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In situ sampling and speciation method for the measurement of dissolved phosphite at ultratrace concentrations in the natural environment.

Chao Han¹, Jinghua Ren², Paul N. Williams³, Zhaode Wang¹, Xu Fang⁴, Di Xu¹,
Xianchuan Xie⁴, Jun Luo^{4*}

¹State Key Laboratory of Lake Science and Environment, Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences, Nanjing 210008, China.

²Geological survey of Jiangsu Province, Nanjing 210018, China.

³Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Belfast BT9 7BL, United Kingdom.

⁴State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing 210093, China.

Graphic Abstract

Abstract: Phosphite (P^{+III}) is of emerging chemical interest as it's importance within the global phosphorus cycle becomes more established. Yet, to date, precise/accurate measurements of P^{+III} in the environment are still lacking due to the inherent analytical challenges linked to its instability/ease of oxidation and ultra-trace concentration in many natural systems. Here, we present the first *in-situ* sampling and speciation analysis method, for dissolved P^{+III} for terrestrial/aquatic media relevant concentrations, using the diffusive-gradient-in-thin-films (DGT) technique, combined with capillary column configured dual ion-chromatography (DIC) for enhanced-sensitivity P^{+III} determination. Method optimization of the DGT elution regime, to simultaneously maximize desorption efficiency and DIC sensitivity, along with the characterization of diffusion coefficients (D) for P^{+III} in agarose cross-linked (APA) polyacrylamide hydrogel, were undertaken prior to full method validation. Laboratory performance testing confirmed DGT- P^{+III} acquisition to be independent of pH (3.0–10) and ionic strength (0–500 mM NaCl). The effective capacity for P^{+III} was $45.8 \mu g cm^{-2}$, while neither P^{+V} (up to $10 mg L^{-1}$) or As^{+V} (up to $1 mg L^{-1}$) impacted the quantitative measurement of DGT- P^{+III} . This novel DGT-DIC method's functionality stems from the herein confirmed speciation preservation provided by the DGT, combined with the double online-preseparation and -preconcentration of P^{+III} gained by DGT and DIC being used in tandem, resulting in quantification limits as low as $7.44 ng L^{-1}$ for a 3-day deployment, even in complex matrixes. Field-testing of the system demonstrated the potential of this method as a geochemical exploration tool for dissolved P^{+III} in soils/aqueous environments.

INTRODUCTION

Phosphorus (P) availability, determines productivity in the majority of global ecosystems,¹ but due to the many different inorganic and organic species in various P oxidation states (P^{+V} , P^{+III} , P^{+I} , P^0 , P^{-III}) that exist in the environment it's characterization is complex.^{2,3} The long-held understanding of P biogeochemical processes is biased. Up until relatively recently inorganic P has been almost exclusively assumed to exist in the +V state in all but the most obscure of natural systems. Meanwhile, the redox reactions involved in (partially)-reduced P compounds [e.g., phosphite (P^{+III}), hypophosphite (P^{+I}), phosphine (P^{-III})] have largely been overlooked. However, recent evidence, arising from the discovery of these reduced-P species in a much wider range of settings,^{1,4} previously thought possible, coupled with the practically ubiquitous ability of microbiota to use reduced P compounds as their sole P source,^{5,6} has led to some questioning of this prevailing consensus. Attention is also being directed at the sampling and analytical methods, with there being an increasing demand for new measurement data, that better reflects the relevant concentrations/bioavailability of terrestrial/aquatic media^{1,7} to calibrate thermodynamic and kinetic models for risk prediction of P speciation and fate in the environment.

Of these reduced P species, P^{+III} is of particular concern within the P redox-cycle. Pasek and co-workers (2008, 2015) have commented on the P geochemistry of the early earth, stating that it was controlled by P^{+III} and P^{+I} , which were much more soluble and reactive than the orthophosphates originally credited with this major role in early earth formation.^{2,8} Thus, P^{+III} and P^{+I} are highly relevant to the origin and development of early life.² Whether today, P^{+III} is considered as a nutrient or contaminant is a moot point. Recent microbiological/biochemical evidence suggests P^{+III} can play a major role in a number of key metabolic processes, which raises interesting questions regarding the nutritional value of P^{+III} in the environment. Several studies have also reported that P^{+III} can be directly or indirectly utilized by plants,^{9,10} microbes,^{5,11} and algae.¹² However, its toxicity that P^{+III} is most recognized

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for, and this is the primary concern for environmental monitoring programmes.

Some P^{+III} species are formed naturally, catalyzed by both abiotic^{3,13,14} and biological processes,^{15,16} while new synthesis pathways continue to be discovered. Anthropogenic sources of P^{+III} into the environment, are poorly characterized, but are also potentially important, given the range of products that feature P^{+III} , such as P fertilizers, plastic stabilizers, water treatment agents, and organophosphorous pesticides.^{15,17} Therefore, the potential for environment enrichment and alteration of the P-cycle is significant. However, our knowledge on the behavior of P^{+III} and its implication for biogeochemical processes is restricted to the paucity of data on its presence and cycling in the environment.

An essential prerequisite for making progress in this field is the means to precisely quantify local distributions and variations of P^{+III} in the environment. Precise measurement of trace P^{+III} (a few sub- μ mol or less) in natural samples is an ongoing challenge because it requires the use of very clean procedures and selective and sensitive methods. Currently procedures available for P^{+III} analysis are based on *ex-situ* sampling and laboratory speciation analysis (e.g., ion-chromatography,^{18,19} and nuclear magnetic resonance spectroscopy¹³), which can easily change the media's properties (such as bioactivity, pH, redox potential, metal hydroxides, etc.) and thereby make the measurements more susceptible to analytical errors during sample handling and preparation.²⁰ Consequently, the miscalculation of P^{+III} in the original sample restricts the scope of the environmental research on P species and the assessment of P^{+III} in the ecosystem due to the destructive character of conventional methods.

A solution is to use the well-established passive sampler technique of diffusive gradients in thin films (DGT).^{21,22} In brief, DGT is used to measure the time-weighted average flux or concentration of chemical species (e.g., P, As, and metals) through a well-defined diffusion layer.²³ A DGT device consists of a binding gel layer, which is overlaid by a diffusive hydrogel and a protective filter membrane.^{24,25} The analyte species of interest diffuse through the sampler, a process that acts to separate and

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clean-up the sample matrix, and are subsequently trapped and concentrated on a resin layer.²⁶ The technique possesses specific advantages, including the low disturbance of the target media during deployment/sample collection and low effective detection limits for trace chemicals.^{27,28} This technique is already being used to measure dissolved inorganic P^{+V} , As^{+III} , and Sb^{+III} in various environments.²⁸⁻³¹ Considering their structural and chemical similarities to P^{+III} ,^{32,33} it is reasonable to assume that the DGT method maybe a potential candidate for *in-situ* P^{+III} determination. To the best of our knowledge, DGT has not yet been applied to P speciation measurements and the diffusion coefficient for P^{+III} in polyacrylamide gels is unknown. Furthermore, capillary column configured dual ion-chromatography (DIC) is a robust and sensitive analytical method, which can provide enhanced-sensitivity for P^{+III} determination.^{19,34} More importantly, a much lower instrumental limit of detection is established at 0.2 $\mu g L^{-1}$ of P^{+III} using DIC in normal mode operation with an injection volume as low as 10 μL , which can perfectly accommodate the DGT method. This paper presents the first DGT-based method coupled with DIC for the in situ determination of environmentally-relevant levels of P^{+III} in the environment. A series of validation experiments were systematically carried out to validate the performance of the new method.

Experimental Section

Materials and Chemicals. All plastic apparatus used for experiments were acid-cleaned in 10% (v/v) hydrochloric acid (HCl) overnight and subsequently rinsed three times in Milli-Q water (18.2 M Ω cm) prior to use. Chemicals of analytical grade or better were purchased from Sigma-Aldrich (USA). Ionic strength was adjusted using sodium chloride (NaCl), while the pH was adjusted by adding 0.1 M HCl or 0.1 M sodium hydroxide (NaOH) and letting the solution equilibrate several times. A typical piston-DGT assembly employing a protective filter membrane (HVLP00010, Millipore), a diffusion layer (APA), and a precipitated zirconia-based binding gels (PZ) was prepared according to reported procedures.²⁸ DGT-assemblies should be

133 deoxygenated with nitrogen for at least 16 h and stored in containers filled with
134 deoxygenated 0.01 M NaCl solution before use.

135 **Analytical Method, Quality Assurance and DGT-concentration Calculations.** A

136 dual ion-chromatography module (ICS 5000, Thermo Fisher, USA) configured with

137 capillary columns, was chosen for P^{+III} measurements. Detailed instrumental

138 conditions are given in **Table S1**. Procedural blanks, matrix spike i.e. P^{+III} standards

139 spiked into waters, elutions, wastewater, etc., and sample triplicates were used for

140 quality assurance purposes of the coupled capillary system with acceptable recoveries

141 ranging between 90% and 115%. To alleviate/mimimise any potential

142 background-signal effects, accumulated masses measured on the binding gels exposed

143 to P^{+III} were blank corrected by subtracting the procedural blank results measured on

144 the blank binding gels that had not been exposed to solutions/matrices. The

145 DGT-measured concentration (C_{DGT}) can be determined by the standard DGT

146 equation using the diffusion coefficients (D) measured in this study (see **Supporting**

147 **Information S1**).

148 **Diffusion Coefficient Measurement.** The diffusion coefficient (D , $cm^2 s^{-1}$) is

149 necessary for calculation of the DGT concentration of target analyte. Since D values

150 for P^{+III} diffusion through the polyacrylamide gel have not been previously reported,

151 they were determined using both a previously described diffusion cell (D_{cell})³⁵ and

152 DGT device (D_{DGT}) method.²⁶ (see **Supporting Information S2**).

153 **Batch Sorption and Elution Experiments.** The set gels were cut into discs of ~

154 2.5 cm diameter. The binding kinetics of P^{+III} to the resin layer was investigated by

155 deploying the PZ-gel discs in 20 mL of solution at $pH\ 7.0 \pm 0.2$ containing 100 μmol

156 L^{-1} P^{+III} in 0.03 M NaCl for 2–120 min. The solutions were immediately shaken on a

157 horizontal shaker during the fixed time deployment. Then, 100 μL of each solution

158 was sampled at known time intervals for DIC analysis, and the differences between

159 the measured and initial concentration of P^{+III} were used to determine the mass of P^{+III}

160 uptake by the resin.

161 To optimize the elution procedure, the resin layer, with 2.5 cm-diameter, was

162 immersed into 10 mL of P^{+III} solution containing 10 $\mu mol\ L^{-1}$ P^{+III} and 0.03 $mol\ L^{-1}$

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NaCl (pH = 7.0 ± 0.2) for 6 h and eluted by NaOH solutions of different concentrations from 0.1–1.0 mol L⁻¹ for at least 24 h. The elution efficiency was expressed as the ratio of the eluted mass to the mass bound in the gels.

The impact of different storage conditions on the stability of P^{+III} adsorbed on the binding gels was also evaluated by deploying PZ-DGT assemblies (three replicates) into 20 mL of filtered freshwater spiked with 25 µmol L⁻¹ P^{+III} for 2 h. After DGT retrievals, the P^{+III}-loaded binding gels were gently washed with deionized water and then tracked for storage using two different procedures: 1) The PZ-gels were directly refrigerated in darkness (i.e., stored as gels) and 2) the adsorbed-P^{+III} on the gels were immediately extracted and then the elutions were refrigerated in darkness (i.e., stored as eluents). The amount of P^{+III} recovered by selective elution with NaOH was immediately determined at different times during preservation.

Capacity and Interfering Effects. The capacity of PZ-DGT for P^{+III} adsorption was determined by deploying the assemblies for 6 h in triplicate in 2 L of well-mixed solutions (at pH 7.0 ± 0.2 and a temperature of ~ 22 °C) containing 0.03 M NaCl and P^{+III} of various concentrations (1–500 µmol L⁻¹). To examine the effects of the PZ-DGT performance across wide pH and ion strength ranges, the assemblies were deployed in 2L of 25 µmol L⁻¹ P^{+III} solutions at varying pH levels (3.0–10.0) or ion strengths (0.0–500 mmol L⁻¹ NaCl), respectively. Besides, to test the effects of the potential competitors, i.e., PO₄³⁻ (P^{+V}), AsO₃⁻ (As^{+V}) on PZ-DGT performance, the assemblies were immersed in 2 L of 25 µmol L⁻¹ P^{+III} solutions containing additional PO₄³⁻ (0.05–10 mg P L⁻¹), AsO₃⁻ (0.001–1 mg As L⁻¹). All testing solutions were constantly stirred at a temperature of ~ 25°C and a stirring rate of ~ 800 rpm min⁻¹ during the 5-hr deployment of PZ-DGT.

DGT Applications. As already described, P^{+III} is heavily used for both agricultural and manufactural applications and is consequently identified in various environmental matrices. To evaluate the performance of the DGT method for P^{+III} determination in field environments, the PZ-DGT devices combined with the DIC technique were deployed in a wide range of environmental samples (e.g. freshwater, wastewater and soils) on the basis of a literature review that established where P^{+III} might be

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present.³⁶⁻³⁸ Details of the sampling sites (**Figure S1-S2**) and protocols have been described in the **Supporting Information S3**. Meanwhile, P^{+III} concentrations in those samples were determined using conventional *ex-situ* sampling and handling methods¹⁵ (see **Supporting Information S4**) for comparison.

3 Results and Discussion

Method Summary. In the coupling strategy, the PZ-DGT is used as a passive sampler by selectively accumulating analytes from the substrates,²⁸ while the coupled DIC is considered as the optimal quantitative method for ultratrace speciation.¹⁹ Accumulated P^{+III} by PZ-DGT is retrieved using the optimal eluting approach. Then, the eluent is injected directly for instrumental analysis without further purification steps. For chromatographic separation, a time span of IC effluents containing target analytes and minimized interferences were roughly separated first in the analytical system (Dimension 1), and then switched to the MAX-100 capillary concentrator column in the capillary system (Dimension 2) where the analyte could achieve a further separation and detection by concentrating the analyte species and lowering detection limits.

Representative chromatograms are shown in **Figure 1**. The retention time of P^{+III} (21.1 min) were verified by injecting eluent samples spiked with P^{+III} . Examples of chromatograms are available in **Figure S3**. Successful separation and quantification of P^{+III} were obtained using the developed eluent gradients; however, differences can be highlighted by the peak numbers and areas in this figure. It is noted that IC seldom peaks in Dimension 1 (**Figure 1-A**) and a larger peak area of P^{+III} occurs in Dimension 2 (**Figure 1-B**) when using the DGT method compared to those found using the traditional method, suggesting that selective *in-situ* preconcentration of P^{+III} and its separation from matrices were achieved using the DGT samplers. These figures verify that PZ-DGT assemblies can be utilized in natural samples with higher sensitivity than conventional sampling methods. This feature is highly desirable for ultratrace and less stable P^{+III} measurement. This method would work exceptionally well in complex matrices. It was also noted that P^{+III} is not resolved from CO_3^{2-}

(black line in shade area in **Figure 1-A**) in natural water in the analytical system owing to their similar affinities toward stationary conditions,³⁹ however, this can be eliminated online by the configured carbonate removal device in the capillary system.⁴⁰

Figure 1. 2D chromatographic separations of P^{+III} in a representative freshwater sample obtained by the traditional method (black line) and DGT method (red line). Separation of P^{+III} in the analytical system (A) and capillary system (B). A time span of 12.0 to 14.0 min (shade zone) containing P^{+III} and minimized disturbing ions was directed on-line from the analytical system to the capillary system.

Uptake and Elution Efficiencies. It was found that the P^{+III} uptake by the PZ-gel disc increased linearly with time for the first 15 min, followed by slow increases up to 40 min (**Figure 2-A**). After that, more than 99.5% of P^{+III} is absorbed by the binding gels. The average binding rate over the first 15 min is $18.5 \text{ ng P cm}^{-2} \text{ min}^{-1}$, much higher than those previously reported by Sun et al. (2014)³⁰ of 6.13 and 11.13 ng cm^{-2} for As^{+V} and As^{+III} for similar binding resins. This observed sufficiently fast uptake of P^{+III} satisfies the basis of DGT theory that the binding layers are effective zero sinks for P^{+III} , further demonstrating the potential for PZ-DGT measurement of P^{+III} .

Accurate quantification of DGT-concentrations for the target analytes relies on their effective and reproducible elution recovery from the binding gels.⁴¹ As mentioned previously, a series of alkaline extractants such as hydroxide, bicarbonates and carbonates have already been used successfully for eluting P^{+III} , P^{+V} and other

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inorganic anions from the stationary phase in previous IC-based methods.³⁹ Also, hydroxide extractants were effective in removing $\text{As}^{+III}/\text{As}^{+V}$, $\text{Se}^{+IV}/\text{Se}^{+VI}$ and P^{+V} bound to Zr-oxide-based binding gels.^{28-30,42} In this study, eluting the PZ-gels loaded with P^{+III} using 2 mL of 1.0 mol L⁻¹ NaOH for 24 h were first tried and then the eluent was directly injected for the DIC analysis, but a very low P^{+III} recovery was achieved which was probably attributed to the strong signal suppression by the high Na^+ in the sample matrix (**Figure S4-A**). As suggested in **Figure 1-B**, the dilution of the eluent with ultrapure water can effectively avoid this matrix effect, and a complete recovery was obtained when Na^+ in the eluted samples were diluted to less than 50 mmol L⁻¹. Therefore, further evaluation of the elution efficiencies using 2 mL of 50 mmol L⁻¹ NaOH was performed, and a consistent elution efficiency of 98.7% with good reproducibility ($\pm 2.79\%$) was obtained for the binding gels loaded with a range of P^{+III} masses (**Figure S4-B**). These elution efficiencies were similar to those determined for other oxyanions (P^{+V} , As^{+III} and As^{+V}) with 1.0 mol L⁻¹ NaOH eluents.^{28,30,42} One of the major drawbacks to those eluents is the necessity of a 2.5 to 20-fold dilution prior to the DIC analysis, which will decrease the sensitivity of trace P^{+III} determination;¹⁹ hence, 2 mL of 50 mM NaOH was consequently chosen as the preferred eluent for the following experiments.

P^{+III} Recovery Under Different Storage Conditions. Effective storage conditions for P^{+III} accumulated by PZ-DGT will ultimately impair the accuracy of P^{+III} quantification and is also a prerequisite for PZ-DGT performance. In our preliminary investigation, we found P^{+III} recovery decreases over time and reaches 50% after a 3-day storage of the retrieved binding discs before elution at room temperature. This variation results from a partial conversion of P^{+III} to P^{+V} in the presence of ambient air.¹⁰ However, P^{+III} recovery remained stable without considerable bias even after the adsorbed P^{+III} was stored for three days as either a binding gel or eluent, as indicated in **Figure 2-C**. After 30 days of refrigeration, P^{+III} recoveries of the retrieved binding gels and eluent decreased over time with a 54.8% and 78.7% retention, respectively. Thus, the refrigerated P^{+III} -contained eluent can avoid the P^{+III} loss more effectively for long-term stored samples. However, considering that normal DIC analysis was

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293 always done within 3 days after the sampling, we recommended storage of the
 294 PZ-gels before elution for the sake of convenience in a routine context.

295 **Figure 2.** Kinetics of P^{+III} bound onto the PZ-gels (A). Elution efficiencies (%) of P^{+III}
 296 from PZ-gels using different NaOH concentrations; ^a and ^b: the eluents were diluted
 297 10-fold and 20-fold respectively with Milli-Q water prior to DIC analysis (B).
 298 Recoveries (%) of P^{+III} loaded-gels which were stored as gels (■) and eluants (●) in a
 299 refrigerator for up to 30 days (C). All error bars represent the standard deviation (SD)
 300 of three replicates (n=3).
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303 **Diffusion Coefficients.** Since robust diffusion coefficients for P^{+III} in solution are
 304 unknown, the experimental measurement of this parameter and the effect of pH levels
 305 and ionic strength were conducted. Detailed D_{cell} measurements of P^{+III} at different
 306 pH levels and ionic strengths were first investigated using the rapid diffusion cell

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method. From the data set in **Table 1**, we did not observe significant influences of pH and ionic strength, although two different P^{+III} species, i.e., $H_2PO_3^-$ and HPO_3^{2-} may exist as a function of pH. The average D_{cell} at pH levels ranging from 3.0 to 10 and ionic strengths ranging from 0.0 mmol L⁻¹ to 500 mmol L⁻¹ was $(6.54 \pm 0.07) \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$. This value was much higher than D_{cell} for P^{+V} ($6.05 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$) and As^{+V} ($6.10 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$), but lower than $7.65 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ of As^{+III} . Meanwhile, the average D_{DGT} for P^{+III} was measured as $(6.92 \pm 0.18) \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, which agreed well with D_{cell} with an acceptance of less than 5.5%, indicating that no measurable effect of the diffusive boundary layer between the diffusive gel and surface solution was found.²⁵ Consistent results between D_{cell} and D_{DGT} have been previously reported in those studies of D -values for oxyanions such as P^{+V} , As^{+III} and As^{+V} , W.⁴³

Table 1 Diffusion coefficients ($10^{-6} \text{ cm}^2 \text{ s}^{-1}$) of P^{+III} determined using a diffusion cell (D_{cell}) and DGT devices (D_{DGT}) at 25 °C.

D_{cell}	D_{DGT}
6.55 ± 0.05^a	6.94 ± 0.24^c
6.53 ± 0.11^b	6.89 ± 0.17^d

^a and ^b diffusion coefficients measured in diffusive gels in synthetic freshwater at various pH (3.1, 4.5, 6.8, 8.2, 9.9) and ionic strengths (0, 10, 30, 100, 500 mM NaCl), which were respectively calculated from **Figure S5** (A-E) and **Figure S6** (A-E), values are means $\pm$ SD, n = 5.

^c and ^d diffusion coefficients measured in DGT devices in synthetic freshwater containing 25 and 50 $\mu\text{mol L}^{-1}$ P^{+III} at pH 6.8 and 30 mM NaCl, which were respectively calculated from **Figure S7-A** and **B**, values are means $\pm$ SD, n = 3.

Effect of pH, Ionic Strength and Competing Ions on P^{+III} Uptake. The influences of pH, ionic strength and competing ions on the DGT performance are presented in **Figure 3**. DGT-measured P^{+III} concentrations (C_{DGT}) agree well with the directly-measured P^{+III} concentrations ($C_{solution}$) in deployment solutions, with the derived ratios of $C_{DGT} / C_{solution}$ ranging from 0.95 to 1.08 with pH levels ranging from 3.0 to 10.0 (**Figure 3-A**). As a diprotic acid, P^{+III} may speciate as HPO_3^{2-} , $H_2PO_3^-$ and H_3PO_3 , and is often measured as total P^{+III} . P^{+III} has a conditional pK_1 value of 1.3 and a pK_2 value of 6.7, and therefore, speciates predominantly as three species over the pH

range of most natural solutions.²⁰ It seems all species can be effectively measured by PZ-DGT, which is especially meaningful since the P^{+III} species is highly pH-dependent.

P^{+III} measurements by PZ-DGT also exhibit no appreciable dependency of ionic strength from 0 to 500 mmol L⁻¹; with the $C_{DGT} / C_{solution}$ values generally ranging between 0.9 and 1.1 (**Figure 3-B**). While the varying ionic strengths presented in environmental samples of freshwater and wastewater had ionic strengths lower than 100 mmol L⁻¹,⁴⁴ seawater had ionic strengths around 500 mmol L⁻¹, verifying that PZ-DGT can effectively measure P^{+III} in a wide range of ionic strengths.

The natural presence of a relatively high concentration of anions particularly P^{+V} and As^{+V} was significantly analogous to the P^{+III} , which may interfere and compete with P^{+III} for binding on PZ interaction sites. Thus, their effects on the DGT uptake of P^{+III} were evaluated in synthetic freshwater with various combinations of anion species. Results are listed in **Figure 3-C** and **D**. There are good agreements between C_{DGT} and the known concentrations in $C_{solution}$, as indicated by $C_{DGT} / C_{solution}$ ratios, which were generally in a range of 0.89 to 1.12. No appreciable effect of increasing concentration on either P^{+V} or As^{+V} was observed, suggesting that the binding affinity of the PZ binding resin towards P^{+III} is strong enough to suppress the influence of the elevated levels of P^{+V} and As^{+V} .

Accordingly, the proposed PZ-DGT method can be highly specific to P^{+III} , which is much more tolerant to pH, ionic strength and many other potential competing anions in the natural matrices, further confirming its wide suitability for measurement of P^{+III} . Similar results were reported by Guan et al. (2015) who found that the PZ-DGT for measurements of oxyanions such as P^{+V} , As^{+V} , Sb^{+V} etc. were very tolerant to variations of pH, ionic strength and potential interfering ions.⁴³

Figure 3. Effects of immersed solution pH (A), ionic strength (B), PO_4^{3-} (C) and AsO_4^{3-} (D) on the ratio of $\text{P}^{\text{+III}}$ concentrations measured by DGT, C_{DGT} , to concentration in deployment solution, C_{solution} . Dotted lines indicate the $\pm 10\%$ accuracy zone, the accuracy requirement of the DGT analysis is satisfied. All error bars represent the SD ($n=3$).

DGT Capacity. To ensure accurate calculation of analyte concentration using the DGT technique, it is essential that the adsorbent capacity is not exceeded during deployment. As **Figure 4** illustrates, the experimental accumulation of $\text{P}^{\text{+III}}$ onto the PZ gels used in DGT measurements initially increased linearly as a function of increased solution concentration and deployment time, and deviated only slightly ($< 5.0\%$) from theoretical accumulation. The measured capacity of PZ-DGT totalled $45.8 \mu\text{g cm}^{-2}$ and $46.4 \mu\text{g cm}^{-2}$, as judged by the significant deviations ($> 39.5\%$) of uptake from linearity at the concentration of 2 mmol L^{-1} or deployment time of up to 128 h. There were slight differences in the capacity values between the two sets of experiments. For such deployed natural situations with the occurring $\text{P}^{\text{+III}}$ concentration of less than $1 \mu\text{mol L}^{-1}$, this capacity was reached after only 231 days of deployment for $\text{P}^{\text{+III}}$, further confirming that PZ-DGT appears to be well suited for long-term deployment to assess natural samples far from saturation.

Figure 4. Accumulated masses of P^{+III} bound onto the PZ-gels (■) within DGT devices deployed in a synthetic freshwater as a function of P^{+III} concentrations (0-5 mmol L⁻¹) (A) and deployment times (0-216 h) (B). The red solid lines represent the theoretical prediction (•) calculated using the eq S2 in S1. All error bars represent the SD (n=3).

DGT Method Detection Limit. The DGT method detection limit (MDL) of P^{+III} at the three-sigma quality level, was derived from the standard deviation of the deployment blanks (0.15 ng per device). The resulting MDL for DGT-measured P^{+III} was 7.44 ng L⁻¹ for a deployment time of 72 h with a typical DGT device (with a 0.80 mm diffusive layer and 0.13 mm filter layer) at 25 °C and pH = 7.0. The MDL is sensitive enough for those reported sub-micromolar levels of natural P^{+III} ,^{1,45} indicating that the PZ-DGT is a robust tool for trace P^{+III} analysis. Furthermore, an enhanced sensitivity for those deployed situations with a P^{+III} concentration lower than the MDL can be achieved by prolonging the deployment period or reducing the thickness of the applied diffusion layer. This data shows that the method described here is very suitable for ultratrace analysis of P^{+III} .

Quantitative Assessment of P^{+III} in Samples of Environmental Interest. As a proof of principle, the present method was processed for a wide set of environmental

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398 samples (freshwater, wastewater and spiked soil). To the best our knowledge, this is
 399 the first attempt to use the DGT technique for detecting P^{+III} speciation in situ in the
 400 environment. Concentrations of P^{+III} measured by the developed method and the
 401 conventional sampling method are summarized in **Table 2**. P^{+III} concentrations in
 402 aquatic systems, i.e., Lake Taihu and wastewater samples measured by two methods,
 403 ranged from 0 to $13.36 \pm 1.85 \mu\text{g L}^{-1}$, which are comparable to previous studies.^{15,36-37}
 404 For all investigated waters, C_{DGT} values are generally higher than the corresponding
 405 C_{solution} for P^{+III} , with the average $C_{DGT} / C_{\text{solution}}$ ratios between 0.91 and 1.74. This is
 406 probably attributed to the diverse conversion pathway of P^{+III} (e.g. oxidation and
 407 microbial metabolism) produced in the conventional method.^{1,46} While, in the spiked
 408 soils, C_{DGT} is found to be lower than C_{solution} , with the average $C_{DGT} / C_{\text{solution}}$ ratios
 409 between 0.51 and 0.77, indicating that only a small subfraction of the entire dissolved
 410 P^{+III} fraction within the dissolved fraction is DGT-available. Indeed, the available
 411 solid P^{+III} fraction is in dynamical equilibrium with porewater P^{+III} and varies under
 412 different biogeochemical environments in soils. It is hypothesized that, the centrifugal
 413 extraction method invariably disturb this dynamic which can assist in measuring those
 414 P^{+III} DGT-inert species such as complexes, colloids, etc., and hence introduce the
 415 additional measurement discrepancies. For these reasons, the labile P^{+III} quantification
 416 by the PZ-DGT method is assumed to be preferred over the traditional extraction
 417 method as it can provide an accurate assessment of P availability in aqueous
 418 environments (freshwater and wastewater) and in soil.

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Comment [1]: Probably best not to use 0 here
but say lower than the method LOD. <X.XX

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Comment [2]: Just a minor point but the
relative standard dev from each of the sample
matrixes if averaged.. would be much lower in
the DGT than solution.

Table 2 Concentrations of P^{+III} ($\mu\text{g L}^{-1}$) measured in environmental samples of interest

Matrix	Location	^a C_{DGT}	^b C_{solution}
<i>Freshwater</i>	MeiLiang Bay	1.10 ± 0.15	0.91 ± 0.28
	Zushan Bay	1.77 ± 0.23	1.02 ± 0.36
<i>Wastewater</i>	Influent	10.65 ± 0.48	7.36 ± 1.85
	Effluent	3.89 ± 0.52	4.29 ± 0.72
<i>Soil</i>	Yixing	140.39 ± 0.85	182.64 ± 9.82
	Taizhou	133.63 ± 5.95	226.26 ± 5.33
	Suqian	110.74 ± 1.21	217.19 ± 20.40

^aC_{DGT} and ^bC_{solution} represent concentrations of P^{+III} measured by the proposed method and the conventional method,⁴⁶ respectively; all values are means ± SD, n = 3.

Conclusions. We developed a new approach, which combines DGT and DIC for the enhanced measurement of P^{+III} in soils/aqueous environments. This technique has considerable benefits over currently available methods. Using a DGT method, its merits of low-disturbance sampling, selectivity and preconcentration were firstly combined for the detection of redox-sensitive P speciation at ultratrace concentrations. This means that potential speciation changes associated with removing samples from their original states and from sample handing and analyzing are largely avoided. This is crucial when considered in the context of potential oxidation of P^{+III} to P^{+V} during sample processing confounding the study of P-redox chemistry and its relevant biogeochemical processes. Also, the DIC has a sensitivity and separation higher than currently available IC-based methods,^{1,18,39} which can further lower the limit of quantification and provide a more accurate estimation of P^{+III}. Employing the combined DGT-DIC method can effectively eliminate the need for many tedious and volume-cost pretreatments (e.g., centrifugation, filtration, and treatment with OnGuard cartridges) to reduce those P^{+III}-compromised variables (e.g., bacteria, minerals). Because of this method's satisfactory application in a wide variety of environments (i.e., pH 3.0–10, ionic strength, 0–500 mM NaCl, and less interferences from other oxyanions), it holds great potential for determining ultratrace P^{+III} in more complicated matrices. Furthermore, PZ-DGT was originally developed for measuring various oxyanions including P^{+V} and As^{+V} simultaneously owing to its high capacity.^{28,43} Combined to a routine procedure,⁴⁷ there is the possibility of using this method for simultaneously capturing the co-distributions of P^{+III} and P^{+V}, which is particularly important in helping to elucidate the complexity of P speciation and biogeochemistry in soil and aqueous environments.

AUTHOR INFORMATION

Supporting Information

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Supplementary text, tables and figures associated with this article can be available free of charge via the Internet at <http://pubs.acs.org>.

Corresponding Author

*Jun Luo Tel: (081)-025-89680632. Email: esluojun@nju.edu.cn.

**Xianchuan Xie Tel: (081)-025-86882207. Email: xchxie@nju.edu.cn.

Author Contributions

The article was written through contributions of all authors.

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