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Combination of DGT and DET can assess redox zonation and mercury methylation in sediments

Environmental impact

In the present study, DGT and DET techniques were used to investigate biogeochemistry and Hg methylation in sediment taken from the Tien River on the Mekong Delta in Vietnam. The robust in-situ techniques revealed that Hg methylation was active during the transition between aerobic and anaerobic sulfate reducing environments. In addition, the depth that showed sulfate reduction was shallower in brackish water sediment than in fresh water sediment, leading to eight times greater methylmercury flux to overlying water in brackish environments. This study shows that co-deployment of various gel-type probes could be extremely helpful in investigating Hg methylation processes coupled with complex biogeochemical reactions and their impact on aquatic environments.

Application of Diffusive Gel-Type Probes for Assessing Redox Zonation and Mercury Methylation in Mekong Delta Sediment

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Abstract

The vertical profiles of PO_4^{3-}, Mn, Fe, S^{2-}, Hg, and CH_3Hg^+ in sediment pore water were investigated using DGT and DET probes in the Tien River, the northern branch of Vietnam's Mekong Delta. Although some of the DGT measurements could be lower than the actual pore water concentrations due to the depletion of the species, the measurements provided information for understanding the redox zonation and Hg methylation. The gradual increases in the measured species concentrations with the sediment depth were observed and the diffusive fluxes of the species to overlying water were expected. Vertical profiles suggested that (1) SO_4^{2-} seemed to be reduced before Fe^{3+}, or the two electron acceptors were reduced simultaneously; (2) the release of PO_4^{3-} was more closely related to S^{2-} than Fe release; and (3) Hg methylation was active in the micro-niche between the aerobic and anaerobic transition zones. Maximum pore water CH_3Hg^+ concentrations were observed at depths just above where the maximum S^{2-} concentrations were detected. Hence, the maximum CH_3Hg^+ concentration was observed near surficial sediments (less than 1 cm from the surface) in brackish water, and the maximum CH_3Hg^+ concentration was observed at a depth of 3 cm in fresh water. The different vertical profiles led to a CH_3Hg^+ diffusive flux eight-times greater in brackish than in fresh water. The present study showed that the *in-situ* application of DGT and DET probes was helpful to understand coupled biogeochemical reactions and mercury methylation by measuring pore water redox species.

Introduction

Since the 1950s, when the Minamata disease in Japan revealed the serious toxicity and environmental persistence of mercury (Hg), understanding of the transport, transformation, fate, and toxicity of Hg in environments and ecosystem has significantly improved.[1-3] However, Hg contamination has continuously increased over the last few decades due to the wide usage of Hg in various industrial processes, the release of Hg from coal-fired power plants, biomass burning, and other elements.[4] Contamination has reached a global scale through Hg transport in the atmosphere, now found in regions as remote and pristine as the Arctic. The human and ecological risks associated with Hg have been recognized as a global problem.[2] As a result, UNEP (United Nations Environmental Programme) organized an inter-governmental treaty, and more than 150 countries adopted the Minamata Convention in October 2013 to regulate the use and trade of Hg.

In aquatic environments, Hg species present in multiple forms, of which monomethylmercury (CH_3Hg^+) is considered the most toxic. The consumption of CH_3Hg^+-contaminated fish is the most significant exposure route to human and ecological top predators.[4] The CH_3Hg^+ in fish is primarily produced by microorganisms in anaerobic sediments.[5,6] The organisms utilize various electron acceptors to create redox zonation (segregation of different terminal electron-accepting processes in separate zones) and release reduced species, such as Mn^{2+}, Fe^{2+}, and S^{2-}.[7,8] In this way, mercury methylation is tightly coupled with the biogeochemical reactions, a relationship that is critical to understanding how these reactions affect CH_3Hg^+ production.[9,10]

Porewater analysis is necessary to study the biogeochemical reactions; sediment centrifugation and filtration following sediment coring is often used.[11] However, the *ex-situ* approach requires lengthy sampling processes including many artifacts, such as physical

suspension of colloidal species, exposure to oxygen, and poor resolution. The vertical profiles of the reduced species in sediment pore water is easily disturbed and could be highly variable within a distance of a few millimeters.[12] Accurate characterization of biogeochemical reactions is important in understanding CH_3Hg^+ production and remobilization processes.[13-17]

To overcome these limitations, diffusive gradient in thin film (DGT) probes and diffusive equilibrium in thin film (DET) probes are often used.[18] The DGT probe employs a series of layers, including a filter membrane, a diffusive hydrogel, and a resin gel in a plastic unit. The filter side is exposed to the environment, and then dissolved metals diffuse through the hydrogel and are accumulated in the resin gel, which acts as a sink. The DET probe has a configuration similar to DGT, but DET does not have resin gel and only employs a diffusive layer and filter.[19] DET allows the contaminant to disperse to the diffusive layer and achieve equilibrium with the water concentrations. The two techniques have been widely used to detect various trace levels of cationic and anionic species in aquatic environments.[12,17,18,20-24]

In the present study, DGT and DET probes were used to investigate *in-situ* biogeochemical reactions and Hg methylation in the Mekong Delta sediment. The Mekong River spans 4,800 km with a watershed area of 795,000 km^2. The river discharges 470 km^3 yr^{-1} of water, making it the 10^{th} largest river in the world by discharge.[25] The Mekong River has water quality problems due to high population density, agricultural activities, and extensive soil erosion in the watershed, which releases nutrients and other contaminants.[26] Millions of people are dependent on the Mekong Delta and are at risk for Hg exposure through fish consumption.[27] Asian countries contribute approximately 50% of the global anthropogenic Hg emissions, of which China accounts for about 60%.[28] Regional neighbors such as Vietnam may also be at risk of Hg contamination.

In the present study, field sampling was conducted to achieve following two objectives: (1) Application of DGT and DET techniques to measure dissolved PO_4^{3-}, Mn, Fe, S^{2-}, CH_3Hg^+, and total Hg (THg) in sediment pore water of the Tien River in Vietnam's Mekong Delta; and (2) use of this data to understand how biogeochemical reactions affect CH_3Hg^+ distribution in sediment pore water. The research will be helpful for improving our current understanding on CH_3Hg^+ production in sediments and analyzing the potential risk associated with CH_3Hg^+ in these areas.

Materials and methods

DGT and DET fabrication

DGT and DET probes were prepared according to the procedure described in previous studies.[18,19,23,29,30] Detailed fabrication processes are presented in the literature, and brief descriptions are provided below and in **Table 1**.

Three types of gel solutions were used to prepare resin and diffusive gels. Gel solutions 1, 2, and 3 were abbreviated as GS1, GS2, and GS3; they consisted of 0.3% agarose cross linker+15% acrylamide gel, 1.5% N,N'-methylene bisacrylamide + 28.5% acrylamide, and 1.5% agarose, respectively, in DI water.

To make resin gels for THg and CH_3Hg^+, 1 g of 3-mercaptopropyl functionalized silica gel (3MFSG, Sigma-Aldrich®) was mixed with 10 mL of GS1. For polymerization, 60 µL ammonium persulfate and 15 µL tetramethylethylenediamine (TEMED) were added to the mixture. The mixture was immediately cast between two glass plates separated by 0.5 mm plastic spacers and allowed to sit at room temperature (22°C) for 2 hours.[29]

To measure S^{2-}, 1 g of finely ground $AgI_{(s)}$ was dissolved in 10 mL of GS1. After adding 60 µL ammonium persulfate and 15 µL TEMED to the mixture, it was immediately

cast between two glass plates separated by 0.5 mm plastic spacers. It is important to keep the $AgI_{(s)}$ protected from sunlight during the entire AgI resin gel fabrication process, as the AgI could be darkened. However, the gel remains stable when stored in the dark.[30]

To make DGT for PO_4^{3-}, ferrihydrite was precipitated, then 24 g of $Fe(NO_3)_3 \cdot 9H_2O$ was dissolved in 600 mL of deionized water to make 0.1 M Fe^{3+} solution. The pH of the solution was raised to 7.0 by adding 0.1 M or 1 M NaOH to precipitate ferrihydrite. After centrifuging the ferrihydrite slurry at 2500 rpm for 10 minutes, the overlying water was discarded and exchanged with new deionized water. The process was repeated five times to remove any impurities from the ferrihydrite. The water content of the final ferrihydrite precipitate slurry was around 50% (± 5). Then 6 g of the ferrihydrite precipitate was mixed with 10 mL of GS2. After adding 160 µL ammonium persulfate and 16 µL TEMED to the mixture, it was immediately cast between two glass plates separated by 0.5 mm plastic spacers. After casting, the gels were hydrated in deionized water for 24 hours and stored in 0.01M $NaNO_3$ solution at 4°C.[23] This ferrihydrite resin gel has a PO_4^{3-} binding capacity of 52 ± 5 µg cm^{-2} with an extraction efficiency of 98 ± 12% (n=7), which capacity was large enough to apply for Mekong Delta.

After preparing the resin gels, 1.5% agarose diffusive gel with a thickness of 0.75 mm was prepared by dissolving 1.5 g of agarose in 100 mL of deionized H_2O on a heating plate. The agarose gel was used to fabricate DGT for THg, CH_3Hg^+, and PO_4^{3-}. Diffusive gel made of GS1 with a thickness of 1.2 mm (0.75 mm multiplied by expansion factor of 1.6) was also prepared and hydrated in DI water for more than 24 hours. The gel was used to fabricate DET for Mn and Fe and DGT for S^{2-}.

The resin and agarose gels were cut to fit into the disk-type (2-cm diameter) and plate-type DGT (1.5 cm × 15 cm × 0.5 cm) holders, which were purchased from DGT Research Ltd (www.dgtresearch.com). The polysulfone filter (Pall Life Sciences) and

142 PVDF filter (Millipore Corp.) with pore sizes of 0.45 μm were used for disk- and plate-type
143 probes, respectively. For the DET, custom-made plate shape plastic units (2 cm × 25 cm ×
144 0.5 cm) were used.
145
146 **Site description and DGT/DET deployment**
147 The Mekong Delta has a tropical monsoon climate. Discharge rate and salinity
148 intrusion are significantly dependent on the seasons. During the dry season (November –
149 April), salt water intrusion extends to 70 km inland due to a low discharge rate (~ 2,000 m^3 s^{-1}
150). However, during the wet season (May – October), salt water extends only a few km
151 inland due to a high discharge rate (~ 40,000 m^3 s^{-1}).[25, 27] Considering these patterns of salt
152 water intrusion, the locations were conservatively selected to cover both fresh and estuarine
153 aquatic environments. As shown in **Fig. 1**, five locations were labeled and numbered as L1
154 – L5. These locations were selected downstream of the Tien River, which is among the
155 main rivers that form the delta in Vietnam and discharge into the South China Sea.
156 In each location, DGT probes for THg and CH_3Hg^+ were deployed in the overlying
157 water during a sampling event conducted in September, 2013. In two selected locations,
158 fresh water (L1) and brackish water (L5), DGT probes for THg, CH_3Hg^+, PO_4^{3-}, and S^{2-} and
159 DET probes for Mn and Fe were deployed. To deploy the probes in overlying water, one
160 end of a 7 mm thick polyethylene line was connected to a navigational buoy, and the other
161 end to a 10 kg concrete block at the bottom of the Tien River. Circular-type DGTs were
162 attached to the line at three different depths (i.e., one close to the air-water interface, one in
163 the middle, and the last at the river's bottom). During the deployment, water temperature,
164 dissolved oxygen, salinity, and conductivity were measured onsite using multi-electrodes
165 (Thermo-Orion® Portable Meter Kit, STARA3295). Plate-type sediment DGTs and DETs
166 were deployed in shallow areas (water with a depth of less than 1.5 m) and in places with a

soft sediment bottom without sea grass. The probes were vertically pushed from the water to the sediment by snorkeling with utmost care to prevent rupture in the filter and diffusive layers. Before deployment, the probes were de-oxygenated by $N_{2(g)}$ for at least 24 hours in the laboratory, and the de-aeration was continued during field deployment by portable nitrogen tanks. The probes were deployed in anoxic sediment within one minute of removal from the de-aerated water.

After two to three days of on-site deployment, the DGTs and DETs were retrieved with careful snorkeling. After retrieval, each probe was carefully rinsed with site waters and stored on ice in a clean Ziploc® bag. Especially for the sediment DET, 1 M NaOH was pipetted at the surface of the filter within one minute after retrieval to stabilize Mn^{2+} and Fe^{2+} by oxidizing the elements.[19] As part of the retrieval process, sediment cores were taken to measure particulate organic carbon in a laboratory. More details about the coring and analysis are discussed in supporting documentation.

Post-deployment laboratory analyses

The DGTs and DETs were transported to laboratories at Daegu University and GIST in South Korea for post-deployment processing. The probes were carefully rinsed with DI water; rein gels for THg, CH_3Hg^+, PO_4^{3-}, and S^{2-}, and diffusive gels for Mn and Fe were removed from the probes. Accumulated species were directly extracted from resin gels in circular-type DGTs. Resin gels in plate-type sediment probes were sliced with 1 cm resolution and soaked in an appropriate extractant. The various extraction and measurement techniques are summarized in **Table 2**.

To extract CH_3Hg^+, 3MFSG gels were soaked in 4 mL of acidic thiourea solution (1.13 mM thiourea + 0.1 M HCl) for 24 hours.[22] The extractant was diluted in 100 mL of

DI water and converted to gaseous CH_3Hg^+ by aqueous phase ethylation using a tetraethylborate solution. The volatile CH_3Hg^+ was then purged and trapped onto Tenax® traps, which were flash-heated in a nitrogen stream. The released Hg species were thermally separated on a GC column, then detected by CVAFS (Model III, Brooks Rand Labs).

To extract THg, 3MFSG gels were soaked in 4 mL of 20% BrCl solution for 24 hours. The excess oxidant was neutralized by adding hydroxylamine hydrochloride solution prior to analysis. Hg in these samples was reduced to elemental Hg by $SnCl_2$ solution, and the elemental Hg was contained in gold traps. The Hg^0 released from the gold traps by thermal desorption was fed into a CVFAS.

To extract PO_4^{3-}, ferrihydrite resin gels were soaked in 1.5 mL of 0.25M H_2SO_4 for 24 hours, and the molybdenum blue method was used to determine PO_4^{3-} colorimetrically. Reagent was prepared by mixing 500 ml of 2.5 M H_2SO_4, 50 ml potassium antimony tartrate solution, 150 ml ammonium molybdate solution, and 300 ml ascorbic acid solution. Then, 0.4 mL of the mixture was added to 5.0 mL of the samples, and absorbance at 880 nm was determined using UV spectrophotometer (MECASIS).

Densitometry was used to determine the S^{2-} levels accumulated in the $AgI_{(s)}$ gel with a slight modification.[30] $AgI_{(s)}$ resin gels with an area of 1.33 cm^2 were prepared and immersed in 12 mL amber vials filled with 10 mL of deaerated DI water. Then, the vials were spiked using S^{2-} with a concentration range from 0.0 – 1.46 μmol by adding 0.0163 M S^{2-} stock solution prepared from $Na_2S{\cdot}9H_2O_{(s)}$ and standardized with iodometric titration. After a 24 hour solution equilibration, the resin gels were removed and placed on the transparent OHP film with the binding side face-up and fixed with transparent tape over the gel to protect the surface. The OHP film was then placed in a flat-bed scanner (Samsung SCX-472x), and the image was recorded with a resolution of 300 DPI and saved as a TIFF

file using Adobe Acrobat Pro 9®. The greyscale intensity (0 – 255) of the scanned image was measured using Adobe Photoshop CS3®. The greyscale intensity of the resin gels was recorded considering the background greyscale intensity of blank $AgI_{(s)}$ resin gels. The $AgI_{(s)}$ resins deployed in the sediments were also placed on OHP film protected by transparent tape and without slicing. They were scanned, and greyscale intensity was recorded. Using the standard curve evaluated above, the mass accumulated in resin was calculated. More information about S^{2-} densitometry is available in the supporting information.

To extract Fe and Mn from the diffusive gel of the DET probe, the gels were soaked in 5 mL of 1 M HNO_3 for 24 hours, and the Fe and Mn were measured using ICP-OES (Optima 7300DV).

DGT data interpretation

The concentrations of species in the water column and sediment pore water were calculated by the following equation[18]:

$$C_b = \frac{M \times \Delta g}{D \times t \times A} \qquad (1)$$

where C_b is the labile metal species concentration in water [M L^{-3}]; M is the mass of the species accumulated in resin [M]; t is the deployment time [T]; D is the diffusion coefficient of the species in the hydrogel [L^2 T^{-1}]; A is the exposed interfacial area [L^2]; and Δg is the total thickness of the diffusion layer [L], including the filter membrane and diffusive gel. The diffusion coefficient of ions and metals depends on the temperature and can be corrected using following equation:

$$\text{Log D} = \frac{\left\{1.37 \times (\text{T} - 25) + 8.36 \times 10^{-4} \times (\text{T} - 25)^2\right\}}{(109 + \text{T})} + \text{Log}\left(\text{D}_{25} \times \frac{(273 + \text{T})}{298}\right) \quad (2)$$

where D and D_{25} are the diffusivity of ions [L^2 T^{-1}] at T°C and 25°C, respectively.

Results

Overlying water quality

In the five locations, the average (±standard deviation) values of pH, dissolved oxygen, and temperature (n=16) were relatively stable during probe deployment and retrieval. These values were 6.79 (±0.3), 5.25 (±0.33) mg L^{-1}, and 28.2°C (±0.3), respectively. Detailed values are available in **Table 3**. The conductivity was also stable at 85.4 (±5.8) µS cm^{-1} from locations 1 to 4, confirming the waters were fresh, although the conductivities at location 5 were varied between 5,500 µS cm^{-1} (~2.5 psu) and 10,310 µS cm^{-1} (~5.2 psu). These measurements suggest that only location 5 (Cua Tieu estuary) was strongly influenced by seawater intrusion from the adjacent South China Sea.

The DGT-measured THg and CH_3Hg^+ in overlying water (September 2013) were comparable to the values in the previous grab sampling event during the dry season (April 2011) at the river.[27] The reported THg and CH_3Hg^+ in filtered overlying water (0.45 µm polyethersulfone) varied from 1.2 to 14 pM and from 0.020 to 0.17 pM, respectively. DGT-measured THg and CH_3Hg^+ varied from 1.16 to 34.5 pM and from 0.0026 to 0.072 pM, respectively. The THg measured by DGT was similar to the grab sampling data, although the DGT-measured CH_3Hg^+ concentrations were approximately two times lower than those measured in the grab sampling. Care should be taken when making the comparison since samplings were conducted during different (wet versus dry) seasons, and the seasonal effect may lead to differences in CH_3Hg^+ concentrations. In addition, during the dry season in

2011, algal bloom was observed in the area, which led to lower dissolved CH_3Hg^+ concentrations in the water.[27] The discrepancy between the CH_3Hg^+ concentrations could be associated with the inter-annual variations in CH_3Hg^+ production in the area.

In the present study, there were no significant and clear horizontal and vertical distribution trends of THg and CH_3Hg^+ observed in the overlying water. The horizontal trends were determined by comparing measurements from each location, and the vertical distributions were determined by comparing measurements at different water depths. Sediment is often considered the source of metals and nutrients, so higher levels of the species in deeper water columns are expected from sediment fluxes. Probably due to the small sample size, it was difficult to observe this trend. More extensive deployment of DGT is necessary to understand seasonal variations and horizontal and vertical distributions of the species in the water column of the Tien River.

Porewater concentrations

The DGT-measured vertical profiles of PO_4^{3-}, Mn, Fe, S^{2-}, THg, and CH_3Hg^+ in fresh water and brackish water are shown in **Fig. 3 (a) – (f)**. The concentrations of THg and CH_3Hg^+ in pore water were 1 – 2 orders of magnitude higher than in the overlying water (**Fig. 2)**, thus, diffusive fluxes of the species from the sediment to overlying water were expected. The concentrations of the measured species gradually escalated with the increased sediment depth, although the vertical depths showing maximum concentrations were different depending on the species. One location was selected in each environment (fresh and brackish waters), therefore the comparisons between the locations were carefully made. Additional studies using replicated sampling locations would provide more valuable information include greater confidence in the comparisons.

In **Fig. 3 (a)**, the PO_4^{3-} concentrations were increased from 0.12 to 0.77 μM in

fresh and increased from 0.18 to 1.52 μM in brackish sediment. The PO_4^{3-} levels in brackish sediment were two times higher than in fresh sediment. Similar vertical profiles were observed for S^{2-} and are shown in **Fig. 3 (d)**. The S^{2-} concentrations were low (0 – 0.3 μM) at the surficial sediments from oxidation by O_2, which was expected. However, the levels increased to the maximum concentrations of 2.6 and 4.1 μM in fresh and brackish sediments, respectively. The S^{2-} levels in brackish sediment were also two times higher than in fresh sediment. This observation was consistent with a previous study that showed higher SO_4^{2-} concentrations in brackish water (946 – 2862 mg L^{-1}) compared to fresh water (~14 mg L^{-1}) and higher acid volatile sulfides in brackish water sediment (3.6±2.6 μmol g^{-1}) compared to fresh water sediment (1.6±1.7 μmol g^{-1}).[27]

The Mn and Fe in **Fig. 3 (b)** and **(c)**, also showed low concentrations at the surficial sediments. The concentrations of Mn and Fe were less than 0.4 mM at the surficial sediments (1 cm) and increased to 0.4 and 7.3 mM in fresh and 0.3 and 3.6 mM in brackish sediment. The increase of Mn and Fe in the pore waters was considered a result of the reduction of iron and manganese oxides to Mn^{2+} and Fe^{2+} respectively.[31] The Fe concentrations were at least an order of magnitude greater than the Mn concentrations. In fresh sediment, the Mn and Fe concentrations were greater than those in brackish sediment, which probably suggests that iron and manganese reduction are more dominant biogeochemical processes in fresh water sediment.[32]

The vertical profiles of THg and CH_3Hg^+ in **Fig. 3 (e)** and **(f)**, were similar, however, they were different from other species. Generally, the higher THg and CH_3Hg^+ concentrations were observed in near-surficial sediments (depth < 6 cm), and lower concentrations were observed in deeper sediments (depth > 6 cm). These characteristic profiles were also observed in previous studies conducted in riverine, estuarine, and marine sediments.[13,14] As shown in **Table S1** and **Fig. S2**, pore water THg concentrations in fresh

sediment (23.7±13.0) were lower than those in brackish sediment (47.9±13.7) pM, although pore water CH_3Hg^+ concentrations were similar (1.18 ±0.61 pM in fresh and 1.24 ±0.67 pM in brackish).

Comparison with other environments

The levels of the measured species were compared with reported values in other areas to assess the level of contamination in the Mekong Delta. Reported PO_4^{3-} concentrations were widely distributed, ranging from 1 to 150 μM in lakes, bays, and intertidal sea grass beds in other areas.[17,23,24,33] Reported S^{2-} concentrations generally varied between 1 and 20 μM in estuarine sediment, and levels as high as 60 μM were also observed.[24,30] Reported Fe and Mn concentrations varied between 0.1 and 0.9 mM and between 0.01 and 0.03 mM; the values in the Mekong were in a similar range as other studies.[12,17] The PO_4^{3-} and S^{2-} levels in the Mekong were in the lower range of the observed levels, and Fe and Mn levels were close to the reported values. The reported CH_3Hg^+ concentrations ranged from 4.63 to 13.9 pM in a salt marsh, 4.63 to 9.26 pM in the bay, and 9.26 to 37.0 pM in a river located in the San Francisco Bay area.[13] The pore water CH_3Hg^+ concentrations in the Mekong Delta sediment were generally lower than the observed values, suggesting the area is less impacted by Hg. These comparisons suggest that the Mekong Delta sediment is not particularly contaminated and more research, including the investigation of multiple locations, is necessary.

Discussion

Redox zonation and nutrient release in sediment

To better understand the redox zonation, the vertical profiles of the species were

normalized by the maximum pore water concentrations of the individual species and re-plotted in **Fig. 4 (a) – (f)**.

In the fresh water sediment, PO_4^{3-} and S^{2-} were first observed at 1 cm directly below the sediment-water interface, and the concentrations gradually increased with depth. The maximum concentrations of the species appeared at approximately 4 – 6 cm and extended to about 10 – 12 cm. Similar profiles were observed for Mn and Fe. The Mn and Fe appeared at depths of 1 and 3 cm respectively, which were slightly deeper than those of PO_4^{3-} and S^{2-}. The concentrations of the species continuously increased, and the maximum concentrations of Mn and Fe were observed in deeper sediment at approximately 9 and 15 cm respectively. The profile of Fe was about 2 cm shifted toward deeper sediment compared to Mn, suggesting that Mn^{4+} was reduced before Fe^{3+}.

In brackish water sediment, the vertical profiles of PO_4^{3-}, S^{2-}, Mn, and Fe were different from fresh water sediment. The profiles of PO_4^{3-} and S^{2-} showed more rapid increase in the pore water, producing sharper vertical gradients at the surficial sediment. The maximum concentrations of PO_4^{3-} and S^{2-} were observed at sediment depths of approximately 4 and 3 cm respectively. The maximum S^{2-} was detected between 2 and 3 cm directly below the surface sediment. Note that the maximum S^{2-} was shown at a depth of about 5 cm in fresh water sediment. The Mn and Fe concentrations also rapidly increased from the interface, and maximum concentrations were detected at an approximate depth of 6 cm; the concentrations then began to decrease. The depths for maximum Mn^{2+} and Fe^{2+} in the brackish sediment were closer to the sediment-water interface compared to those in the fresh water sediment.

The particulate organic matter measured by loss on ignition (550^{o}C) at surficial 8 cm sediments were higher in brackish water sediment (7.81±0.44%) compared to fresh water sediment (5.85±1.3%) (**Table S3**). The higher organic matter concentrations (i.e., energy

for microorganism metabolism and higher SO_4^{2-} in brackish water probably increased the activities of anaerobic microorganisms and induced more intensive biogeochemical reactions in surficial sediments.

It is generally assumed that electron acceptors (EA), such as O_2, NO_3^-, $MnO_{2(s)}$, $Fe(OH)_{3(s)}$, and SO_4^{2-}, are sequentially reduced in order from the most energy-yielding to the lowest energy-yielding EA when microorganisms decompose organic matter as an electron donor.[34] However, in the fresh and brackish water sediments, the vertical profiles of Fe and S^{2-} (shown in **Fig. 4)** suggested that SO_4^{2-} seemed to be reduced before Fe^{3+}, or the two electron acceptors were reduced simultaneously. Theoretical calculations in realistic environmental conditions, and several field observations suggest that simultaneous reduction of Fe^{3+} and SO_4^{2-} is thermodynamically possible under a wide range of sedimentary environmental conditions and that SO_4^{2-} reduction may occur before Fe^{3+} reduction.[7,24]

In addition, the release of PO_4^{3-} seems tightly coupled with the release of S^{2-} in the two sediments (see **Fig. 5**). The PO_4^{3-} is believed to be strongly adsorbed in iron oxide and, when reduced, Fe^{2+} and PO_4^{3-} tend to release simultaneously.[35] However, the simultaneous release of PO_4^{3-} and S^{2-} has also been observed.[24] A previous study showed that the Fe^{2+} and PO_4^{3-} concentrations in sea grass-sediment pore water did not coincide when the two species were compared in a two-dimensional graph, although they seemed well related in a one-dimensional graph.[17] In marine environments, S^{2-} appears to induce phosphate release from marine microorganisms.[36] In addition, evidence shows that PO_4^{3-} release may originate from benthic microorganisms via polyphosphate metabolism, rather than iron reduction and adsorbed-PO_4^{3-} release.[37] More research is necessary to understand the coupled biogeochemical reactions that release PO_4^{3-}, Fe^{2+}, and S^{2-} in sediment pore water.

Mercury methylation in sediments

As shown in **Fig. 4 (c) and (f)**, the profiles of THg and CH_3Hg^+ were similar, however, they were distinct compared to other species in sediment pore water. In fresh water sediment, the concentrations of THg and CH_3Hg^+ increased with sediment depth, and maximum concentrations were observed at a depth of approximately 3 – 4 cm. The concentrations then decreased with the increase of sediment depth. In contrast, two distinct peaks of maximum THg and CH_3Hg^+ concentrations were observed in brackish water sediment pore water. The first peak materialized directly below the water-sediment interface at a depth of approximately 0 – 1 cm, and the second peak was observed at a depth of roughly 6 – 7 cm. The first CH_3Hg^+ maximum in fresh and brackish water sediments was detected directly above the area where the S^{2-} maximum concentrations began to build up. The second CH_3Hg^+ maximum in brackish water sediment corresponds to the area where the Mn and Fe maximum concentrations were observed.

Several processes for microbial uptake of Hg^{2+} procures CH_3Hg^+ in an aquatic environment.[1] A passive diffusion mechanism of uncharged, dissolved Hg complexes such as HgS^0 is probably the most widely studied process.[38,39] The mechanism is strongly dependent on the level of dissolved HgS^0 in anoxic water, which is highly dependent on S^{2-} concentrations. The HgS^0 concentrations are dominant species at S^{2-} concentrations greater than 10^{-9} M. However, at S^{2-} concentrations greater than 10^{-5} M, the HgS^0 species shift to charged, non-bioavailable complexes, such as HgS_2^{2-} and HgS_2H^-.[39,40] Hence, the decrease of bioavailable Hg^{2+} species (and, therefore, low CH_3Hg^+ concentrations) in the presence of a high S^{2-} environment (>10^{-5} – 10^{-4} M) has been observed in estuarine and marine environments.[41,42]

This study's observations of the first CH_3Hg^+ maximum near surficial sediments immediately before S^{2-} maximum probably support the previous observations and suggest that the CH_3Hg^+ production in the Mekong Delta sediment is coupled with SO_4^{2-} reduction.

It is well established that DGT can underestimate pore water concentrations of a species when resupply kinetics of a species from solid are slow and when the species pool is small.[43] Considering the use of DGT may deplete pore water S^{2-} concentrations, and the acid volatile sulfides were relatively low in the two sediments, the actual pore water S^{2-} concentrations could be higher than the calculated values. It is possible that the elevated S^{2-} concentrations in sediment pore water reduced the bioavailable HgS^0 in deeper sediments, which decreased Hg^{2+} methylation in the pore water. The alternative is that the sediment layer between the sulfide and Fe maximum (4 – 14 cm for fresh sediment and 3 – 7 cm for brackish sediment) could be enriched with solid FeS (i.e., AVS) that limits the microbial Hg^{2+} methylation.[44]

The second CH_3Hg^+ peak in brackish water sediment seems to be more related to iron reduction processes. Iron and manganese oxides appear to reduce significantly at a depth of approximately 6 cm, and Hg seems to be methylated simultaneously during the reduction reactions. In some studies, iron-reducing bacteria can produce CH_3Hg^+ [6], and the production and mobility is tied to the Fe redox cycling in the sediment.[14]

Flux calculations

Estimating the diffusive flux of THg and CH_3Hg^+ from sediment overlying water is important for assessing the sediment contamination and managing Hg risks in a body of water. The diffusive flux at the sediment water interface was calculated using the following equation:

$$\text{Flux} = -\frac{\theta D_w}{1 - \ln(\theta^2)} \frac{dC}{dx} \qquad (3)$$

where D_w is the diffusivity of THg or CH_3Hg^+ [L^2 T^{-1}]; θ is the porosity of sediments [unitless]; dC is the THg or CH_3Hg^+ concentration difference between water column (C_w) and sediment pore water (C_{pw}) [M L^{-3}]; and dx is the average sediment depth used to measure C_{pw}

[L]. **Table 4** summarizes the flux calculations. The first 1-cm depth-averaged pore water THg and CH_3Hg^+ concentrations were used for C_{pw}, and the depth-averaged overlying water THg and CH_3Hg^+ concentrations shown in **Fig 2 (b)** and **(c)** were used for C_w. In fresh and brackish sediments, the calculated THg fluxes to overlying water were 4.3 and 23.6 ng m^{-2} d^{-1} respectively, and the CH_3Hg^+ fluxes were 0.33 and 2.92 ng m^{-2} d^{-1} respectively. The CH_3Hg^+ fluxes were about 8 – 12% of the THg fluxes to overlying water. Although the surface 10-cm averaged THg concentrations in brackish sediment were only two times greater than in the fresh sediment (**Table S2**), the calculated THg diffusive fluxes were five times greater in the brackish sediment. This observation was even more drastic for CH_3Hg^+. The CH_3Hg^+ concentrations in the two sediment pore waters were similar (in **Table S2**); nonetheless, the flux to overlying water was eight times higher in brackish than in fresh water sediment. The grab sampling of the surficial sediments may not have captured the sharp concentration gradients of CH_3Hg^+ in sediment pore water, and may have calculated biased diffusive fluxes. Measuring pore water CH_3Hg^+ concentrations with high resolution is considered important for estimating diffusive fluxes of the species in sediment.

Diffusive fluxes of THg (ng m^{-2} d^{-1}) were reported as 1.7 – 30 in a bay [9] and 710 – 1590 in an estuary.[45,46] Diffusive fluxes of CH_3Hg^+ (ng m^{-2} d^{-1}) were reported as 0.16 in a lake; 10.1 in a river; 0.03 – 27.4 in a Delta; and 15.1 – 42 in a bay.[13] Direct comparisons of the estimated fluxes might not be possible since the fluxes could be highly heterogeneous depending on the biogeochemical conditions of the sites. Nevertheless, the estimated fluxes of THg and CH_3Hg^+ in the Mekong Delta were in the lower range of the reported values, which further suggests that the area has relatively low risk.

Conclusions

458 DGT and DET techniques were applied to the Tien River in Vietnam's Mekong Delta
459 to assess Hg contamination and to understand how redox zonation affects Hg methylation.
460 Elevated S^{2-} concentrations were detected in the shallower depth in brackish compared to
461 fresh sediments, suggesting that copious SO_4^{2-} was reduced in near surficial sediments in
462 brackish sediments. This redox status seemed to drive pore water CH_3Hg^+ maximum in the
463 shallower depth with higher concentrations, which resulted in a CH_3Hg^+ flux approximately
464 eight times higher in the brackish than fresh sediments. Accurate measurement of pore
465 water CH_3Hg^+ concentrations without disturbance would be critical for estimating such
466 diffusive fluxes of the species in aquatic environments. The release of PO_4^{3-} seems to be
467 related with S^{2-} release, suggesting PO_4^{3-} release may be more related to sulfate reduction
468 than iron reduction, a process commonly correlated with PO_4^{3-} release.
469 For better quantitative use of DGT, future research should be directed to accurately
470 estimate dissolved chemical species in pore water.[43] The application of DETs for redox
471 sensitive species such as PO_4^{3-} and S^{2-} could be an appropriate approach, as it minimizes the
472 decrease of the species during pore water collection and processing. For THg and CH_3Hg^+,
473 deployment of multiple DGT probes with different diffusive thicknesses[12] would be effective
474 in estimating actual porewater concentrations when DGTs are deployed in environments
475 where the resupply kinetics of the species are slow.[12] In addition, fine resolution (~ mm)
476 measurements of Hg in sediment pore water could provide notable information on the Hg
477 biogeochemical reactions that have not been observed and reported. Lastly, further studies
478 are necessary in the Mekong Delta to understand which biogeochemical conditions (e.g.,
479 sediment organic matter) mainly control Hg methylation, and samplings in replicated
480 locations are necessary to obtain site representative information.
481

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Table 1 Summary of DGT and DET used in the present study. For all circular type overlying probes and plate type sediment probes, polysulfone and Millipore Durapure PVDF (hydrophilic polyvinylidene fluoride) with 0.45 μm pore size were used, respectively.

Target Species	Probe Type	Resin Gel Composition	Diffusive Gel	Extractant	Extraction Efficiency	Reference
CH_3Hg^+	DGT	2 g of 3MPFS in 10 mL of GS1	GS3[c]	1.13 mM Thiourea + 0.1M HCl	0.91	22, 29
THg	DGT	2 g of 3MPFS in 10 mL GS1	GS3	20% BrCl [b]	1.0	29, 47
PO_4^{3-}	DGT	6 g of FPF in 10 mL GS2	GS3	0.25 M H_2SO_4	0.98 [c]	23
S^{2-}	DGT	1 g of $AgI_{(s)}$ in GS1	GS1	NA	-	30
Fe, Mn	DET	NA	GS1	1.0 M HNO_3	-	19

a. Summary of acronyms: 3MPFS=3-mercaptopropyl functionalized silica gel (Sigma Aldrich); FPF=freshly precipitated ferrihydrite slurry; GS1=gel solution 1 (15 mL agarose solution from DGT research + 37.5 mL 40% acrylamide solution + 47.5 mL H_2O); GS2=gel solution 2 (100 mL solution containing 28.5 g acrylamide + 1.5 g bisacrylamide); GS3=gel solution 3 (1.5 g arose gel in 10 mL DI water)

b. 27 g KBr+ 38 g $KBrO_3$ in 2.5 L concentrated HCl

c. Re-evaluated in the present study

498 **Table 2** Summary of diffusion coefficients used to convert DGT accumulated mass to
499 pore water concentration.

Species	D (10^{-6} cm^2 s^{-1})		Reference
	25°C	29°C	
THg [a]	4.0	4.41	29
CH_3Hg^+	5.26	5.80	
PO_4^{3-}	6.05	6.67	23
S^{2-}	14.8 [b]	16.3	30

500 a. Assumed mostly consist of Hg^{2+}

501 b. A value at 18°C

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516 **Table 3.** Summary of location information and water quality measurements.
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ID	latitude	longitude	water depth (m)	deployment time (days)	values during deployment – values during retrieval			
					pH	conductivity ($\mu S\ cm^{-1}$)	DO ($mg\ L^{-1}$)	temperature (^oC)
L1	10.31916	106.02000	5.7	2.79	7.32 – 6.87	79.80 – 80.14	5.52 – 5.61	28.0 – 28.0
L1 [a]	10.32333	106.03000	-	2.85	7.10 – 7.23	84.40 – 79.75	5.80 – 4.94	28.1 – 28.5
L2	10.31583	106.20055	27.7	2.73	7.09 – 6.14	81.21 – 86.57	4.97 – 5.49	27.7 – 28.1
L3	10.34805	106.35055	8.4	2.18	6.97 – 6.91	87.78 – 84.01	5.01 – 5.24	27.8 – 28.0
L4	10.30750	106.50361	8.6	2.10	6.98 – 6.88	94.23 – 95.71	4.93 – 5.75	28.5 – 28.2
L5	10.26000	106.75527	6.5	2.03	7.01 – 7.36	9657 – 5500	5.32 – 4.91	28.0 – 28.0
L5 [a]	10.26888	106.75083	-	2.07	7.23 – 6.56	10310 – 3890	5.23 – 4.64	28.5 – 28.4

518 a. Locations that sediment pore water DGTs and DETs were deployed.

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Table 4 Fluxes of CH_3Hg^+ from sediment to overlying water using the surface 1 cm averaged pore water CH_3Hg^+ concentrations determined by DGT and equation (3). In fresh and brackish water sediments, diffusion coefficients [3] of THg and CH_3Hg^+ were 4.41×10^{-6} and 5.8×10^{-6} cm^2 s^{-1} at 29°C, respectively, and porosities (θ) were 0.58 and 0.79, respectively. The dx was 0.5 cm.

Environment	-dC (=C_{pw} – C_w)		Flux	
	THg (ng L^{-1})	CH_3Hg^+ (pg L^{-1})	THg (ng m^{-2} d^{-1})	CH_3Hg^+ (ng m^{-2} d^{-1})
Fresh	2.1 (=3.7 – 1.6)	112 (=116 – 4)	4.3	0.33
Brackish	5.8 (=8.6 – 2.8)	455 (=464 – 9)	23.6	2.92

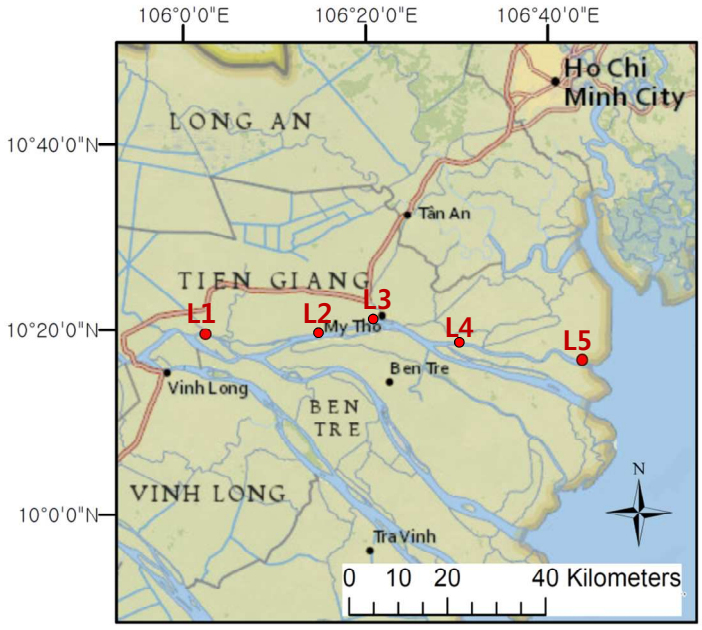

Fig. 1 Map of the Mekong River Delta showing the location of DGT and DET deployment locations. DGTs were deployed in the overlying waters of L1 – L5. DGTs and DETs were deployed in the sediments of L1 (fresh) and L5 (brackish).

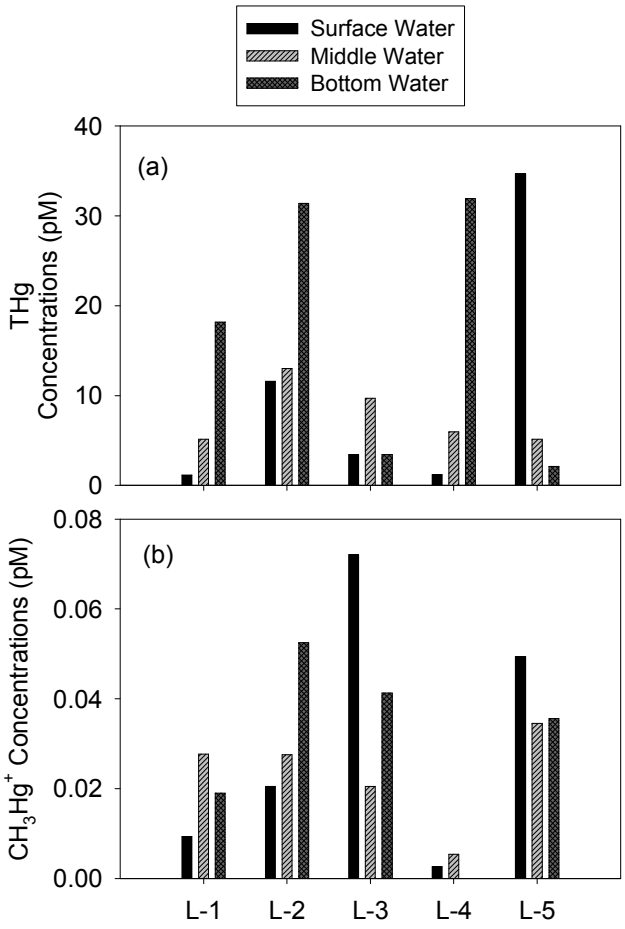

Fig. 2 The vertical and horizontal distribution of DGT measured (a) THg and (b) CH_3Hg^+ concentrations in the water column of Tien River, Mekong Delta, Vietnam.

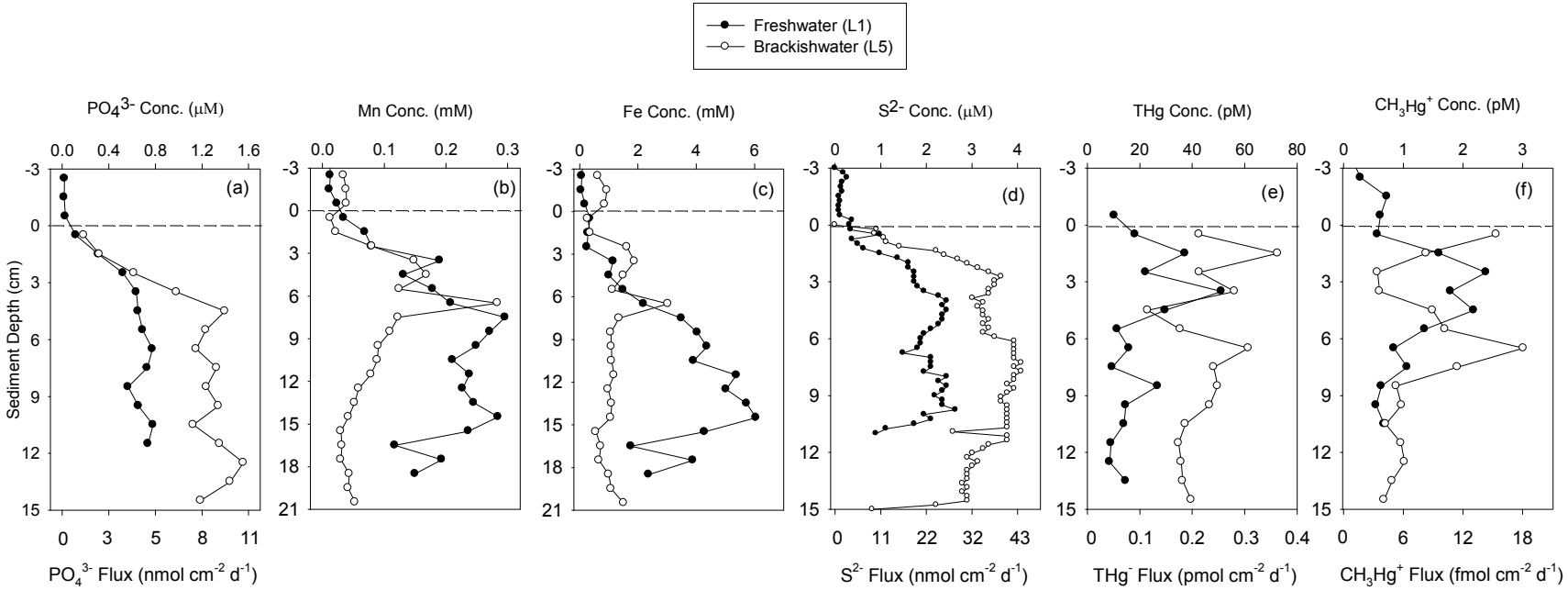

Fig. 3 The vertical pore water concentrations of DGT or DET measured (a) PO_4^{3-}, (b) Mn, (c) Fe, (d) S^{2-}, (e) THg, and (f) CH_3Hg^+ in fresh water (solid circles) and brackish water (hollow circles) sediments of the Tien River, Mekong Delta, Vietnam. Note that the DGT measurements were also shown as flux (=M/At, where M is the mass accumulated in resin, A is the exposed area, and t is the deployment time)

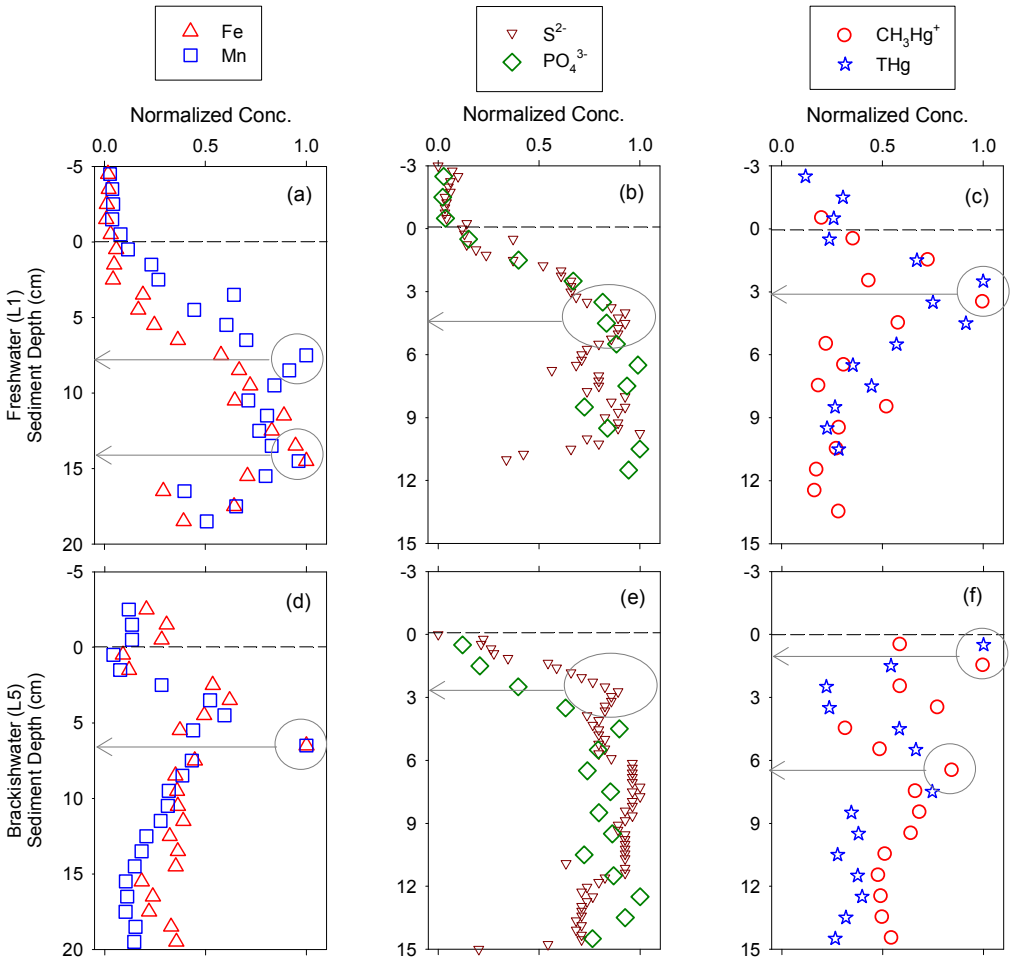

Fig. 4 The normalized vertical pore water levels of (a)/(d) Mn, Fe, (b)/(e) PO_4^{3-}, S^{2-}, and (c)/(f) THg, CH_3Hg^+ measured by DGT or DET in the Tien River, Mekong Delta, Vietnam. The arrows indicate the sediment depths correspond to maximum concentrations of the species.

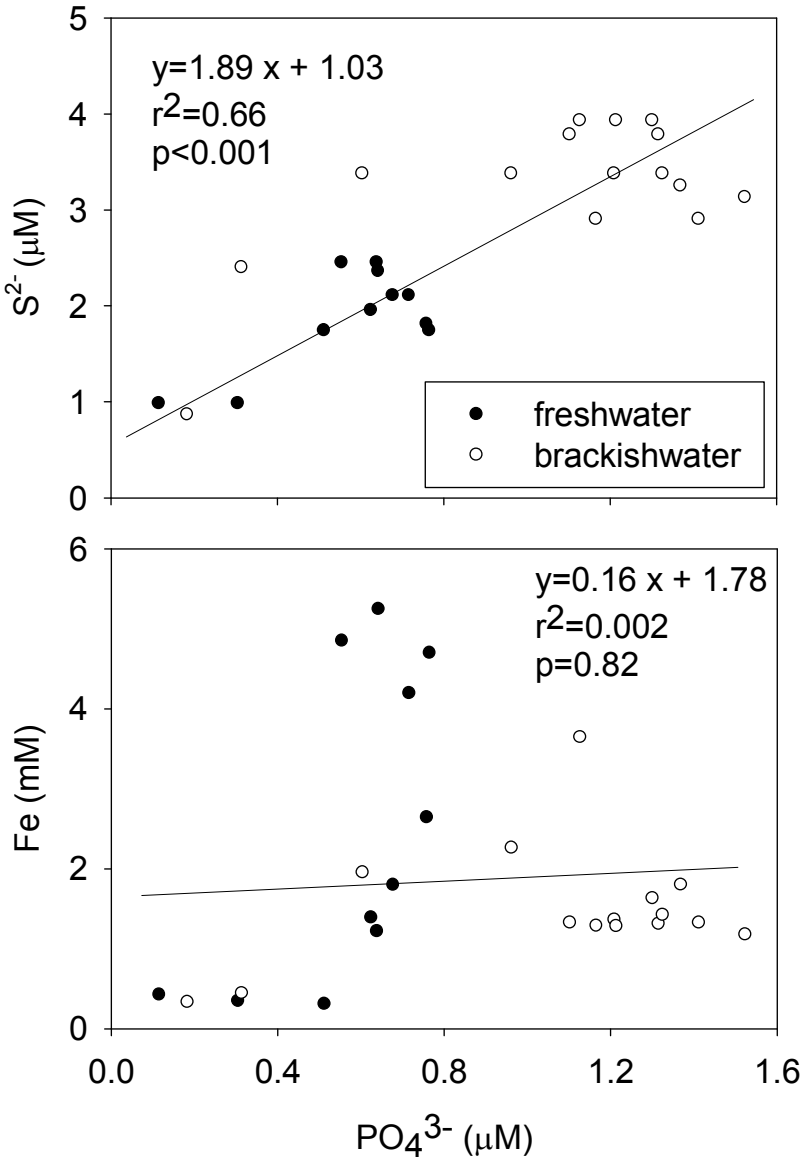

Fig. 5 The correlation between PO_4^{3-} and S^{2-}/Fe in sediment pore water of Tien River, Mekong Delta, Vietnam.

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