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# Evaluation of Titanium Dioxide as a Binding Phase for the Passive Sampling of Glyphosate and Aminomethyl Phosphonic Acid in an Aquatic Environment

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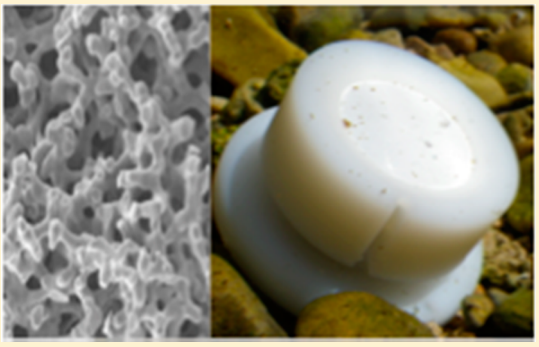
**ABSTRACT:** Glyphosate is the most widely used herbicide on a world scale for the last 40 years, for both urban and agricultural uses. Here we describe the first passive sampling method for estimating the concentration of glyphosate and AMPA (aminomethyl phosphonic acid, one of its major degradation products) in surface water. The sampling method is based on a newly developed configuration of the diffusive gradient in thin-film (DGT) technique, which includes a $TiO_2$ binding phase, already in use for a wide range of anions. Glyphosate and AMPA were retained well on a $TiO_2$ binding phase, and elution in a 1 mL of 1 M NaOH led to recoveries greater than 65%. We found no influence of pH or flow velocity on the diffusion coefficients through 0.8 mm polyacrylamide gels, although they did increase with temperature. $TiO_2$ binding gels were able to accumulate up to 1167 ng of P for both glyphosate and AMPA, and linear accumulation was expected over several weeks, depending on environmental conditions. DGT sampling rates were close to 10 mL $day^{-1}$ in ultrapure water, while they were less than 1 mL $day^{-1}$ in the presence of naturally occurring ions (e.g., copper, iron, calcium, magnesium). These last results highlighted (i) the ability of DGT to measure only the freely dissolved fraction of glyphosate and AMPA in water and (ii) the needs to determine which fraction (total, particulate, dissolved, freely dissolved) is indeed bioactive.



Passive sampling of aquatic environments has become increasingly common over the last 20 years for a wide range of contaminants.[1−3] Efforts were first concentrated on the passive sampling of hydrophobic and metallic contaminants dissolved in the water.[2,4] The sampling of hydrophilic contaminants started to be investigated more recently,[3,5] and that of very hydrophilic organic and ionic compounds (e.g., pharmaceuticals, perfluorinated compounds, acidic herbicides) is the most recent, with the first studies published only a few years ago.[6−10]

Among the most polar and ubiquitous contaminants, *N*-phosphonomethyl glycine (i.e., glyphosate or PMG) is heavily used for both agricultural and noncrop applications[11,12] and is thus measured with its major degradation product AMPA (aminomethyl phosphonic acid) in all water bodies.[12−15] Several papers have discussed how spot samples and passive sampling could spatially and temporally improve the reliability of the data set, by providing an estimation of the dissolved fraction.[16,17] The question is even more important for ionic compounds such as PMG and AMPA (amphoteric, highly polar; p$K_a$ PMG, 0.7, 2.2, 5.9, 11; p$K_a$ AMPA, 0.9, 5.6, 10.2) because speciation with metal cations could plays an important role in their mobility, availability, and ecotoxicity.[18−20]

The speciation of inorganic pollutants is often studied using the diffusive gradient in thin-film (DGT) technique.[2] Chen et al. recently developed this technique for polar and ionic organic compounds, since they are able to diffuse across the diffusive gel (unlike medium polar or nonpolar compounds).[10,21] In addition, several papers showed the benefits of using a titanium dioxide ($TiO_2$) binding phase over conventional iron oxide receiving phase for trapping anionic species in aquatic media, including phosphates.[22−24] Many studies showed the importance of measuring freely dissolved phosphates, because it is assumed to be the reactive fraction (filterable reactive phosphates).[25] Considering similarities between PMG and phosphates in terms of chemical structure and reactivity, we could expect comparable fate and behavior in the environment. The need for a passive sampler for such compounds is thus of importance, taking into account recent work showing that the

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joint presence of PMG and copper reduced their respective toxicity on invertebrates (earthworms),[19,26] suggesting thus that complexes are less bioactive.

This work aims to evaluate the applicability of the DGT approach for determining freely dissolved PMG and AMPA in water. First, we adapted and validated a robust and sensitive analytical method, based on published studies,[27,28] which has now been normalized by the International Organization for Standardization (ISO). Second, affinities with $TiO_2$ material and elution recoveries were investigated in relation to the specific affinity of $TiO_2$ for phosphorylated compounds.[29−32] Then, diffusion coefficient measurements were performed using a diffusion cell, and finally, the gel capacity as well as the influence of natural freshwater ions on DGT uptake were measured.

## EXPERIMENTAL SECTION

**Chemicals.** Acrylamide, ammonium hydroxide (25% $NH_3$), ammonium persulfate, 9-fluorenylmethylchloroformate (FMOC-Cl), formic acid, sodium borate, sodium chloride, sodium ethylenediaminetetraacetic acid ($Na_2EDTA$), sodium hydroxide (NaOH), tetramethylethylenediamine (TEMED), titanium(IV) oxide ($TiO_2$, particle size <325 mesh or 44 $\mu$m), and triethlyamine (TEA) were purchased from Sigma-Aldrich (Schnelldorf, Germany). Methanol (MeOH), acetonitrile (ACN), and ethyl acetate (EtAC) were obtained from Sharlau (HPLC grade, AtlanticLabo, Bruges, France). Ultrapure water (UPW, resistivity >18 MΩ) was produced with a Synergy UV system from Millipore (Billerica, MA, U.S.A.). Analytical standards were purchased from Dr. Erhenstorfer GmbH (Augsburg, Germany, purity >98%): glyphosate; AMPA; glyphosate-$^{13}C_2$,$^{15}N$; AMPA-$^{13}C$,$^{15}N$. Monomolecular stock solutions were prepared in UPW (100 $\mu$g mL$^{-1}$) and stored at 4 °C for less than 6 months. Working solutions (10, 20, 100, and 1000 ng mL$^{-1}$) were also prepared in UPW and stored at 4 °C for up to 3 months. DGT gel cross-linker 2% aqueous solution was purchased from DGT Research Ltd. (Lancaster, U.K.).

**Theory of DGT.** DGT is composed of a receiving phase ($TiO_2$) separated from the aquatic medium by a diffusive hydrogel (controlling the contaminant uptake rate), protected by a prefilter. The time-weighted averaged water concentration ($C_w$) can be calculated using the mass of analyte accumulated in the DGT receiving phase ($M_s$), the thickness of the diffusive gel ($\Delta g$), the diffusion coefficient in the selected diffusive material ($D$), the duration of exposure ($t$), and the exposure window area ($A$):

$$C_w = \frac{M_s \Delta g}{DtA} \quad (1)$$

$D$ can be determined using a diffusion cell (see next section), or using a calibration experiment (see Calibration Experiment section). $D$ depends mainly on temperature and can be deduced from a reference value using the water viscosity ($\eta$):[24]

$$\frac{D_1\eta_1}{T_1} = \frac{D_2\eta_2}{T_2} \quad (2)$$

The sampling rate ($R_s$) is defined as the volume of cleared water per unit time. It can be represented as the slope of $f(t) = (M_s/C_w)$, expressed as follows:

$$R_s = \frac{DA}{\Delta g} \quad (3)$$

**Diffusion Cell Experiment.** A diffusion cell was set up according to the recommendations of Zhang and Davison and Chen et al.[10,33] Two 0.38 L poly(vinyl chloride) compartments were separated by 1.77 cm$^2$ opening window containing the hydrogel. Each compartment was filled with UPW and stirred vigorously using magnetic stirrers to minimize the diffusive boundary layer effect. The room temperature was 22 ± 0.5 °C. We also carried out experiments at 11 and 33 ± 0.5 °C using a water bath containing either ice or hot water. The pH was adjusted using NaOH or HCl. The leakage flow between the two compartments of the cell was measured periodically and was always <0.2 mL day$^{-1}$, i.e., <3% of $D$ for a 0.8 mm polyacrylamide gel. All diffusion cell experiments included a 0.45 $\mu$m poly(ether sulfone) prefilter. The source compartment concentration was checked at the beginning and at the end of the experiment (≈300 $\mu$g L$^{-1}$), whereas the receiving compartment concentration was measured every 40 min for 200 min ($n$ = 6). The method for determining diffusion coefficients has been described previously by Zhang and Davison using eq 1.[2]

**Calibration Experiment.** Laboratory calibrations were performed in 3 L plastic containers in a dark room. Temperature was 20 ± 2 °C. A preliminary experiment showed no losses of PMG or AMPA for 1 week under these conditions. Duplicate DGT-$TiO_2$ were removed after 0.14, 1, 2, 4, and 6 days. Stirring was performed with magnetic stirrers. Calibrations were performed in both UPW (pH 6.5) and synthetic water (pH 7.4). Analyte concentrations were set at 50 $\mu$g L$^{-1}$. The composition of the synthetic freshwater is reported in Table 1.

**Table 1. Composition of Synthetic Freshwater**[a]

| ion | reference value | $t_0$ | $t_f$ |
|---|---|---|---|
| $Ca^{2+}$ | 94 | 108 | 117 |
| $Mg^{2+}$ | 8.7 | 27 | 30 |
| $Na^+$ | 16 | 116 | 125 |
| $K^+$ | 3.8 | 35 | 38 |
| $Cl^-$ | 28 | 196 | 213 |
| $HPO_4^{2-}$ and $H_2PO_4^-$ | 0.19 | 0.11 | 0 |
| $HCO_3^-$ | 256 | 350 | 428 |
| $SiO_2$ | 11 | 14 | 15 |
| $NO_3^-$ | 50 | 52 | 54 |
| $Fe^{2+}$ | 0.27 | 0.04 | 0.06 |
| $Cu^{2+}$ | 3 | 0.26 | 0.31 |
| $SO_4^{2-}$ | 33 | 112 | 118 |

[a]The reference value is based on the 3rd quartile of concentrations measured in 1000 French rivers over the 2004−2009 period (French monitoring network). Concentrations are expressed in milligrams per liter. $t_0$ and $t_f$ are the concentrations at the beginning and at the end of the calibration experiment.

**Passive Sampler Procedure.** Diffusive and binding gels were prepared according to the method described by Zhang and Davison and adapted by Bennett et al. and Panther et al. for the $TiO_2$ binding phase.[2,23,34] Several minor modifications concerned the use of poly(ether sulfone) prefilters (0.45 $\mu$m, 25 mm, Sigma-Aldrich, France) instead of cellulose nitrate and a different titanium oxide supplier (i.e., Aldrich, with similar particle size and purity). DGT housings were purchased from


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Table 2. MS/MS Parameters

| analytes | quantitative transition | DP (V)[a] | CE (V)[b] | CXP (V)[c] | qualitative transition | DP (V)[a] | CE (V)[b] | CXP (V)[c] |
|---|---|---|---|---|---|---|---|---|
| PMG-FMOC | 390 > 168 | −15 | −20 | −15 | 390 > 150 | −15 | −40 | −15 |
| AMPA-FMOC | 332 > 110 | −20 | −15 | −10 | 332 > 136 | −20 | −20 | −15 |
| PMG-FMOC ($^{13}C$,$^{15}N$) | 393 > 171 | −15 | −20 | −15 | 393 > 153 | −15 | −40 | −15 |
| AMPA-FMOC ($^{13}C$,$^{15}N$) | 334 > 112 | −20 | −15 | −10 | 334 > 138 | −20 | −20 | −15 |

[a]Declustering potential. [b]Collision energy. [c]Cell exit potential.

DGT Research Ltd. Immediately after exposure, binding gels were transferred into 15 mL polypropylene tubes (Greiner Bio One, Sodipro, France) and eluted with 1 mL of 1 M NaOH for 24 h. The extracts collected were then diluted with 4 mL of 1.5% (v/v) formic acid prior to derivatization and preconcentration (see next section).

**Derivatization Step.** The method used here followed the recommendations of the project ISO 16308:2014 (Water Quality—Determination of PMG and AMPA—Method using high-performance liquid chromatography (HPLC) with tandem mass spectrometric detection). Briefly, 5 mL of freshwater sample was transferred into 50 mL polypropylene tubes and spiked with 50 $\mu$L of both PMG and AMPA $^{13}C$,$^{15}N$ isotopes at 20 ng mL$^{-1}$ (surrogates). Then, 325 $\mu$L of 50 mM sodium borate and 200 $\mu$L 0.1 M $Na_2EDTA$ were added. The sample was homogenized and left to stand for 5 min. Amounts of 4.5 mL of ACN and 600 $\mu$L of FMOC-Cl (50 mg mL$^{-1}$) were added, and the sample was left for 30 min in the dark at room temperature (formation of FMOC derivatives). Then, ACN was evaporated off with $N_2$ for roughly 1 h (until sample volume was <5 mL). After that, the derivatized extract was transferred to a 15 mL graduated glass tube and liquid−liquid extractions were performed with 3 × 1.5 mL ethyl acetate. The remaining ethyl acetate was removed using a $N_2$ stream for 15 min. An amount of 100 $\mu$L of formic acid 5% was added, and the sample volume was adjusted to 5 mL and homogenized. The sample extract was then loaded onto Oasis HLB cartridges (3 mL, 60 mg, 30 $\mu$m particle size, Waters, Guyancourt, France) after a conditioning step (1 mL MeOH followed by 1 mL formic acid 0.1%). After sample loading, the cartridge was washed with 1 mL of 0.1% formic acid and 1 mL of UPW, dried with $N_2$, and eluted in with 2 mL of ammonium hydroxide/UPW/MeOH 2:30:68 (v/v/v). The collected extract was then evaporated until the volume stabilized at 0.5 mL. Finally, the volume was adjusted to 1 mL with UPW, and 50 $\mu$L was injected into the HPLC system.

**Apparatus.** Analyses were performed by HPLC−ESI-MS/MS (Dionex Ultimate 3000, ABSciex API 2000). Reversed-phase separation was chosen (Waters X-Bridge $C_{18}$ 3.5 $\mu$m, 2.1 mm × 50 mm protected by a precolumn X-Bridge $C_{18}$ 2.1 mm × 10 mm; Le Pecq, France). The mobile phase was composed of 0.1% TEA (A) and ACN (B). The analytical gradient was as follows: 92:8 (A/B) from 0 to 1.5 min, increasing B linearly to 95% between 1.5 and 3 min, maintaining B at 95% for 1.5 min, then decreasing B linearly to the initial conditions between 4.5 and 6 min, and finally equilibrating the column for 5 min (11 min run). The mobile phase flow rate was set at 400 $\mu$L min$^{-1}$, and the column oven temperature was 40 °C. Mass acquisition was performed using selected reaction monitoring (SRM) and negative ESI mode (mass parameters are listed in Table 2). A positive chromatogram is shown in Figure 1. External quantification was performed using a six-point calibration (from 0.05 to 10 ng mL$^{-1}$). PMG and AMPA standards were derivatized according to the method described in the Derivatization Step section. Limits of quantification (LOQ) were validated at 0.03 ng mL$^{-1}$ for both compounds, and LOQ were 5 times higher.

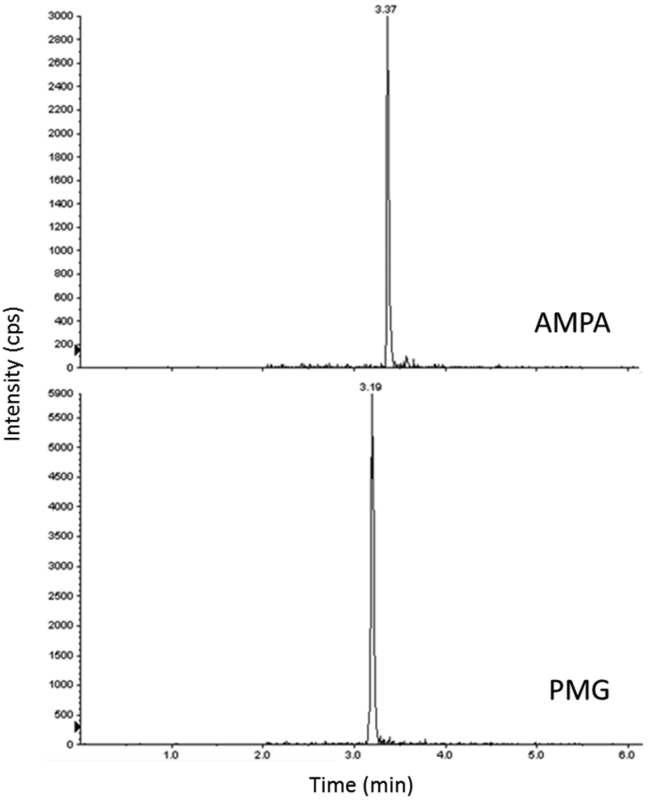


**Figure 1.** HPLC−ESI-MS/MS chromatogram, Waters X-Bridge $C_{18}$ 3.5 $\mu$m, 2.1 mm × 50 mm, precolumn X-Bridge $C_{18}$ 2.1 mm × 10 mm. Gradient conditions: (A) TEA 0.1%, (B) ACN; 92:8 from 0 to 1.5 min, 5:95 at 3 min, 5:95 from 3 to 4.5 min. Detection MS AMPA 332 > 110 PMG 390 > 168.

**Quality Assurance and Quality Control.** Calibration linearity (from 0.05 to 1.5 ng mL$^{-1}$), specificity (standard additions at five different levels measured in river water not significantly different from theoretical values), extraction recoveries (93.7% ± 3.8% and 92.1% ± 5.5% for PMG and AMPA, respectively), and limits of quantification (0.03 ng mL$^{-1}$ for both compounds) of the method were validated according to French standard NF T90-210. The quantification of isotope-labeled surrogates ensured the accuracy of each analysis (derivatization step and HPLC analysis). Additionally, derivatization and passive sampler blanks were analyzed (always <LOD). The periodic control of two calibration points (0.1 and 1.5 ng mL$^{-1}$, every 10 samples) and one blank ensured accuracy of analysis, while surrogates ensured the purity of each sample.


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## RESULTS AND DISCUSSION

**Retention and Elution Recovery of PMG and AMPA on $TiO_2$.** The first aim of this study was to evaluate the affinity of PMG and AMPA for the $TiO_2$ binding gels and then measure the elution efficiency using the commonly used elution fraction of 1 mL of 1 M NaOH.[23,34] Six polypropylene tubes were filled with 12 mL of 1 ng $mL^{-1}$AMPA and PMG, and one $TiO_2$ binding gel (25 mm diameter, 0.4 mm thickness) was added to three of them. Sample volumes of 5 mL were taken to evaluate the concentration at $t_0$, and the tubes were then shaken for 24 h at room temperature to equilibrate the system. At the end of the experiment, 5 mL were sampled again in order to determine the concentration in water at $t_{final}$ and the gels were eluted for 24 h using 1 mL of 1 M NaOH. The derivatization step described in Experimental Section was applied to all water samples. No significant variations of AMPA or PMG concentrations were observed in the three controls between $t_0$ and $t_{final}$, whereas $t_{final}$ concentrations from tubes containing $TiO_2$ were found to be lower than the limit of detection (LOD). Thus, we were able to determine the elution efficiency for both analytes (Table 3) and found acceptable values (i.e., 69% and 86% for AMPA and PMG, respectively). This first experiment showed the relevance of using the $TiO_2$ material to retain PMG and AMPA. Chelation seems to be the major interaction process between the phosphonic group and the titanium dioxide surface (similar to the interactions described for phosphates).[29−32]

**Table 3. Elution Recoveries of AMPA and PMG from $TiO_2$ Binding Phase with 1 M NaOH ($n$ = 3)**

| | AMPA | PMG |
|---|---|---|
| accumulated mass (ng) | 9.8 ± 2.4 | 6.9 ± 1.5 |
| eluted mass from gel (ng) | 6.8 ± 0.8 | 5.9 ± 0.7 |
| elution recovery (%) | 69 ± 19 | 86 ± 21 |

**Influence of pH, Flow Velocity, and Temperature on Diffusion Coefficient.** Diffusion coefficient measurements were first performed using a diffusion cell in order to rapidly determine the influence of the major parameters on analyte diffusion itself (Figure 2). First, the experimental data (between 2 and 5 × $10^{-10}$ $m^2$ $s^{-1}$) are in good agreement with those determined for some antibiotics through agarose (0.58−6.24 × $10^{-10}$ $m^2$ $s^{-1}$) and for phosphates through polyacrylamide (6.05 × $10^{-10}$ $m^2$ $s^{-1}$ at 25 °C) in previous studies.[35,36] Second, we found a higher diffusion coefficient for PMG through polyacrylamide than through agarose. Thus, agarose matrix was not investigated more deeply. Third, we did not observe a large influence of pH (between 5 and 8.5) on $D$. Only one p$K_a$ is in the pH range tested (5.9 and 5.6 for PMG and AMPA, respectively, deprotonation of the phosphonic group), which seems to have limited impact on analyte transfer. pH could, however, affect the PMG and AMPA binding on $TiO_2$. However, previous research showed no effect of pH on phosphate measurements by DGT, although two forms of phosphate were represented in the pH range tested.[34,36−38] As we could expect the same interaction processes between phosphates, PMG, AMPA, and $TiO_2$, further investigations could make a focus on this point. Fourth, similar diffusion coefficients were found under quiescent and stirred conditions. As the diffusive boundary layer thicknesses is the same for PMG, AMPA, and inorganic species (although organic compounds have higher steric hindrance), a 0.8 mm gel is sufficient to ignore the influence of hydrodynamics.[2] Finally, an increase of $D$ was measured for both PMG and AMPA with increasing temperature, also in agreement with the literature.[2] From this set of data, we estimated a $D$ value at 20 °C of 2.95 ± 0.35 × $10^{-10}$ and 3.50 ± 0.25 × $10^{-10}$ $m^2$ $s^{-1}$ for PMG and AMPA, respectively. It is interesting to note that diffusion coefficients decrease with increasing molar mass, also in agreement with $D$ values measured for phosphates in earlier studies.[36−38]

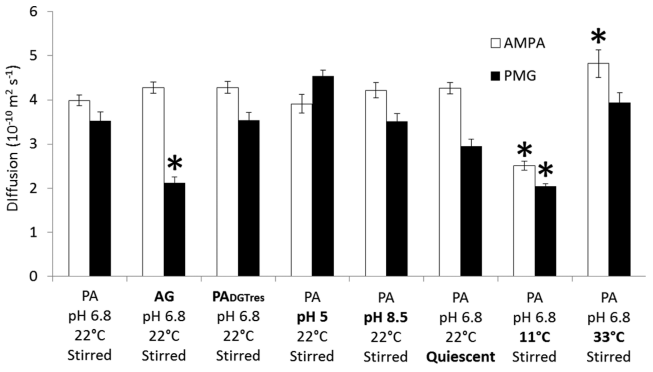


**Figure 2.** Diffusion coefficients of PMG and AMPA through self-made PA (polyacrylamide, noted PA), PA obtained from DGT Research (noted $PA_{DGTres}$), and agarose obtained from DGT Research (noted AG). Variable conditions of pH (5, 6.8, 8.5), temperature (11, 22, 33 °C), and turbulence (stirred, quiescent) evaluated for PA only. Stars highlight $D$ values that are different (Kruskal−Wallis, $p$ < 0.01) from the reference (PA, pH 6.8, 22 °C, stirred).

**Gel Binding Capacity.** A 6 day DGT-$TiO_2$ calibration in highly concentrated medium (1 mg $L^{-1}$ of both PMG and AMPA, high level of environmental concentration) allowed the determination of the maximum capacity of $TiO_2$ binding gels in environmental conditions. The mass of analyte accumulated in the receiving phase reached a plateau after only 2 days in the operating conditions we fixed. A nonlinear regression was used to model the experimental data and precisely determine the value of the plateau for each compound (Figure 3, 2673 ± 99 and 2424 ± 77 ng per device for PMG and AMPA, respectively). The observed capacity of one gel is thus 1167 ng of P. Panther et al. determined a 30-fold higher value using Metsorb-DGT (i.e., $TiO_2$-based receiving phase).[34] This could be accounted for by (i) the different P concentration applied

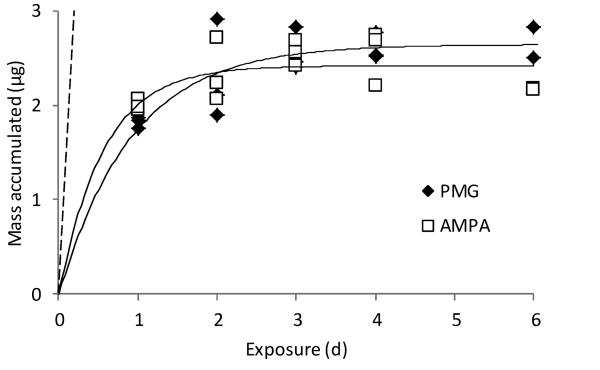


**Figure 3.** DGT-$TiO_2$ capacity experiment for AMPA and PMG 1 mg $L^{-1}$ over 6 days under environmental conditions. Dashed line is the theoretical flux of PMG.


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(isotherms study would be needed to determine if the plateau is actually the maximum capacity or a given equilibrium state) and (ii) the different $TiO_2$ supplier (Graver Technology vs Sigma-Aldrich) implying a different specific surface area (data not available from either supplier), initial moisture content, etc., and by the different steric hindrance of the studied analytes. Indeed, the smaller molar mass of phosphate could make a wider range of interaction sites available. However, Randon et al. showed no influence of the functional group (e.g., methyl vs phenyl) associated with the phosphonic acid on the amount adsorbed on $TiO_2$ particles.[39] The global mass ratio analyte/$TiO_2$ is around 0.8‰ (considering 6.5 mg of $TiO_2$ per gel), which is far from the mass ratio generally considered with polymeric receiving phases with high specific surface areas generally used to retain organic compounds (e.g., about 1% for poly(styrene−divinylbenzene) 1000 $m^2$ $g^{-1}$). Thus, a special attention should be paid on accumulation linearity depending on the exposure conditions.

**Influence of Naturally Occurring Ions.** Natural ions (e.g., phosphate, chloride, sulfate, carbonate, nitrate, calcium, magnesium, iron, copper) may interfere and compete with $TiO_2$ for chelation with PMG and AMPA (i.e., cations) and with PMG and AMPA for binding on $TiO_2$ interaction sites (i.e., anions). Thus, two calibrations were set up simultaneously in order to measure the effect of natural ions, by comparing the accumulation of PMG and AMPA in DGT-$TiO_2$ from UPW and from a synthetic freshwater (Figure 4) with realistic freshwater ion concentrations. First, we observed that the first sampling point (0.14 day or 3.36 h) is in agreement with the overall kinetics, meaning that the binding is fast enough to consider appropriated $TiO_2$ receiving phase for the sampling of PMG and AMPA. Sampling rates (i.e., $R_s$'s, slope of $f(t) = M_s/C_w$, eq 3) measured in UPW were very similar to theoretical ones (i.e., 8.3 vs 10.0 and 11.6 vs 11.9 mL $day^{-1}$ for PMG and AMPA, respectively, eq 3 with $D$ measured with the diffusion cell, $A$ = 3.14 $cm^2$ and $\Delta g$ = 0.08 cm). On the other hand, $R_s$'s measured in synthetic freshwater medium were about 30 times lower than those measured in UPW (i.e., 0.28 and 0.39 mL $day^{-1}$ for PMG and AMPA, respectively). These observations are well-illustrated by the comparison with the theoretical flux (Figure 4). This finding can be explained by several parameters: the difference in ionic strength or pH, the charge effect due to the low ionic strength of UPW,[33,40] and the effect of competing ions in the synthetic water. First, as pH and ionic strength do not seem to have a strong effect on phosphates accumulation, it is likely that they have no effect on PMG and AMPA, as $pK_a$'s are similar and the interactions involved should be the same.[34,36−38] Second, as sampling rates measured in UPW are very close to the theoretical ones based on the diffusion experiments, we could conclude that the effect of charge is negligible. Third, as we do not observe any lag phase or plateau, it is likely that competition between PMG, AMPA, and other anions did not occur. Therefore, the difference in sampling rates observed in UPW and synthetic water should be explained by competition phenomena between metal cations and TiO2 for chelation with PMG and AMPA. Indeed, multivalent cations (e.g., copper, iron, calcium, magnesium) complex strongly with PMG and AMPA[19,26,27] and could thus decrease the available DGT-$TiO_2$ concentration. Considering the log $K$ values mentioned by Freuze et al. and calculated by Popov et al. (e.g., 15.6 and 2.6 for $Cu^{2+}$ with PMG and AMPA, respectively),[27,41] together with our operating conditions (neutral pH, large excess of $Ca^{2+}$, $Fe^{2+}$, $Cu^{2+}$), the M + L + H forms are thermodynamically favored, and we thus predicted that no free form of either PMG or AMPA would be find in the aqueous media. However, as a small part of the contaminants was sampled in synthetic water ($R_s$'s in synthetic water were about 3.5% that determined in UPW), it is likely that the contaminant amount accumulated in DGT-$TiO_2$ only came from complexes that dissociated within the diffusive gel (ML + $TiO_2$ → M + $TiO_2$L), because interactions with $TiO_2$ could be stronger than those occurring with all other cations in the solution.[2] Panther et al. showed very small differences between the amounts of phosphate accumulated in UPW and in synthetic waters, but metals were not included in the naturally occurring ion list.[34] Otherwise, as the receiving phase was far from saturation, we were not able to evaluate the effect of commonly found anions (e.g., phosphates, carbonates, sulfates, chlorides) on the maximum mass accumulated. Such differences in analyte amount sampled in both operating conditions may equally question the amount of analyte bioavailable in the environment. Speciation of organic and ionizable contaminants should therefore be addressed in order to be able to monitor the most relevant contaminant fraction (i.e., dissolved, particulate, complexed, etc.). Indeed, the entire dissolved fraction of organic contaminants is generally considered bioavailable, but we show here that only a small subfraction within the dissolved fraction is DGT-available. Thus, approaches comparable to those developed for phosphates and other inorganic species, including the concepts of freely dissolved, filterable reactive, and total reactive fractions, will be required to better understand and predict the ecotoxicology of

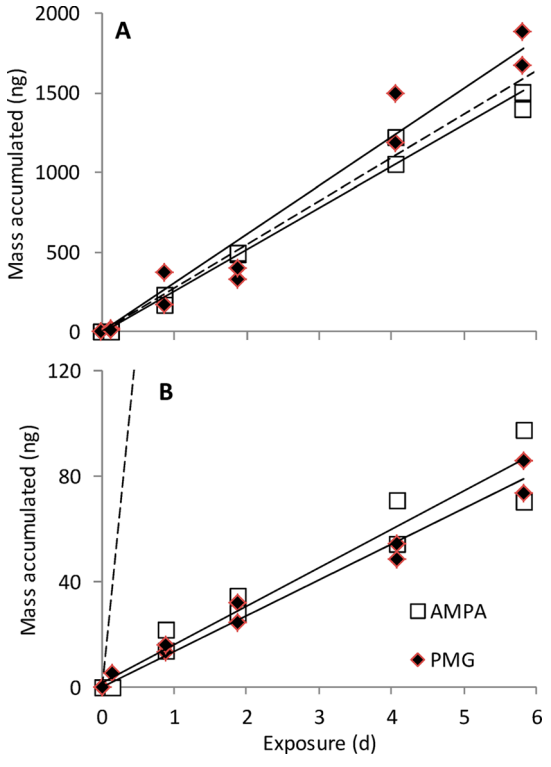


**Figure 4.** Calibration of DGT-$TiO_2$ for AMPA and DGT in UPW (A) and synthetic freshwater (B). Mass accumulated expressed as a function of exposure duration. Dashed line is the theoretical flux of PMG. The concentrations of PMG and AMPA in the microcosm were between 28 and 39 $\mu g$ $L^{-1}$.



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contaminants such as PMG and AMPA, depending on the ionic environment of such analytes.[25,42]

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### Notes

The authors declare no competing financial interest.

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