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Assessment of the DGT technique in digestate to fraction twelve trace elements

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Abstract

This study proposes an evaluation of the diffusive gradients in thin films technique (DGT) for studying trace elements in digested sewage sludge samples. Twelve elements were monitored by Chelex (Al, Cd, Co, Cr (III), Cu, Fe, Mn, Ni, Pb) and zirconia-DGT (As, Mo, Se) samplers exposed from 4 hours to 9 days. Twenty-four hours' deployment time was suitable for most of the studied elements. However, short deployment led to insufficient element accumulation or non-establishment of steady state while long deployment (from 18 to 144h depending on the element) led to saturation of the binding gels and/or competing effects with other major elements. In addition, this study showed that the matrix of the digested sewage sludge lowers the accumulation of some trace elements in the DGT samplers, leading to labile concentrations underestimation of roughly 10-30% (depending on the element). Moreover, compared to the conventional total dissolved elements measurement, DGT technique allowed to quantify 7 out of 12 labile elements whereas only 3 out of 12 dissolved elements were quantified. These results highlight the potential of DGT technique to assess labile trace elements in digestate samples, provided a careful adaptation of the deployment time as well as an evaluation of the matrix effect is performed.

Keywords: Digested sewage sludge; Passive sampling; Matrix interferences; Metals; Metalloids; Speciation.

Introduction

Knowledge regarding trace elements' speciation is fundamental to assess their bio-accessibility in digestate. Given the complexity of the matrix and wide diversity of metal species (*e.g.* complexes, precipitates...) encountered in digestate, fractionation approaches are commonly used for metal speciation purposes [1].

Few studies [2–4] attempted to fractionate trace elements in digested sewage sludge by chemical sequential extraction procedures to determine the degree of leachability of different trace elements' species. Zhu et al. [4] underlined that sequential extraction methods could be used for environmental risk assessment of digestate as a soil fertilizer. However, Bacon and Davidson [5] have questioned the usefulness of sequential extraction procedures to fractionate trace elements. The authors highlighted some limitations in quantifying trace elements associated with several mineral phases extracted during these procedures. Such limitations include the re-distribution of the element among the mineral phases and precipitation during the extraction, the non-selectivity of the reagents to the targeted phases and their incomplete extraction [5].

To overcome the limitations of sequential extraction procedures, in a recent paper, Thanh et al. [6] identified the diffusive gradients in thin films technique (DGT) as a promising technique to determine bio-accessible metal concentrations in anaerobic bioreactors. This technique allows sampling labile trace elements after diffusion through a gel and accumulation on a binding gel in the DGT device [7]. The labile elements comprise free ions and weakly bound complexes and thereby would represent the most readily bio-accessible species of trace elements [7]. Recently, Bourven et al. [8] demonstrated a link between DGT-labile Cd concentrations and biogas production as well as enzymatic activities during whey anaerobic digestion. However, DGT use in digestate is only emerging and, to our knowledge, only Takashima et al. [9] has used the DGT

technique to measure labile Co and Ni species in a digested sewage sludge filtrate. Currently, no methodological development has been performed to adapt this technique to the digestate matrix. Moreover, the use of DGT is not straightforward in such complex matrix (*e.g.* multi-element contamination, high organic content) and requires preliminary validation or adaptation of the procedure.

We sought to investigate the potential of DGT as a fractionation tool for twelve trace elements (Al, As, Cd, Co, Cr (III), Cu, Fe, Mn, Mo, Ni, Pb and Se) in anaerobic digestate. Experiments were performed to validate the principles of the method in this complex biological matrix and to investigate potential organic matter interferences on trace elements' accumulation in DGT devices. Moreover, to discriminate large labile complexes from small ones, we performed fractionation based on the size of trace elements by using two different diffusive layers in our DGT devices.

The outcomes of this research work will highlight the benefits and limitations of using the DGT tool to assess labile trace elements in digestate samples and we offer recommendations to help establishing robust DGT deployment methods in digestates.

1. Materials and methods

1.1 Digested sewage sludge sample

Digested sewage sludge was collected from a municipal waste-water treatment plant in Limoges, France. About 20 L of sample was collected in June and September 2017. The sample was collected in polypropylene (PP) tanks up to maximum capacity and closed with a lid to limit sample oxidation from dioxygen in the air. Later, they were stored at 4°C for less than 24 hours before starting the experiments.

1.2 DGT preparation

Two different DGT samplers were used during this study: Chelex-DGTs for cationic species (Al, Cd, Co, Cr (III), Cu, Fe, Mn, Ni and Pb) and zirconia-DGTs (Zr-DGTs) for anionic species (As, Mo and Se). The selectivity of Chelex-DGT sampler over the oxidation state of Cr species was previously demonstrated by Ernstberger et al. [10]. Each DGT consisted of a binding gel, a diffusive gel and a filter membrane enclosed in a piston type holder, the latter purchased from DGT Research (Lancaster, UK). Chelex binding gels were prepared according to the procedure described by Zhang et al. [11], whereas Zr binding gels were made according to Devillers et al. [12].

Unless stated otherwise, the DGT samplers were equipped with a standard polyacrylamide diffusive gel (15% acrylamide and 0.3% agarose-derived cross linker, 0.77 mm thick), prepared according to Zhang et al. [11]. In addition, the use of restricted diffusive gels (15% acrylamide and 0.75% bisacrylamide cross linker, 0.75 mm thick) with pore size <1 nm [13] was investigated. The gels were prepared following a procedure slightly modified from Scally et al. [14]. The polymerization was performed by mixing 200 µL of 10% (m/V) freshly prepared ammonium persulfate (Fisher Scientific) and 8 µL of tetramethylethylenediamine (TEMED)

(Aldrich) with 10 mL of gel solution (15% acrylamide and 0.75% bisacrylamide cross linker).

The full procedure is described in supporting information.

Protective membranes of 0.4 μm pore size Nuclepore® in polycarbonate (0.02 mm thickness, Whatman, UK) or 0.2 μm pore size cellulose acetate membrane (0.12 mm thickness, Whatman, UK) was placed on the top of the diffusive gel.

1.3 Experimental set-up

1.3.1. Optimization of DGT samplers' deployment time

About 20 L of digested sludge was poured into a PP container and continuously stirred with an overhead plastic propeller at 30 rpm. A Tinytag data logger (TG-4100, Gemini Data Loggers, UK) was used to record the temperature in the sample. To avoid changes of trace elements speciation, the sample was kept in anaerobic conditions by covering its surface with paraffin oil and a plastic film.

Two different experiments were performed: a “short term” one to validate the establishment of steady state conditions in the samplers, and a “long term” one to increase the sensitivity of the method. In detail, triplicate devices of both Chelex and Zr were deployed for 4, 8, 18 and 24 hours (“short term” experiment) or for 24, 48, 72, 144 and 216 hours (long term experiment). A representation of the experimental set-up is shown in Figure 1. Before starting the experiment, the devices were immersed overnight in nitrogen flushed ultrapure water to remove oxygen from them.

Fig 1. On the left, the pilot scale tank containing the digested sludge. On the right, a scheme of the deployment time of the Chelex and Zr-DGT samplers.

1.3.2. Potential interference from digestate matrix on trace elements accumulation

To evaluate the potential interference from the digestate matrix on the diffusion and accumulation of trace elements in the binding gels, the Chelex and Zr-DGT samplers were exposed in triplicate to the digestate sample for 24 hours to load their diffusive gels with the digestate matrix. The pre-exposed diffusive gels were then recovered to build new DGT samplers with new Chelex and Zr binding gels (henceforth named “soiled” DGT samplers). Additionally, triplicate DGT samplers were built with new diffusive and binding gels as control in the experiment.

All Chelex-DGT samplers (control and soiled) were immersed in 1.5 L of 10^{-2} M NaCl solution spiked with cationic elements (Cd (II), Co (II), Cu (II), Ni (II) and Pb (II)) for 4 hours under continuous stirring. Al (III), Cr (III), Fe (II) and Mn (II) were not added in the synthetic solution since they tend to precipitate. The control and “soiled” Zr-DGT samplers were deployed for 4 hours under continuous stirring in a second beaker, containing 1.5 L of 10^{-2} M NaCl spiked with anionic elements (As (III), Mo (VI) and Se (IV)) and flushed with N_2 to avoid oxidation of the

elements. The total concentrations of the elements were chosen to be either quantifiable or comparable to the studied digestate samples. The conditions of the experiments (pH, temperature and element concentration) are summarized in Tables S1 and S2.

To check the contamination of the binding gel brought by the “soiled” diffusive gel, three blank DGT samplers were built with “soiled” diffusive gels and new Chelex and Zr binding gels. The blanks were stored at room temperature ($20\pm1^{\circ}\text{C}$) in a moistened plastic bag and disassembled after 4 hours alongside the other samplers.

For statistical analysis of the results, a F-test was performed using Microsoft Excel 2013 to determine the variances of the two sets of samples, then the two-tailed t-test was applied at 95% confidence interval.

1.3.3. Size fractionation of labile elements

Fractionation of labile elements based on their size was investigated through the simultaneous deployment of DGT samplers equipped with restricted or standard diffusive gels. The Chelex and Zr-DGT samplers were deployed for 24 hours in 20 L of digested sludge sample continuously stirred at 30 rpm. The deployment time was chosen according to the results obtained from the experiment described in 1.3.1.

1.4 Analytical procedures

1.4.1 DGT-labile concentration

After retrieval, DGT samplers were rinsed with ultrapure water and disassembled to recover the binding gels. The accumulated mass (m) of trace elements in each DGT sampler was determined after elution of the binding gel. The Chelex binding gels were eluted in 2 mL of 1 M HNO_3 for 24 hours and the Zr binding gels in 2 mL of $5\cdot 10^{-3}$ M NaOH and 0.5 M H_2O_2 for 24 hours. Then the concentration of trace elements in the eluents (C_e) were quantified by the inductively coupled

plasma mass spectrometry (ICP-MS) or microwave plasma atomic emission spectroscopy (MP-AES) (see section 1.4.3). The accumulated mass is determined according to equation (1) [15]:

$$m = \frac{C_e \times V_e}{f_e} \quad , \quad \text{Eq. (1)}$$

where V_e is the volume of the eluents (2 mL) and f_e is the elution factor (values are reported in Table S3).

The concentration of labile trace elements, C_{DGT} , in the sample is then derived using equation (2) based on Fick's first law [16]:

$$C_{DGT} = \frac{m \times \Delta_{MDL}}{D \times t \times A} \quad , \quad \text{Eq. (2)}$$

where Δ_{MDL} is the thickness of the material diffusion layer (*i.e.* diffusive gel plus membrane), t is the time of DGT samplers' exposure in the sludge, D is the coefficient of diffusion of the considered element in the diffusion layer and A is the geometric area of the DGT holder window (3.14 cm^2). The values of D were corrected for the average temperature (T) recorded every 10 min by a Tinytag data logger during each deployment using Stokes–Einstein relation [13] as follows:

$$\frac{D_1 \times \eta_1}{T_1} = \frac{D_2 \times \eta_2}{T_2} \quad , \quad \text{Eq. (3)}$$

where η is the viscosity of the water taken from the NIST chemistry WebBook [17]. The values of D at 25°C used in our study for a standard diffusive gel are summarized in Table S4 in supporting information. The D values for the restricted gel are equal to 70% of the D for a standard gel, based on the work of Scally et al. [14] and Shiva et al. [18] as summarized in Table S5.

1.4.2. Physicochemical analysis

The pH was measured with a Mettler Toledo pH electrode. The total solids (TS), volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS) were measured according to the French standard AFNOR NF T90-105 method. The supernatant recovered during the TSS and VSS procedure was used to estimate dissolved trace elements (see section 1.4.3).

1.4.3. Sample treatment and trace elements analysis

At the beginning and at the end of each experiment, an aliquot of digested sewage sludge was sampled to measure the total and dissolved elements' content. About 5 g of raw sample (total content) or 2 mL of supernatant (dissolved content), recovered after centrifugation at 3.000 g for 20 min, were digested with 6 mL of 69% HNO₃ and 3 mL of 37% HCl in a microwave oven (Multiwave GO, Anton Paar GmbH) at 180°C for 60 min.

Digested samples were further diluted with ultrapure water and analyzed by ICP-MS (Agilent 7700X) except for Fe which was analyzed by MP-AES (Agilent 4210). During the ICP-MS analysis, internal standards were added: ¹¹⁵In for Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se and ²⁰⁹Bi for Pb. Blanks (*i.e.* ultrapure water adjusted to 2 % HNO₃) were analyzed every 10 samples. Moreover, quality controls at 5 and 10 µg/L were added to check the performance of the analysis. The recovery was equal or above 86% for each element among all analyses performed by ICP-MS or MP-AES.

1.4.4. Method's limits of detection

The method's limits of detection were determined for each procedure (*i.e.* digestion or DGT handling) to account for sample contamination. For the acid digestion procedure, ultrapure water blanks were treated alongside samples with the procedure described in 1.4.3. Blank DGT devices were prepared in duplicate and treated alongside exposed devices during the “short” and “long

term” experiments (see section 1.3.1). The method’s limit of detection (MLD) and quantification (MLQ) were calculated according to IUPAC as the average plus three or ten times the standard deviation of the blanks for MLD and MLQ, respectively.

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2. Results and discussions

2.1 Sample characterization

The characteristics (*i.e.* pH, TS, VS, TSS and VSS) of the samples collected for the short and long term experiments are summarized in Table S6. For each parameter, the difference in percentage is low (ranging from 4% to 7%).

The total and dissolved element concentrations of the samples is reported in Table S7. Dissolved element concentrations were below the MLQ except for As, Fe and Mn. A small discrepancy between the samples is observed for the dissolved Fe (9% difference) whereas a high discrepancy for the dissolved As (75% difference) and Mn (31% difference). Regarding the total element concentrations, only Se is not quantified in the samples. A small discrepancy is observed for Fe and Mn ($\leq 9\%$ difference) between the samples, whereas a discrepancy higher than 10% is observed for the other elements.

2.2 Validation of DGT principle

2.2.1. Steady state establishment

During the “short term” experiment, Cd, Cu, Mo and Pb were below the MLD whereas Al, Cr (III) and Se were below the MLQ of DGT deployment. Therefore, these elements are not discussed further in this section. According to DGT theory, steady state is rapidly established in the sampler ($\leq 1\text{h}$, [19]) and the accumulated mass should behave linearly over time.

The mass of elements accumulated over time on the Chelex and Zr-DGTs is reported in Figure 2. We observe a linear accumulation trend from 0 to 24h for Co, Mn and Ni. Therefore, the system (DGT-digestate) is rapidly in steady state and Eq. (2) holds for these elements regardless of the deployment time (until 24h at least). We also observe a linear accumulation trend for As and Fe

from 0 to 18h and from 4 to 24h, respectively. For As, it indicates that the steady state is rapidly reached and that Eq. (2) holds up to 18h deployment. Deviation from linearity after 18h is likely caused by competing effect. Indeed, Zr-binding gels are known to bind both As and P [20] that are chemical analogous (in the form of arsenate AsO_4^{3-} and phosphate PO_4^{3-}). Consequently, P could have replaced As on the binding gel. This hypothesis is supported by data shown in Figure S1 where P displays the same linear behavior as As, but its accumulated mass on the Zr-binding gel was about 40-fold higher than As up to 24 h deployment time.

Fe presents a unique behavior since we observed linearity only after 4h, indicating delayed establishment of steady state in the sampler. Such behavior can be explained by the properties of Fe complexes (partially labile complexes) or by interactions between Fe and the diffusive gel [21]. Such properties indicate that Eq. (2) does not hold at 4h deployment and its use will result in an underestimation of C_{DGT} . Indeed, we calculated C_{DGT} from the regression line and compared to the value estimated with Eq. (2) using 4 and 24h deployment and we found that C_{DGT} is highly underestimated at 4h (*i.e.* 70% less) than 24h deployment (*i.e.* 16% less).

We observed the establishment of steady state in the samplers for all the quantified elements, therefore the principle of DGT are validated for short deployments ($\leq 24\text{h}$) in the studied digestate matrix. However, the non-significant accumulation of Al, Cd, Cu, Cr (III), Mo, Pb and Se during this “short term” experiment suggests that these elements may be countered by deploying the DGT samplers longer.

Fig 2. The mass of elements accumulated on DGT samplers at different time of deployment during the “short term” experiment. In red, values between MLD_{DGT} and MLQ_{DGT} .

2.2.2. Optimization of the deployment time

To overcome the above mentioned limits of DGT samplers’ deployment time, a “long term” experiment was performed. Increasing the deployment time up to 216h did not enable the detection of labile Cd, Cu and Mo. Indeed, the concentration of these elements under labile form are lower than 0.4, 70 and 20 ng/L, respectively (MLD for 216h DGT deployment).

The results of the “short” and “long term” experiments for the other studied elements are shown in Figure 3. Except for As, Mn, Pb and Se, all quantified elements show linear accumulation over time up to 48h (Cr (III), Fe, Ni), 72h (Al) or 144h (Co). Labile concentration of these elements can be therefore calculated with Eq. (2) using deployment time up to the above-mentioned values. Linearity breaks can result from the formation of pockets of gas observed between the DGT membrane and the diffusive gel (Figure S2) that reduce the effective surface area of the DGT samplers. These pockets of gas likely derive from endogenous microorganisms. However, such hypothesis only holds for deployment times longer than 144h since it should not be element dependent. For shorter deployment times, saturation of the binding gel appears a more realistic hypothesis. When saturation is reached, the accumulated mass of these elements rapidly decreases because of competing effect between elements. For example, the competing effect of Mg (element likely present in high amount in sewage sludge [2]) to Mn binding on the Chelex resin gel was studied by Jiménez-Piedrahita et al. [22]. Our results show that Mn does not accumulate linearly during the “long term” experiment (after 24h).

Finally, increasing the deployment time enabled the quantification of Al, Cr (III), Pb and Se in samplers compared to the “short term” experiment. However, Pb does not linearly accumulate over time and quantification of labile concentration using Eq. (2) could be inappropriate. Moreover, the quantified Pb values are close to MLQ of DGT (from 1 to 3 fold). Such associated uncertainty can explain the nonlinear accumulation of Pb.

During the “short term” experiment, we observed an accumulation of As in the samplers over time, whereas not anymore during the “long term” experiment. Such behavior is consistent with the competing effect of P already highlighted and discussed in section 2.2.1.

Regarding Se, we cannot state that its accumulation trend is linear after 24h deployment time ($R^2 < 0.6$). Consequently, this element cannot be correctly estimated using Eq. (2).

Fig 3. Accumulated mass of elements versus deployment time during the “short” (green rhombus) and “long term” (blue circles) experiments. In red, values between MLD_{DGT} and MLQ_{DGT} . The 24h point is an average between the two experiments.

2.2.3. Impact of digestate matrix on accumulated labile elements

To check the interference of the digestate matrix on the trace elements accumulation by DGT samplers, some diffusive gels were pre-exposed for 24h to the digestate before deployment in a well-defined spiked solution as described in 1.3.2. Since As (III) and Mo (VI) were below the MLQ of the DGT blanks, these elements are not further discussed in this section.

The mass of the elements accumulated by the control and “soiled” DGT samplers are presented in Figure 4.

Fig 4. Accumulated mass of trace elements by the control and “soiled” DGT samplers in 4 hours deployment time.

Except for Se (IV) and Cd (II), we observed that the accumulated mass of the elements measured by the control DGT samplers is significantly higher ($p < 0.05$) than the one measured by the “soiled” DGT samplers. In particular, the “soiled” DGT devices accumulate 11%, 18%, 24%, 28% less Co (II), Ni (II), Pb (II), Cu (II), respectively, compared to the control DGT devices. Such low accumulation could be even more pronounced in the digested sludge since its pH is higher than the one measured in the spiked solution of this study ($4 < \text{pH} < 6$, Table S1). A high pH is favorable for element binding to organic matter [23], at least for cations. In fact, organic matter is known to diffuse within diffusive gels [16,24–26]. We hypothesize that organic matter accumulated on the diffusive gel during pre-exposure and promoted element sorption onto the gel, resulting in a delay of element diffusion as already observed by Davison et al. [27] for Cu with river or soil organic matter.

Here, we showed that DGTs pre-exposure to the matrix of the digestate lowers the accumulation of most of the studied trace elements, leading to underestimation of the labile element concentrations in the medium.

2.3 DGT as a fractionation tool in digestates

2.3.1. Sensitivity of DGT method

The limit of detection and quantification of the method for DGT (MLD_{DGT} and MLQ_{DGT}) are given in Table 1. Compared to the instrumental limit of quantification (which only counts for the analytical sensitivity of the ICP-MS or MP-AES), the MLQ_{DGT} is at least two times higher (data not shown), meaning that some contamination of the samplers occurred during the samplers handling.

Additionally, we compared the MLQ_{DGT} to MLQ for dissolved element ($\text{MLQ}_{\text{dissolved}}$, Table 1). It arises that DGT greatly increased the sensitivity for element monitoring in the digested sludge

than the conventional method (*i.e.* dissolved elements measurement). In particular, the MLQ_{DGT} for Al, Cd, Co, Cr (III), Pb and Se is more than 1000 lower than the $MLQ_{dissolved}$. For the other elements the ratio decreases in the following order $Fe > Ni > Cu > Mn > As > Mo$. This high sensitivity is inherent to the sampling method since DGTs concentrate analytes whereas dissolved elements measurement requires acid digestion of the sample and subsequently its dilution. However, we must stress that both methods do not target the same chemical fraction since the labile fraction targeted by DGT represents only a part of the dissolved elements. Besides, from a monitoring point of view, DGT appears a very interesting method since it allowed to quantify several of the labile elements during the experiments (Table S8) whereas it was not possible for most dissolved elements (Table S7). Therefore, we consider DGT as a sensitive method to monitor trace elements in digested sludge.

Table 1. DGT method limit of detection (MLD_{DGT}) and quantification (MLQ_{DGT}) for a 24h deployment at 19°C (average of recorded values during all deployments). The values are calculated using Eq. (2). The ratio between the MLQ for dissolved elements and the MLQ_{DGT} is also reported.

Element	MLD_{DGT} ($\mu g/L$)*	MLQ_{DGT} ($\mu g/L$) [#]	Ratio $MLQ_{dissolved} / MLQ_{DGT}$
Al	2	4	1197
As	0.2	0.4	247
Cd	0.004	0.009	1288
Co	0.004	0.008	1383
Cr (III)	0.04	0.08	1707
Cu	0.7	2	543
Fe	0.9	2	898
Mn	0.1	0.3	320
Mo	0.2	0.4	74

Ni	0.3	0.7	852
Pb	0.02	0.04	4719
Se	0.02	0.04	35742

*MLD=average blanks+3 σ blanks (n=10)

#MLQ=average blanks+10 σ blanks (n=10)

2.3.2. Fractionation with restricted gels in digestate matrix

A comparison between the labile concentrations of trace elements measured in DGT samplers with restricted and standard gels is reported in Table 2. Cu, Mo, Pb, Cd and Se are not shown because their concentration was below the MLQ_{DGT}.

Statistical analysis indicates that the labile concentration of Al, Co, Cr (III) and Mn measured by the DGT samplers with restricted gels is not significantly different from the one measured with standard gels ($p>0.05$). It means that no large labile complexes of these elements are present in the studied digestate.

However, the labile concentration of Fe was significantly lower ($p<0.01$) when measured with restricted gels (70% less) than standard gels, indicating the presence of some large labile Fe complexes (*i.e.* size>1 nm).

Surprisingly, a significant higher concentration of labile As and Ni was estimated with restricted gels ($p < 0.02$) than standard gels. Such results are not consistent since restricted gels have smaller pore size (*i.e.* < 1 nm) than standard gels (*i.e.* > 5 nm) and it should not allow diffusion of a higher amount of labile elements. Such discrepancy could derive by the use of a non-adapted D value for the restricted gels. In fact, the values reported in Table S5 for D in the restricted gel are estimated in synthetic inorganic solutions, whereas in this study we demonstrated that the diffusion of trace elements is affected by the matrix of digestate. Therefore, we do not exclude that D in the restricted gel could be different in our sample compared to the D estimated in synthetic inorganic solutions. Finally, the interest of size fractionation with restricted gels foreseen above still have to be demonstrated.

Table 2. The ratio between C_{DGT} measured in DGT samplers with restricted gel and the DGT samplers with standard gel.

	Al	As	Co	Cr(III)	Fe	Mn	Ni
$C_{\text{labile restricted}}/C_{\text{labile standard}}$	0.9	1.3	1.1	1.1	0.7	1.1	1.3

2.4 Practical implementation for other digestate samples

In the studied digestate, the “short” and “long term” experiments revealed the following optimal deployment times for each element (Figure 5):

Fig 5. Suitable deployment times for the studied digested sludge.

A 24h deployment appears a good compromise to allow quantification of most elements.

However, these results cannot be generalized to any digestate sample given the variable composition of digestate in terms of trace elements and organic compounds which may interfere with elements' accumulation in DGTs. Therefore, preliminary tests to optimize the deployment time are strongly recommended. In general, we advise to avoid long deployment time because saturation of the binding gel can occur due to the presence of other major compounds. Very short deployment time (*i.e.* <4h) should also be avoided, since the mass of trace elements may not accumulate in the device or the steady state is not established.

The studied digestate matrix altered accumulation of labile elements in DGT devices by 10-30% for Co (II), Ni (II), Pb (II), Cu (II). Such alteration was due to diffusion of organic matter in the sampler from the digestate matrix. This behavior is probably expected in most digestate samples given their high organic matter content [28,29]. Further studies are needed to determine the diffusion rate of trace elements in the presence of digestate matrix. From such work one should be able to correct for matrix effect with the aim to accurately determine labile trace elements concentrations. Unless this, it is safe to limit interpretation of labile concentration established

with DGTs to general trends (*e.g.* evolution over time, order of magnitude) in order to limit misinterpretation of the absolute DGT labile trace elements concentrations.

Finally, size fractionation by coupling the restricted and standard gels was investigated in this study. Our results show the presence of large labile complexes for Fe (>1 nm) and small labile complexes for Al, Co, Cr (III) and Mn (<1 nm). However, these results must be confirmed and cannot be generalized at this stage.

2.5 Interpretation of DGT fractionation

One of the main objective when performing trace element fractionation is to predict their bio-accessibility. The DGT technique demonstrated to perform well mostly in natural waters and soils [7]. Currently, data regarding the relationship between DGT-labile element concentrations and their bio-accessibility in digestate are very sparse. To our knowledge, only the study of Bourven et al. [8] addressed this topic. They showed, in the context of whey anaerobic digestion, that DGT-labile Cd content is linked to the initial alteration of biogas production and enzymatic activities (*i.e.* β -galactosidase and TTC-dehydrogenase). However, such correlation was absent after 21 days of anaerobic digestion. DGT based fractionation of Cd appears, therefore, encouraging to predict its bio-accessibility, but not straightforward. Similar works could be performed for several trace elements and in various digestates. Therefore, new studies are required to fully establish the extent to which DGT fractionation can be used to predict elements bio-accessibility in digestates.

Conclusions

This study investigated the potential of DGT as a fractionation tool for trace elements in digested sewage sludge. Ensuring reliability of sampling is a prerequisite to the further use of DGT in digestate matrices. Our results suggest that DGT-labile trace elements sampling in digestate is feasible providing the deployment time is carefully tested and interpretation is limited to general trends (*e.g.* evolution over time, order of magnitude).

This study also showed that the DGT technique increases the sensitivity of trace elements monitoring compared to the dissolved element measurement by acid digestion. Moreover, DGT technique does not require sample treatment such as liquid-solid separation by centrifugation, preventing changes in trace elements speciation. These advantages over other fractionation methods already open a wide field of investigation for trace elements speciation in digestates.

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Conflict of interest

The authors declare no conflict of interest.

Appendix. Supporting information

The supporting information is available at the following link (to be mentioned).

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Graphical Abstract

Highlights

- DGT principle is validated in a digestate matrix;
- Deployment time must be carefully adapted depending on the targeted elements;
- Digestate matrix can induce underestimation of labile elements concentration;
- Sensitivity of DGT is better than conventional dissolved elements measurement.