Environmental Technology & Innovation 33 (2024) 103526

Contents lists available at ScienceDirect

Environmental Technology & Innovation

journal homepage: www.elsevier.com/locate/eti

Diffusive gradients in thin films (DGT) as a robust and reliable technique to measure bioavailable metals in anaerobic digestates

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ARTICLE INFO

Keywords:
Anaerobic digestate
Bioavailability
DGT
Feedstock type
Method precision
Trace metals

ABSTRACT

Applying diffusive gradients in thin films (DGT) to digestates as a standardized method requires addressing unclear precision of the method and the lack of information on compounds in digestates responsible for metal binding. We tested the precision of the method on seven different digestates by measuring Co, Ni, Fe and Mn accumulated in the DGT devices. Next, we tested gels with two different pore sizes in parallel (restricted gel with smaller and open gel with bigger pore size) to identify organic complexes that bind trace metals. Finally, we tried to understand how the feedstock affects DGT concentrations. The precision of the method was acceptable as the RSDs of the DGT technical triplicates for Co were lower than 10% for all seven digestates. For the other metals, slightly higher RSDs were detected in a few samples: Mn RSD varied more than 10% in only one digestate sample (27% RSD), Ni RSD in two samples (19% twice) and Fe RSD in three (with 18% maximum RSD). The measurements of Co, Ni and Fe after diffusion through the restricted gel compared to the open gel showed a significant difference in maximum three out of seven samples. The investigated metals were found in higher concentrations on the DGT resins exposed to digestates originating from manure, energy crops and food waste than in DGT resins exposed to samples originating from wastewater treatment plant digesters. Overall, open DGT appears to be applicable for Co, Ni, Fe and Mn bioavailability measurements in digestates originating from various feedstocks.

1. Introduction

Microorganisms, like all living organisms, require trace metals (TMs) as these are crucial constituents of enzymes. When TM deficiency occurs, the proper synthesis of enzymes can be hindered, resulting in lower microbial activity and limited growth. This deficiency problem has been reported for anaerobic digesters that treat a variety of substrates, such as crops (Hinken et al., 2008), food waste (Ariunbaatar et al., 2016; De Vrieze et al., 2013), slaughterhouse waste (Karlsson et al., 2012; Ortner et al., 2015), grain stillage (Gustavsson et al., 2013), waste activated sludge (Yu et al., 2015) and different types of industrial and municipal wastewater (Fermoso et al., 2008; Muñoz Sierra et al., 2017; Ye et al., 2022). Generally, total metal concentration is a parameter for assessing metal

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https://doi.org/10.1016/j.eti.2024.103526
Received 27 July 2023; Received in revised form 1 January 2024; Accepted 1 January 2024
Available online 3 January 2024

deficiency in digesters, but it does not represent actual metal bioavailability to the microorganisms.

The bioavailability of TMs, reflecting the ability of microorganisms to take up TMs, is dependent on the chemical form (species) in which TMs occur in the environment (free ions, adsorbed, precipitated, organic or inorganic complexes, etc.). There are many techniques that focus on measuring metal ions that are free or form small and/or labile complexes, thought to be the most available to microorganisms for uptake: Donnan Membrane Technique (Bartacek et al., 2008; Weng et al., 2011), ion selective electrode (Lisak, 2021), adsorptive stripping voltammetry (Jansen et al., 2005; Pesavento et al., 2009), ultrafiltration (Forsberg et al., 2006; Liu et al., 2013) and diffusive gradients in thin films (DGT). Metal speciation models (e.g., MINTEQ or WHAM) can be used to predict the fractions of inorganic and organic complexes of metals occurring in solution, based on input data on the composition of the solution. An overview of these techniques can be found in the work of Thanh et al. (2016) and van Hullebusch et al. (2016).

The application of DGT has been extended over the years, because of the ease of in situ species separation during sampling and undisturbed sample testing. It is considered to be a prospective tool for environmental samples (Wei et al., 2022) that is - until now, mainly applied on surface waters, soils and sediments. The DGT sampler works as a dynamic analyte accumulation device, which is based on analyte diffusion through a diffusive gel, followed by analyte accumulation on a binding gel. The accumulated amount is further used to calculate the concentration of the analyte. The type of diffusion and binding gel defines what kind of element, or compound will be accumulated. Diffusive gels are made of different pore sizes, and this can be used to exclude organic complexes that bind trace metals. The restricted gel (RES) has a smaller pore size, hence it retards organic complexes in comparison to the open gel (OP) through which these molecules diffuse. When these two gels are applied in parallel, it can be estimated to what extent metals are bound to organic complexes (Österlund et al., 2012). This information is highly relevant in ecosystems, like anaerobic digestion, in which organic complexation plays an important role in metal bioavailability. More details on the working principle of DGT are presented elsewhere (Davison, 2016; Davison and Zhang, 2012; Zhang and Davison, 2015).

With very few exceptions (Bourven et al., 2017; Laera et al., 2019a; b; Takashima, 2018), DGT devices have not been investigates yet as tools to measure the bioavailability of trace metals in anaerobic digestate. The DGT accumulates free metal ions, metals contained in inorganic complexes and, to some extent, metals complexed by organic compounds. An assessment of DGT in digestate samples in relation to deployment time, type of gel (restricted and unrestricted type of gel), and matrix influence was carried out only in one sample (Laera et al., 2019a). In other studies, DGT was used to measure Ni and Co bioavailability in digestate samples (Takashima, 2018), or it was applied to measure Cd in anaerobic digestate, which was spiked with different Cd concentrations (Bourven et al., 2017). In general, there is a lack of literature on the reproducibility and reliability of these devices when being deployed in digestate samples, but also insufficient data on DGT deployment in various digestates, which is essential as first step towards standardization of this technique for trace metal characterization in this type of samples.

The aim of this study was to assess the precision of open and restricted DGT gels on seven digestate samples, and to understand how different feedstock affects the DGT results. The influence of the origin of the digestates on the DGT concentrations was investigated by direct comparison, as well as by comparing ratios of DGT-extractable metals (i.e. labile metal concentrations) to the total metal content in the digestate. Measurements from restricted and unrestricted gel DGTs were used to determine the effect of organic matter on metal availability. The analyzed trace metals were Co, Ni, Fe, Cu, Zn and Mn as they are essential components of enzymes involved in anaerobic reactions.

2. Material and methods

2.1. Sampling and characterization of digestates

Trace metals were quantified in digestate samples from seven full-scale digesters. Three samples came from wastewater treatment plant (WWTP) digesters (defined as WWTP1, WWTP2 and WWTP3). The WWTP1 and WWTP2 samples were taken from digesters that were fed waste activated sludge. The sample labeled as WWTP3 was digestate from a digester treating A-sludge. A-sludge is produced in the A-stage of the adsorption/bio-oxidation process, which is a modified two-stage wastewater treatment process relying on waste activated sludge with a very low sludge retention time, in comparison with conventional activated sludge systems (Boehnke et al., 1997). The four other samples were from digesters treating agricultural waste containing manure, energy crops and food waste as main feedstock (defined as MIX1, MIX2 and MIX3), while one sample came from a digester treating only food waste (FW). For the sake of clarity, when discussed as a group, samples originating from WWTP digesters were defined as WWTP samples and the other four were

Table 1
General characteristics of seven different digestates. TS represent total solids, TSS represent total suspended solids, VSS represent volatile suspended solids, S^{2-} represent sulfides.

Sample	Digester feed	pH	TS (g/L)	TSS (g/L)	VSS (g/L)	S^{2-} (mg/L)
WWTP1	Waste activated sludge	7.80	39.0	20.0	15.0	117
WWTP2	Waste activated sludge	7.59	29.4	27.0	15.8	97
WWTP3	A-sludge	8.36	37.7	34.7	21.7	178
MIX1	Mix of manure, energy crops and food waste	8.49	42.1	19.6	14.5	208
MIX2	Mix of manure, energy crops and food waste	8.53	52.6	49.7	26.3	425
MIX3	Mix of manure, energy crops and food waste	8.09	41.2	29.1	22.4	115
FW	Food waste	8.09	38.3	19.3	13.9	324

grouped as MIX samples. All samples were kept at 4 °C, filled until the top of the containers to minimize air exposure until analysis.

The pH of the samples was measured by a Thermo Scientific Orion Star A211 pH meter. The measurements of total solids (TS), total suspended solids (TSS) and volatile suspended solids (VSS) were performed according to the Standard Methods (APHA, 2005). The total sulfides were determined by the iodometric method (Horáková et al., 1986). All general sample characteristics are presented in Table 1. The pH of the different samples ranged from 7.6 to 8.5. Total solids of all samples varied in a range of 29.41 to 52.56 mg/L. Sulfides, which are typically the major metal binding species in the digestors, were the highest in MIX2 and FW samples.

2.2. Diffusive gradients in thin films (DGT)

2.2.1. Types of gels

All DGT samplers were purchased from DGT® Research Ltd. (Lancaster, UK). Two types of samplers were used, one type with open pore diffusive gel and the other with restricted diffusive gel (Table 2.). The characteristics of both samplers are the same, varying only in the type and thickness of the diffusive gel. The size of the pores is not precisely defined, but in the OP gel it is considered to be 5 nm and in the RES gel around 1 nm (Österlund et al., 2012). Significant differences between metal concentrations measured after diffusion through OP and RES gels were identified by comparing their averages using an unpaired t-test at a significance level of 0.05.

2.2.2. Experimental set-up

To ensure the appropriate deployment time of DGTs for different digestate samples, one sample (MIX2) was chosen and tested with OP DGT at different deployment times: 6, 13 and 23 h. This test was conducted in a plastic container, filled with around 800 mL of digestate sample. The DGTs were fixed in duplicate to the bottom of the container with double-sided tape and shaken at 55 rpm to ensure homogeneity of the sample and minimize the diffusive boundary layer. At the time points mentioned above, the samplers were taken out and further prepared and quantified as described in Section 2.3 of Material and methods. Based on the results (see Results Section 3.1), 23 h was chosen as deployment time for all further DGT experiments. These subsequent experiments were performed in the same way as the previous test, but with triplicates of open and restricted DGT exposed to the digestate together in the same container. Separately, a blank was run in triplicate in the same way as the digestate samples, but just with MiliQ® water instead of the digestate sample.

For calculation of the metal concentration extracted by the DGTs, the standard DGT equation (Eq. (1)) was applied (Zhang and Davison, 2000):

$$c_{DGT} = \frac{M\Delta_g}{D\mathrm{A_p}t} \tag{1}$$

C_{DGT} (μmol) is the time averaged concentration of the metal, M is the mass of the accumulated metal in the resin (nmol), Δ_g is the thickness of the diffusive gel and filter membrane (cm), D is the diffusion coefficient of the specific metal (cm^2/s), A_p is the area of the filter membrane (cm^2), and t is the deployment time (s). The diffusion coefficients for OP DGTs were taken from the official DGT Research website (https://www.dgtresearch.com/), while the RES DGTs coefficients were calculated from a ratio between OP and RES coefficients at pH 7 at 25 °C, as reported by Shiva et al. (2015). The mass of the accumulated metal (μmol) was calculated using Eq. (2):

$$M = \frac{C_e\left(\mathrm{V_{HNO3}} + \mathrm{V_{gel}}\right)}{f_e M_w} \tag{2}$$

where C_e is the concentration of metals in the 1 M HNO_3 elution solution (g/L), V_{HNO3} is the volume of HNO_3 added to the binding gel (L), V_{gel} is the volume of the binding gel (L), f_e is the elution factor for each metal and $M_w$is the molar mass of each metal (g/mol). The values of f_e, V_{gel}, Δ_g, A_p and OP and RES diffusion coefficients used for calculation of the DGT concentration are reported in the Supplementary material.

2.3. Determination of metal contents

2.3.1. Reagents

All solutions used were purchased from the Chem-Lab NV and were of analytical grade. The nitric acid (67–69%) used for the samples that were measured using ICP-MS was of Pico-Pure grade from the same manufacturer. All glassware was rinsed with 1% nitric acid solution before usage, and then with deionised and Mili-Q® water.

Table 2
Characteristics of open pore and restricted DGT.

	Binding gel	Diffusive gel	Membrane filter
Open pore DGT	0.4 mm Chelex	0.78 mm APA (polyacrylamide cross linked with agarose derivative (15% monomer with 0.3% cross-linker)	0.45 μm polyethersulphone
Restricted DGT	0.4 mm Chelex	0.78 mm RES (polyacrylamide cross linked with bisacrylamide)	0.45 μm polyethersulphone

2.3.2. Sample preparation

DGT samples.

Once the samplers were taken out from the digestates, they were rinsed with MiliQ® water. Subsequently, the resin gels were taken out of the DGT samplers and soaked in 1.5 mL of 1 M HNO_3. The obtained solution was diluted 10 times with 1% HNO_3 in which metals were quantified.

Total metal content in the digestates.

All seven digestate samples were treated by the microwave assisted *aqua regia* method. The samples were dried for 24 h at 105 °C, ground in a mortar and around 0.2 g of each sample was measured and placed in microwave tubes. After addition of 7.5 mL 65% nitric acid and 2.5 mL 37% hydrochloric acid, the samples were left to rest for 30 min, then followed by sonification for 20 min. Once the sonification was finished, the samples were digested in the CEM MARS 6 microwave at 175 °C for 15 min at the maximum microwave power of 1800 W. Once cooled down, the digested samples were filtered through ashless filter paper (MN 640 m, Macherey-Nagel GmbH & Co.) and diluted to 50 mL in volumetric flasks with Milli-Q® water. All samples were analysed in triplicate. Parallel to the samples, about 0.2 g of soil reference material ``BodemB`` was treated using the same procedure. Recovery of metals from the reference material varied in the range of 104% to 121%.

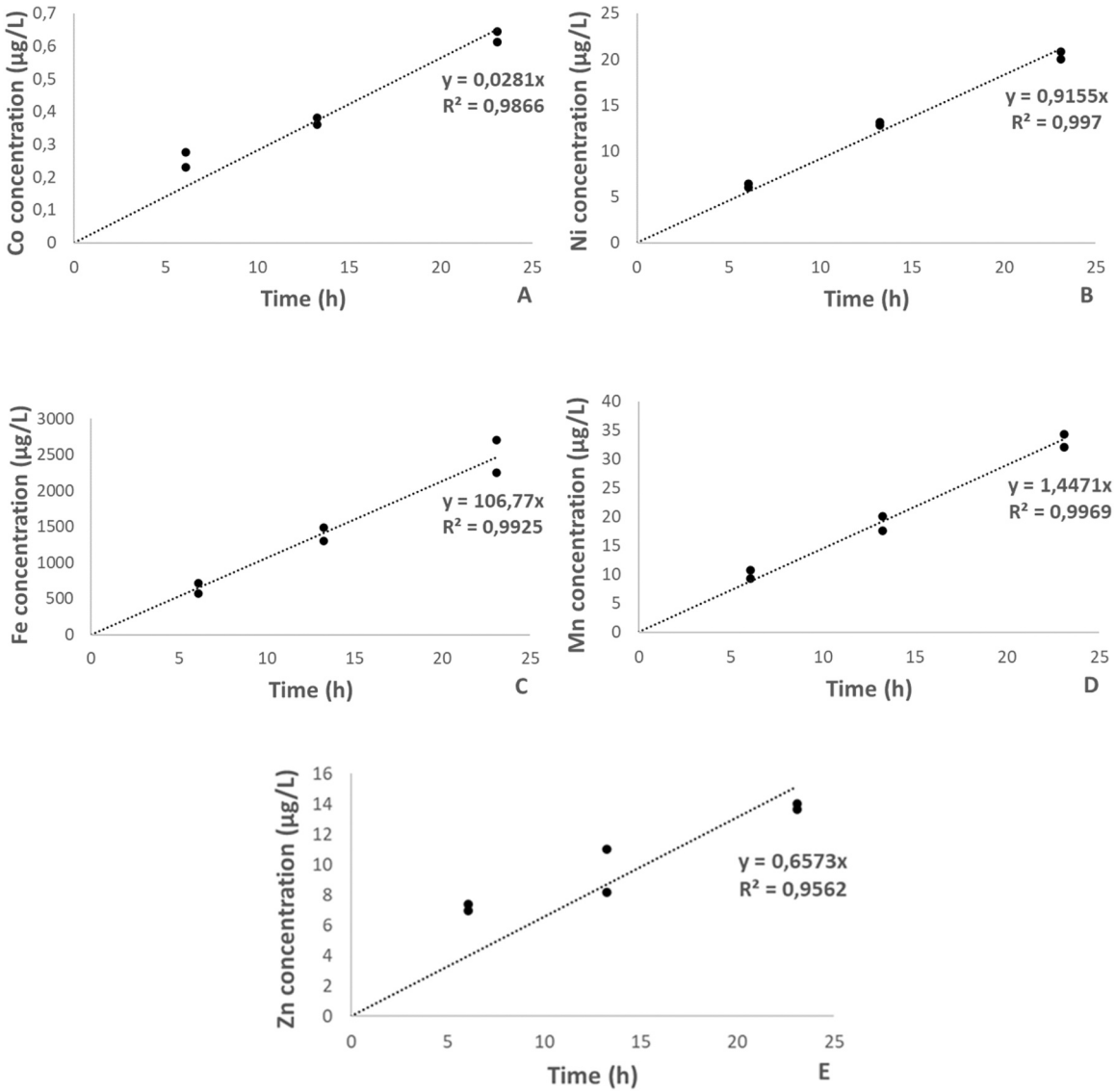

Fig. 1. Co (A), Ni(B), Fe(C), Mn(D) and Zn(E) concentrations measured from eluted DGT discs after 5, 13 and 23 h deployment. All time points were measured in duplicates.

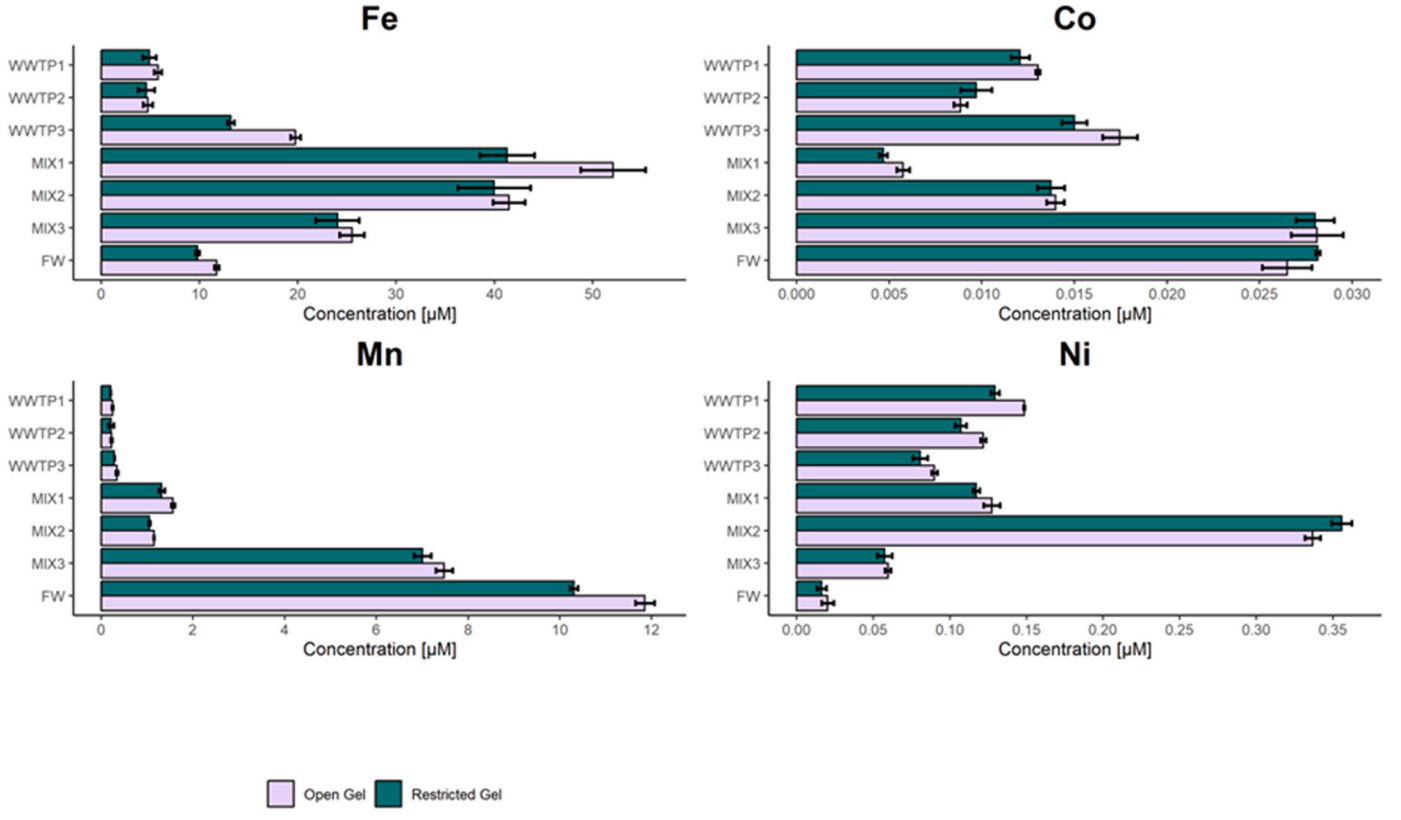

Fig. 2. Concentrations of Fe, Co, Mn and Ni collected from seven digestate samples (WWTP1, WWTP2, WWTP3, MIX1, MIX2, MIX3 and FW) by DGT after diffusion over restricted (RES) and open (OP) gels. Average values of technical triplicate are presented with standard deviations as error bars.

2.3.3. Instrumentation

A Thermo Scientific model iCAP 7000 series inductively coupled plasma-optical emission spectrometer (ICP-OES) with axial view configuration was used to measure Mn, Co, Ni, Cu and Zn in the *aqua regia* digested samples. The following emission wavelengths (nm) were used for element analysis: Mn 257.61, Co 228.616, Ni 221.647, Cu 324.754 and Zn 213.856. A Varian model Vista-MPX CCD Simultaneous ICP-OES with radial view configuration was used to measure Fe at 238.204 nm in the *aqua regia* digestion and the DGTgels. A Perkin-Elmer model NexION 350D Inductively coupled plasma mass spectrometer (ICP-MS) was used to measure Mn, Co, Ni, Cu and Zn in the DGT gel samples. The chosen isotopes were: ^{55}Mn, ^{59}Co, ^{58}Ni, ^{63}Cu and^{64}Zn. For all measurements, Rh was used as internal standard. To check the accuracy and precision of the calibration curves for both ICP-OES and ICP-MS, a Quality Control (QC) sample was included in each batch of samples. The recovery of QC on ICP-OES varied from 96% to 116%, and on ICP-MS from 88% to 107%. The limits of detection (LOD) and limits of quantification (LOQ) were calculated and their values for all metals are reported in Supplementary material.

3. Results

3.1. Determination of deployment time of DGT devices

To assure that we applied appropriate deployment time, i.e., time frame in which an increase of the deployment time is linear to an increase in metal accumulation in the DGT gel, we tested OP DGT devices on MIX2 sample for 5, 15 and 23 h. All metals measured showed a very high linear dependency of metal accumulation against time, with R^2 ranging from 0.956 for Zn to 0.992 for Fe. These results imply that- (1) the DGT device with OP gel can be used to measure metals in digestates ranging from very low (Co) to high (Fe) concentrations, and (2) even at shorter deployment times, such as 5 h, results are satisfactory (Fig. 1). We chose 23 h as the deployment time for our experiments, to ensure we can detect potentially very low metal concentrations.

3.2. Feasibility of DGT application to digestate samples in terms of precision and potential contamination

The results of DGT blanks showed that contamination contributed substantially to Zn and Cu measurements. Along with the fact that the blank triplicates showed high variability, high observed masses of Zn and Cu accumulated on the blanks could not be neglected. The median masses accumulated on the blank DGT gel (in ng) were: Fe 115; Cu 10.0; Co 0.70; Mn 27.3; Zn 445; Ni 0.70. These masses affected the digestate samples at a maximum of 4% for Fe, 20% for Co, 21% for Mn and 2% for Ni. Since Zn and Cu showed an accumulation in blanks of masses similar to the measured digestate samples, these two elements were not considered further.

To assess method precision, we chose a 10% relative standard deviation (RSD) as sample variation threshold, as suggested by Davison (2016). For all metals measured, sample variation was satisfactory in relation to this threshold. Out of 14 RSDs calculated per metal, (i.e. seven digestates measured with OP and RES DGT devices, each device in triplicate), there was no RSD higher than 10% for Co. Mn had one RSD above the threshold (27% relative standard deviation), whereas Ni had two (twice 19%) and Fe three (10%, 14% and 18%) RSDs above the threshold. When comparing the two types of diffusive gel, RES gel showed a higher variation compared to OP gel for Fe in six out of seven samples. Similarly, Ni variation was higher in RES gel in four samples and was lower in one sample. In two other samples, the RSD of both gels for Ni were the same. Unlike Fe and Ni, Co and Mn both had RSDs higher in OP gel in three samples, while in the other three samples, the RES gel showed a higher variation.

3.3. DGT bioavailability of metals

To assess bioavailability of metals in the digestates, we looked at the OP DGTs, as they have been more often used in the literature. The concentrations of metals measured varied to a high degree. As presented in Fig. 2, the highest concentrations were obtained for Fe (5–52 μM), followed by Mn (0.2–11.9 μM), Ni (0.02–0.34 μM) and Co (0.006–0.028 μM). Variation of the Mn concentration had a factor of about 50 between digestate samples; MIX3 and FW samples showed considerably higher concentrations than the other samples, with a maximum of 11.9 μM for FW and a minimum of 0.22 μM for WWTP2. The Ni concentration variation factor was 20, within the range of 0.02 to 0.34 μM. Even lower variation was found for Fe, with a factor of 10, with samples MIX1, MIX2 and MIX3 having the highest concentrations (maximum 52 μM for MIX1), while the lowest concentrations were measured in the WWTP1 and WWTP2 samples (5.8 μM and 4.7 μM, respectively). The lowest variations were found for Co with a factor 5 between the samples (from 0.06 to 0.3 μM).

3.4. Metal measurements with restricted (RES) and open pore (OP) gel

Restricted and open pore gels were used in parallel to measure the presence of organic complexes in the digestates (Fig. 2). Statistically significant differences between the metal concentrations extracted using both samplers were found in 54% of the digestate samples. More precisely, the concentrations of Mn differed significantly in 71% of the samples. For other metals, less than 50% of the digestate samples showed statistically significant differences (Fe 43%, Co 29%, Ni 43%). With respect to the digestate sample type, in the samples WWTP3 and MIX1, 3 out of 4 metals had averages that were statistically significant between both gel types, while other samples had only none to two. A ratio between metal concentrations obtained after separation on both gel types (C_{RES}/C_{OP}) can reflect to what extent metals are bound in organic complexes having a size of 1–5 nm. From the samples that showed statistically significant

differences, the presence of organic complexes is assumed to play only a minor role for Fe since the ratio $\mathbf{C}_{RES}/\mathbf{C}_{OP}$ was found to be the smallest for that metal. Other metals were bound by organic complexes by less than 20% (Table 3).

3.5. Total metal content in the digestates

Measured analytical triplicates showed very low variability with a maximum RSD of 8%. Based on the concentration range, we grouped the investigated metals in three sections (Fig. 3). In general, measured concentrations were as expected in terms of concentration range: Ni and Co were measured in concentrations ranging up to 35 mg/kg TS, Mn in hundreds of mg/kg TS and Fe varied in a significantly higher range of 10.3 to 61.9 g/kg TS.

The total metal content appeared to be sample dependent, namely, WWTP samples on average had higher total metal contents than MIX samples. This observation is most explicit for Fe, which was measured in the range of 40.4 to 61.9 g/kg TS in WWTP samples and from 10.3 to 36.2 g/kg TS in MIX samples. The lowest difference in metal concentration between these two groups was found for Mn, with an average of 286 and 418 mg/kg TS for WWTP and MIX samples, respectively.

3.6. Influence of digestate type on DGT-extractable metal contents

As total metal content is currently the basic parameter analyzed to assess metal availability in digestates, it is worth relating it to DGT-extractable concentrations in order to: (1) determine the share of DGT available metals relative to the total metal amount and (2) look for possible patterns on which bioavailability in relation to the feedstock can be predicted. Overall, it is clear that the DGT available fraction represents only a very small portion of the total amount of metals, ranging from 0.017 to 1.545% (with exception of 3.95% for one sample of Mn). The total amount of Fe compared to other metals was found to be very high. The WWTP samples evidently had higher total Fe concentrations than MIX samples, which can be expected because of Fe addition for chemical phosphate removal in WWTP processes. Interestingly, Fe was more available in MIX samples (Fig. 4b). Similarly, Ni was more available in MIX samples even though the total amount was higher in WWTP samples. At the same time, total Mn content showed similar values between samples, but also much higher DGT extractability in MIX samples. On the other hand, Co did not differ among the various digestate samples in neither total concentration nor in relative DGT extractability (Figs. 4a and 4b). These results once more confirm that total metal concentration is not an appropriate parameter to assess metal (bio)availability.

4. Discussion

4.1. DGT as a reliable technique to assess bioavailable metals in anaerobic digestate

The previous work on DGT applied in digestate samples provided very little information on method precision. The determination of reproducibility of the measurements is the first step towards using DGT as a device to routinely assess metal bioavailability in digestates. In the study conducted by Takashima (2018), the RSD for Ni ranged from 9% to 19%, while Co had a very high RSD from 20% to 100%. It is unclear whether this low reproducibility originated from too little measurements being done or low concentrations being measured. Our work showed consistency with the work done by Laera et al. (2019a), who also observed high reproducibility for Co, Fe, Mn and Ni. Despite the fact that some of our RSDs were above the recommended 10%, this threshold is proposed for measurements in solutions, which are, unlike the digestate matrix, completely homogenous. Hence, a higher variation between the samples was expected, which was also observed by Laera et al. (2019a) for As, Co, Mn and Pb. Variations being slightly higher than 10% (RSD) should also be acceptable since DGT measured concentrations of metals (important for anaerobic processes) vary with a factor of 1000 between digestate samples, thus they should not be affected by the RSD to a high extent.

From our results and the above-mentioned literature, it can be concluded that Fe, Co, Mn and Ni can be measured routinely in triplicates in digestates. On the other hand, based on our observations, special care in handling devices and avoidance of contamination of blanks are of utter importance to enable accurate Cu and Zn measurements. Potential Zn and Cu contamination from the environment has already been mentioned previously in the work of Davison (2016). Accordingly, we propose extensive measurements of DGT blanks and inclusion of these data in the reports as an important contribution to the quality of the DGT measurements. Another important parameter to consider is the deployment time of the DGT devices. Our experiment, where we tested different deployment times, showed that 23 h is an appropriate time for Co, Fe, Mn and Ni, which is in accordance with the studies done by Laera et al. (2019a); Takashima (2018).

Table 3
Ratios of metal concentrations extracted by DGT samplers after diffusion through RES and OP gels ($\mathbf{C}_{RES}/\mathbf{C}_{OP}$), illustrating to what extent metals may be bound in organic complexes having a size of 1–5 nm. Results are shown as mean±standard deviation, n = 3.

	WWTP1	WWTP2	WWTP3	MIX1	MIX2	MIX3	FW
Fe	0.91 ± 0.14	0.89 ± 0.18	0.68 ± 0.025*	0.82 ± 0.076*	0.96 ± 0.10	0.93 ± 0.10	0.81 ± 0.026*
Mn	0.85 ± 0.052*	0.85 ± 0.24	0.86 ± 0.060*	0.87 ± 0.046*	0.91 ± 0.015*	0.96 ± 0.034	0.87 ± 0.017*
Co	0.91 ± 0.038	1.0 ± 0.097	0.87 ± 0.062*	0.80 ± 0.062*	0.99 ± 0.063	1.0 ± 0.063	1.1 ± 0.055
Ni	0.86 ± 0.019*	0.87 ± 0.033*	0.88 ± 0.055	0.90 ± 0.042	1.0 ± 0.025*	0.93 ± 0.083	0.74 ± 0.20

* a ratio with a significant difference

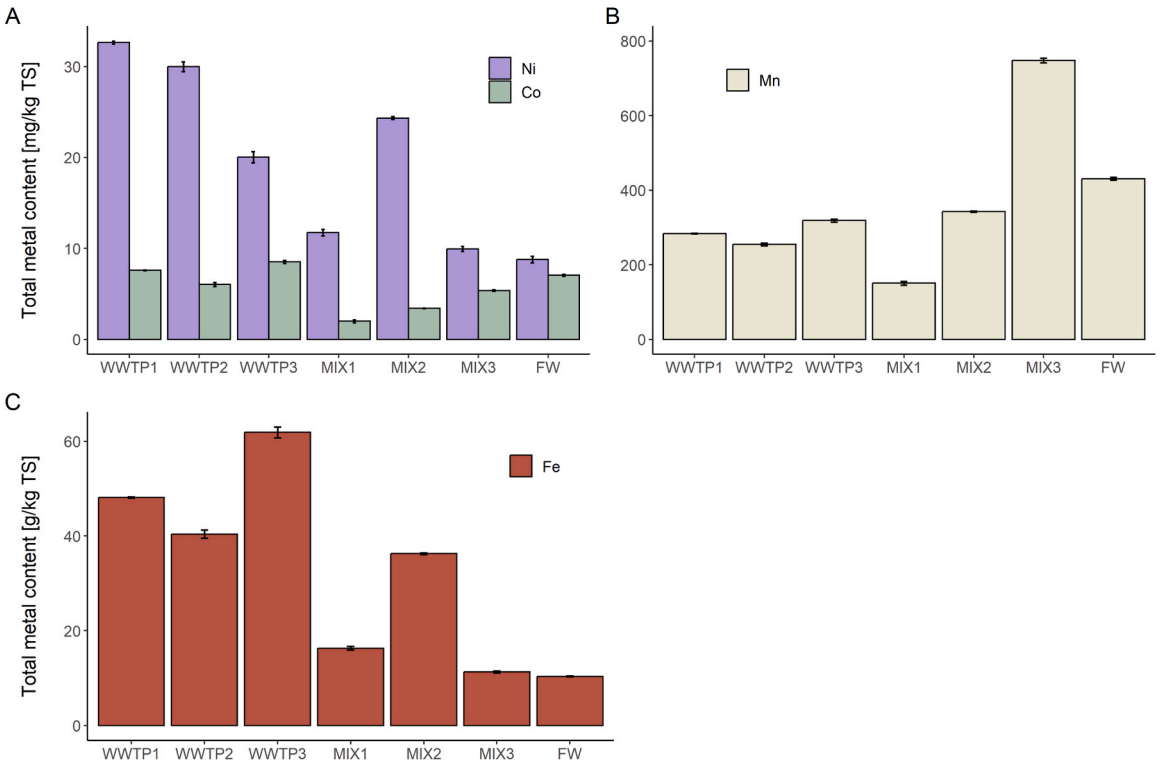

Fig. 3. The total metal content of each metal in each digestate sample. Each value is an average of triplicate analysis with error bars representing standard deviations. Metals are grouped based on the concentration range for easier comparison.

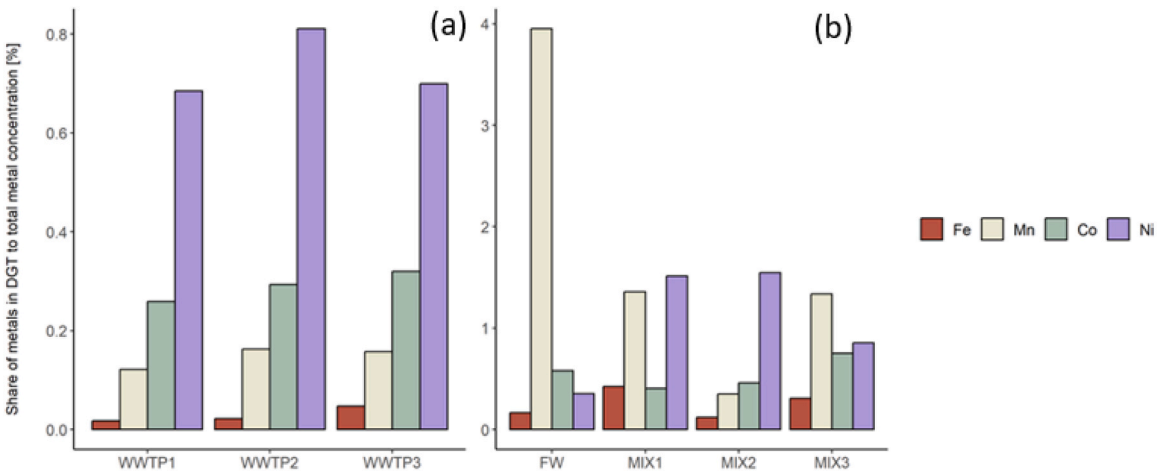

Fig. 4. Ratio of DGT-extractable to total concentration of metals in digestate samples originating from wastewater treatment plants (a) and agriculture (b).

4.2. The ratio of RES to OP DGT measurements to determine the presence of organic complexes

The difference in pore sizes of restricted and standard open gels used in DGT extractions is evaluated as a way to determine the presence of organic complexes containing trace metals. Through a restricted gel, only free metals and inorganic metal complexes are expected to diffuse, while in open gels, organic complexes such as humic and fulvic acid complexes should also be able to diffuse (Davison, 2016). The assessment of precision of RES gels is less reported in the literature than the OP gels. The application of both gels to differentiate inorganic metal complexation has been applied already on fresh waters in several studies. In one study (Baeyens et al., 2011), no substantial difference between RSDs obtained through the two different gels was seen for Fe, Mn and Ni (RSDs all below

10%). Only in one location, RES gel showed significantly higher RSD than OP gel for Co measurement (50 to 12%). This is in accordance with our work for commonly investigated metals, i.e,. Fe, Mn, Co and Ni.

In the work of Baeyens et al. (2011), the presence of metal-binding organic complexes, derived from the difference between RES and OP gel extractions, was pronounced in two out of three samples of fresh water for Fe (RES/OP: 0.15 and 0.36) and Mn (RES/OP: 0.30 and 0.32). For Co and Ni, one sample showed a noticeable difference in RES/OP ratio of 0.23 and 0.38, respectively. In contrast, Österlund et al. (2012) did not observe large organic compounds binding Cu and Ni, since the RES/OP ratio was on average 0.80, 0.89 and 0.84 for Cu and 0.82, 0.89 and 0.84 for Ni for three different locations. Interestingly, the results of digestate metal fractionation conducted by Laera et al. (2019a) showed that only Fe was affected by large organic complexes (RES/OP was 0.70). This corresponds with our results, which showed the smallest ratio of RES/OP among all metals for Fe (from 0.68 to 0.82).

The value of the diffusion coefficient (D) is one of the crucial parameters for metal concentration calculation, thus it affects the results to a high degree. There is no measured D for the digestate matrix for any metal so far, so using diffusion coefficients that were obtained from synthetic solution leads to possible systematic errors. In an earlier study (Laera et al.), D values for a restricted gel were approximately 70% of the D values for a standard gel. In our study, we used the data obtained from a synthetic solution (Shiva et al., 2015). Application of D values, even though likely inappropriate, resulted in the Co value higher in RES than in OP gel in FW sample. Therefore, as long as the D values of metals in the digestate are not known, i.e., presence of a high concentration of organic matter is not taken into account for both types of gels, conclusions about the presence of organic complexes based on the gel difference should be drawn cautiously. The interpretation based on the exclusion of organic complexes in RES gels should be used as a first approximation.

4.3. Influence of sample type on DGT measurements

The concentration of essential trace metals needed in anaerobic digestion depends on many factors: type of reactor, feedstock, operating parameters (temperature, pH, hydraulic retention time), presence of different compounds in the system and their equilibriums (mainly sulfides as precipitating and organic complexes as chelating agents), but also the reason for supplementation (e.g., optimize methane production, reduce VFA, start the reactor, explore metal deficiency in a system, reduce inhibition of a toxic compound). Due to these wide range of research aims, different studies have expressed metal requirements in different ways. One way is to define the amount of metals per g of TSS (Ketheesan et al., 2016), TS (Ariunbaatar et al., 2016; Schmidt et al., 2014) or dry matter (Roussel, 2013). Another way is to report the concentration of a metal in the medium in mg/L (Gustavsson et al., 2013; Takashima et al., 2011; Zandvoort et al., 2002). Consequently, the ability to compare data is often limited, due to a lack of data needed for conversion of data from one unit (parameter) to another. Another difficulty is that experimental studies study metal speciation under dynamic conditions, whereas model-based data are useful for delineating effects of precipitation, adsorption, complexation processes, etc. but they predict these concentrations only in equilibrium (Thanh et al., 2016).

Each of the four metals in WWTP samples have a very similar ratio of DGT extractable to total concentrations (Fig. 4a). This could imply that metals are bound by the same compounds. Accordingly, similar DGT extractability and metal (bio)availability can be expected for other WWTP samples. As for agricultural (food waste) samples, only Co showed very similar ratios, while other metals showed large variations, especially Mn.

We propose as a rule of thumb that Fe, Co, Ni and Mn extractability by DGT and their (bio)availability can be expected to be similar for other WWTP digestate samples, and DGT extractability and (bio)availability of Co can also be predicted in digestate samples originating from a mixture of manure, energy crops and food waste.

5. Conclusion

Our work demonstrated that regular DGT samplers show good precision for Co, Ni, Fe and Mn when applied to digestate samples of different origin, while Zn and Cu could not be analyzed after DGT extraction due to high blank contamination. Furthermore, we suggest a deployment time of 23 h. The application of restricted and open gels to assess the occurrence of organic complexes showed statistically significant differences for only half of the digestate samples, indicating that this method needs further verification once the diffusion coefficients for the digestate matrix are known. The DGT extractable fraction was a very small portion (less than 2% on average) of total metal content regardless of digestate sample type. Digested sewage sludge samples showed very similar DGT concentrations for all analyzed metals. In contrast, samples of digested mixtures of manure, energy crops and food waste had similarities only among Co concentrations. Overall, application of DGT as a tool to measure metal bioavailability in bioreactors has great potential due to the ease of in situ species separation during sampling and the dynamic nature of measurement that are able to capture real bioavailability.

CRediT authorship contribution statement

Du Laing Gijs: Resources, Conceptualization, Supervison, Writing – review & editing. **De Vrieze Jo:** Formal analysis, Resources, Writing – review & editing. **Bartacek Jan:** Conceptualization, Resources, Supervision, Writing – original draft. **Kouba Vojtěch:** Supervision, Writing – review & editing. **Ilic Aleksandra:** Conceptualization, Data curation, Investigation, Visualization, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

Many thanks to Roseline Blanckeart for her assistance with the ICP-MS and ICP-OES analyses. This research was funded by the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 676070. This communication reflects only the authors' views and the Research Executive Agency of the EU is not responsible for any use that may be made regarding the information it contains.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eti.2024.103526.

References

APHA, 2005. Standard Methods for the Examination of Water & Wastewater. American Public Health Association, Washington, D.C.
Ariunbaatar, J., Esposito, G., Yeh, D.H., Lens, P.N.L., 2016. Enhanced anaerobic digestion of food waste by supplementing trace elements: role of selenium (VI) and iron (II). Front. Environ. Sci. 4 (8).
Baeyens, W., Bowie, A.R., Buesseler, K., Elskens, M., Gao, Y., Lamborg, C., Leermakers, M., Remenyi, T., Zhang, H., 2011. Size-fractionated labile trace elements in the Northwest Pacific and Southern Oceans. Mar. Chem. 126 (1), 108–113.
Bartacek, J., Fermoso, F.G., Baldó-Urrutia, A.M., van Hullebusch, E.D., Lens, P.N.L., 2008. Cobalt toxicity in anaerobic granular sludge: influence of chemical speciation. J. Ind. Microbiol. Biotechnol. 35 (11), 1465–1474.
Boehnke, B., Diering, B., Zuckut, S.W., 1997. Cost-effective wastewater treatment process for removal or organics and nutrients. Water Eng. Manag. 144 (7), 18–21.
Bourven, I., Casellas, M., Buzier, R., Lesieur, J., Lenain, J.F., Faix, A., Bressolier, P., Maftah, C., Guibaud, G., 2017. Potential of DGT in a new fractionation approach for studying trace metal element impact on anaerobic digestion: the example of cadmium. Int. Biodeterior. Biodegrad. 119, 188–195.
Davison, W., 2016. Diffusive Gradients in Thin-Films for Environmental Measurements. Cambridge University Press, Cambridge.
Davison, W., Zhang, H., 2012. Progress in understanding the use of diffusive gradients in thin films (DGT)- back to basics. Environ. Chem. 9 (1), 1–13.
De Vrieze, J., De Lathouwer, L., Verstraete, W., Boon, N., 2013. High-rate iron-rich activated sludge as stabilizing agent for the anaerobic digestion of kitchen waste. Water Res. 47 (11), 3732–3741.
Fermoso, F.G., Collins, G., Bartacek, J., Lens, P.N.L., 2008. Zinc deprivation of methanol fed anaerobic granular sludge bioreactors. J. Ind. Microbiol. Biotechnol. 35 (6), 543–557.
Forsberg, J., Dahlqvist, R., Gelting-NystrÖm, J., Ingri, J., 2006. Trace metal speciation in brackish water using diffusive gradients in thin films and ultrafiltration: comparison of techniques. Environ. Sci. Technol. 40 (12), 3901–3905.
Gustavsson, J., Shakeri Yekta, S., Sundberg, C., Karlsson, A., Ejlertsson, J., Skyllberg, U., Svensson, B.H., 2013. Bioavailability of cobalt and nickel during anaerobic digestion of sulfur-rich stillage for biogas formation. Appl. Energy 112, 473–477.
Hinken, L., Urban, I., Haun, E., Urban, I., Weichgrebe, D., Rosenwinkel, K.H., 2008. The valuation of malnutrition in the mono-digestion of maize silage by anaerobic batch tests. Water Sci. Technol. 58 (7), 1453–1459.
Horáková, M., Lischke, P., Grünwald, A., 1986. Chemické a fyzikální metody analýzy vod, 1. vyd. ed. SNTL, Praha.
van Hullebusch, E.D., Guibaud, G., Simon, S., Lenz, M., Yekta, S.S., Fermoso, F.G., Jain, R., Duester, L., Roussel, J., Guillon, E., Skyllberg, U., Almeida, C.M.R., Pechaud, Y., Garuti, M., Frunzo, L., Esposito, G., Carliell-Marquet, C., Ortner, M., Collins, G., 2016. Methodological approaches for fractionation and speciation to estimate trace element bioavailability in engineered anaerobic digestion ecosystems: an overview. Crit. Rev. Environ. Sci. Technol. 46 (16), 1324–1366.
Jansen, S., Steffen, F., Threels, W.F., van Leeuwen, H.P., 2005. Speciation of Co(II) and Ni(II) in anaerobic bioreactors measured by competitive ligand exchange−adsorptive stripping voltammetry. Environ. Sci. Technol. 39 (24), 9493–9499.
Karlsson, A., Einarsson, P., Schnürer, A., Sundberg, C., Ejlertsson, J., Svensson, B.H., 2012. Impact of trace element addition on degradation efficiency of volatile fatty acids, oleic acid and phenyl acetate and on microbial populations in a biogas digester. J. Biosci. Bioeng. 114 (4), 446–452.
Ketheesan, B., Thanh, P.M., Stuckey, D.C., 2016. Iron deficiency and bioavailability in anaerobic batch and submerged membrane bioreactors (SAMBR) during organic shock loads. Bioresour. Technol. 211, 136–145.
Laera, A., Buzier, R., Guibaud, G., Esposito, G., van Hullebusch, E.D., 2019a. Assessment of the DGT technique in digestate to fraction twelve trace elements. Talanta 192, 204–211.
Laera, A., Buzier, R., Guibaud, G., Esposito, G., van Hullebusch, E.D., 2019b. Distribution trend of trace elements in digestate exposed to air: Laboratory-scale investigations using DGT-based fractionation. J. Environ. Manag. 238, 159–165.
Lisak, G., 2021. Reliable environmental trace heavy metal analysis with potentiometric ion sensors - reality or a distant dream. Environ. Pollut. 289, 117882.
Liu, R., Lead, J.R., Zhang, H., 2013. Combining cross flow ultrafiltration and diffusion gradients in thin-films approaches to determine trace metal speciation in freshwaters. Geochim. Et. Cosmochim. Acta 109, 14–26.
Muñoz Sierra, J.D., Lafita, C., Gabaldón, C., Spanjers, H., van Lier, J.B., 2017. Trace metals supplementation in anaerobic membrane bioreactors treating highly saline phenolic wastewater. Bioresour. Technol. 234, 106–114.
Ortner, M., Rameder, M., Rachbauer, L., Bochmann, G., Fuchs, W., 2015. Bioavailability of essential trace elements and their impact on anaerobic digestion of slaughterhouse waste. Biochem. Eng. J. 99, 107–113.
Österlund, H., Gelting, J., Nordblad, F., Baxter, D.C., Ingri, J., 2012. Copper and nickel in ultrafiltered brackish water: labile or non-labile? Mar. Chem. 132-133, 34–43.
Pesavento, M., Alberti, G., Biesuz, R., 2009. Analytical methods for determination of free metal ion concentration, labile species fraction and metal complexation capacity of environmental waters: a review. Anal. Chim. Acta 631 (2), 129–141.
Roussel, J., 2013. Metal behaviour in anaerobic sludge digesters supplemented with trace nutrients. University of Birmingham.

Schmidt, T., Nelles, M., Scholwin, F., Pröter, J., 2014. Trace element supplementation in the biogas production from wheat stillage – optimization of metal dosing. Bioresour. Technol. 168, 80–85.
Shiva, A.H., Teasdale, P.R., Bennett, W.W., Welsh, D.T., 2015. A systematic determination of diffusion coefficients of trace elements in open and restricted diffusive layers used by the diffusive gradients in a thin film technique. Anal. Chim. Acta 888, 146–154.
Takashima, M., 2018. Effects of thermal pretreatment and trace metal supplementation on high-rate thermophilic anaerobic digestion of municipal sludge. J. Environ. Eng. 144 (3), 04018009.
Takashima, M., Shimada, K., Speece, R.E., 2011. Minimum requirements for trace metals (iron, nickel, cobalt, and zinc) in thermophilic and mesophilic methane fermentation from glucose. Water Environ. Res. 83 (4), 339–346.
Thanh, P.M., Ketheesan, B., Yan, Z., Stuckey, D., 2016. Trace metal speciation and bioavailability in anaerobic digestion: a review. Biotechnol. Adv. 34 (2), 122–136.
Wei, T.-J., Guan, D.-X., Li, X.-Y., Hao, Y.-L., Teng, H.H., Yang, J.-F., Xu, Y.-Y., Li, G., 2022. Analysis of studies on environmental measurements using diffusive gradients in thin-films (DGT) from 1994 to 2020. J. Soils Sediment. 22 (4), 1069–1079.
Weng, L., Vega, F.A., Van Riemsdijk, W.H., 2011. Strategies in the application of the Donnan membrane technique. Environ. Chem. 8 (5), 466–474.
Ye, M., Sun, B., Zhu, A., Song, L., Ha, J., Qin, Y., Li, Y.-Y., 2022. Characterization of trace metal impact on organic acid metabolism and functional microbial community in treating dairy processing wastewater with thermophilic anaerobic membrane bioreactor. Bioresour. Technol. 359, 127495.
Yu, B., Zhang, D., Shan, A., Lou, Z., Yuan, H., Huang, X., Yuan, W., Dai, X., Zhu, N., 2015. Methane-rich biogas production from waste-activated sludge with the addition of ferric chloride under a thermophilic anaerobic digestion system. RSC Adv. 5 (48), 38538–38546.
Zandvoort, M.H., Osuna, M.B., Geerts, R., Lettinga, G., Lens, P.N.L., 2002. Effect of nickel deprivation on methanol degradation in a methanogenic granular sludge bioreactor. J. Ind. Microbiol. Biotechnol. 29 (5), 268–274.
Zhang, H., Davison, W., 2000. Direct in situ measurements of labile inorganic and organically bound metal species in synthetic solutions and natural waters using diffusive gradients in thin films. Anal. Chem. 72 (18), 4447–4457.
Zhang, H., Davison, W., 2015. Use of diffusive gradients in thin-films for studies of chemical speciation and bioavailability. Environ. Chem. 12 (2), 85–101.