of this technique to assess the status of groundwater sediments in South and Southeast Asia could provide those tasked with the identification of safer drinking water sources with a useful tool. Two recent reviews by Fendorf and co-workers and Polizzotto and co-workers emphasised the importance of understanding the biogeochemistry of groundwater sediments and anthropogenic influences on arsenic mobilisation in groundwater so that safer sources of drinking water can be identified, helping to improve the human health outcomes for millions of people.
Conclusions

This study has demonstrated the utility of combining Metsorb DGT and colourimetric DET to investigate the distributions of arsenic and iron(II) in sediment porewaters. The use of sieved, homogenised sediment mesocosms, coupled with the relatively high-resolution measurements of porewater iron(II) and arsenic by DET and DGT, allowed mechanistic interactions between the two chemical species to be clearly observed and interpreted, while avoiding many of the caveats of traditional sediment sampling techniques. Furthermore, the use of homogenised and sieved sediment resulted in highly reproducible profile shapes for both iron(II) and arsenic, allowing simpler interpretation of the sediment biogeochemistry. Future research will focus on the application of this experimental approach to investigating the effect of anoxia on the concentration profiles of porewater arsenic and iron(II), and the use of the combined DGT–colourimetric DET technique for in situ studies of porewater arsenic and iron(II) in natural sediments. Further work should also be focussed on the application of this technique to the assessment of groundwater and sediment biogeochemistry in areas impacted by high concentrations of naturally mobilised arsenic.

Acknowledgements

The authors acknowledge the financial support of the NSW Environmental Trust (Research Project APP2006-RD-0059). We also thank the School of Environment, Griffith University, for provision of a scholarship for W.W.B. and the University of Wollongong for provision of a scholarship for H.L.P. The authors also thank Graver Technologies (www.gravertech.com) for the provision of the Metsorb product used in this study.
References


Madigan MT, Martinko JM, Parker J, Brock TD. Brock Biology of Microorganisms 2009 (Pearson Benjamin Cummings: San Francisco).


