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High-resolution two-dimensional quantitative analysis of phosphorus, vanadium and arsenic, and qualitative analysis of sulfide, in a freshwater sediment

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Environmental context. Chemical characterisation of sediment microniches can reveal diagenetic processes that may not be detected by larger-scale analysis. With the development of a new preparation method for a binding phase gel, the technique of diffusive gradients in thin films has been used to demonstrate links between the diagenesis of sulfide, phosphorus, vanadium and arsenic at microniches. Knowledge of these processes may improve predictions of past deposition climates where trace elements are considered as paleoredox proxies.

Abstract. Recently introduced techniques that can provide two-dimensional images of solution concentrations in sediments for multiple analytes have revealed discrete sites of geochemical behaviour different from the average for that depth (microniches). We have developed a new preparation method for a binding phase, incorporated in a hydrogel, for the diffusive gradients in thin films (DGT) technique. It allows co-analysis of sulfide and the reactive forms of phosphorus, vanadium and arsenic in the porewaters at the surface of the device. This gel, when dried and analysed using laser ablation mass spectrometry, allows the acquisition of high-resolution sub-millimetre-scale data. The binding phase was deployed within a DGT device in a sediment core collected from a productive lake, Esthwaite Water (UK). Localised removal of phosphate and vanadium from the porewaters has been demonstrated at a microniche of local sulfide production. The possible removal processes, including bacterial uptake and reduction of vanadate to insoluble V^{III} by sulfide, are discussed. Understanding processes occurring at this scale may allow improved prediction of pollutant fate and better prediction of past climates where trace metals are used as paleoredox proxies.

Additional keywords: DGT, early diagenesis, laser ablation, microniche, phosphate.

Introduction

Recent developments in analytical techniques have enabled two-dimensional analysis in sediments of oxygen, pH, pCO_2, sulfide, Fe, Mn and trace metals.[1–7] Several of these techniques have identified microniches, characterised by geochemical behaviour significantly different from the average for the given depth. These niches are generally attributed to elevated microbial activity at discrete sites with high concentrations of labile organic matter.[8] If processes associated with microniches are demonstrated to be significant in terms of the accumulation of specific elements in sediments, some reassessment of our current understanding of 'bulk' sediment diagenesis may be required. Processes at microniches may affect: our understanding of pollutant fate, the interpretation of trace metal data for the prediction of past climates, or the prediction of reaction rates for redox processes. Sulfide, metal oxide and arsenic data can inform all of these subjects and phosphate may provide information relevant to the growth stage of the microniche bacteria.

Vanadium is likely to be in the measurable form $H_2VO_4^-$ in the pH range 5–9 and the E_h (redox potential) range 0–1 V.[9] Reduction of V^V to V^{IV} can occur with organic materials as electron donors,[10,11] with reduction to V^{III} requiring a stronger reductant. V^{III} is rapidly hydrolysed in aqueous solution, resulting in the precipitation of relatively insoluble V^{III} oxyhydroxides.[9] Sulfide can reduce V^{IV} to V^{III}, but when studied under laboratory conditions, the reaction was slow at natural sulfide concentrations.[9] However, the authors considered that effects such as surface catalysis may increase reaction rates under natural conditions.

Removal of As from anoxic porewaters, observed by Huerta-Diaz et al.,[12] was attributed to coprecipitation with FeS and/or adsorption on FeS. Microniches may have implications for As and anionic metal oxides such as vanadate, molybdate, or perrhenate, which are known to have a tendency to accumulate in strongly reducing sediments.[13–16] Diffusion of these oxyanions into sulfidic microniches within the oxic, suboxic and weakly reducing zones may enable fixation where no net removal is predicted. Provided these reactions are not fully reversible, these discrete environments may account for a significant part of their bulk sediment accumulation. Phosphate release from organic matter (OM) degradation occurring in microniches may be overcompensated by uptake from OM-reducing microorganisms that are in the exponential growth phase. Tezuka's[17] observation of no phosphate regeneration at low substrate nitrogen (C : N of 60 : 1) was attributed to the capacity of bacteria to store excess P within their cells.

It has been recognised that a lack of congruent analysis makes it difficult to cross-interpret data obtained from different

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probes.[8] Data obtained simultaneously from the same probe can be easier to interpret, as assumptions about the deployment conditions and nature of microniches do not have to be made. Here, we describe a new method for the preparation of a binding phase gel for diffusive gradients in thin films (DGT) based on iron oxide, which is suitable for the binding of soluble reactive species of phosphorus, vanadium and arsenic. This gel is combined with a previously described sulfide binding phase of silver iodide to provide visual identification of sulfidic microniches. Analysis of the combined gel using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) gives two-dimensional data at high-resolution (sub-millimetre). We detail the principles of DGT, preparation of the combined phase gel, deployment procedure, LA-ICP-MS procedure, and results obtained from analysis of a sulfidic microniche.

Principles of DGT

Immobilisation of analytes diffusing through a filter membrane and a diffusive gel from the porewater to the binding layer results in a concentration gradient through the diffusive layers. The concentration of solute accumulated in the binding gel (M) per unit area (A, cm^2), divided by the deployment time (t, s), gives the time-averaged solute flux (F) through the diffusive layers (typical units mol cm^{-2} s^{-1}, Eqn 1).

$$F = M/At \quad (1)$$

DGT utilises Fick's law of diffusion to determine the average concentration at the DGT interface (C_{DGT}) from the mass of analyte on the binding layer (Eqn 2):

$$F = D_d C_{DGT}/\Delta g \quad (2)$$

where Δg is the diffusive layer thickness and D_d is the diffusion coefficient in the gel. Combining Eqns 1 and 2 allows calculation of C_{DGT} (Eqn 3).[18]

$$C_{DGT} = \frac{M\Delta g}{D_d At} \quad (3)$$

In sediments, C_{DGT} is the average concentration at the surface of the DGT device during the deployment. It is only the same as undisturbed porewater concentrations when resupply from the solid phase is sufficient to prevent their depletion. In most cases, resupply will be insufficient for full buffering and partial resupply will be controlled by the desorption kinetics (characteristic response time[19,20]) and partition coefficients of the analyte. Various cases of resupply are considered by Zhang et al.[21] Where a solute is being locally generated within the sediment at rates higher than the average for that depth, there will be local elevation in the porewater concentration. The elevated concentration and comparatively high rate of resupply will contribute to a higher DGT signal.[22] DGT-observed elevations in concentration can therefore be indicative of localised release processes and conversely, observed minima in concentrations may be indicative of other processes such as scavenging.

Materials and methods

Preparation of diffusive gel

Diffusive gels 0.4 mm thick were prepared using established procedures,[23] from gel solution prepared from the following reagents: 37.5% acrylamide solution (40% w/w, BDH, Electran, Lutterworth, UK), 47.5% 18.2 MΩ cm Milli-Q (MQ) water and 15% DGT cross-linker (2% w/w, DGT Research Ltd, Lancaster, UK). Chemical polymerisation was initiated by ammonium persulfate (APS; 10% w/w, BDH Electran) and catalysed by TEMED (99%, N,N,N,N-tetramethylethylenediamine, BDH Electran).

Preparation of the combined silver iodide–iron oxide binding layer

Preparing the AgI phase

A homogeneous gel, where AgI is precipitated into a precast gel, first suggested by Teasdale et al.,[24] was reported by Devries and Wang.[4] The method presented in this work is a modification of the Devries and Wang procedure using the gel solution described above.[25] Silver nitrate (0.153 g; BDH, AnalaR) was dissolved in 0.45 mL of MQ water. Gel solution (8.55 mL) and 36 μL of 5% w/w APS were added to the dissolved $AgNO_3$ and well mixed between each addition (TEMED catalyst was not required). Following addition of the last solution, the gels were cast immediately between glass plates spaced by a 0.25-mm plastic spacer, resulting in a 0.4 mm-thick gel after hydration. Once cast, the gels were allowed to polymerise at room temperature ($20 \pm 2°C$) in dark conditions for ~1 h. Following polymerisation, the glass plates were opened so that the gel remained adhered to one plate. The plate and gel were then immersed in a solution of ~0.2 M potassium iodide (16.6 g in 500 mL, Acros Organics, ACS reagent grade, Loughborough, UK). The gel became white within a few minutes and was peeled off from the glass plate (this procedure ensures the precipitate in the gel is distributed evenly). Gels were then kept in the KI solution overnight to allow full development, before being rinsed several times in MQ water to remove excess reagents.

Addition of the iron oxide phase

An iron oxide gel, prepared using ferrihydrite slurry as the binding agent, was first reported by Zhang et al.[18] for the measurement of dissolved phosphorus. Inevitable heterogeneity in the particle size and distribution makes this gel unsuitable for high-resolution analysis. The method presented here provides a homogeneous gel by precipitating ferrihydrite directly in the gel. Four grams of ferric nitrate ($Fe(NO_3)_3 \cdot 9H_2O$, BDH, AnalaR) were dissolved into ~40 mL of MQ water. Three AgI gel sheets were placed into the solution and the volume made up to 100 mL. The gels were allowed to equilibrate for a minimum of 2 h. Following equilibration, the gels were rinsed for a few seconds in MQ water before being introduced, one by one, into a 0.05 M solution of sodium bicarbonate (BDH, AnalaR) that was previously adjusted to pH 6–7 by addition of ~1 mL of nitric acid (70%, BDH, AristaR). Gels gave the appearance of total precipitation of orange-brown ferrihydrite within 5 min, but were left in the solution for ~30 min to ensure complete development. Prepared gels were rinsed several times in MQ water to remove excess reagents.

The presence of carbonate in the ferrihydrite binding layer from the buffer solution may lead to an increase in the rate of conversion of ferrihydrite to goethite.[26] As this may change the binding properties of the gel, it is recommended that the ferrihydrite phase be freshly prepared before deployment. Should the sulfide scans not be required, the AgI gel can be replaced with a DGT diffusive gel to give a binding layer containing only ferrihydrite.

Calibration procedure

Discs of binding phase gel (4.9 cm^2) were exposed to known molar amounts of solutions of phosphate, vanadate and arsenate (thus giving known molar amounts bound per cm^2). Standards were prepared from the salts sodium hydrogenarsenate heptahydrate (Sigma, Gillingham, UK, 98%), sodium orthovanadate (Aldrich, Gillingham, UK, 99.8%) and disodium hydrogen orthophosphate dodecahydrate (BDH, GPR). Discs were placed into vials containing 14 mL of each standard solution and shaken in an end-over-end stirrer for 72 h. No analytes were detectable in the solutions after this time, indicating complete uptake by the gels. Following exposure, gels were placed onto a polysulfonate filter and dried for laser analysis.

Deployment procedure

DGT sediment probes (DGT Research Ltd) were used to contain the gel layers. The binding layer was placed onto a polysulfonate backing filter (0.45-μm pore size, Pall Corporation, Portsmouth, UK) to aid removal of this layer from the probe assembly after deployment. The filter also supported the dimensional integrity of the binding phase during handling and drying. The binding layer was positioned in the probe housing, and then overlaid by the diffusive gel and a filter, which prevented fouling of the diffusive gel. All layers were held within the plastic housing by means of a close-fitting plastic window. The exposed area of the probe was 150 × 18 mm. Prior to deployment, all probes were deoxygenated for 24 h by submerging in a 0.01 M sodium chloride solution (BDH, AnalaR) through which oxygen-free nitrogen gas was continuously bubbled.

Sediment probes were deployed in sediment from Esthwaite Water (UK), a small, productive, softwater lake, which is seasonally anoxic and has a sediment whose porewater chemistry is dominated by iron and manganese.[27] Intact cores of the sediment and overlying water were collected in May 2007 from the deepest basin using a Jenkin corer. Oxygen was present in the overlying water and it was maintained during the experiment by bubbling with air. Probes were deployed for a period of 6 days ($T = 20 \pm 2°C$). After deployment, the gels were cut from the probe window with a Teflon-coated razor blade. The binding phase was placed between clean plastic sheets and refrigerated before being dried. The drying procedure is provided as accessory materials.

Laser ablation procedure

Laser ablation (LA) was undertaken using a New Wave UP-213 coupled to a Thermo Electron X Series (Hemel Hempstead, UK) inductively coupled plasma mass spectrometer (ICP-MS). A beam diameter of 100 μm was selected to achieve the maximum analyte signal. Ablation intensity was set at 43% with a firing time of 4 s and an inter-sample dwell time of 10 s. These parameters were chosen to ensure peaks were easily defined from the background by the instrument software. The distance between ablation spots was 333 μm. The procedure for extracting the transient peaks from the time series of counts followed the method described by Warnken et al.[5] (the exact procedure is provided as accessory materials).

Obtaining qualitative sulfide data

Qualitative sulfide data from the deployed gels was obtained by computer imaging densitometry (CID). Gels were scanned, after ablation, using a flatbed scanner (Hewlett Packard ScanJet 2100C). Files were saved as greyscale tagged image file format (.TIF) to enable conversion of the images to background-corrected text files using Scion Image image-processing software (Scion Corp., freeware available from http://www.scioncorp.com, accessed March 2007). Data were then plotted as colourmaps using Matlab (MathWorks, Cambridge, UK). Scanning of the gels after laser ablation allowed the ablation grid to be mutually visualised with sulfide and avoided potential contamination of gel handling before laser analysis. Future work should include the quantification of the sulfide data. CID is not a suitable technique for sulfide calibration as the ferrihydrite contributes to a high background greyscale value. Optimisation of laser ablation techniques to include S will be required.

Results and discussion

Analytical performance

Fig. 1 shows the time series of counts for the analytes and internal standard for the gel used for the 0.14 nmol cm^{-2} calibration for As and V (1.4 nmol cm^{-2} for P). Good analytical precision was achieved for all analytes and the internal standard (residual standard deviation (RSD) < 10%). ^{57}Fe was chosen as the internal standard for the present work as its mass is closer to those of the analytes than ^{13}C (used previously for cationic metals in gels).[5] Furthermore, the peak count-to-background ratio for Fe was significantly higher than for C. Internal standardisation was performed on a line-by-line basis, with each data point adjusted according to the average of the internal standard for the line. All data were then standardised against the average peak areas of the standard gels. Analytical precision tended to improve with internal standardisation. The RSD decreased by 0–7.6% in 11 cases and increased by 1% and 2.1% in two cases. As analysis of the blank did not produce resolvable analyte peaks, method detection limits (MDL) were determined using three times the standard deviation of the lowest concentration standard. Time-averaged detectable C_{DGT} concentrations for a deployment of 6 days were 9.8, 0.16, and 0.39 nM for P, V and As respectively. This corresponds to an MDL per ablation spot of 39, 0.65 and 1.6 fmol for P, V and As respectively. Calibrations were linear for all analytes ($r^2 > 0.995$, $n = 4$ for V and As, $n = 8$ for P).

In situ profiles

Deployed gels were analysed in a 1.2 × 0.25-cm area around a sulfidic microniche located ~11 cm below the sediment water interface. The ablation grid spacing was 333 μm, giving 280 data points for the gridded area (Fig. 2). The 2-D images of concentration and 1-D concentration–depth profiles (obtained by averaging data at 1-mm vertical intervals) show elevated sulfide directly associated with depleted P and V. Arsenic shows no clear depletion in the 2-D image, but the concentrations in the vertical profile are generally lower in the vicinity of the microniche.

Sulfidic microniches previously observed by DGT techniques have been attributed to sulfate-reducing bacteria at discrete sites of OM that can be utilised by these bacteria.[4] Several studies[17,28–30] have revealed that bacterial decomposition of OM can reduce ambient P levels owing to enhanced uptake during the bacterial growth phase. There is no net release of P until death of the bacteria. Our observed depletion of P at the sulfidic microniche (Fig. 2) is consistent with active decomposition of OM. This is supported by a good inverse correlation between the mm-averaged greyscale values (sulfide) and P (Table 1). Although a pH decrease at microniches of decomposing OM has been observed,[31] this should have little impact on the adsorption

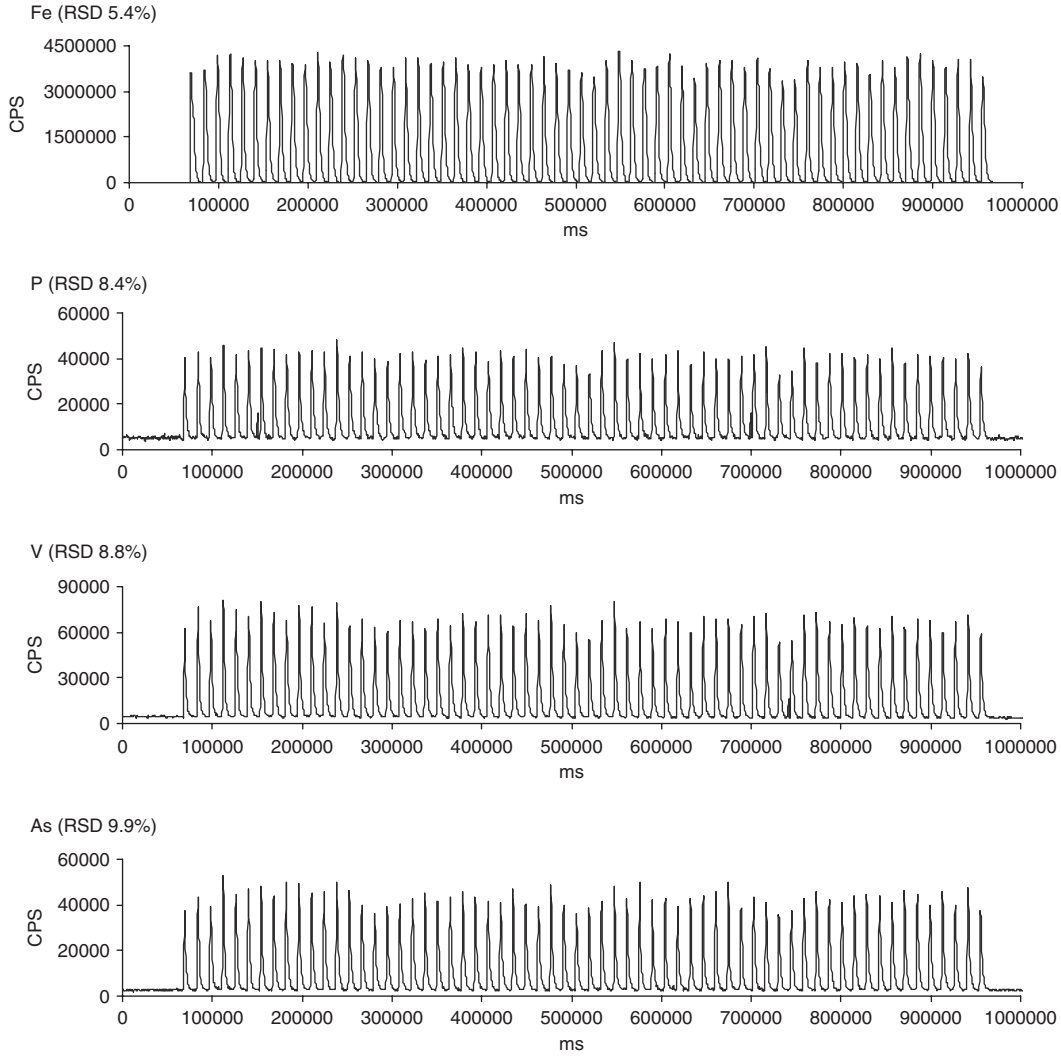

Fig. 1. Time series of counts per second (CPS) recorded by the mass spectrometer detector for a 64-spot ablation grid with 200-μm spacing of a standard gel exposed to 1.4 nmol cm^{-2} of P and 0.14 nmol cm^{-2} of V and As. Fe is used as the internal standard. Values for the residual standard deviation (RSD) are for the resolved peak areas before internal standardisation.

capacity of the binding phase, as lowering the pH will alter the ambient conditions further away from the point of zero charge of ferrihydrite. Zhang et al.[18] found no change in the P adsorption behaviour of ferrihydrite slurry gel over a pH range of 2–10.

The potential of V to accumulate or be retained by sediments under strongly reducing conditions has been recognised.[10,15] V is also known to be incorporated into enzymes of nitrogen-fixing bacteria.[32,33] Bacterial cultures isolated from crude oil-contaminated land have been shown to bioaccumulate vanadium from agar medium at high V concentration.[34] Microorganisms capable of reducing vanadium have been isolated and the possibility of microbial reduction causing precipitation of V in anoxic environments has been recognised.[35] Several studies have suggested V concentrations are reduced by phytoplankton uptake or biogenic adsorption in surface layers of oceans[36,37] or lakes.[38] Observations of depletions of V in oxic waters of the Mississippi River Delta were initially attributed to these processes.[39] However, a more recent study suggests that mixing of surface waters with V-depleted bottom waters may explain depletions in overlying water.[40] Comparison of the mm-averaged data (Fig. 2) for V and P gives an indication that the data are closely correlated (Table 1). Given this close correlation and evidence for both the microbial uptake of phosphate and vanadium's microbial role, it is more likely that the vanadium observations at the microniche are attributed to microbial activity. However, without evidence for the utilisation of V by sulfate reducers, V profiles in Fig. 2 could be due to sulfide reduction and subsequent scavenging or by a combination of this processes and biological utilisation.

Strains of bacteria capable of reducing arsenate and sulfate concomitantly have been isolated. Arsenate can also be bacterially or inorganically reduced to arsenite, which has similar retention properties on iron oxide as arsenate. Arsenite can subsequently react with sulfide to produce thioarsenite. Thioarsenite can, in turn, coprecipitate with iron sulfide or precipitate as arsenic sulfides (Nicholas et al. and references therein[41]). Fox and Doner[15] concluded that As can be accumulated or retained in sediments under strongly reducing conditions, but in moderately reducing conditions, mobilisation may occur. The mm-averaged data suggest that there may be some link between the elevated microbial activity (and thus sulfide) at the microniche and the depleted As concentrations. However, owing to the lack of a direct association between sulfide and As in the 2-D data, there is no clear evidence for the processes described.

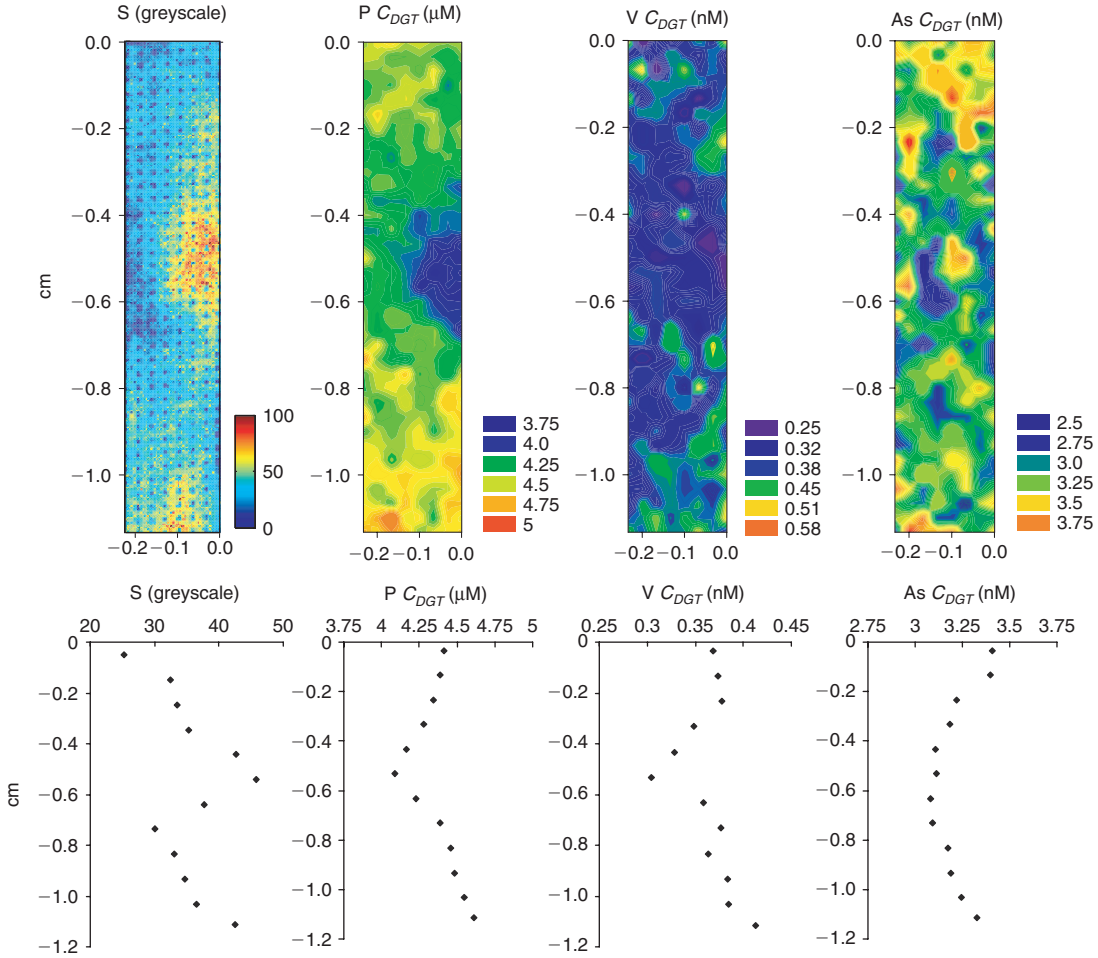

Fig. 2. Analyte profiles at a microniche. 2-D images show greyscale density for sulfide and DGT resolved concentrations (C_{DGT}) for P, V and As. The ablation grid (8 columns, 35 rows) can be seen on the sulfide scan with each point representing a data point for the 2-D C_{DGT} figures. 1-D plots show concentration–depth profiles obtained by averaging the data into 1-mm deep vertical slices (24 ablation spots per point, except the lowest point where the bottom two rows of ablation spots are averaged). Averaged greyscales values are affected, owing to the ablation spots having low values. The elevated sulfide at the very bottom of the analysed area is possibly associated with a general elevation in sulfide at that depth, as such a feature was present across the entire width (18 mm) of the DGT probe for that depth coordinate (A. Stockdale, W. Davison and H. Zhang, unpubl. data). Distances are in cm and are relative to the top right hand corner of the area.

Table 1. Pearson product–moment correlation coefficients (PMCC) of mm-averaged data in Fig. 2 ($n = 11$)

Analyte 1	Analyte 2	PMCC
Greyscale	Phosphorus	−0.68
Greyscale	Vanadium	−0.75
Greyscale	Arsenic	−0.61
Phosphorus	Vanadium	0.89
Phosphorus	Arsenic	0.49
Vanadium	Arsenic	0.43

Much is known about molybdenum geochemistry and it can provide an example of why microniche geochemistry may be important in the diagenesis of some elements. Molybdate (MoO_4^{2-}) can be reduced by sulfide in a stepwise manner to form intermediate oxythiomolybdates (MoO_3S^{2-}, $MoO_2S_2^{2-}$, $MoOS_3^{2-}$) on the reaction pathway towards the formation of tetrathiomolybdate (MoS_4^{2-}).[42] It has also been proposed that this sulfidation does not follow first order kinetics and that a three-fold increase in ΣH_2S concentration may result in a 100-fold change in reaction rates.[42] Furthermore, the sulfidic forms have been shown to be more susceptible to scavenging.[43] Recently, evidence for the analogous intermediates of (oxy)thioarsenates ($AsO_xS_{3-x}^{3-}$, $x = 0$–3) has been presented.[44] Should the rate and scavenging behaviour be analogous to that observed for molybdenum, arsenic transformations at sulfidic microniches may be significant, compared with the average for that depth. Helz et al.[43] have raised the possibility that Mo fixation within highly sulfidic microenvironments (such as those observed by Widerlund and Davison[25] and Motelica-Heino et al.[45]) may be misinterpreted as fixation at low sulfide concentrations within the bulk sediment. This may also apply to As and anionic metal oxides.

Processes occurring in microniches may have wider implications in understanding pollutant fate and the use of trace elements in providing paleoredox information. If the depleted V concentration is caused by bacterial uptake, death of the bacteria may release the V, with no net accumulation over the duration of the

bacterial lifecycle. However, if sulfide reduction and subsequent scavenging is the sole process responsible for the depleted concentration, microniches, if significantly distributed, may provide an additional sink for V. In using trace elements, including vanadium, as paleoredox proxies[46] in predictions of past deposition climates, it may be necessary to consider sediment microniche processes. Bioturbation, with associated microniches, will be expected in all but pristine (i.e. no or low OM input) or euxinic environments. Fixation at microniches could be misinterpreted as fixation within ambient anoxic or suboxic sediment. This highlights the importance of using multiple indicators when predicting past deposition climates.

Conclusions

The present work reports the development and deployment of a combined phase AgI–ferrihydrite DGT gel and demonstrates the effectiveness of the method in yielding new geochemical information about sediment microniches. Phosphate uptake associated with elevated activity of sulfate-reducing bacteria has been demonstrated. Unequivocal interpretation of the vanadium and arsenic data is more difficult, as the literature indicates that more than one mechanism may be possible. Further work is required to determine if bacterial uptake, use by bacteria as an electron acceptor, reduction by sulfide and subsequent scavenging, or other processes are responsible for the observed depletions associated with sulfide production.

Accessory materials

Details of drying procedure and settings for the TRA software: this material is available free of charge via the Internet at http://www.publish.csiro.au.

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