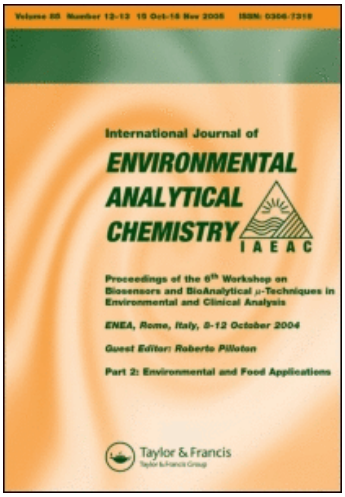

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

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Online publication date: 22 October 2010

To cite this Article Ding, Shiming , Sun, Qin and Xu, Di(2010) 'Development of the DET technique for high-resolution determination of soluble reactive phosphate profiles in sediment pore waters', International Journal of Environmental Analytical Chemistry, 90: 14, 1130 — 1138
To link to this Article: DOI: 10.1080/03067310903434733
URL: http://dx.doi.org/10.1080/03067310903434733

Intern. J. Environ. Anal. Chem.
Vol. 90, Nos. 14–15, 10–20 December 2010, 1130–1138

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Development of the DET technique for high-resolution determination of soluble reactive phosphate profiles in sediment pore waters

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(*Received 3 May 2009; final version received 20 October 2009*)

The DET (diffusive equilibrium in thin films) technique is developed to measure soluble reactive phosphate (SRP) profiles in sediment pore waters at a millimetre resolution. The analytical procedure includes equilibration of the gels in sediments, section of the gels after retrieval from the sediments, back elution of phosphorus in the gels, and analysis of SRP in the eluents. Recovery of phosphorus is improved from back elution with 0.25 M nitric acid relative to deionised water. SRP concentrations in pore waters of different sediments measured by DET probes agree well with those directly measured by the colorimetric method. Pore water profiles obtained simultaneously using gel probes and other techniques (including Rhizon and dialysis peeper) also show comparability at similar resolutions. The DET probes were used to investigate pore water SRP profiles in the sediments of two contrasting regions (algal-dominated and macrophyte-dominated) in Lake Taihu. An increasingly upward movement of SRP was observed in subsurface pore waters of the algal-dominated region coupled with an increase in water temperature from March to May. Peak-shape distribution of SRP and horizontal heterogeneity was observed in pore waters of the macrophyte-dominated region, which is most likely caused by the activity of submerged macrophyte roots.

Keywords: diffusive equilibrium in thin films; pore water; phosphorus; high resolution; lake; sediments

1. Introduction

Phosphorus (P) is commonly the limiting nutrient for the growth of primary producers in lacustrine ecosystems [1]. P availability in the water column is thus regarded as the most important factor determining the trophic state of lakes, where the major fractions of P come from external sources. However, a reduction in external P input does not always show a linear shift in lake trophic level [2]. This delay in recovery is often linked to the release of P from sediments to the overlying water column [3,4].

P remobilisation in sediments can generate a steep gradient in pore water profiles, which is a good indicator of internal loading processes taking place in recent sediments. Therefore understanding the remobilisation and calculation of P fluxes at the sediment–water interface (SWI) requires a precise measurement of the gradient. Conventional pore

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ISSN 0306–7319 print/ISSN 1029–0397 online

DOI: 10.1080/03067310903434733
http://www.informaworld.com

water sampling techniques, such as centrifugation, squeezing, sippers and dialysis, are generally limited in their resolution (typically 1 cm) [5]. Use of microelectrodes can achieve a much higher resolution [6], but the electrodes are very fragile and expensive. The DET (diffusive equilibrium in thin films) technique is a new approach to *in situ* measurement of pore water constituents at a relatively high resolution [7]. It uses a thin film of gel for equilibration with solutes in pore waters, relying on a similar equilibration principle as dialysis, but the complete equilibration is achieved in hours rather than days or weeks [8]. DET has been used extensively in determination of metal or anion profiles in pore waters of sediments [9,10], and few studies focus on pore water, Jarvie *et al.* [11] and Monbet *et al.* [12], for example, measured soluble reactive phosphate (SRP) in pore waters of river-bed and lake sediments, respectively. Insufficient recovery of P resulting from back elution of the gels with deionised water after equilibration in pore waters [8] is considered as a major problem causing the difficulty in DET application. Another limitation is an insufficient volume of the following eluent used for analysis of SRP concentration by the traditional colorimetric (molybdenum blue) method [10].

In this study, nitric acid (0.25 M) was tested as an eluting agent to improve P recovery from back elution of the DET gels. Soluble reactive phosphate (SRP) in the following eluent was analysed in batch with a miniaturised photometrical method. The established DET technique was further used to investigate pore water SRP profiles in algal- and macrophyte-dominated regions of Lake Taihu, respectively.

2. Experimental

2.1 *Gel preparation*

Gel preparation was conducted according to Docekalova *et al.* [13]. A gel containing 1.5% agarose was prepared by dissolving it in a certain volume of deionised water. The mixture was covered and heated in a boiling water bath until all the agarose was dissolved. The hot solution was immediately pipetted into a sandwich between two preheated glass plates, with plastic spacers placed between the glasses to control the thickness of gel at 0.4 mm. The gel sheet was left at room temperature. After complete solidification it was immersed in excess deionised water and left for 24 h. The dimension of the gel kept stable on hydration, but its water content increased to ~99%. The gel sheet was stored in 0.01 M $NaNO_3$ after removing from the assembly.

2.2 *Selection of eluting agents*

The pre-cast gel was cut into 2.5 cm diameter discs, with the volume of each disc being typically 0.2 mL. The gel discs were immersed into a range of standard phosphate solutions (0.1–5 mg L^{-1}) and left for 24 h to receive complete equilibration. They were back-equilibrated either in 1.8 mL deionised water or 0.25 M HNO_3 after retrieving from the solutions. The mixtures were centrifuged at 10,000 rpm for 10 min to ensure that all the liquids came in contact with the gels. The gel discs were subsequently left in the deionised water or 0.25 M HNO_3 for 16 h. A volume of 0.2 mL solution was then sampled for SRP determination.

2.3 *Test of SRP recovery for extracted pore water samples*

A total of 30 surface sediment samples were collected from different lakes in China. The pore waters were extracted by centrifugation of the sediments at 5,000 rpm for 10 min. The following extracts were filtered through a 0.45 μm filter (Cellulose acetate membrane, Whatman). A gel disc was immersed in 20 mL of each pore water sample, and left for 16 h to achieve complete equilibration. Once retrieved, the gel was back-equilibrated in 1.6 mL 0.25 M HNO_3. The solution was centrifuged at 10,000 rpm for 10 min and left for 16 h. An appropriate volume of the solution was sampled for SRP determination. Concentration of SRP in each pore water sample was also determined after equilibration with the gels.

2.4 *Preparation of DET probes for sediment deployment*

The plastic assemblies designed for loading gels were purchased from DGT Research (UK, www.dgtresearch.com). The assembly has a concave plate on the face. Gel was generally laid on the concave plate and covered with a filter membrane. A plastic frame was attached on the face to fix the gel system. Agarose gels pre-cut into 3.5 × 18 cm were placed on the back of the probes to reduce vertical pore water transport along the probe surface during the deployment [14]. This assembly increased contact between the sediment and gel without significant gel deformation except along its bottom edge. A 0.45 μm cellose nitrate membrane covered the gel and was back-fixed by the frame. The probes were immersed into 0.01 M $NaNO_3$ and bubbled with nitrogen for 24 h before deployment.

2.5 *Test of SRP recovery for pore water profiles*

Two sediment cores (12 cm diameter) were sampled from Lake Taihu and transported to the laboratory at low temperature (~15°C). One DET probe was inserted into each core, with ~3 cm being exposed above the SWI. The probes were left to equilibrate for 24 h. Once retrieved, the probes were washed with deionised water to remove adhering sediments. The gel and membrane of each probe were cut out along the plate edge. They were placed on a clean flat board, and the membrane was discarded. The gel was cut into two portions at the exact location of the SWI. Each portion was then sectioned into 1 cm long strips. Each strip was further sectioned into ~3 mm pieces and placed in preweighted 1.5 mL microcentrifuge tubes for weighing. The weight of each gel piece was determined as the difference. The precise sizes of the pieces were used to calculate their exact positions in the depth profile. A 0.5 mL of 0.25 M HNO_3 was added to each tube. The tube were centrifuged at 10,000 rpm for 10 min and left for 16 h. An appropriate volume of the solution was then sampled for SRP determination.

During the deployment of the probes in each sediment core, the sampling devices of minipeeper and Rhizon were simultaneously used to test the fidelity of measured profiles. Rhizon is a suction sampler originally used for sampling fluids from unsaturated soils [15]. It has recently been applied to sample pore water with a vertical resolution of 1 cm [16]. Peeper is a passive sampler relying on dialysis equilibration between fluid-filled cells and the surrounding sediments, which has been extensively used to sample pore water over the past 30 years [17,18]. Its equilibration time normally needs several weeks, depending on sediment properties and the depth of dialysis cell [19]. Its vertical resolution depends on cell width and separation, being typically ≥1 cm [19]. The minipeeper used in this study has 54 vertical disposed dialysis cells, with 0.15 cm between each. Each dialysis cell has a

surface size of 3×0.25 cm (length × width) and a thickness of 0.2 cm. The above sizes produce a vertical resolution of 0.4 cm.

Rhizon samplers were inserted into one sediment core through small holes predrilled in the tube wall, and the pore waters at different depth sediments were collected. Two minipeepers were simultaneously inserted into another core when deploying the DET probe. They were retrieved after 24 and 48 h, respectively, and solutions in dialysis cells were sampled. Pore water samples collected with Rhizons and minipeepers were used for SRP analysis.

2.6 In situ *measurement*

Lake Taihu is the third largest freshwater lake in China. It is a shallow, eutrophic lake, with the surface area 2338 km^2, mean depth 1.9 m, and the maximum depth 2.6 m [20]. The lake is located in the Changjiang (Yangtze) delta, the most industrialised area in the country. Economic development has affected its water quality via inputs of industrial and domestic wastewater, and additional contributions from agricultural non-point sources since 1980s [21]. Frequent algal blooms have been reported in the northern part of the lake since 1980s, while large submerged plants dominate the east and southeast of the lake [21]. A steady increase in total P in the water column is reported especially in the north region of Lake Taihu since 1999, despite a marked decrease in external inputs from 1996 [22]. The increase in total P is thus considered as a result of internal P loading that plays an important role in P cycling in the lake.

Sediment cores were collected in two contrastive regions (algal- and macrophyte-dominated, respectively) of Lake Taihu. The algal-dominated region located in its north region has being receiving sewage input since 1980s. The macrophyte-dominated region is located in its southeast region. Aquatic plants in this region are mostly submerged macrophyte *Elodea nuttalli* and *Potam ogeton macckianus* [23]. Taihu Laboratory for Lake Ecosystem Research reported concentration of total P in sediments and annual total P in the water column in the two sites as 1830 and 571 $\mu g\,g^{-1}$, and 0.24 and 0.06 $mg\,L^{-1}$, respectively (unpublished data).

Two sediment cores were collected from each site in March, April and May in 2007, respectively. The cores were left in the dark under ~15°C. Each core was inserted with a DET probe and left for equilibration for 24 h, with about 10 cm water maintaining above the SWI. On retrieval, the probe was processed according to the above procedure.

2.7 *Analytical method*

SRP was analysed with a miniaturised photometrical method, modified from the standard molybdenum blue method [24,25]. The method allows simultaneous determination of up to 96 samples by a microtiter plate reader, while the consumed sample volume decreases to below 250 μL [25]. The detection and quantitation limits were 0.003 and 0.01 $mg\,L^{-1}$, respectively, and are similar to those determined by the traditional colorimetric method.

3. Results and discussion

3.1 *Selection of eluting agents*

After equilibration in the standard phosphate solutions, the DET gels were back-eluted-with deionised water or 0.25 M HNO_3. The recovery rate with deionised water

was 90 ± 3% (Figure 1), and is similar to the 89 ± 3% as reported by Krom *et al.* [8]. Incomplete recovery indicated a weak binding of agarose with phosphate. Back elution with 0.25 M HNO_3 achieved complete recovery of phosphate (101 ± 2%), showing that residual phosphate retained by the gel could be desorbed by the acid. The eluting time was determined at 16 h, according to acceptable variation of phosphate recovery for the different standard phosphate solutions. This time is longer than the 2 h used by Krom *et al.* [8] but shorter than the 36 h used by Monbet *et al.* [12], when deionised water was used as the eluting agent. This time is also shorter than the 24 h used by Jarvie *et al.* [11], when sulphuric acid (0.25 M) was the eluting agent. A further increase in HNO_3 concentration to 0.5 M might increase the eluting efficiency and shorten the eluting time, but simultaneously produces gel debris and causes interference to phosphate detection.

SRP recovery rates from DET determination of 30 extracted pore water samples were 99 ± 6% (Figure 2), demonstrating that the eluting procedure established above could be used in pore water SRP determination. Analytical uncertainty is mainly from the samples with a SRP concentration lower than 0.1 mg L^{-1}, exhibiting an average recovery rate of 97 ± 9%. As there is an inevitably 10-fold dilution for back elution of phosphorus-equilibrated gels, the limit of quantitation for DET determination is at least 0.1 mg L^{-1}. The present DET procedure is thus suitable for analysis of pore water SRP with its concentration higher than 0.1 mg L^{-1}.

3.2 *Test of SRP recovery for pore water profiles*

Pore water SRP profiles in two sediment cores were measured with the established DET procedure. Equilibration of the minipeepers in sediments for 24 and 48 h, respectively, produced similar SRP profiles. However, the SRP in the latter profile has a much smoother change with depth, reflecting that diffusive equilibration was generally reached within 48 h (Figure 3). This time is much shorter than for other peeper samplers reported in the literature [26,27].

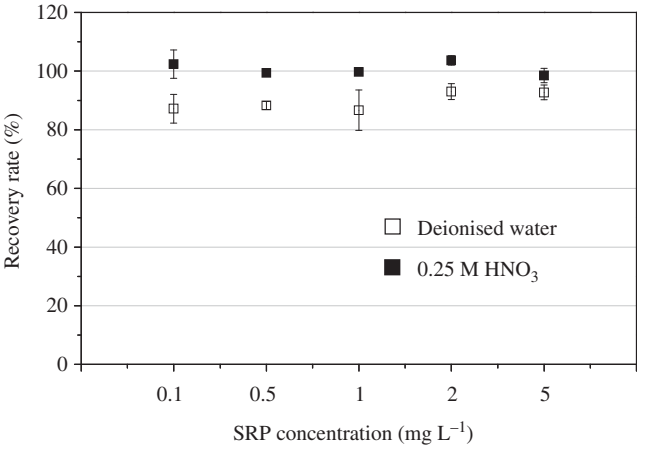

Figure 1. Recovery of phosphate from back elution of the gels with deionised water and 0.25 M HNO_3, respectively, after equilibration of the gels into standard phosphate solutions.

The pore water SRP profile obtained with DET is similar to that with Rhizon (Figure 3) although their comparison is relatively rough due to a limited resolution of the Rhizon. The profiles obtained with DET and minipeepers have similar vertical resolutions, both exhibiting concentration gradients below the SWI. However, the gradient obtained with DET is somewhat relaxed around the concentration peak (between 0 and 7 cm below the SWI). The profile relaxation is possibly caused by lateral diffusion in the gels due to

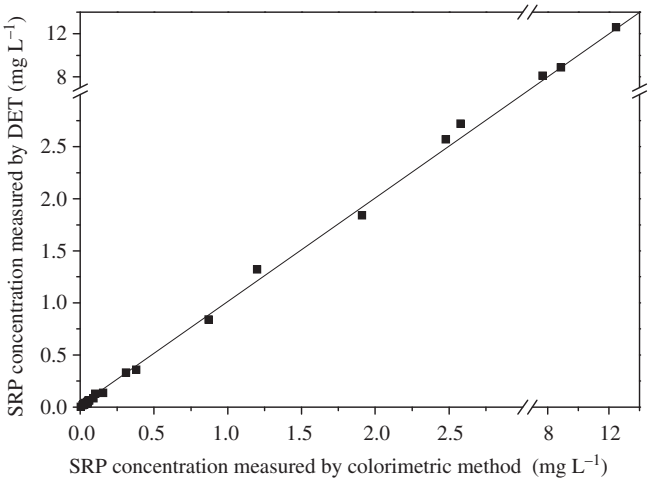

Figure 2. Relationship between SRP concentrations in different pore waters measured directly by colorimetric method and measured by DET.

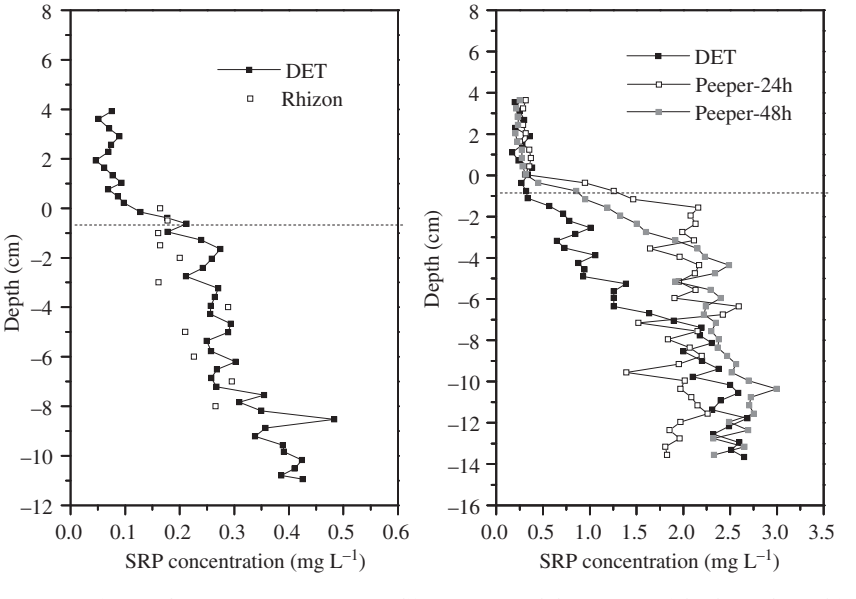

Figure 3. Comparison of pore water SRP profiles measured by DET with those by Rhizon and minipeeper.

their continuous medium. This process occurs during the deployment in sediments in the absence of sources and sinks responsible for vertical structure, and in the absence of external sources after the removal from the sediments [19]. The profile relaxation is also likely to be caused by horizontal heterogeneity in sediments, or use of an unsuitable procedure for DET analysis. In this study, the probes were washed with deionised water to remove adhering sediments after retrieval from sediments, a procedure used by Krom *et al.* [8]. However, Mortimer *et al.* [10] found that this would cause errors for measurement of solute distributions in marine pore waters due to dilution of the pore waters. Instead, the authors removed adhering sediments from the probes by wiping the probes with tissues.

3.3 *Investigation of pore water SRP profiles in sediments of Lake Taihu*

Average pore water SRP concentration shows a downward increasing trend ($0.57\,mg\,L^{-1}$ in March, $0.93\,mg\,L^{-1}$ in April and $2.17\,mg\,L^{-1}$ in May) from SWI up to 12 cm depth in the algal-dominated region (Figure 4). The downward increasing concentration of SRP in all profiles suggests that sediment P is a potential source of P to the water column. Concentration gradient depth in the profiles ranges from the depth of 2 cm below the SWI to the bottom in May, which is wider than those from 6 cm below the SWI to the bottom in March and April. Therefore the upward movement of SRP likely becomes stronger with the increase in water temperature from March to May. SRP profiles are in general the same for the two parallels in all sampling times, reflecting the reliability of the present DET processing. The lateral diffusion in the gels (Figure 3) should thus be attributed to profile relaxation or sediment horizontal heterogeneity. The reason needs further verification.

SRP concentrations in the sediments of the macrophyte-dominated region are much lower than those of algal-dominated region (Figure 5). Average concentration of SRP

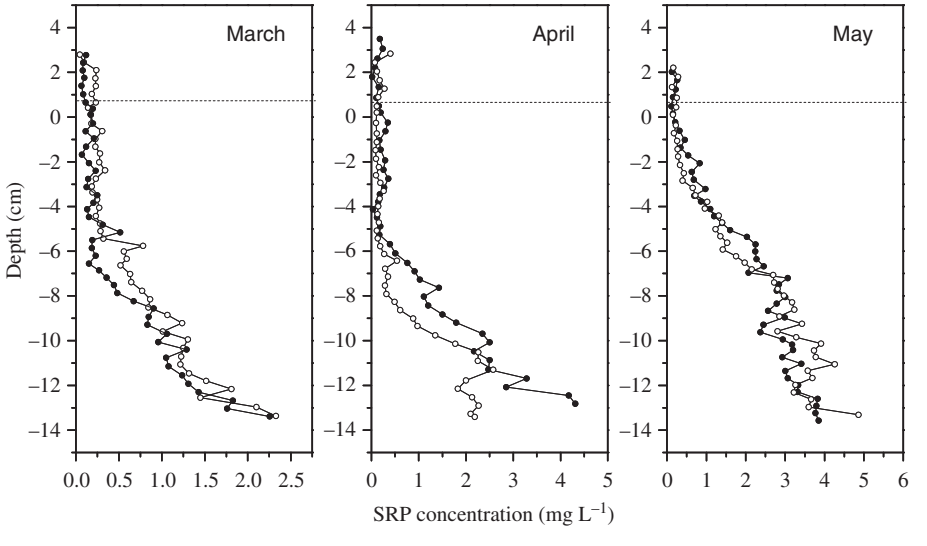

Figure 4. Pore water SRP profiles in sediments of the algal-dominated region in Lake Taihu. The hollow and filled circles show two parallel profiles at each sampling time.

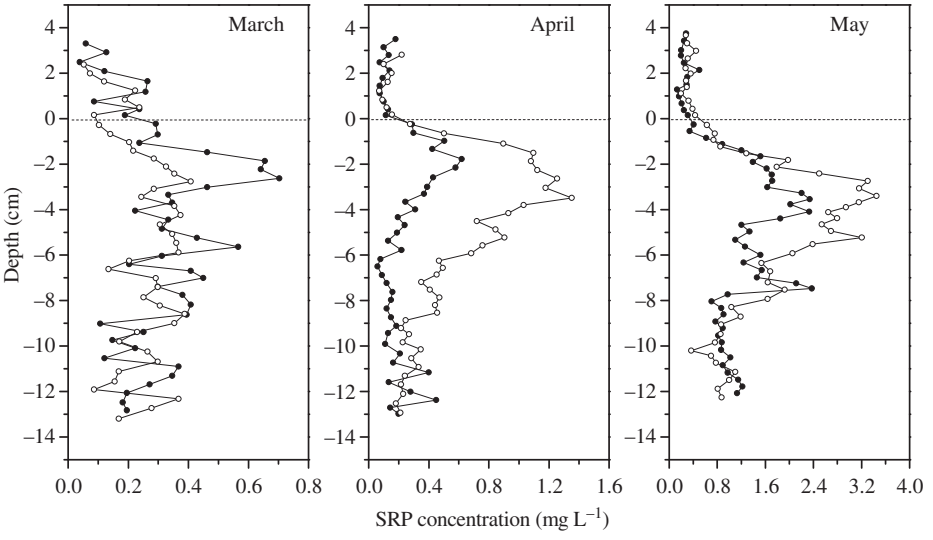

Figure 5. Pore water SRP profiles in sediments of the macrophyte-dominated region in Lake Taihu. The hollow and filled circles show two parallel profiles at each sampling time.

across the profiles also shows a much smaller increase with the increase in water temperature, varying from 0.27 mg L^{-1} in March, 0.36 mg L^{-1} in April, to 1.21 mg L^{-1} in May. All the SRP profiles display peak-shape distributions, exhibiting concentration maximum at about 2 cm below the SWI in March and 4 cm below the SWI in both April and May. Concentration gradient depth is generally similar for the two parallel profiles in each sampling time, ranging from the SWI to below 4 cm in March, to below 7 cm in April and to below 8 cm in May. However, their SRP concentrations are evidently different at the gradient depth. For example, higher SRP concentration maximum in the two parallel profiles is 0.70 mg L^{-1} in March, 1.35 mg L^{-1} in April, and 3.45 mg L^{-1} in May, being 1.8, 2.1, and 1.5 folds of the correspondent lower maximum. Sediment heterogeneity has been recently emphasised by others, and is considered as a mineralised consequence of organic-rich particles unevenly distributed in surface sediments [6,28–30]. The observed concentration gradient regions are generally consistent with the distribution of submerged macrophyte roots, suggesting that SRP profiles and their horizontal heterogeneity were most likely caused by the activity of roots.

4. Conclusions

In this study, the DET technique was developed to measure SRP profiles in sediment pore waters at a millimetre resolution. The established procedure includes back elution of SRP in the gels with 0.25 M nitric acid and in batch analysis of SRP in the following eluent with a miniaturised photometrical method. Both the DET measurements of SRP in extracted pore water samples and *in situ* pore water profiles produced acceptable results, despite the profile relaxation possibly due to the lateral diffusion in the gels. In site DET measurements on the two contrasting regions (algal- and macrophyte-dominated) of Lake Taihu reflected distinctive features in spatiotemporal variations of SRP profiles.

Acknowledgements

This research work was funded by the National Natural Science Foundation of China (40601087, 40730528) and the National High Technology Research Development Plan (863) (2007AA06Z411). The authors thank the three anonymous reviewers for their comments and suggestions to this manuscript.

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