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

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## Abstract

12 This study proposes an evaluation of the diffusive gradients in thin films technique (DGT) for

13 studying trace elements in digested sewage sludge samples. Twelve elements were monitored by

14 Chelex (Al, Cd, Co, Cr (III), Cu, Fe, Mn, Ni, Pb) and zirconia-DGT (As, Mo, Se) samplers

15 exposed from 4 hours to 9 days. Twenty-four hours' deployment time was suitable for most of

16 the studied elements. However, short deployment led to insufficient element accumulation or

17 non-establishment of steady state while long deployment (from 18 to 144h depending on the

18 element) led to saturation of the binding gels and/or competing effects with other major elements.  
19 In addition, this study showed that the matrix of the digested sewage sludge lowers the  
20 accumulation of some trace elements in the DGT samplers, leading to labile concentrations  
21 underestimation of roughly 10-30% (depending on the element). Moreover, compared to the  
22 conventional total dissolved elements measurement, DGT technique allowed to quantify 7 out of  
23 12 labile elements whereas only 3 out of 12 dissolved elements were quantified. These results  
24 highlight the potential of DGT technique to assess labile trace elements in digestate samples,  
25 provided a careful adaptation of the deployment time as well as an evaluation of the matrix effect  
26 is performed.

## **Keywords**

27 Digested sewage sludge;

28 Passive sampling;

29 Matrix interferences;

30 Metals;

31 Metalloids;

32 Speciation.

## 33 **Introduction**

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

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

47 To overcome the limitations of sequential extraction procedures, in a recent paper, Thanh et al.  
48 [6] identified the diffusive gradients in thin films technique (DGT) as a promising technique to  
49 determine bio-accessible metal concentrations in anaerobic bioreactors. This technique allows  
50 sampling labile trace elements after diffusion through a gel and accumulation on a binding gel in  
51 the DGT device [7]. The labile elements comprise free ions and weakly bound complexes and  
52 thereby would represent the most readily bio-accessible species of trace elements [7]. Recently,  
53 Bourven et al. [8] demonstrated a link between DGT-labile Cd concentrations and biogas  
54 production as well as enzymatic activities during whey anaerobic digestion. However, DGT use  
55 in digestate is only emerging and, to our knowledge, only Takashima et al. [9] has used the DGT

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

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

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

## 71 **1. Materials and methods**

### 72 1.1 Digested sewage sludge sample

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

### 78 1.2 DGT preparation

79 Two different DGT samplers were used during this study: Chelex-DGTs for cationic species (Al,  
80 Cd, Co, Cr (III), Cu, Fe, Mn, Ni and Pb) and zirconia-DGTs (Zr-DGTs) for anionic species (As,  
81 Mo and Se). The selectivity of Chelex-DGT sampler over the oxidation state of Cr species was  
82 previously demonstrated by Ernstberger et al. [10]. Each DGT consisted of a binding gel, a  
83 diffusive gel and a filter membrane enclosed in a piston type holder, the latter purchased from  
84 DGT Research (Lancaster, UK). Chelex binding gels were prepared according to the procedure  
85 described by Zhang et al. [11], whereas Zr binding gels were made according to Devillers et al.  
86 [12].

87 Unless stated otherwise, the DGT samplers were equipped with a standard polyacrylamide  
88 diffusive gel (15% acrylamide and 0.3% agarose-derived cross linker, 0.77 mm thick), prepared  
89 according to Zhang et al. [11]. In addition, the use of restricted diffusive gels (15% acrylamide  
90 and 0.75% bisacrylamide cross linker, 0.75 mm thick) with pore size <1 nm [13] was  
91 investigated. The gels were prepared following a procedure slightly modified from Scally et al.  
92 [14]. The polymerization was performed by mixing 200 µL of 10% (m/V) freshly prepared  
93 ammonium persulfate (Fisher Scientific) and 8 µL of tetramethylethylenediamine (TEMED)

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

95 The full procedure is described in supporting information.

96 Protective membranes of 0.4  $\mu\text{m}$  pore size Nuclepore® in polycarbonate (0.02 mm thickness,

97 Whatman, UK) or 0.2  $\mu\text{m}$  pore size cellulose acetate membrane (0.12 mm thickness, Whatman,

98 UK) was placed on the top of the diffusive gel.

### 99 1.3 Experimental set-up

#### 100 1.3.1. *Optimization of DGT samplers' deployment time*

101 About 20 L of digested sludge was poured into a PP container and continuously stirred with an

102 overhead plastic propeller at 30 rpm. A Tinytag data logger (TG-4100, Gemini Data Loggers,

103 UK) was used to record the temperature in the sample. To avoid changes of trace elements

104 speciation, the sample was kept in anaerobic conditions by covering its surface with paraffin oil

105 and a plastic film.

106 Two different experiments were performed: a “short term” one to validate the establishment of

107 steady state conditions in the samplers, and a “long term” one to increase the sensitivity of the

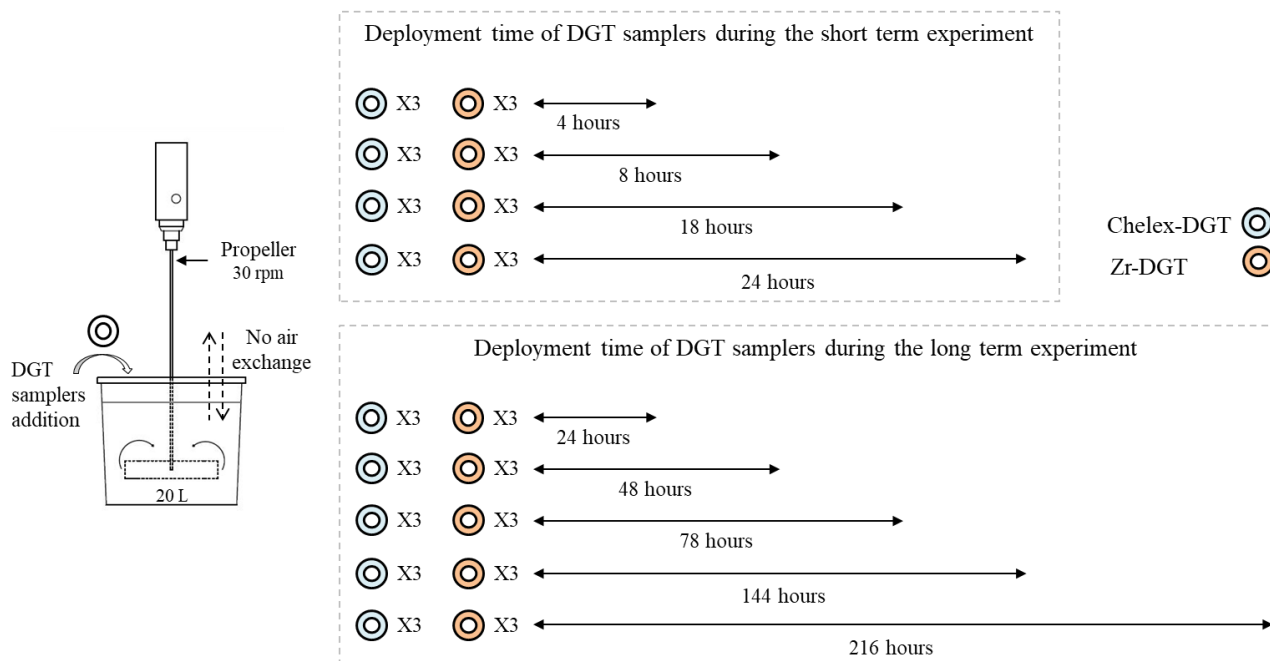
108 method. In detail, triplicate devices of both Chelex and Zr were deployed for 4, 8, 18 and 24

109 hours (“short term” experiment) or for 24, 48, 72, 144 and 216 hours (long term experiment). A

110 representation of the experimental set-up is shown in Figure 1. Before starting the experiment,

111 the devices were immersed overnight in nitrogen flushed ultrapure water to remove oxygen from

112 them.



113

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

116 *1.3.2. Potential interference from digestate matrix on trace elements accumulation*

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

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



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

132 To check the contamination of the binding gel brought by the “soiled” diffusive gel, three blank  
133 DGT samplers were built with “soiled” diffusive gels and new Chelex and Zr binding gels. The  
134 blanks were stored at room temperature ( $20\pm 1^\circ\text{C}$ ) in a moistened plastic bag and disassembled  
135 after 4 hours alongside the other samplers.

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

### 139 *1.3.3. Size fractionation of labile elements*

140 Fractionation of labile elements based on their size was investigated through the simultaneous  
141 deployment of DGT samplers equipped with restricted or standard diffusive gels. The Chelex and  
142 Zr-DGT samplers were deployed for 24 hours in 20 L of digested sludge sample continuously  
143 stirred at 30 rpm. The deployment time was chosen according to the results obtained from the  
144 experiment described in 0.

## 145 1.4 Analytical procedures

### 146 *1.4.1 DGT-labile concentration*

147 After retrieval, DGT samplers were rinsed with ultrapure water and disassembled to recover the  
148 binding gels. The accumulated mass ( $m$ ) of trace elements in each DGT sampler was determined  
149 after elution of the binding gel. The Chelex binding gels were eluted in 2 mL of 1 M  $\text{HNO}_3$  for  
150 24 hours and the Zr binding gels in 2 mL of  $5\cdot 10^{-3}$  M NaOH and 0.5 M  $\text{H}_2\text{O}_2$  for 24 hours. Then  
151 the concentration of trace elements in the eluents ( $C_e$ ) were quantified by the inductively coupled

152 plasma mass spectrometry (ICP-MS) or microwave plasma atomic emission spectroscopy (MP-  
153 AES) (see section 0). The accumulated mass is determined according to equation (1) [15]:

$$154 \quad m = \frac{C_e \times V_e}{f_e} \quad , \quad \text{Eq. (1)}$$

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

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

$$159 \quad C_{DGT} = \frac{m \times \Delta_{MDL}}{D \times t \times A} \quad , \quad \text{Eq. (2)}$$

160 where  $\Delta_{MDL}$  is the thickness of the material diffusion layer (*i.e.* diffusive gel plus membrane),  $t$  is  
161 the time of DGT samplers' exposure in the sludge,  $D$  is the coefficient of diffusion of the  
162 considered element in the diffusion layer and  $A$  is the geometric area of the DGT holder window  
163 ( $3.14 \text{ cm}^2$ ). The values of  $D$  were corrected for the average temperature ( $T$ ) recorded every 10  
164 min by a Tinytag data logger during each deployment using Stokes–Einstein relation [13] as  
165 follows:

$$166 \quad \frac{D_1 \times \eta_1}{T_1} = \frac{D_2 \times \eta_2}{T_2} \quad , \quad \text{Eq. (3)}$$

167 where  $\eta$  is the viscosity of the water taken from the NIST chemistry WebBook [17]. The values  
168 of  $D$  at  $25^\circ\text{C}$  used in our study for a standard diffusive gel are summarized in Table S4 in  
169 supporting information. The  $D$  values for the restricted gel are equal to 70% of the  $D$  for a  
170 standard gel, based on the work of Scally et al. [14] and Shiva et al. [18] as summarized in Table  
171 S5.

172 *1.4.2. Physicochemical analysis*

173 The pH was measured with a Mettler Toledo pH electrode. The total solids (TS), volatile solids  
174 (VS), total suspended solids (TSS) and volatile suspended solids (VSS) were measured according  
175 to the French standard AFNOR NF T90-105 method. The supernatant recovered during the TSS  
176 and VSS procedure was used to estimate dissolved trace elements (see section 0).

177 *1.4.3. Sample treatment and trace elements analysis*

178 At the beginning and at the end of each experiment, an aliquot of digested sewage sludge was  
179 sampled to measure the total and dissolved elements' content. About 5 g of raw sample (total  
180 content) or 2 mL of supernatant (dissolved content), recovered after centrifugation at 3.000 g for  
181 20 min, were digested with 6 mL of 69% HNO<sub>3</sub> and 3 mL of 37% HCl in a microwave oven  
182 (Multiwave GO, Anton Paar GmbH) at 180°C for 60 min.

183 Digested samples were further diluted with ultrapure water and analyzed by ICP-MS (Agilent  
184 7700X) except for Fe which was analyzed by MP-AES (Agilent 4210). During the ICP-MS  
185 analysis, internal standards were added: <sup>115</sup>In for Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Se and  
186 <sup>209</sup>Bi for Pb. Blanks (*i.e.* ultrapure water adjusted to 2 % HNO<sub>3</sub>) were analyzed every 10 samples.  
187 Moreover, quality controls at 5 and 10 µg/L were added to check the performance of the analysis.  
188 The recovery was equal or above 86% for each element among all analyses performed by ICP-  
189 MS or MP-AES.

190 *1.4.4. Method's limits of detection*

191 The method's limits of detection were determined for each procedure (*i.e.* digestion or DGT  
192 handling) to account for sample contamination. For the acid digestion procedure, ultrapure water  
193 blanks were treated alongside samples with the procedure described in 0. Blank DGT devices  
194 were prepared in duplicate and treated alongside exposed devices during the "short" and "long

195 term” experiments (see section 0). The method’s limit of detection (MLD) and quantification  
196 (MLQ) were calculated according to IUPAC as the average plus three or ten times the standard  
197 deviation of the blanks for MLD and MLQ, respectively.

## 198 **2. Results and discussions**

### 199 2.1 Sample characterization

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

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

### 210 2.2 Validation of DGT principle

#### 211 2.2.1. *Steady state establishment*

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

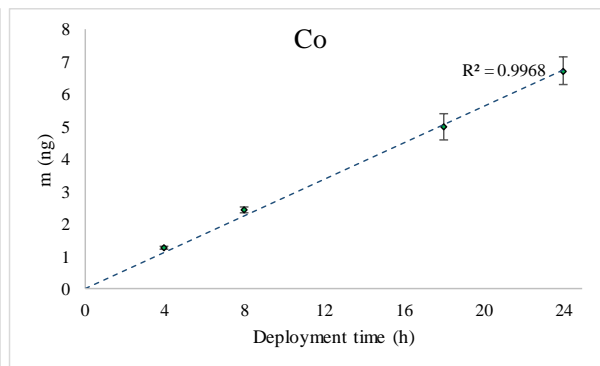
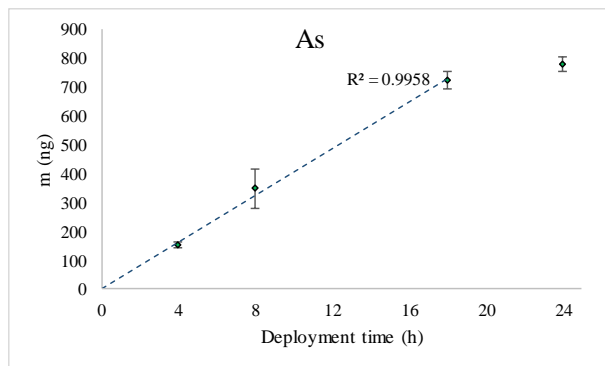
216 The mass of elements accumulated over time on the Chelex and Zr-DGTs is reported in Figure 2.  
217 We observe a linear accumulation trend from 0 to 24h for Co, Mn and Ni. Therefore, the system  
218 (DGT-digestate) is rapidly in steady state and Eq. (2) holds for these elements regardless of the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

220 from 0 to 18h and from 4 to 24h, respectively. For As, it indicates that the steady state is rapidly  
221 reached and that Eq. (2) holds up to 18h deployment. Deviation from linearity after 18h is likely  
222 caused by competing effect. Indeed, Zr-binding gels are known to bind both As and P [20] that  
223 are chemical analogous (in the form of arsenate  $\text{AsO}_4^{3-}$  and phosphate  $\text{PO}_4^{3-}$ ). Consequently, P  
224 could have replaced As on the binding gel. This hypothesis is supported by data shown in Figure  
225 S1 where P displays the same linear behavior as As, but its accumulated mass on the Zr-binding  
226 gel was about 40-fold higher than As up to 24 h deployment time.

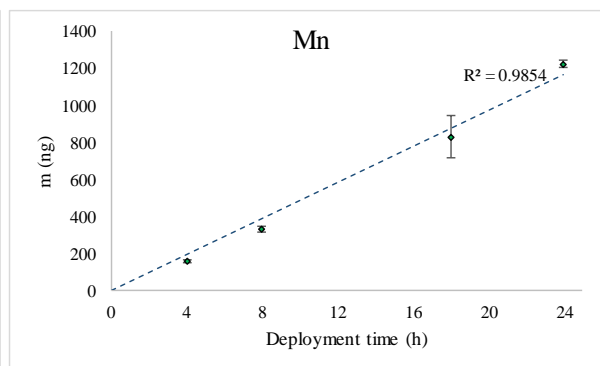
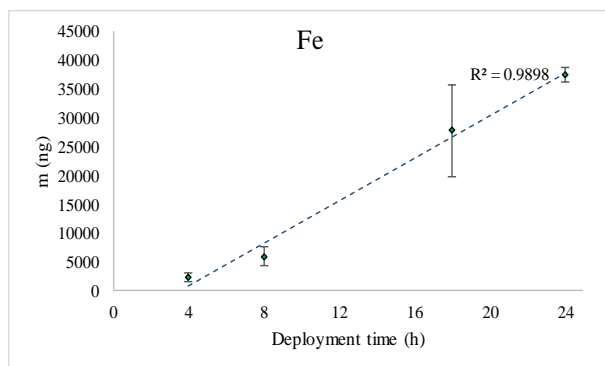
227 Fe presents a unique behavior since we observed linearity only after 4h, indicating delayed  
228 establishment of steady state in the sampler. Such behavior can be explained by the properties of  
229 Fe complexes (partially labile complexes) or by interactions between Fe and the diffusive gel  
230 [21]. Such properties indicate that Eq. (2) does not hold at 4h deployment and its use will result  
231 in an underestimation of  $C_{\text{DGT}}$ . Indeed, we calculated  $C_{\text{DGT}}$  from the regression line and compared  
232 to the value estimated with Eq. (2) using 4 and 24h deployment and we found that  $C_{\text{DGT}}$  is highly  
233 underestimated at 4h (*i.e.* 70% less) than 24h deployment (*i.e.* 16% less).

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

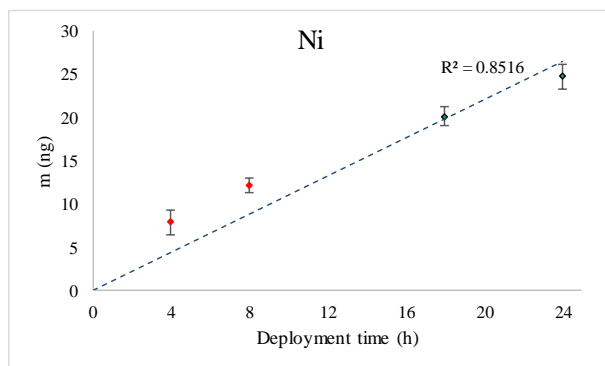
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240



241



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

244 **2.2.2. Optimization of the deployment time**

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

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

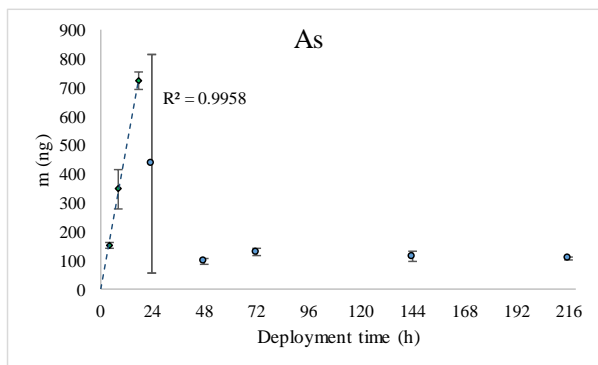
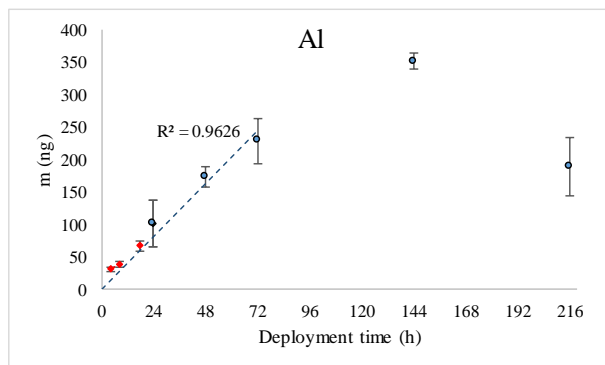
263 Finally, increasing the deployment time enabled the quantification of Al, Cr (III), Pb and Se in  
264 samplers compared to the “short term” experiment. However, Pb does not linearly accumulate  
265 over time and quantification of labile concentration using Eq. (2) could be inappropriate.  
266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

268 During the “short term” experiment, we observed an accumulation of As in the samplers over  
269 time, whereas not anymore during the “long term” experiment. Such behavior is consistent with  
270 the competing effect of P already highlighted and discussed in section 0.

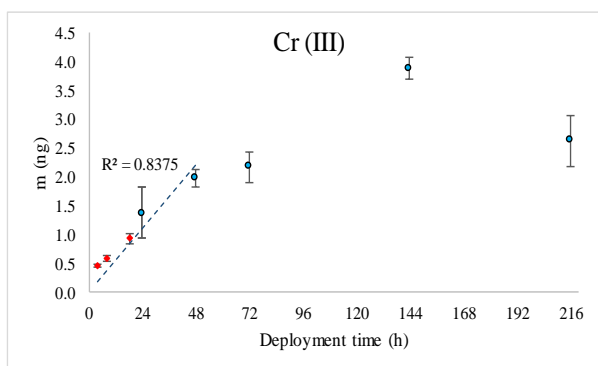
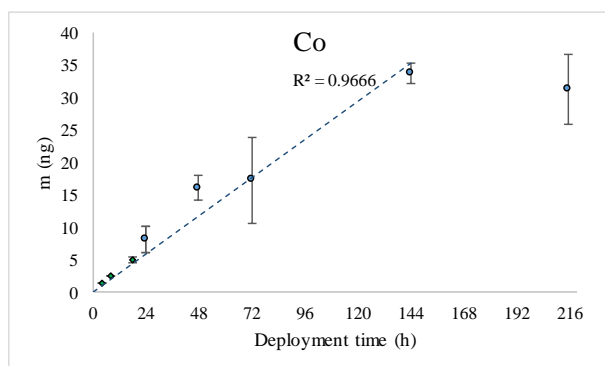


271 Regarding Se, we cannot state that its accumulation trend is linear after 24h deployment time  
272 ( $R^2 < 0.6$ ). Consequently, this element cannot be correctly estimated using Eq. (2).

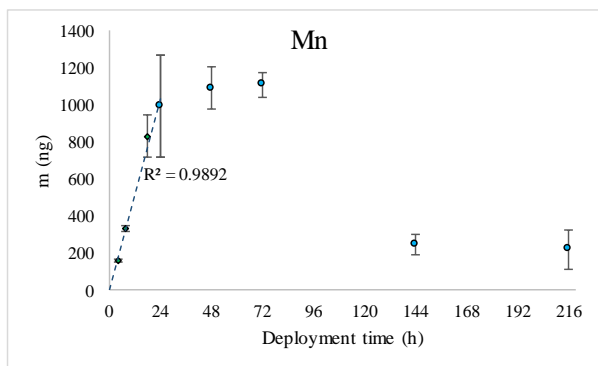
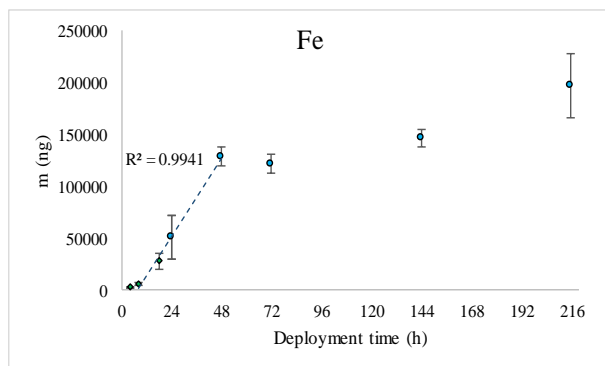
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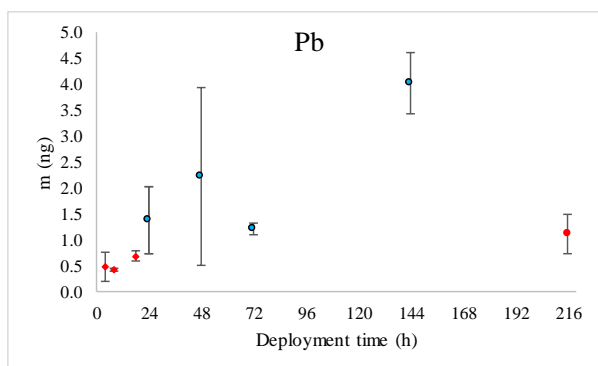
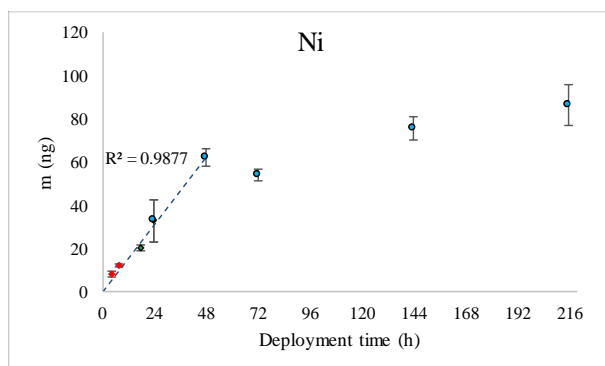
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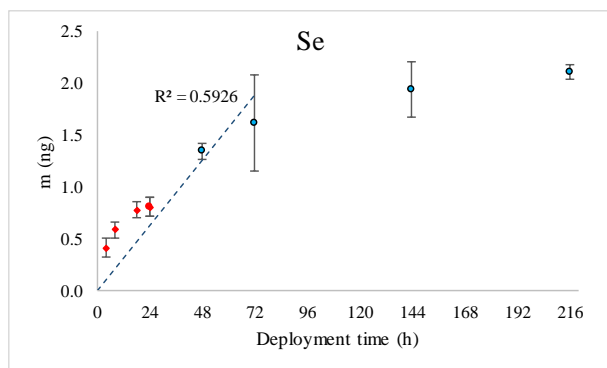


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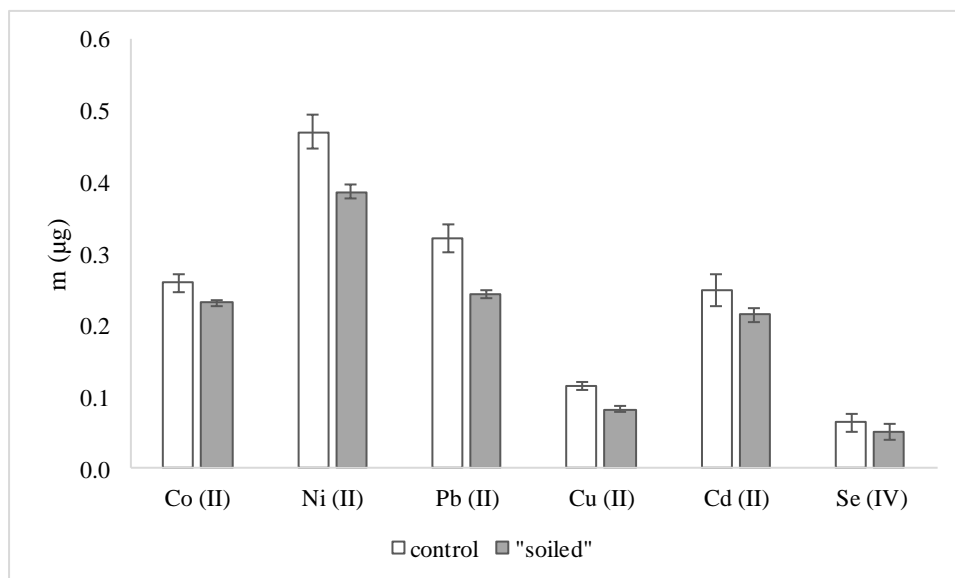
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278 **Figure 3.** Accumulated mass of elements versus deployment time during the “short” (green rhombus) and “long term” (blue  
 279 circles) experiments. In red, values between  $MLD_{DGT}$  and  $MLQ_{DGT}$ . The 24h point is an average between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

280 *2.2.3. Impact of digestate matrix on accumulated labile elements*

281 To check the interference of the digestate matrix on the trace elements accumulation by DGT  
 282 samplers, some diffusive gels were pre-exposed for 24h to the digestate before deployment in a  
 283 well-defined spiked solution as described in 0. Since As (III) and Mo (VI) were below the MLQ  
 284 of the DGT blanks, these elements are not further discussed in this section.

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



287

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

289 Except for Se (IV) and Cd (II), we observed that the accumulated mass of the elements measured  
290 by the control DGT samplers is significantly higher ( $p < 0.05$ ) than the one measured by the  
291 “soiled” DGT samplers. In particular, the “soiled” DGT devices accumulates 11%, 18%, 24%,  
292 28% less Co (II), Ni (II), Pb (II), Cu (II), respectively, compared to the control DGT devices.  
293 Such low accumulation could be even more pronounced in the digested sludge since its pH is  
294 higher than the one measured in the spiked solution of this study ( $4 < \text{pH} < 6$ , Table S1). A high pH  
295 is favorable for element binding to organic matter [23], at least for cations. In fact, organic matter  
296 is known to diffuse within diffusive gels [16,24–26]. We hypothesize that organic matter  
297 accumulated on the diffusive gel during pre-exposure and promoted element sorption onto the  
298 gel, resulting in a delay of element diffusion as already observed by Davison et al. [27] for Cu  
299 with river or soil organic matter.

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

## 303 2.3 DGT as a fractionation tool in digestates

### 304 2.3.1. *Sensitivity of DGT method*

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

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

312 than the conventional method (*i.e.* dissolved elements measurement). In particular, the  $MLQ_{DGT}$   
313 for Al, Cd, Co, Cr (III), Pb and Se is more than 1000 lower than the  $MLQ_{dissolved}$ . For the other  
314 elements the ratio decreases in the following order  $Fe > Ni > Cu > Mn > As \gg Mo$ . This high  
315 sensitivity is inherent to the sampling method since DGTs concentrate analytes whereas dissolved  
316 elements measurement requires acid digestion of the sample and subsequently its dilution.  
317 However, we must stress that both methods do not target the same chemical fraction since the  
318 labile fraction targeted by DGT represents only a part of the dissolved elements.

319 Besides, from a monitoring point of view, DGT appears a very interesting method since it  
320 allowed to quantify several of the labile elements during the experiments (Table S8) whereas it  
321 was not possible for most dissolved elements (Table S7). Therefore, we consider DGT as a  
322 sensitive method to monitor trace elements in digested sludge.

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

Element	$MLD_{DGT}$ ( $\mu\text{g/L}$ )*	$MLQ_{DGT}$ ( $\mu\text{g/L}$ ) <sup>#</sup>	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	<del>3252</del>
Pb	0.02	0.04	<sup>4719</sup> <sub>327</sub>
Se	0.02	0.04	35742

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\*MLD=average blanks+3 $\sigma$  blanks (n=10)

#MLQ=average blanks+10 $\sigma$  blanks (n=10)

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328 *2.3.2. Fractionation with restricted gels in digestate matrix*

329 A comparison between the labile concentrations of trace elements measured in DGT samplers  
330 with restricted and standard gels is reported in Table 2. Cu, Mo, Pb, Cd and Se are not shown  
331 because their concentration was below the MLQ<sub>DGT</sub>.

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

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

339 Surprisingly, a significant higher concentration of labile As and Ni was estimated with restricted  
340 gels (p<0.02) than standard gels. Such results are not consistent since restricted gels have smaller  
341 pore size (*i.e.* <1 nm) than standard gels (*i.e.* >5 nm) and it should not allow diffusion of a higher  
342 amount of labile elements. Such discrepancy could derive by the use of a non-adapted D value for  
343 the restricted gels. In fact, the values reported in Table S5 for D in the restricted gel are estimated  
344 in synthetic inorganic solutions, whereas in this study we demonstrated that the diffusion of trace

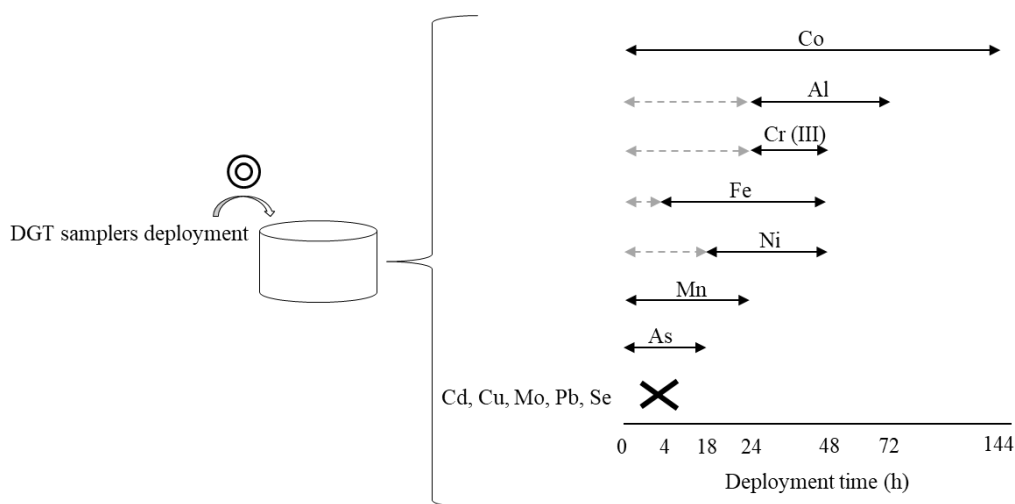
345 elements is affected by the matrix of digestate. Therefore, we do not exclude that D in the  
 346 restricted gel could be different in our sample compared to the D estimated in synthetic inorganic  
 347 solutions. Finally, the interest of size fractionation with restricted gels foreseen above still have to  
 348 be demonstrated.

349 **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
$C_{labile\ restricted}/C_{labile\ standard}$	0.9	1.3	1.1	1.1	0.7	1.1	1.3

### 350 2.4 Practical implementation for other digestate samples

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



353  
 354 **Figure 5.** Suitable deployment times for the studied digested sludge.

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

356 However, these results cannot be generalized to any digestate sample given the variable  
 357 composition of digestate in terms of trace elements and organic compounds which may interfere  
 358 with elements' accumulation in DGTs. Therefore, preliminary tests to optimize the deployment  
 359 time are strongly recommended. In general, we advise to avoid long deployment time because

360 saturation of the binding gel can occur due to the presence of other major compounds. Very short  
361 deployment time (*i.e.* <4h) should also be avoided, since the mass of trace elements may not  
362 accumulate in the device or the steady state is not established.

363 The studied digestate matrix altered accumulation of labile elements in DGT devices by 10-30%  
364 for Co (II), Ni (II), Pb (II), Cu (II). Such alteration was due to diffusion of organic matter in the  
365 sampler from the digestate matrix. This behavior is probably expected in most digestate samples  
366 given their high organic matter content [28,29]. Further studies are needed to determine the  
367 diffusion rate of trace elements in the presence of digestate matrix. From such work one should  
368 be able to correct for matrix effect with the aim to accurately determine labile trace elements  
369 concentrations. Unless this, it is safe to limit interpretation of labile concentration established  
370 with DGTs to general trends (*e.g.* evolution over time, order of magnitude) in order to limit  
371 misinterpretation of the absolute DGT labile trace elements concentrations.

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

## 376 2.5 Interpretation of DGT fractionation

377 One of the main objective when performing trace element fractionation is to predict their bio-  
378 accessibility. The DGT technique demonstrated to perform well mostly in natural waters and  
379 soils [7]. Currently, data regarding the relationship between DGT-labile element concentrations  
380 and their bio-accessibility in digestate are very sparse. To our knowledge, only the study of  
381 Bourven et al. [8] addressed this topic. They showed, in the context of whey anaerobic digestion,  
382 that DGT-labile Cd content is linked to the initial alteration of biogas production and enzymatic

383 activities (*i.e.*  $\beta$ -galactosidase and TTC-dehydrogenase). However, such correlation was absent  
384 after 21 days of anaerobic digestion. DGT based fractionation of Cd appears, therefore,  
385 encouraging to predict its bio-accessibility, but not straightforward. Similar works could be  
386 performed for several trace elements and in various digestates. Therefore, new studies are  
387 required to fully establish the extent to which DGT fractionation can be used to predict elements  
388 bio-accessibility in digestates.

### 389 **3. Conclusions**

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

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



400 **Conflict of interest**

401 The authors declare no conflict of interest.

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407 **Appendix. Supporting information**

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

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