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Guidelines for the use of Diffusive Gradients in Thin Films for measuring metal fluxes in sediment

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Guidelines for the use of Diffusive Gradients in Thin Films for measuring metal fluxes in sediment

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Corrigendum April 2021: Correction to the instructions for gel stock solution preparation in Section 2.2.1.2 (page 4; water volume corrected from 41.5 mL to 47.5 mL)

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Correspondence about the details of any method or procedure should be directed to the author(s).

This series presents detailed descriptions of methods and procedures relating to chemical and biological measurements in the marine environment. Most techniques described have been selected for documentation based on performance in ICES or other intercalibration or intercomparison exercises: they have been carefully evaluated and demonstrated to yield good results when correctly applied. They have also been subject to review by relevant ICES working groups, but this is not to be construed as constituting official recommendation by the Council.

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Contents

Foreword

Increasing public and regulatory concern over the chemical and ecological status of water bodies has led to a higher demand for performing extensive water quality assessments. However, regulatory frameworks often only focus on monitoring contaminants in the water column, and scarce regulatory attention has so far been dedicated to assessing sediment quality. Contaminants accumulated in the sediment may be released into the overlying water, or reach higher trophic levels due to transfer across the aquatic food chain. Thus, quality assessments of water bodies should include an evaluation of the occurrence of contaminants in sediments and the associated ecological risk.

During the last decades, efforts have been made to integrate sediment quality assessments into regulatory frameworks. As a consequence, robust and reliable methodologies for assessing sediment quality are now required. Among the existing methods for assessing metal contamination in sediments, the Diffusive Gradients in Thin films (DGT) technique has shown potential, due to its ability to measure metal fluxes from the pore water and labile sediment phases. Laboratory and field studies indicate that the DGT-labile metal flux provides robust predictions of metal bioaccumulation and toxicity to benthic organisms (Roulier *et al.* 2008; Dabrin *et al.* 2012; Simpson *et al.* 2012; Amato *et al.* 2014, 2015, 2016, 2018; He *et al.* 2018). DGT could thus be a useful tool for improving sediment quality assessments.

1   Introduction

This technical annex provides advice on the use of the Diffusive Gradients in Thin films (DGT) technique for evaluating metal contamination in sediment, and on the interpretation of DGT data. DGT has been extensively validated and is currently one of the most used techniques for passive sampling of metals in freshwater and marine sediments (Lehto, 2016; Österlund *et al.* 2016).

These guidelines are intended to assist with (i) the preparation of DGT probes (Section 2.2; unless purchased from specialised suppliers), (ii) the use of DGT probes in the laboratory and in the field (Section 2.3), and (iii) the preparation and analysis of DGT samples (Section 3). The guidelines are based on a review of relevant scientific publications, and also contain useful tips on technical aspects provided by experienced users (Zhang 2003; Jolley *et al.* 2016). More details on the theory underpinning the DGT technique can be found in the cited literature.

Currently, there are two DGT devices available: (i) a piston device, suitable for water, soil, and, to some extents, sediment deployments; and (ii) a planar device, specifically designed for sediment deployments. These guidelines focus only on the use of the planar DGT device, which allows improved sampling conditions and a more detailed assessment of vertical profiles of DGT-labile metal fluxes.

The DGT device can be loaded with different sorbents which are selective for specific substances. The focus of these guidelines is on applications of DGT for the detection of cationic metals (e.g. Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Pb^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}) using Chelex as the accumulative phase (the most commonly used for measurements of divalent metals).

2   Diffusive Gradients in Thin Films (DGT)

2.1   Principles and Theory

DGT is an *in situ* technique which allows the accumulation of a wide range of dissolved substances in aqueous media (e.g. pore water). Measurable substances depend on the receiving phase used, and include (but are not limited to) cationic metals, metalloids, anions, and organic compounds. For the determination of metal cations, Chelex is the most commonly used binding agent. DGT exploits Fick's first law of diffusion to link the accumulated amount of substance to its aqueous concentration (Davison and Zhang, 2012).
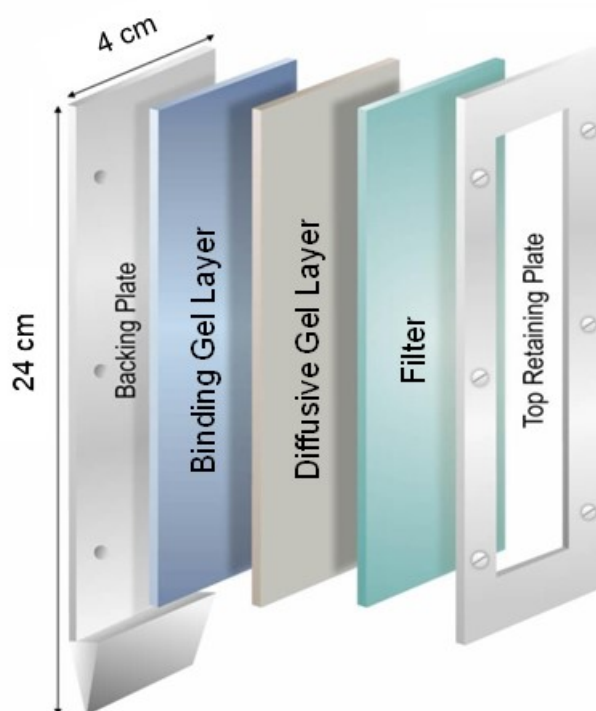
Figure 1. Schematic representation of the DGT assembly (not to scale).

The DGT technique relies on the use of an *in situ* device which is comprised of a binding gel, a diffusive gel, and a filter membrane (Figure 1). Once the device is introduced in the sediment, metals dissolved in the pore water diffuse across the filter membrane and the diffusive gel, and accumulate on the binding gel, driven by the gradient that exists between the concentration in the pore water adjacent to the binding gel layer (virtually zero) and the concentration in the bulk of the pore water (Zhang *et al.* 1995). The metal concentration at the device/pore water interface decreases overtime, inducing the release into the pore water of metals that are weakly bound to the sediment particulate phase. This process is regulated by the sediment/water equilibrium partitioning constants. Overall, DGT measurements of pore water concentrations rely on the ability of the sediment particulate phase to sustain pore water concentrations. The mass of metal accumulated on the DGT binding gel can be used to calculate pore water concentrations, if (i) pore water concentrations are fully sustained by release from the particulate phase of the sediment, and (ii) a limiting steady-state flux is assumed to

exist over the entire deployment time (Harper *et al.* 1998; Ciffroy *et al.* 2011; Nia *et al.* 2011). Alternative scenarios may occur, which are discussed in Section 5. Post-deployment analysis of the binding gel is used to determine the mean flux of DGT-labile species accumulated on the device during the deployment time (Section 3 and 4).

2.2 DGT probe

The DGT probes can either be purchased directly from commercial suppliers (such as DGT Research Ltd., UK[1]) or built in-house.

To avoid contamination, it is recommended that all procedures (gel preparation, probe assembly, etc.) take place inside a laminar flow cabinet with filtered air. All equipment used for DGT gels synthesis, handling, and storage should be cleaned by soaking in 10% HNO_3 for 24 hours and thoroughly rinsed with high purity water (18.2 MΩ cm).

Clean gloves should be worn at all times.

Detailed procedures for the preparation of DGT probes by individual laboratories are described in the following subsections: gel preparation (Section 2.2.1), DGT Probe-preparation and assembly (Section 2.2.2), and storage and handling (Section 2.2.3).

2.2.1 Gel preparation

The most commonly used gel for preparing DGT probes is an APA-type polyacrilamyde gel. Gels are synthesized by casting the gel solution between two rectangular glass plates separated by a plastic spacer. The spacer is placed round 3 edges of the plates (one long side is used for pouring the gel solution between the plates). The thickness of the spacer determines the thickness of the gel. Swelling after hydration occurs with polyacrylamide gels, which must be taken into account.

The following subsections provide instructions for the preparation and storage of binding and diffusive gels: required chemicals (Section 2.2.1.1), gel stock solution preparation (Section 2.2.1.2), diffusive gel preparation (Section 2.2.1.3), and Chelex binding gel preparation (Section 2.2.1.4).

2.2.1.1 Chemicals

- Acrylamide solution (40%)

- DGT gel cross-linker solution (2%; DGT Research Ltd, UK[1])

- Ammonium persulphate (10% w/w, prepared daily)

- N,N,N′,N′-Tetramethylethylenediamine (TEMED)

- Chelex-100 (sodium form, 200-400 mesh)

2.2.1.2 Gel stock solution preparation

The gel stock solution is prepared by mixing 47.5 mL of ultrapure water (18.2 MΩ cm) with 37.5 mL of acrylamide solution (40%). Once the solution is well mixed, add 15 g of DGT gel cross-linker solution, mix thoroughly and store in the fridge (4 °C). When kept outside the fridge, the solution must be constantly shaken.

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[1] www.dgtresearch.com

2.2.1.3 Diffusive gel preparation

1. Acid wash and rinse with ultrapure water a set of glass plates (pairs of 150 mm × 60 mm × 5 mm, and 150 mm × 50 mm × 5 mm) and 0.5 mm thick spacers (the thickness of the fully hydrated gel will be 0.8 mm).

2. Allow glass plates and spacer to dry in a laminar flow cabinet.

3. Place a spacer on top of 3 edges of the large plate, place the small plate on top of the spacer, and then clip the plates together using paperclips. Allow a 10 mm offset on the edge without spacer.

4. In a 50 mL centrifuge tube containing a magnetic stirring bar, mix 10 mL of gel stock solution with 70 µL of ammonium persulphate solution (10% w/w) and mix well. After approximately 3 min, add 25 µL of TEMED solution and mix for an additional 3 min.

5. Pipette the solution between the two plates in a smooth and controlled fashion. Care should be taken to avoid air bubbles forming in the casting mould.

6. Place the assembly (horizontally) in an oven set to about ~ 42–46°C. Allow the gel to settle for approximately 1 hour until the solution no longer appears liquid.

7. Rinse the edges of the assembly with ultrapure water and pull apart the plates. Carefully remove the gel using a plastic spatula and place it in a clean container.

8. Allow the gel to hydrate in ultrapure water for at least 24 hours, changing the water at least four times during this period (this process will require approximately 1 L of water in total). Store the gels in NaCl solution (0.1–0.01 M). The pH of the solution with the gel should be around 6.5–7.

2.2.1.4 Chelex binding gel preparation

1. Acid wash and rinse with ultrapure water a set of glass plates (pairs of 150 mm × 70 mm × 5 mm, and 150 mm × 60 mm × 5 mm) and 0.25 mm thick spacers (the thickness of the fully hydrated gel will be 0.4 mm).

2. Allow glass plates and spacer to dry in a laminar flow cabinet.

3. Place a spacer on top of 3 edges of the large plate, place the small plate on top of the spacer, and then clip the plates together using paperclips. Allow a 10 mm offset on the edge without spacer.

4. Add 4 g of wet Chelex resin to a centrifuge tube (50 mL) containing a magnetic stirring bar (it may be useful to make a slurry by adding ultrapure water to allow the resin to hydrate, and then pouring away the excess water). Add 10 mL of gel stock solution under vigorous stirring, followed by 50 µL of ammonium persulphate. Mix for approximately 3 min and add 15 µL of TEMED.

5. Mix the solution vigorously, ensuring that the resin beads are suspended. Pipette the solution between the two plates in a smooth and controlled fashion. Care should be taken to avoid air bubbles forming in the casting mould.

6. Place the assembly (horizontally) in an oven set to about ~ 42–46°C. Allow the gel to settle for approximately 1 hour until the solution no longer appears liquid.

7. Rinse the edges of the assembly with ultrapure water and pull apart the plates. Carefully remove the gel using a plastic spatula and place it in a clean container. If the plates are difficult to pull apart keep rinsing the edges of the assembly to

promote hydration and let it rest for a minute. Repeat this operation until plates become easier to pull apart.

8. Allow the gel to hydrate in ultrapure water for at least 24 hours, changing the water at least four times during this period (this process will require approximately 1 L of water in total). Store the gels in NaCl solution (0.1–0.01 M). The pH of the solution with the gel should be around 6.5–7.

2.2.2 DGT Probe-Preparation and Assembly

During DGT probe preparation and assembly, ensure that contamination is minimal by adopting all the laboratory practices described above. All containers and surfaces that may come in contact with the gels should be cleaned (by soaking in 10% HNO_3 for 24 hours and thoroughly rinsing with high purity water).

2.2.2.1 DGT assembly

The DGT probe for application in sediment (planar probe) consists of a plastic unit (total dimensions of 24 cm x 4 cm x 0.5 cm), which is comprised of two pieces, a backing plate and a top plate. The backing plate contains a pocket (16.2 cm x 2.8 cm x 0.12 cm) in which the binding gel, the diffusive gel, and a filter membrane are placed. A handle on the top of the base plate facilitates probe handling, and a bevelled edge at the bottom of the plate allows easier deployment and reduces disturbance of the sediment. The gels and filter membrane are held in place using the top plate with an open window of 1.8 cm x 15 cm.

2.2.2.2 Preparation of the sediment DGT probe

The DGT probe is assembled as shown in Figure 1. The following procedure is required to prepare a DGT probe:

1. Cut the filter membranes and gels using a Teflon-coated razor (scissors or knife) to fit in the DGT probe (16.2 cm x 2.8 cm x 0.12 cm). Wet the filter membranes with ultrapure water.

2. Lay the binding gel on the base plate of the probe with the side where Chelex beads settled facing up. Overlay the binding gel with the diffusive gel and then place a 0.14–mm thick filter membrane on top of the diffusive gel. Polysulphone membranes should be used instead of cellulose nitrate membranes, to avoid introduction of nitrates into the device.

   Note: During synthesis, Chelex beads settle on one side of the gel. The side with the beads is much rougher than the other side. Normally this should be visible when the gel is placed on a flat surface. If it is difficult to find the rough side, take a piece of clean tissue paper and dip it on a small area of the gel to take up the excess water on the surface. The roughness due to resin particles on the resin side should now be more visible. Alternatively, after the plates are pulled apart (and before the gels are cut to size), mark the gel to identify on which side the beads have settled (for instance, by removing a corner of the binding gel using a Teflon-coated razor).

3. Ensure that no air bubbles are trapped between the gel layers and that each layer is correctly aligned with the base plate. The total thickness of the gel layers and filter is approximately 1.34 mm for DGT. This allows some compression within the 1.2 mm space available in the pocket where the gel layers and filter are placed, and provides a good seal around the exposure window.

4. Put the window plate of the probe on top of the assembly, press them together gently, and clip it into place with the male connectors.

2.2.3   Storage and handling

- When handling all chemicals, ensure safety precautions are in place and adhered to.
- It is essential that DGT probes are kept isolated from potential sources of contamination at all times, except when being exposed at the sampling site.
- Avoid physical contact with the receiving phase or membrane of the passive sampling devices, since this might affect the results. When handling, use powder-free vinyl gloves.
- Store the probes at 4 °C, in sealed clean plastic bags containing a few drops of 0.01 M NaCl solution.
- Check the probes about once a week to make sure they are under moist conditions. Add a few more drops of trace metal clean 0.01 M NaCl solution if necessary.

2.3   Sampling

2.3.1   Pretreatment

Prior to deployment, the DGT probe must be de-oxygenated to avoid a potential alteration of redox conditions within the sediment, which can occur due to introduction of oxygen in anaerobic sediment layers. The following instructions should be followed to achieve this step:

1. Prepare an acid-washed plastic bottle (1 L, wide neck) to accommodate the probes and plastic tubing for introducing gas.

2. Prepare a 0.01 M NaCl solution, add 5–10 g Chelex-100 into the solution, and stir overnight. Alternatively use extra pure NaCl.

3. Fill the acid washed bottle with the previously prepared NaCl solution and immerse the probe in the solution. Introduce nitrogen or argon gas through the solution for 24 hours. Add some Chelex to the solution to reduce the risk of probe contamination. Make sure the bottle is capped while it is bubbling, or carry out the degassing in a glove bag. Deploy the probe immediately after degassing.

Note: DGT blank probes (Section 2.4) should be de-gassed together with regular probes.

2.3.2   DGT deployment

Deployments are commonly made *in situ* or in sediment cores. The latter deployment is typically performed within a laboratory or on board a research vessel (*ex situ* deployment).

2.3.2.1   Deployment *in situ*

Before performing *in situ* deployments, the depth of the overlying water should be considered. In shallow water, standing on, or in the immediate vicinity of, the deployment point must be avoided to prevent disturbance of the sediment. In the case of deeper water, scuba divers, extension devices, or landers will be required.

The probe should be as vertical as possible during the insertion into the sediment. Very compact sediments may be difficult to perforate with the DGT probe, and the presence

of roots could make insertion in the sediment more challenging. Testing the sediment with a ruler can help identify the most suitable area to insert the probe.

The presence of large particles, shells and debris may tear the DGT membrane and gel during deployment. To prevent this, a ruler can be inserted into the sediment, and the DGT probe can then be slid into the sediment with the sampling window facing the ruler. Once the probe is properly inserted, carefully remove the ruler and allow the sediment to collapse against the probe.

Care should be taken to ensure that the probe has been inserted at a sufficient depth to avoid water currents displacing the probe.

Figure 2 shows a photo of a DGT probe inserted in a sediment site.

Figure 2. DGT probe inserted in sediment

2.3.2.2   Deployment *ex situ*

For deep-water environments, *in situ* deployment of passive sampling devices can be very challenging or not feasible. As an alternative, deployment can be performed in collected sediment cores, mesocosms or box sediments (Fones *et al*. 2004; Kankanamge *et al.* 2017; Yu *et al*. 2017; Sun *et al.* 2019). The sediment can be collected using a Box or Nioz corer. Once at the surface, the sediment cores should be carefully transferred into an oxygenated water tank. DGT probes are then inserted in the sediment core.

Note: During deployment *ex situ*, the sampling conditions are modified, as the sediment is slightly disturbed and removed from the seabed. Ideally, equipment should be used to mimic the natural environment, such as tide simulations, overlying water flow rates, light cycles, ambient temperature fluctuations, and groundwater movement.

2.3.3   Deployment procedure (*in situ* and *ex situ*)

The deployment procedure is common to both *in situ* and *ex situ* deployments:

1. Make a mark (a fine line) on the side of the probe at the desired distance from the sediment/water interface (SWI) to the top of the window of the DGT probe. The height of the mark will depend on whether fluxes in the overlying water should be measured or not. Always ensure that at least ¾ of the device is inserted into the sediment to avoid potential loss of the device itself due to displacement.

2. Push the probe gently and smoothly into the sediment until the mark is in line with the SWI. Keep the probe as vertical as possible during the insertion.

3. Note down the temperature of the pore water (and/or overlying water near the SWI) and deployment time.

4. The length of time the DGT is deployed should be long enough to ensure that the time required to reach a steady-state flux within the DGT device is negligible compared to the overall duration of the deployment. However, long exposures can lead to increasing uncertainty in data interpretation due to (i) potential depletion of the DGT-labile metal reservoir in the immediate vicinity of the DGT probe, (ii) displacement of metals from the Chelex resin due to competition effects, and (iii) biofouling, which may act as an additional binding phase. As a general rule, a 24 hour exposure should allow a steady-state flux to be established for practically the entire duration of the deployment, while avoiding significant contribution from undesired factors affecting DGT measurements. However, deployments of up to 3 days can also be appropriate. For further details consult, e.g. Harper *et al*. 1998; Ciffroy *et al*. 2011; Nia *et al*. 2011; Jolley *et al*. 2016; and Koppel *et al*. 2019.

Note: blank probes (Section 2.4) should be exposed to the air and rapidly placed in a clean plastic bag.

2.3.4   DGT probe retrieval (*in situ* and *ex situ*)

The retrieval procedure is common to both *in situ* and *ex situ* deployments:

1. Pull the probe out of the sediment and rinse the surface with ultrapure water as shown in Figure 3. Make sure there are no particles left on the surface of the probe, especially around the sampling window area.

Figure 3. Rinsing of a DGT probe after retrieval.

2. Place the probe in a cleaned and labelled plastic bag and keep it at 4°C. Care should be taken to avoid contamination of the probe during transport and storage.

3. Record the temperature of the pore water (and/or overlying water near the SWI) and retrieval time.

4. Exposed DGT probes can be stored in a refrigerator prior to analysis. There is no specific time limit for how long the probes can be stored. However, care should be taken to avoid gels becoming dry, as this can cause the gels to stick to each other and become difficult to separate.

Note: the blank probes that were exposed to air during the deployment should be rinsed with the same ultrapure water used for rinsing deployed probes, and then placed back in the plastic bag.

2.4 Blanks and contamination

Ensure that all the steps necessary to minimize contamination are taken while preparing gels, assembling/handling DGT probes, and during sample preparation (Section 2.2). Direct contact with the DGT probes should be minimized as much as possible. Always wear clean, powder-free, vinyl gloves when handling the probe to avoid contamination.

Procedural blanks should be used in each of the sampling locations. The blank probe should be exposed to the air at the sampling site during deployment and retrieval of the sampling device set, but only during the time frame the probes exposed to sediment are being manipulated. Prior to deployment and after retrieval, blank probes must be handled in the same way as the set of probes exposed to sediment.

3 Sample preparation and Analysis

3.1 Elution of target elements

After the DGT devices/probes have been retrieved, accumulated metals can be eluted as follows (the slicing steps are illustrated in Figure 4):

1. Make a cut at the SWI mark using a Teflon coated blade.

2. Cut the gels and the filter membranes along the window edges without disassembling the probe.

3. Carefully lift the gels together with the filter membranes out of the window and lay them on a clean flat surface. Peel the top filter membrane and the diffusive gel off and leave the resin gel on the bottom filter membrane.

4. Cut the resin gel at the resolution required (no less than 1 mm).

5. Put each gel slice into a micro centrifuge tube (1.5 ml) and add 0.5–1 mL 1 M HNO_3 solution, ensuring that the gel slices are fully immersed. Elute for at least 24 hours.

The resin gel can also be dried onto a filter membrane using a gel dryer. It can then be analysed using ICP-MS-laser ablation at the required resolution.
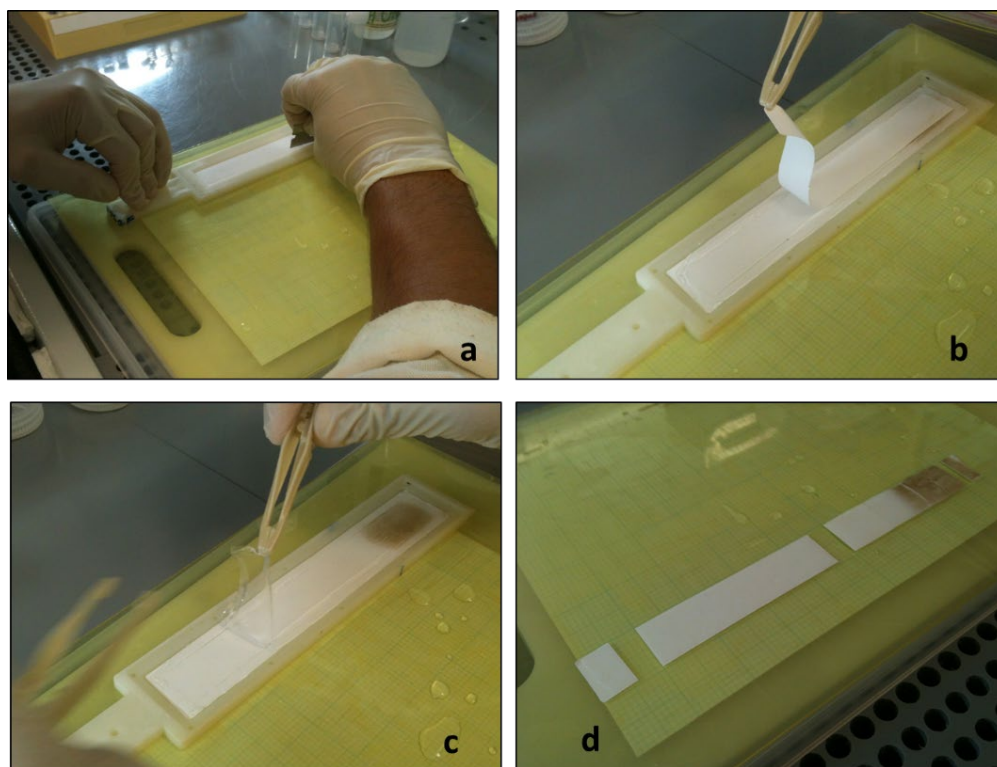

Figure 4. Steps involved in the slicing procedure of DGT binding gels: (a) the gels and the filter membrane are cut along the frame window edges; (b) the filter membrane is removed; (c) the gels are removed; (d) the resin gel on the filter membrane is sliced to achieve the desired resolution.

3.2   Analysis and detection

The analysis of metals in DGT extracts is usually performed using spectrometric or spectroscopic technics. Flame or graphite furnace atomic absorption spectroscopy used to be the primary method for the analysis of metals. Multi-element techniques like inductively coupled plasma, coupled with either an atomic emission spectrometer (ICP-AES) or mass spectrometer (ICP-MS) allow much more rapid analysis of a wide range of metals. Alternatively, if elution was not performed, PIXE or ICP-MS laser ablation can also be carried out at the required resolution (Davison *et al.* 1997; Stockdale *et al.* 2009).

If the analysis is conducted with ICP-MS, gel slices eluted in 1M HNO_3 require a 1:10 dilution with high purity water to avoid interferences during the analysis.

It should be ensured that the limits of detection of the analytical technique selected meet the requirements of the respective monitoring programme.

4 Calculations

DGT measurements of pore water concentrations rely on (i) a fast release of weakly bound metals from the particulate phase of the sediment into the pore water, (ii) a sufficiently large reservoir of such metals capable of sustaining pore water concentrations for the entire duration of the deployment, and (iii) a limiting steady-state flux over the entire deployment time. If such sampling conditions do not apply, the concentration measured by DGT will be different from the actual concentration in the pore water. The DGT pore water concentration can still be used as long as the difference with actual pore water concentrations is understood. However, this may lead to misinterpretation of results.

Metal accumulation on the DGT device depends on the same biogeochemical processes which influence metal speciation and bioavailability in sediment. Therefore, the metal flux (g m^{-2} s^{-1}) measured by DGT can be used as a measure of the ability of the sediment to release weakly bound and potentially bioavailable metals (Amato *et al*. 2014, 2015, 2016, 2018; He *et al*. 2018). The general concept of using DGT thus relies on the premise that a higher flux measured by DGT corresponds to a greater risk of metal exposure for benthic organisms.

4.1 DGT concentration and flux

First, calculate the mass (µg) of metal accumulated in the resin gel layer (M) using:

$$M = \frac{C_e(V_{HNO_3}+V_{gel})}{f_e} \qquad \text{Eq. (1)}$$

where C_e is the concentration of metals in the 1 M HNO$_3$ elution solution (µg L^{-1}), V_{HNO_3} is the volume of HNO$_3$ added to the resin gel (L), V_{gel} is the volume of the resin gel , and f_e is the elution factor for each metal, typically 0.8 (Zhang *et al*. 1995).

The concentration of metal measured by DGT (C$_{DGT}$; µg L^{-1}) can then be calculated using (Zhang *et al.* 1995; Davison and Zhang 2012):

$$C_{DGT} = \frac{M\Delta g}{DAt} \qquad \text{Eq. (2)}$$

where Δg is the thickness of the diffusive gel (0.078 cm) plus the thickness of the filter membrane (0.014 cm), D is the diffusion coefficient of metal in the gel (cm^2 s^{-1}; Zhang, 2003; Scally *et al*. 2006), t is deployment time (s), and A is the exposure area (cm^2).

The DGT flux can be calculated as follows:

$$J = \frac{M}{At} \qquad \text{Eq. (3)}$$

4.2 Limits of detection

Detection limits depend on the duration of the exposure, the temperature of the sediment, the slicing procedure (i.e. exposed surface area), the volume of acid used for the extraction, and the sensitivity of the analytical instrument used. Indicatively, for a 24 hour deployment, at a temperature of 15°C, using a surface area of 1.8 cm^2 (equivalent to a 1 cm thick horizontal slice), 0.5 mL of HNO$_3$ (for the extraction of the DGT sample), and an ICP-MS with LOD of 0.01 µg L^{-1}, the LOD for a standard DGT assembly is approximately 0.004 µg L^{-1}.

LODs under specific sampling conditions (e.g. temperature, deployment time, exposed surface area) can be estimated by calculating the lowest mass in the resin that can be detected by the analytical method used (obtained by replacing C_e with the LOD of the instrument in Equation 1 - do not forget to include the dilution factor if applicable), and substituting the obtained mass in Equation 2. C_{DGT} will be equal to the LOD of the method at the desired sampling conditions (i.e. temperature, deployment time, exposed surface area). Analogously, the lowest detectable flux can be estimated by replacing the calculated mass in Equation 3 (at the desired exposed surface area and deployment time).

4.3   Calibration and standards

Calibrations are usually performed using multi-element stock solutions, and at least a 4-point calibration covering the range of concentrations expected in the samples. Multi-element solutions are commercially available, and may be used provided that they are of a similar matrix to the analyte. A crosscheck solution from a separate batch, a different supplier, or an internal reference standard, should be used to check the calibration. Differences should not exceed 10%.

4.4   Quality assurance

At present, no DGT reference material is available for testing the performance of the analytical procedure required for analysing DGT samples. To our knowledge, no intercalibration exercise or proficiency testing has been documented so far.

5 Recommendations for the use of the DGT technique for sediment risk assessment

5.1 DGT fluxes or concentrations?

DGT measurements can be expressed as a flux (Equation 3 in Section 4.1; Amato *et al.* 2014; He *et al.* 2018) or a concentration (Equation 2 in Section 4.1; Roulier *et al.* 2008; Song *et al.* 2018; Zhang *et al.* 2019).

To express DGT measurements as concentrations in pore waters, the following assumptions must be met: (i) a fast resupply of solute from the solid phase, (ii) a sufficient pool of labile metals, and (iii) a limiting steady-state flux over the entire deployment time. These conditions may not be satisfied in some sediment deployments, and thus the concentrations measured by DGT can result in inadequate estimates of pore water concentrations (Harper *et al.* 1998; Nia *et al.* 2011).

In absence of the conditions required to match the DGT assumptions, DGT concentrations in pore waters can still be used, as long as the differences are understood between the concentrations 'experienced' by the DGT device and the real pore water concentrations. However, it should be noted that the calculation of concentrations adds further uncertainty, and frequently results in misleading interpretations of DGT data. Thus, expressing DGT measurements as a flux of metal ($g\ m^{-2}\ s^{-1}$), instead of a concentration in pore waters, is recommended here. Thresholds or guidelines can be derived based on the assumption that sediments with greater fluxes of metals pose a greater risk to benthic organisms than sediments with lower fluxes of metals (Simpson *et al.* 2012; Amato *et al.* 2014, 2015, 2016, 2018; He *et al.* 2018).

5.2 A multi-compartment approach

One of the most important characteristics of the DGT technique is the ability to simultaneously measure fluxes of metals released in different compartments of the sediment (oxic, sub-oxic, anoxic) and in the overlying water. The exposure to metals will differ for each biological species according to the organisms borrowing and feeding behaviours. Therefore, relationships between DGT fluxes and biological responses have been investigated in different compartments representing different exposures (bulk sediment, SWI and overlying water).

In some studies, the strongest relationships were obtained using fluxes measured at the SWI (± 1 or 2 cm), which represent a combined exposure to metals released in the pore- and overlying water (Simpson *et al.* 2012; Amato *et al.* 2014, 2015, 2016, 2018; He *et al.* 2018). As a large proportion of benthic organisms live in close proximity to the SWI, and/or are affected by the dynamics occurring in this compartment (e.g. burrowing activities, solute exchange between pore and overlying water, particle deposition), using fluxes measured at the SWI may be the most appropriate way of investigating the exposure to metals of benthic organisms.

6 References

Amato, E.D., Simpson, S.L., Jarolimek, C.V., and Jolley, D.F. 2014. Diffusive gradients in thin films technique provide robust prediction of metal bioavailability and toxicity in estuarine sediments. Environmental Science and Technology, 48: 4485–4494. https://doi.org/10.1021/es404850f

Amato, E.D., Simpson, S.L., Belzunce-Segarra, M.J., Jarolimek, C.V., and Jolley, D.F. 2015. Metal fluxes from porewaters and labile sediment phases for predicting metal exposure and bioaccumulation in benthic invertebrates. Environmental Science and Technology, 49: 14204–14212. https://doi.org/10.1021/acs.est.5b03655

Amato, E.D., Simpson, S.L., Remaili, T.M., Spadaro, D.A., Jarolimek, C.V., and Jolley, D.F. 2016. Assessing the effects of bioturbation on metal bioavailability in contaminated sediments by Diffusive Gradients in Thin films (DGT). Environmental Science and Technology, 50: 3055–3064. https://doi.org/10.1021/acs.est.5b04995

Amato, E.D., Marasinghe Wadige, C.P.M., Taylor, A.M., Maher, W.A., Simpson, S.L., and Jolley, D.F. 2018. Field and laboratory evaluation of DGT for predicting metal bioaccumulation and toxicity in the freshwater bivalve *Hyridella australis* exposed to contaminated sediments. Environmental Pollution, 243: 862–871. https://doi.org/10.1016/j.envpol.2018.09.004

Ciffroy, P., Nia, Y., and Garnier, J.M. 2011. Probabilistic multicompartmental model for interpreting DGT kinetics in sediments. Environmental Science and Technology, 45: 9558–9565. https://doi.org/10.1021/es104221b

Dabrin, A., Durand, C.L., Garric, J., Geffard, O., Ferrari, B.J. and Coquery, M., 2012. Coupling geochemical and biological approaches to assess the availability of cadmium in freshwater sediment. Science of the Total Environment, 424: 308–315. https://doi.org/10.1016/j.scitotenv.2012.02.069

Davison, W., Fones, G.R., and Grime, G.W. 1997. Dissolved metals in surface sediment and a microbial mat at 100-μm resolution. Nature 387: 885–888. https://doi.org/10.1038/43147

Davison, W., and Zhang, H. 2012. Progress in understanding the use of diffusive gradients in thin-film – back to basics. Environmental Chemistry, 9: 1–13 https://dx.doi.org/10.1071/EN11084

Fones, G.R., Davison, W., and Hamilton-Taylor, J. 2004. The fine-scale remobilization of metals in the surface sediment of the North-East Atlantic. Continental shelf research, 24: 1485–1504. https://doi.org/10.1016/j.csr.2004.05.007

Harper, M.P., Davison, W., Zhang, H., and Tych, W. 1998. Kinetics of metal exchange between solids and solutions in sediments and soils interpreted from DGT measured fluxes. Geochima et Cosmochimica Acta, 62: 2757–2770. https://doi.org/10.1016/s0016-7037(98)00186-0

He, Y., Guo, C., Lv, J., Hou, S., Zhang, Y., Zhang, Y., and Xu, J. 2018. Predicting trace metal bioavailability to chironomids in sediments by diffusive gradients in thin films. Science of the Total Environment, 636: 134–141. https://doi.org/10.1016/j.scitotenv.2018.04.285

Jolley, D.F., Mason, S., Gao, Y., and Zhang, H. 2016. Practicalities of working with DGT. *In* Diffusive Gradients in Thin-Films for Environmental Measurements, Cambridge Environmental Chemistry Series, pp: 263-290. Ed. by W. Davison. Cambridge University Press. https://doi.org/10.1017/cbo9781316442654.011

Kankanamge, N.R., Bennett, W.W., Teasdale, P.R., Huang, J. and Welsh, D.T. 2017. Comparing *in situ* colorimetric DET and DGT techniques with *ex situ* core slicing and centrifugation for measuring ferrous iron and dissolved sulfide in coastal sediment pore waters. Chemosphere, 188: 119–129. https://doi.org/10.1016/j.chemosphere.2017.08.144

Koppel, D.J., Adams, M.S., King, C.K., and Jolley, D.F. 2019. Diffusive gradients in thin films can predict the toxicity of metal mixtures to two microalgae: validation for environmental monitoring in Antarctic marine conditions. Environmental Toxicology and Chemistry, 38: 1323–1333. https://doi.org/10.1002/etc.4399

Lehto, N. 2016. Principles and application in soils and sediments. *In* Diffusive Gradients in Thin-Films for Environmental Measurements, Cambridge Environmental Chemistry Series, pp: 123–145. Ed. by W. Davison. Cambridge University Press. https://doi.org/10.1017/CBO9781316442654.008

Nia, Y., Garnier, J.-M., Rigaud, S., Hanna, K., and Ciffroy, P. 2011. Mobility of Cd and Cu in formulated sediments coated with iron hydroxides and/or humic acids: A DGT and DGT-PROFS modelling approach. Chemosphere, 85: 1496–1504. https://doi.org/10.1016/j.chemosphere.2011.08.045

Österlund, H., Widerlund, A. and Ingri, J. 2016. Applications in natural waters. *In* Diffusive Gradients in Thin-Films for Environmental Measurements, Cambridge Environmental Chemistry Series, pp: 123–145. Ed. by W. Davison. Cambridge University Press. https://doi.org/10.1017/CBO9781316442654.007

Roulier, J.L., Tusseau-Vuillemin, M.H., Coquery, M., Geffard, O., and Garric, J. 2008. Measurement of dynamic mobilization of trace metals in sediments using DGT and comparison with bioaccumulation in *Chironomus riparius*: First results of an experimental study. Chemosphere, 70: 925–932. https://doi.org/10.1016/j.chemosphere.2007.06.061

Scally, S., Davison, W., and Zhang, H. 2006. Diffusion coefficients of metals and metal complexes in hydrogels used in diffusive gradients in thin films. Analytica Chimica Acta, 558: 222–229. https://doi.org/10.1016/j.aca.2005.11.020

Simpson, S.L., Yverneau, H., Cremazy, A., Jarolimek, C.V., Price, H.L., and Jolley, D.F. 2012. DGT-induced copper flux predicts bioaccumulation and toxicity to bivalves in sediments with varying properties. Environmental Science and Technology, 46: 9038–9046. https://doi.org/10.1021/es301225d

Song, Z., Shan, B., and Tang, W. 2018. Evaluating the diffusive gradients in thin films technique for the prediction of metal bioaccumulation in plants grown in river sediments. Journal of hazardous materials, 344: 360–368. https://doi.org/10.1016/j.jhazmat.2017.10.049

Stockdale, A., Davison, W., and Zhang, H. 2009. Micro-scale biogeochemical heterogeneity in sediments: A review of available technology and observed evidence. Earth-Science Reviews, 92: 81–97. https://doi.org/10.1016/j.earscirev.2008.11.003

Sun, H., Gao, B., Gao, L., Xu, D., and Sun, K. 2019. Using Diffusive Gradients in Thin films (DGT) and DGT-induced fluxes in sediments model to assess the dynamic

release of copper in sediment cores from the Three Gorges Reservoir, China. Science of the Total Environment, 672: 192–200. https://doi.org/10.1016/j.scitotenv.2019.03.400

Yu, J., Ding, S., Zhong, J., Fan, C., Chen, Q., Yin, H., Zhang, L., and Zhang, Y. 2017. Evaluation of simulated dredging to control internal phosphorus release from sediments: focused on phosphorus transfer and resupply across the sediment-water interface. Science of the Total Environment, 592: 662–673. https://doi.org/10.1016/j.scitotenv.2017.02.219

Zhang, H., Davison, W., Miller, S., and Tych, W. 1995. *In situ* high resolution measurements of fluxes of Ni, Cu, Fe, and Mn and concentrations of Zn and Cd in porewaters by DGT. Geochimica et Cosmochimica Acta, 59: 4181–4192. https://doi.org/10.1016/0016-7037(95)00293-9

Zhang, H., 2003. DGT for measurements in waters, soils, and sediments. DGT Research Ltd: Lancaster, UK.

Zhang, Y., Yang, J., Simpson, S.L., Wang, Y. and Zhu, L. 2019. Application of Diffusive Gradients in Thin films (DGT) and Simultaneously Extracted Metals (SEM) for evaluating bioavailability of metal contaminants in the sediments of Taihu Lake, China. Ecotoxicology and environmental safety, 184: 109627. https://doi.org/10.1016/j.ecoenv.2019.109627

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