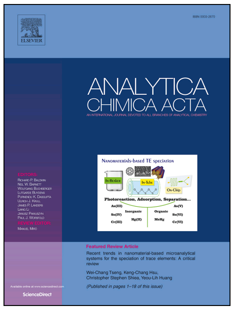

Use of diffusive gradient in thin films for *in situ* measurements: A review on the progress in chemical fractionation, speciation and bioavailability of metals in waters

Amauri Antonio Menegário, Lauren N. Marques Yabuki, Karen S. Luko, Paul N. Williams, Daniel M. Blackburn

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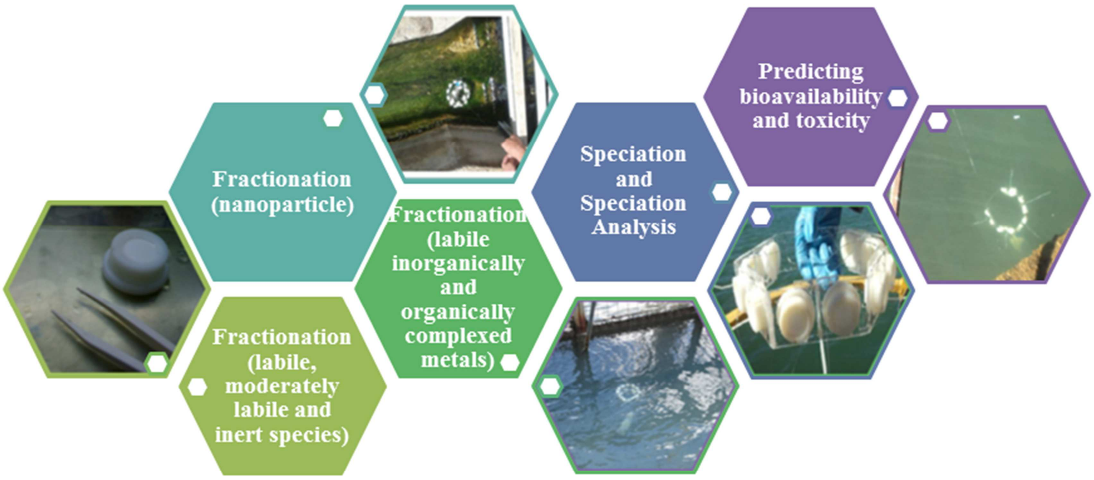
Fractionation (nanoparticle)
Fractionation (labile, moderately labile and inert species)
Fractionation (labile inorganically and organically complexed metals)
Speciation and Speciation Analysis
Predicting bioavailability and toxicity

Use of diffusive gradient in thin films for *in situ* measurements: a review on the progress in chemical fractionation, speciation and bioavailability of metals in waters

Abstract

Chemical fractionation, speciation analysis and bioavailability of metals and metalloids in waters have received increased attention in recent years. However, this interest is not matched by progress in improving species integrity during standard ‘grab’ sample collection, processing and storage. Time-averaged, low disturbance sampling, *in situ*, of trace element species, in particular, is a more reliable approach for environmental chemical surveillance and methods based on the diffusive gradients in thin films (DGT) technique stand out as one of the most widely used of the passive sampler classes, and hence will be the primary focus of this review. The DGT technique was initially developed to sample metals and semi-metals in freshwaters, and later was extended to include marine settings as well as the measurement of metal fluxes in sediments/soils. Nowadays, DGT based technologies are used extensively in a variety of geochemical and environmental health research disciplines. This review specifically surveys the application of the DGT measurement for fractionation and speciation analysis (as defined by IUPAC) of metal or metalloids *in aqua*. Use of DGT in fresh, estuarine and marine waters, as well as effluents has improved the knowledge base of *in situ* data related to fractionation processes (*e.g.* labile and inert species; organic and inorganic species; dissolved and nanoparticles), and speciation analysis. This supports not only the calculations underpinning numerous software speciation models for cation and anion behavior, but also our understanding of the bioavailability and toxicity of these species. The measurement of metals by DGT are easy to obtain, which is core to its popular use, but often the results require sophisticated interpretation and a wide spectrum of chemical knowledge to really explain in full, which is why the method has and continues to capture the interest of researchers.

keywords: DGT, Passive sampler, Metal speciation, Trace element, Labile species

Summary

1. Introduction and relevance for *in situ* speciation (fractionation, speciation analysis and bioavailability)

Metallic elements and species in natural waters can be *free*, complexed (*e.g.* by humic substances) or adsorbed by suspended solids. The metals in the *free* or labile form in most cases are more reactive, possess a smaller mass enabling faster diffusive transport, and have a higher toxicity [1]. Some organometallic species, for example, methylmercury (MeHg) or tributyltin (TBT) are considerably more toxic than their inorganic counterparts, Hg(II) and Sn(IV) respectively. These exceptions to the rule, relate to a very specific case of molecular mimicry, which confers upon these species the ability to be transported freely within living systems via pathways that are intended for biologically essential organic compounds [2]. Additionally, metalloid (*e.g.* Sb, As and Se) toxicity varies in relation to valency characteristics, for example trivalent As(III) and Sb(III) are more harmful than their pentavalent As(V) and Sb(V) counterparts [3]. Therefore, analytical techniques to selectively determine these fractions are essential for the study of hazard risk associated with metals in aquatic environments [4]. Differentiation of organometallic species from their inorganic form or separation based on valence followed by species quantification normally requires the use of a combination of techniques, firstly to separate the target analytes/molecules and then to measure them (*e.g.* gas chromatography coupled with inductively coupled plasma mass spectrometry, GC-ICP-MS) [5]. Although approaches based on the use of coupled techniques have been increasing in recent years, less attention has been afforded to the preservation of species integrity during sample collection and processing. In this sense, the use of sophisticated and state-of-the-art techniques (*e.g.* GC-ICP-MS) to selectively determine highly toxic but stable species at low environmental concentrations (*e.g.* determination of TBT in water or sediments) can be considered indeed an evolution in analytical chemistry.

However, determination of labile metal fractions, or selective determination of metalloid redox species in both the laboratory and field settings cannot always replicate the true conditions as accurately as we as a research community would ideally like.

Ensuring that the correct protocols for collection and sample preparation are followed is critical if contamination and transformation (changes in the distribution of species) of a sample during collection, handling, transport and storage, are to be minimised. It is worth noting, that even the most rigours preservation techniques will only ever slow down the inevitable on-going chemical and biological changes that occur after collection, with the complete preservation of samples being nearly impossible [6]. In this context, passive samplers may be considered an effective alternative compared to traditional grab sampling collection techniques since the analytes are being sampled *in situ*, with low environmental disturbance. In addition to other advantages, passive samplers can integrate multiple levels of speciation data to provide a better overall measurement of metal bioavailability. Particularly for determination of trace element species, methods based on the DGT technique are the most widely used globally [7], and hence form the focus of this review.

The DGT technique was developed in 1994 [8] and was initially applied to sample metals and semimetals in freshwaters *in situ*. In 1998, the range of DGT applications was extended to include the measurement of metal fluxes in sediments and soils [7,9–11]. Since then, DGT based technologies have been used extensively in a variety of geochemical and environmental health research disciplines. In addition to the ability to sample species selectively, DGT provides a time-weighted measure of concentration, acts to stabilise and pre-concentrate target analytes, while providing an effective alternative to multiple repeat single sampling events that not only take-up resources (time and expense) but are risk points for contamination. All these attributes are key for the quantification of metals at ultra-trace concentrations (ppb or ppt) in the environment. When measured with plasma based analytical techniques such as optical emission spectrometry (ICP-OES) or mass spectrometry (ICP-MS) multiple element parameters can be obtained simultaneously [7].The DGT technique is based on the immersion of a polypropylene device comprising of two pieces, the piston and the cap. The piston works as a support for the gel-layers that are placed inside the devices (firstly a membrane, then diffusive matrix and finally a functionalised binding layer), which may vary according to the method and the target analyte being sampled. The piston base and membrane/diffusive layer stack is then enclosed by a tight-fitting cap, which guarantees that the pathway of ion transport from the bulk solution to the inner layers occurs specifically through an exposure window of a defined area. The diffusive layer forces ion transport to occur exclusively by molecular diffusion, thus allowing analyte concentration to be determined by applying Fick’s First Law of diffusion [7].

According to the principles of the technique, analyte concentration in the solution can be determined by the equation below:

$$C_b = (M\,\Delta g).(D\,t\,A)^{-1} \qquad \text{Eq. (1)}$$

where $\boldsymbol{C_b}$ is the *free* or labile concentration of metals in the deployment media/sample matrix, $\boldsymbol{M}$ is the recovered mass of the analyte, $\boldsymbol{\Delta g}$ is the diffusive gel thickness, $\boldsymbol{D}$ is the diffusion coefficient of the analyte in the gel, $\boldsymbol{t}$ is the deployment time and $\boldsymbol{A}$ is the exposure window area [7]. Eq. (1) is based on a steady-state condition and satisfactorily holds for most environments and deployment scenarios. Nevertheless, there are exceptions for some conditions, mainly for short deployments (less than 4 h), systems with very high dissolved organic carbon (DOC) concentrations, and water matrixes with low ionic strengths. The issue of allowing sufficient time for a diffusive gradient of solute to form within the DGT devices is quite a straight-forward concept to grasp, but the subtle effects of either, metals and humic substances binding to diffusive layers or the generation of weak charges on the diffusive gel to occur are more complicated measurement discrepancies to identify, mitigate and understand [12–14].

Until now, various types of materials (e.g. polyacrylamide gel [8], agarose gel [15], dialysis membrane [16], Nafion® membrane [17], chromatography paper [18], filter paper [19]) have been evaluated as diffusive layers within the DGT samplers. The first material used in DGT to fabricate the binding layer, was the polyvalent metal chelating resin Chelex-100® (Bio Rad). The replacement of Chelex-100® with other binding phases may vary according to desired binding species and also the matrix, in which DGT will be deployed. Since then, various binding agents have been developed to measure different analytes in waters. Table 1 lists several proposed binding agents (in groups of analytes) for use in DGT samplers.

From its development in 1994 up until the present time (August, 2016) more than 715 papers can be found by entering the keywords *diffusive gradients in thin films* on Web of Knowledge Index. Published studies of DGT applications in waters total ca. 550 papers (found by entering the keywords *water diffusive gradients in thin films*) and this has helped greatly to improve our understanding of fractionation patterns (labile fraction, organic and inorganic labile fraction) in many different water systems.

In the literature, it is possible to find a wide variety of interpretations or uses of the terms of metal speciation being applied to the DGT measurement, although, these approaches do not always exclusively fit within the IUPAC definition [20]. For example, the use of DGT for studies of speciation and bioavailability was recently (2015) reviewed by Zhang and Davison [21]. The review examines and discusses key publications in the last 20 years, giving an interesting environmental perspective to DGT theory (related to measurements of metal complexes) and the capability of DGT to obtain *in situ* kinetic information. Also, the relationships between DGT measurements in soils and plant uptake were discussed in depth.

The focus of the present review is to consider from a practical perspective DGT approaches *in situ* and highlight the advantages and analytical limitations of the method. We hope that this focus can also inform analytical knowledge concerning sample storage and preservation, and encourage more grab sampling measurements to have some form of passive sampler data validation, as part of confirmatory protocols.

Over the last two decades, the term "*speciation*" (borrowed from biological sciences) has become an important measurement concept in analytical chemistry, although because it is a broad term it can have a diffuse meaning. Thus, aiming to avoid confusion, the International Union of Pure and Applied Chemistry (IUPAC) suggested to differentiate the terms 1) speciation, 2) speciation analysis and 3) fractionation as: 1) "distribution of an element between defined chemical species in a system"; 2) "an analytical procedure to identify and/or quantitative measurement of one or more chemical species in a sample"; 3) "classification of an analyte or a group of analytes from a sample according to physical or chemical properties"[20]. Although, the above definitions seem explicit, ambiguity in their use is still common in the published literature. Nevertheless, in the present review, we have considered the IUPAC concepts as key criterion to separate the various DGT approaches into different topics. Key papers (n = 90) focused on speciation and metal (or metalloids) in waters were selected. The findings are summarised and discussed concerning the DGT techniques' capability and potential for i) *in situ* fractionation (*e.g.* labile and inert species; organic and inorganic species; dissolved and nanoparticles), ii) speciation analysis and iii) the support the measurements can provide for software speciation models for cation and anion behaviour.

2. Water analysis - Main concepts and characteristics related to DGT technique for fractionation, speciation and speciation analysis.

Fig. 1 and Fig. 2 show the frequency of these approaches when different definitions of the concepts are employed. It can be clearly seen from these illustrations, that the sections are considerably different when different concepts (for fractionation and speciation) are adopted and, according to IUPAC definitions, most of the DGT approaches relate to fractionation of labile species.

2.1. Fractionation of labile, moderately labile and inert species

An essential attribute of the DGT technique is the possibility of *in situ* fractionation (although, normally, fractionation is called speciation in DGT papers) by determination of selective labile species based on their diffusion within a thin gel and on their interactions with a solid phase (generally, a resin bound in a porous gel support). As the process takes place *in situ,* the species of interest are protected against decomposition during the stages of transport and storage. The first two publications featuring DGT [7,8] clearly explain the fractionation properties of the technique, and how small dissolved species are differentiated in at least three degrees of lability: labile, partially labile and inert species. These measurements depend on both gel thickness and the rate of diffusion through the gel [7]. Normally, labile species are thus assumed to be those only measured using a DGT sampler with a standard 0.8 mm thick diffusive gel configuration. In the particular case of partially labile complexes, changing diffusive gel thicknesses yields information about the lability of the species and when interpreting DGT measurements it is necessary to take into account the changes of metal flux and lability degree [22]. Analytical expressions for the metal flux, the lability degree, and the concentration profiles in DGT devices have been recently reported and are encouraging DGT users to collect more kinetic and dynamic information from the environment [23,24]. Nevertheless, changing diffusive gel thicknesses yields information about the lability of the species. Based on this diffusive layer thickness driven concept of DGT-lability, there have been a number of published DGT papers to date [22–24]. This approach looks very exciting, and is stimulating interest in the use of DGT to obtain further kinetic and lability information. When considering a partially labile species situation, the measurement can be more accurately achieved by varying the diffusion layer thickness. Differences relating to the lability of metal and ligand complexes (ML) and solitary *free metal* (M) species allows them to be discriminated. This is possible to determine if both ML and M are being measured. In this case, the edge of the range (totally labile and non-labile) is represented. In the case of partially labile species, if only M is measured, the dissociation of the ML complex can be promoted by using a binding phase with strong adsorption properties. In this last scenario, the kinetics of the ligand exchange determines the fraction of ML that will be measured.

Adoption of this relatively simple fractionation procedure (measurement of labile, moderately labile and inert species) can be considered as a significant improvement to traditional filtering based approaches used to quantify the dissolved metal fraction in waters, providing a potentially more reliable *in situ* measure of the bioavailable metal species and toxicity.

2.1.1. DGT labile species and speciation modelling

Comparing DGT results with speciation modelling software can be a very insightful way to interpret the measurements, in addition to acting as a complementary technique for purposes of data validation. The Windermere Humic Aqueous Model (WHAM) based on the humic ion binding model VI, Visual MINTEQ (vMINTEQ) based on the NICA- Donnan model and Stockholm Humic model (SHM as a variety of WHAM) are the most popular speciation modelling platforms that feature alongside DGT in the published literature.

Meylan et al. [25] compared DGT results for Cu and Zn with voltametric measurements and predictions performed with speciation programs WHAM, vMINTEQ and SHM. The predictions of *free* and inorganic Cu were overestimated, attributed to the three models not considering a strong enough binding of Cu to DOC. Zn concentrations calculated by these models were in agreement with DGT and voltametric results.

Unsworth et al. [26] obtained results by DGT for Cd, Cu, Ni and Pb and these findings were compared to the equilibrium distribution of species calculated using WHAM and vMINTEQ models. In the river Wyre, the DGT concentrations for Cu were 13% of the filtered metal ($[M]_{tf}$), consistent with the model predictions (20% of $[Cu]_{tf}$). The Pb concentration measured by DGT was only a small fraction (3% of $[Pb]_{tf}$) showing that most of the Pb was in a non-labile form. The Cd concentration measured by DGT was similar to that predicted by WHAM (c^{dyn}_{max} with Fe as a colloid input into the model) and for vMINTEQ the result was lower than expected because this model predicted a higher proportion of Cd-humic species probably due to slow dissociation kinetics of Ni compared to other metals. For Ni, DGT concentrations were lower than in both the model predictions (predictions of measured species assuming complete lability - c^{dyn}_{max}) probably due to the slow dissociation kinetics of Ni compared to other metals.

Observations of DGT-labile concentrations and dynamic metal concentrations for Cd, Cu, Pb, and Zn in aquatic systems influenced by historical mining activities are studied in the work of Balistrieri and Blank [27]. They showed an agreement between labile concentrations and chemical speciation software (WHAM VI) for Cu and other chemical speciation models (i.e. SHM) to calculate Pb speciation. DGT was applied to fractionate labile Al, Pb, Cu, Fe, Zn, Cd, Ni, Co and Mn in a stream near to a neutralized acid mine drainage effluent reservoir. vMINTEQ was used to assess the inorganic fraction (all inorganic species), which was assumed to represent the labile pool. When the results were compared to those obtained by DGT, there was good agreement, suggesting the underlying interpretation of the model was correct for most metals. The authors’ report a minor labile fraction just for Fe, Pb, Cu and Al

(DGT-labile concentration 5 %, 12 %, 42 % and 50 % of total dissolved, respectively) as these elements readily form organic complexes or exist in particulate form [28].

2.1.2. DGT labile species and filtration

As filtration separates a fraction of an element based on differences in size, with the common size fraction discriminator being, 0.45 µm; representing the divide between dissolved plus colloidal species and undissolved particulates. Dissolved species are potentially more bioavailable as they can pass through cell membranes more easily than particulate fractions. Thus, filtered fractions can be also used to represent a bioavailable fraction when colloid formation is considered low to insignificant. By comparing the metal concentrations in filtered fractions with DGT measured availability, the contribution of colloids or inert species can be quantified. In addition, the expression of bioavailability can be more precisely represented by the DGT measurements, if values are related in some way back to the filtered fraction. Thus, DGT maybe employed successfully as a substitution to filtration. Although more complex than filtration, in situations when the pre-concentration of the analyte is required, DGT may be a helpful tool.

DGT measurements were performed by Lucas et al. [29] in an estuary with 4 different DGT devices (Chelex, Ferrihydrite, carbon and Purolite®). Ten elements (As, Au, Co, Cr, Cu, Mn, Mo, U, V and Zn) were determined. Carbon and Purolite® were used to sample DGT-labile Au, ferrihydrite for labile As and Chelex-100® for cations. The DGT-measurements of Au were in agreement with total dissolved concentrations (filtered sample) from downstream sites. DGT- labile concentrations of Mn and Zn were similar to grab sample concentrations (C_{DGT}/Grab ranging from 97% to 117%). On the other hand, DGT concentration of Au, Cu, Co, Cr, U, V, Mo and As, were lower than total dissolved concentrations at the upstream site, probably due to formation of colloids or complexes binding with DOC which was not sampled by DGT. Senila et al. [30] presented the content of dissolved and labile metals in waters of the Aries River catchment (Romania). The labile metal fractions, expressed as % of total dissolved metal concentrations (using the DGT technique), were found to be 28-88% for Cu, 43-72% for Zn, 73-85% for Fe, and 33-70% for Mn. Denney et al. [31] present results for Cd and Cu concentrations in two Tasmanian (Australia) river catchments using DGT. Concentrations of Cu and Cd measured by DGT were equal to dissolved (0.45 μm filtered) concentrations for the Ring and Stitt rivers, implying organic complexes or colloidal species to be of little relevance to the transfer of metals in these specific water-bodies. However, conversely in the Que and Savage rivers (Victoria, Australia) DGT concentrations of Cu and Cd were around 30 - 50% of the dissolved metal values, highlighting a distinctively different fluvial biogeochemistry. Warnken et al. [32] compared concentrations of Al, Fe, Mn, Ni, Cu, Cd, Pb, and Zn measured using DGT ($[Me]_{DGT}$) deployed *in situ* in 34 headwater streams in Northern England with filtered samples. Except for Zn and Cd, concentrations measured by DGT were similar to or lower than concentrations of the filtered samples.

DGT deployments, used in parallel with competing ligand exchange techniques, were used to study the lability and mobility of complexes of humic acid and either Zn(II), Cd(II), Pb(II), and Cu(II), by varying the diffusive layer thickness [33]. Here, Zn(II) and Cd(II) humic acid species tended to be more labile than Pb(II) or Cu(II) complexes [33]. Other, adaptations to the DGT method that have improved our understanding of *in situ* speciation trends include, the use of sodium polyacrylate as a binding phase to selectively retain labile Cu^{2+} and Cd^{2+}, while selectively not capturing metal-EDTA complexes [34]. The same binding phase was also used to scavenge Ni^{2+} ions, while Ni-EDTA and Ni-humic substance complexes were not retained [35]. Munksgaard and Lottermoser [36] measured runoff waters with DGT, finding Zn in the dissolved phase (0.45 μm filtered sample) was nearly entirely in the labile fraction (ratio between DGT and filtered samples was approximately 100%), while Cu was only partially labile (DGT/filtered = 25 – 46%). In a study reported by Yabuki et al. [37] concerning the use of DGT in Amazonian rivers (Amazon and Negro river) with high organic matter and low ionic strength, for determination of Al, Cd, Co, Cu, Mn, Ni and Zn. DGT-labile measurements were lower or similar to dissolved concentrations (except for Co and Mn in the Negro river; Ni and Zn in the Amazon River). The study of Shi et al. [38] compared the concentrations of dissolved and DGT-labile V and their relationship with DOC in the Churchill River estuary system (Manitoba, Canada) during spring pre-freshet, freshet and summer base flow. Dissolved V concentration at summer base flow was approximately 5 times higher than spring high flow while for DGT-labile V a converse trend was observed (greater values found during the spring high flow). This difference can be explained when DOC concentrations are considered, and highlight again the importance of accounting for the possible effects of DOC complexation when undertaking field sampling.

On the basis of the examples discussed above, the results obtained by DGT (normally lower than the total dissolved concentration) can suggest that in most environmental systems the labile fraction is less than the dissolved fraction when *in situ* measurements are performed. However, this assumption needs to be considered carefully. Firstly, DGT provides a time-integrated concentration, and thereby is a fundamentally different measurement to that of a single time-point, grab sample, and this can result in measurements yielding different values. The extent of this variation is site/time specific, and reflects changing weather conditions, temperature and inputs/discharges to the water body. Also, DGT results cannot represent quantitatively the real labile fraction when large complexes are formed in the system, as diffusion coefficients are considerably smaller (as compared with those of free ions) and consequently the DGT concentrations are underestimated (by not accounting of the inert fraction). Other errors caused by changing some DGT parameters (e.g. DGT geometry [39]; diffusive boundary layer, DBL [40]; biofouling [41]) is not a focus of this paper but can be found discussed in a recent DGT review, where Galceran and Puy [14] have interpreted the DGT measurements with appropriate physicochemical models giving valuable comprehension about the system's behavior. To some extent, errors associated with the formation of different complexes can be overcome by fractionation of labile inorganically and organically complexed metals, as discussed in the next item.

2.1.3. DGT labile species and other techniques for measuring lability

In the study by Twiss and Moffett [42], DGT devices were compared to an independent speciation technique (competitive ligand exchange - adsorptive cathodic stripping voltammetry (CLE-ACSV)). The results revealed that at least 10−35 % of the organically complexed Cu measured by CLE-ACSV were comparable to DGT labile measurements. While, Dunn et al. [43] found traditional 0.45-µm filtered solution and DGT-labile measurements to vary substantially, with the later registering between *ca*. 20-30% of the former for Cu, Pb, Zn and Ni. Additionally in another study, DGT was compared to voltametry and competitive ligand exchange, to sample labile Cu and Zn and was considered a robust and efficient technique for *in situ* measurements [25]. The majority of DGT binding layers are solid, gel-based supports; however this is not an exclusive requisite for the method. Li et al. [44] developed a sampler configuration that uses an aqueous solution of poly 4-styrenesulfonate (PSS) as the binding phase, containing the liquid within a cellulose dialysis membrane (CDM), which also serves as the diffusive layer. The method was validated in seawater and freshwater sites. In keeping with the predicted chemistries of the two matrixes, the freshwaters possessed higher DOC values, and also recorded lower DGT concentrations when compared with the seawater sites. The labile fraction of metals in freshwater was 3.5 – 4.8 % and 4.9 – 8.2 % for Cd and Cu, respectively. For seawater, the labile fraction was 6.5 – 7.2 % and 42 – 46 % for Cd and Cu, respectively. These results clearly highlight the importance of the Cu and Cd interaction with DOC with these metals, and Cu in particular, being very extensively complexed to organic matter in the freshwater sites. Dakova et al. [45] compared the concentrations obtained with DGT and SPE (obtained with a solid phase extraction procedure based on silica spheres modified with 3-aminopropyltrimethoxysilane) in water sampled from the Black Sea. The ratios between DGT/SPE for Cd and Ni were relatively high (0.6 to 0.8), suggesting that the binding of these metals by inorganic complexes or kinetically labile organic complexes was not a predominant environmental process in this setting. However, the DGT/SPE ratios were much lower for Cu and Pb (0.2 - 0.4), highlighting a stronger complexation of Cu and Pb by the dissolved organic matter (2.9 mg L^{-1}).

2.2. Fractionation (organic and inorganic/nanoparticles)

2.2.1. Labile inorganically and organically complexed metals

Whether the metal is complexed or not with humic substances is of great interest for the study of the speciation of trace metals in environmental samples, because it impacts on the mobility and even on the bioavailability of the analyte species. Yet, the relationship is not simply binary, bound versus unbound, but also depends on the rate of dissociation of the complexes formed by humic substance and the metal.

In 2000 [46] and 2001 [47] DGT was suggested as a potential tool for the separation of inorganic and organic complexes of Cd and Cu formed with humic and fulvic acids. This fractionation is based on the use of diffusive layers with different pore sizes, each one placed in a standard DGT device (Fig. 3). One diffusive layer (called open pore gel) and another one, with smaller pore size (called restrictive gel) are used simultaneously. As inorganic species are small, it diffuse freely and consequently faster through gels, but organic larger complexes formed by Fulvic (FA) and Humic (HA) acids diffuse less freely (and consequently more slowly than small complexes) in the gels. When two similar devices (same area, thickness and binding phase) containing restrictive and open pore gel and are immersed simultaneously in the same solution, for the same time, the organic and inorganic species have very distinct diffusive coefficients in each type of diffusive gel; therefore the accumulated mass in each binding disc will vary significantly, allowing the calculations of the concentration of each fraction by eluting the different masses retained during immersion as follow:

$$C_{inorg} = ({}^{o}M / {}^{o}D_{org} - {}^{r}M / {}^{r}D_{org}) / [k ({}^{o} D_{inorg} / {}^{o}D_{org} - {}^{r}D_{inorg} / {}^{r}D_{org}) \qquad \text{Eq. (2)}$$

$$C_{org} = ({}^{o}M / {}^{o}D_{\ inorg} - {}^{r}M / {}^{r}D_{inorg}) / [k ({}^{o} D_{org} / {}^{o}D_{inorg} - {}^{r}D_{org} / {}^{r}D_{inorg}) \qquad \text{Eq. (3)}$$

Where:

$\boldsymbol{C_{inorg}}$ is the concentration of the inorganic species;

$\boldsymbol{C_{org}}$ is the concentration of the organic species;

$\boldsymbol{{}^{o}M}$ is the accumulated mass in the device congaing the open pore gel;

$\boldsymbol{{}^{o}D_{org}}$ is the diffusion coefficient of the organic specie in the open pore gel;

$\boldsymbol{{}^{r}M}$ is the accumulated mass in the device congaing the restrictive pore gel;

$\boldsymbol{{}^{r}D_{org}}$ is the diffusion coefficient of the organic specie in the restrictive pore gel;

$\boldsymbol{{}^{o}D_{inorg}}$ is the diffusion coefficient of the inorganic specie in the open pore gel**;**

$\boldsymbol{{}^{r}D_{inorg}}$ is the diffusion coefficient of the inorganic specie in the restrictive pore gel;

$\boldsymbol{k}$ is a constant, that depends on DGT parameters, $\boldsymbol{S}$ area and $\boldsymbol{\Delta g}$ thickness of the DGT devices and the deployment time $\boldsymbol{t}$ **,** which are maintained constant.

On the basis of this approach, even though it ideally needs analyte concentrations to be relatively high [47], for the first time inorganic and organic complexes formed from humic and fulvic substances can be evaluated by using an *in situ* sampler and consequently compared with measurements performed in the laboratory. Thus, again DGT promotes progress on fractionation. After being initially applied to just the fractionation of Cu and Cd, many further studies have taken the method and extended the scope of the work, broadening the range of studied elements. Zhang [48] extended their initial proposal [46] for fractionation of Zn and Ni in freshwater. Fractionation of organic and inorganic Pb were successfully performed in an synthetic system containing either FA, HA or nitrilotriacetic acid (NTA) over the pH range 4 - 8 using three types of DGT devices (with diffusive gels of different pore sizes), when the diffusion coefficient of each species in each gel type was considered [49]. Determination of labile inorganic and organic species of Al and Cu in model synthetic solutions and river water samples were evaluated by Tonello et al [50]. For the model Cu solutions, the most labile fraction consisted of just inorganic species, but, significant amounts of labile organic complexes of Cu were also present. For the river water samples analysed in the laboratory, less than 45% of the analytes were present in labile forms (most were organic species). For the *in situ* measurements, the labile inorganic and organic fractions were larger than those obtained in the laboratory analyses. These differences could have been due to errors incurred during sample collection and storage showing the challenge associated with this type of fractionation measurement *in situ*. Data from the fractionation of labile inorganic and organic complexed metals obtained using DGT have been compared and/or supplemented with ultrafiltration (TFU), solid phase extraction (SPE) [50], competing ligand exchange (CLE) methods [25,51], anodic stripping voltammetry and computer speciation (WHAM) [47,48,52]. Chakraborty et al. [51] state that DGT estimates lower concentrations of labile metal complexes than CLE, but the association of these two techniques was found to be very valuable in determining diffusion coefficients for labile metal-humic complexes in quasi-labile systems. Similarly, Tonello et al. [50] in a procedure based on ultrafiltration data proposed to determine diffusion coefficients of the analytes, Cu and Al, in water samples and model solutions containing both *free metal* (M) and complexes (metal – humic substances binding). When compared to SPE in the sampling of organic-rich waters, the measurements of DGT showed good agreement for Al and Cu, with there being only a small variation in the measurement, likely due to differences in time-scales of each method. The effect of HA on the metal uptake of Cd, Cu, Ni, and Pb in DGT was evaluated by Docekal et al [53]. The authors tested HA substances at approximately equimolar and higher concentrations with respect to metals ions and reported a considerable reduction in the DGT metals uptake increasing in the sequence of Cd, Ni, Pb, Cu. They suggested that HA species diffuse through the permeable gel and affect predominantly the interaction of metal ions with specific iminodiacetic groups of the resin by competitive reactions. Therefore, in this sense, reduced mobility of larger molecular species of metal-humic acid complexes plays a minor role. Balch and Gueguen [54] confirmed diffusion of four humic substances (500-1750 Da) through the DGT system (diffusing across the diffusive gel membrane) at rates ranging from 3.48×10^{-6} $cm^2\ s^{-1}$ to 6.05×10^{-6} $cm^2\ s^{-1}$. The authors' report similar behaviour for DOC (2.48×10^{-6} $cm^2\ s^{-1}$ to 5.31×10^{-6} $cm^2\ s^{-1}$). Van der Veeken and Van Leeuwen report that HA even appears to accumulate in polyacrylamide gels, with enrichment factors typically on the order of 10 [55–59]. Thus, similarly to questions raised by Docekal et al. [53] this behaviour has consequences for understanding DGT data on metal fluxes from aquatic media containing FA and HA. Therefore, Eq. (1) and Eq. (2), now, must be taken carefully when performing fractionation of organic and inorganic metals compounds.

2.2.2. Nanoparticle fractionation

The basis of DGT measurement to discriminate species according to size lies in fact that the diffusion of *free ions* occur more rapidly than larger chemical species such as metal complexes with humic substances. Therefore, similarly, the discrimination of colloids and nanoparticles from other species will depend on diffusion of these particles through the gel and their sufficiently rapid release upon interaction with the binding layer. In theory, as shown in Fig. 3, very small nanoparticles could be distinguished/detected by DGT by using different types of gel and varying the concentrations and type of cross-linker (e.g. acrylamide cross-linked with an agarose derivative (APA), acrylamide cross-linked with bis-acrylamide (restricted gel – RG)) [60]. Forsberg et al. [61], when comparing DGT and ultrafiltration in the Baltic Sea have demonstrated that concentrations of Cd, Mn and Zn measured by DGT were similar to the concentrations measured in 1 kDa ultrafiltered samples probably due to the weak tendency of these metals to form organic complexes. For Cu and Ni the ultrafiltered concentrations exceeded the DGT-labile concentrations indicating the existence of inert colloids, which may also be strong organic complexes. The authors' suggest that DGT may only be measuring particles smaller than 1kDa (approximately 2 nm). This agreement between DGT and ultrafiltered concentrations (1kDa fraction) in other types of water was also reported for Mg in the study of Dahlqvist et al [62] in freshwater. Van der Veeken, Pinheiro and Van Leeuwen [55] reported that in solutions containing *free* Pb and Pb bound to monodisperse carboxylated latex nanospheres (diameters of 81 and 259 nm, using APA and restricted gels) there was more accumulation of Pb in solutions containing smaller particles than expected from the *free ion* alone. Davison and Zhang [12] counter this by referring to evidence in which NPs with diameters <2 nm could diffuse and be measured with better sensitivity than NPs up to a 5−6 nm diameter.

Recent studies by Pouran et al [63] concerning nanoparticle discrimination have employed fine-pored membranes rather than the conventional 0.45-mm filter. The authors' proposed locating a 1000 molecular weight cut off dialysis membrane (MWCO) (thickness ≈ 0.05 mm) in front of the diffusive gel layer to prevent diffusion of nanoparticles of ZnO (ZnO NPs). The mass of Zn accumulated using open-pore gels reflects the concentrations of ZnO NPs and Zn^{2+}, while the accumulated mass in devices with dialysis membranes provides an estimation of the concentration of just Zn^{2+}. The experiments carried out with Chelex and Metsorb as binding layers show that Chelex offers potentially better performance characteristics. However this approach has received criticism in Zhang and Davison [21]. Based on non-published studies (performed by Garmo, Peters, Davison and Zhang), Zhang and Davison [21] documented that the use of ultra thin membranes in DGT devices may cause radial diffusion effects. Although the above mentioned review [12] highlighted that there were still key knowledge gaps concerning nanoparticle measurement by DGT. Measurements of nanoparticles can be affected by natural colloids, and size discrimination by DGT can be not totally controlled.

2.3. Speciation and speciation analysis (or selective determination of a species)

Given the distinct interaction with the biota and environment that each species of the same element may encounter, coupled with their unstable behaviours when stored, DGT, as an *in situ* sampling technique, can be a useful tool for the selective determination of species. By knowing the characteristics of the speciation of the element in natural waters, it is possible to choose a suitable DGT configuration according to its binding properties, resulting in a species targeted or selective sampler that has preferable uptake of one form over the other (*e.g.* cations instead of anions, organometallic complexes instead of metallic forms). During the evolution of DGT's development, there have been numerous works framed around alternative binding layers and the uptake of specific species, with these studies focusing in the main on the most toxic elemental forms.

In this section, we have followed the IUPAC [20] speciation definition *"distribution of an element between defined chemical species in a system"*. Therefore, we assumed that DGT alone cannot be considered a complete speciation tool, as it can only be configured to be selective to one species but will only be able to provide the full range or distribution of species present in a system with the aid of speciation modelling software or auxiliary separation technique, such as HPLC-ICP-MS.

2.3.1. DGT U species and speciation modelling

For U species selective determination by DGT, the approach is based on using a binding phase with a cationic functional group, thus the anionic forms of the element are targeted, combined with a binding phase that aims to capture the cationic $(CO_3)^{2-}$ dissociated species. The knowledge of which species is retained by which type of biding phase was achieved with the aid of a speciation modelling software.

Li et al [64] investigated the application of a paper based DGT using DE81® as a binding layer which may have preferentially sampled $UO_2(CO_3)_2^{2-}$ while Chelex-100 sampled the fraction of U species dissociated from $(CO_3)^{2-}$ during its transport in the diffusion layer; both methods of U speciation were only appropriate to alkaline fresh waters. Li et al. [65] proposed a new binding layer – Dowex resin® – for U uptake and compared it to other already described binding layers, Chelex-100® and DE81®. Among the binding layers, U concentration provided by Dowex devices was the most selective when deployed in the same site and compared to the total U concentration while DE81® was the least. Additionally, DGT has been tested for radionuclides, with Chelex-100® found to be suitable for sampling Eu^{3+} in pH close to neutrality, UO_2^{2+} for pH of at least up to 10.7 and for NpO_2^+ up to at least pH 11.7 [66]. In these papers, the authors' highlighted the importance of further studies to better understand the selectivity of these binding layers for U species and have also emphasized the importance the role DGT plays as a complementarily tool to better understand U speciation.

2.3.2. DGT Pb and Mn species and speciation modelling

Similarly to the U speciation method, but this time focusing on Pb and Mn, a selective binding phase was also used in parallel with a speciation modelling software. *Saccharomyces cerevisae* immobilized in agarose gel has also been reported as an alternative binding layer for cationic Pb species in fresh and seawaters, providing a detection limit of the method of 0.75 µg L^{-1} (calculated for a 72-h deployment). The speciation modelling software CHEAQS was combined with the DGT results to assess Pb speciation in solution, indicating the presence of almost exclusively cationic species in circumneutral pH values, i.e. Pb^{2+}, $Pb(NO_3)^+$, and $Pb(OH)^+$[67]. Speciation of Mn in an acid mine drainage catchment was performed by DGT. The labile Mn fraction was assessed by using DGT devices assembled with Chelex-100® resin as a binding phase and, when modelled with CHEAQS software, it was possible to predict that DGT selectively sampled *free* Mn^{2+} and a portion of the $MnSO_4$(aq). Negative Mn species sampling was performed by changing the binding phase to a DE81 membrane. Devices assembled with P81 membrane in association with CHEAQS modelling identified Mn^{2+} and $Mn(OH)^{(+)}$ species in samples with low Ca concentration [68]. For the next item we have selected only papers related to speciation that matches IUPAC definition of speciation analysis [20]: "an analytical procedures to identify and/or quantitative measurement of one or more chemical species in a sample".

2.3.3. Cr redox speciation analysis by DGT

As Cr(III) and Cr(VI) are found in cationic and anionic forms, respectively, in freshwater conditions, the most common approach for studies focused on the speciation analysis of these analytes are based on their redox speciation, by assembling the devices with a binding phase known to be selective/specific for each target species. Thus, the combination of a binding phase selective for cations and another binding phase selective for anions seems to fit satisfactorily the aims of this study field. Another approach is to use DGT along with other techniques, as DET and diphenyl-carbohydrazide (DPC) methods, or even by using a selective eluting agent, as further detailed. Although all the proposed methods were highly efficient in terms of limit of detection, only DGT/DET provides an *in situ* sampling of the species, which is especially appealing for speciation analysis studies, by avoiding changes in the equilibrium of the species present in the system during sample storage.

The first DGT layout proposed included the cation exchange resin Chelex-100®, which is expected to only retain cationic species. Soon, ferrihydrite was used to sample anionic species, being firstly proposed for dissolved phosphorus sampling in soils [10]. Only in 2002, Chelex-100 was shown to selectively sample Cr(III), while the other species of interest, Cr(VI), was not retained, being instead sampled by DET [69]. Giusti and Barakat [70] have successfully combined Chelex-100-DGT with a DPC method to selective sample Cr(III) and Cr(VI), respectively. Cr(VI) was selectively sampled by polyquaternary ammonium salt (PQAS) while Cr(III) was not. The method agreed with the determination of the conventional colorimetric DPC method, but possessed a lower detection limit [71]. Later, the speciation analyses of Cr was performed by combining a DGT device assembled with a sodium poly(aspartic acid) solution as the binding layer to sample Cr(III), obtaining a detection limit of the method of 3.18 μg L^{-1} and a DGT device assembled with PQAS as the binding layer to sample Cr(VI), achieving a limit of detection of the method of 2.92 μg L^{-1} [72]. N-Methyl-D-glucamine (NMDG) resin was also reported as a Cr(VI) selective binding layer, presenting negligible accumulation of Cr(III)[73]. Another analysis speciation method was reported using a different approach, wherein the same binding layer, zirconium gel, retains both Cr(III) and Cr(VI). The separation of the species is performed by eluting with NaOH, which is able to exclusively elute Cr(VI)[74]. Cr(VI) and Cr(III) speciation analysis can be performed by the complementary use of DGT devices assembled with a DE81 binding layer and agarose diffusive phase to sample Cr(VI), while Cr(III) is retained by Chelex-100 [75].

2.3.4. As redox speciation analysis by DGT

Due to the pressing environmental and human health concerns relating to the toxicities of As' inorganic species, this element has become one of the most studied using redox speciation analysis by DGT, alongside Hg and Cr. A very common path for As speciation analysis in DGT is to combine a selective binding phase with a binding phase able to sample the total As inorganic fraction, thus the concentration of one the species is given by the difference of both retained fractions. Also, the speciation analysis of As has also been performed by harnessing differences in the properties of the diffusive layers, as detailed bellow.

The binding layer 3-Mercaptopropyl-Functionalized silica gel has been successfully proposed by Bennett et al [76] to selectively retain As(III), achieving detection limits of the method of 0.03 µg L^{-1} over 72 h deployments. The authors' also suggest the complementary use of DGT devices assembled with Metsorb binding phases to sample total As, so As(V) concentration can be calculated from the difference between total As and As(III) concentration. Panther et al. [77] proposed an innovative approach in speciation analyses of the inorganic species of As using different properties of diffusive medias instead of the binding layer. While the diffusion coefficient for both inorganic species of As on the conventional polyacrylamide diffusive gel are similar, by using the negatively charged Nafion® membrane a significant increase in

As(III) species is achieved. Therefore, the concentration of both species can be known similarly to the approach for fractionation of organic and inorganic species (Eq. (1) and Eq. (2)). Bennett et al. [78] have used the DGT technique to study the mobilization of As between sediments and freshwater along the transitions of water redox conditions. Mercapto-silica DGT was utilized to selectively measure As(III) and DET to measure Fe(II) concentration. The authors' highlight the effectiveness of combining both techniques, ensuring the sampling is achieved with minimal disturbance to the sediment, thus avoiding the occurrence of many speciation artefacts, *e.g.* the oxidation of both As(III) to As(V) and Fe(II) to Fe(III), which could significantly change the results for the mobility interpretation. A method for inorganic As speciation analyses was proposed by combining ferrihydrite for total As and the novel binding layer Amberlite IRA-910 to sample As(V), thus As(III) concentration is obtained by the difference. This method approach has been validated for speciation analyses in river waters [79].

2.3.5. Hg speciation analysis by DGT

A detection limit of 1 pg of MeHg for the overall method was obtained for the use of DGT combined to a 3-mercaptopropyl-functionalized silica gel [80]. In 2014, 3-mercaptopropyl-functionalized silica gel was again applied to MeHg measurement but this time attention was given also to the diffusive layer which apparently influenced this ions speciation. The standard polyacrylamide gel was replaced with an agarose gel, which unfortunately also showed affinity to MeHg and therefore proved to be an unsuitable diffusive layer replacement [81]. Although both methods were able to measure MeHg, the selective sampling of MeHg by DGT was not achieved, as in both cases the required gas chromatography methods to separate MeHg from other species were not used.

The above scheme is a common approach to DGT sampling of MeHg and has been reported elsewhere for quantifications of four mercury species (Hg^{2+}, CH_3Hg^+, $C_2H_5Hg^+$, and $C_6H_5Hg^+$) sampled by DGT assembled with ion-exchange resins containing thiol-functionalized sub-groups (Duolite GT73® and Ambersep GT74®) and chemical determination by liquid chromatography (LC) and cold vapor atomic fluorescence spectrometry (CV-AFS)[82]; for CH_3Hg^+ and Hg^{2+} sampled by DGT devices assembled with thiol-functionalized resin layer and determined by ion chromatography coupled to ICP-MS [83]. This approach was also used for As speciation analysis [84]. Further, the separation of MeHg from Hg(II) was done exclusively by DGT, wherein a *Saccharomyces cerevisae* immobilized in agarose gel combined to an agarose diffusive layer efficiently retained MeHg while Hg(II) remained in the solution. The coupling of DGT and CV-AFS has achieved a method limit of detection of 0.44 ng L^{-1} (48 h deployment) [85].

2.3.6. Other speciation analysis by DGT and new trends

Polyvinyl Alcohol was used as binding layer (PVA DGT) to measure selectively *free* Cu^{2+} ion concentrations in water samples (river water and industrial wastewater). The results of PVA DGT have been compared with cupric ion selective electrode (Cu-ISE) measurements, with presented recoveries in percentages equivalents found to be low (in spiked river water, recovery of 24.99 and 26.32 %; in spiked industrial wastewater, 4.21 and 5.10 % for PVA DGT and Cu-ISE, respectively)[86]. A recent development of DGT methods has been to use ion imprinted binding layers, wherein the analyte is sorbed to the ligand to improve species retention, this approach has been successfully carried out for Cd(II) [87], Pb(II) [88].

Although, application of DGT for *in situ* speciation analyses is well established, it still needs to be extended to other species. Approaches already stated (*e.g.* by coupled techniques), such as speciation analysis of organometallic Sn and organometallic Pb, are still notable gaps in the DGT literature.

2.4. Predicting bioavailability and toxicity

Currently there is a growing body of research focusing on the use of the DGT technique for predicting bioavailability and toxicity. Labile and small complexes are the forms of a metal most likely to be able to pass through cell membranes and therefore they are commonly the most bioavailable and harmful to biota. As these forms are the ones sampled by the DGT technique, some studies have been published to evaluate the possibility to use DGT to predict bioavailability and toxicity to human [89–91]. As bioavailability strongly depends on the species present in the system, selective binding phases DGT based approach turns out to be a powerful tool to assess the bioavailability/toxicity of target species.

2.4.1. Toxicity and DGT

Tusseau-Vuillemin et al. [92] have investigated the relevance of DGT to measure Cu lethality on *Daphnia magna* in mineral water spiked with various organic ligands (EDTA, NTA and glycine). These experiments showed that Cu-EDTA complexes are inert while Cu-NTA and Cu-glycine complexes appear as fully labile or partly labile, respectively. Humic acids, fresh and aged algae extracts were also used to represent the natural organic matter and these three forms were not toxic to *Daphnia magna* but DGT results (using open pore hydrogel) showed that Cu complexes were partially labile. Nevertheless, the fraction of labile complexes were significantly reduced (mainly for humic acids and aged algae Cu complexes), when DGT’s with restricted gels were used, suggesting that , DGT devices configured with restricted gels seem to be effective at measuring bioavailable Cu in natural water bodies. Apte et al. [93]

have studied natural fresh waters with different concentrations of Cu and DOC for studies of toxicity to an algae (*Chlorella sp.* 12), a cladoceran (*Ceriodaphnia cf. dubia*) and a bacterium (*Erwinnia sp.*) and compared with Cu lability using DGT. Cu labile and toxicity measurements were tested with 20 µg L^{-1} Cu spiked natural water observing a reasonable relationship between bacterial response and Chelex-labile Cu concentrations. Another test with 40 µg L^{-1} Cu spiked natural water has shown growth inhibition effects that are related to the measured Chelex-labile Cu concentrations. The algal toxicity studies were not presented concordant results with DGT technique probably due to an insufficient labile concentration to cause a significant effect on algal growth.

2.4.2. Bioavailability

A trend that can be observed related to the development of new methods based on the DGT technique is the prediction of bioavailability based on the comparison of the DGT results with bioindicator animal models for specific analytes. These studies commonly aim to determine the labile fraction of an element in varying matrices and understand how the bioavailability of this fraction changes in the environment (*e.g.* DOC rich waters). DGT may be a very helpful tool for this, since it can provide either short or medium-term exposure data. Also, DGT standardises many variables which are highly variable but inherent to the studies involving living organisms. Despite of all the above-described DGT advantages on the study of bioavailability, the development of a DGT method and its validation by an established biological model remains a challenge, given the difficulty of the interpretation of the data obtained from the two different techniques. Metal accumulation in passive samplers (like DGT) tend to exhibit similar uptake as that displayed by organisms (i.e. diffusion through a cellular membrane). However, inconsistent results when comparing both techniques can be found. The main efforts made for the improvement of this approach are detailed bellow.

Luider et al. [94] have compared the labile Cu determined by DGT technique with uptake of Cu in trout gills. In this study, there was an influence of organic matter in Cu uptake by trout gills as well as Cu concentrations determined by DGT. Similar results for Cu binding to organic matter were measured by DGT and fish gill bioindicators. Divis et al. [95] have reported results of total and dissolved concentrations (obtained through regular water sampling), DGT technique and (bio)available concentration calculated by the aquatic moss species *Fontinalis antipyretica* in river water. The authors demonstrated that the concentrations of Cd, Cr, Pb and Zn measured by the DGT technique are comparable with the (bio)available concentrations, except for Cu and Ni which showed significant differences between DGT and (bio)available concentrations possibly due to different incorporation mechanisms and uptakes of Ni and Cu to the *Fontinalis Antipyretica*.

Martin and Goldblatt [96] have conducted studies to assess the behavior, fractionation, and bioavailability of Cu in a stream system rich in DOC (7 – 17 mg L^{-1}) and elevated levels (~50 µg L^{-1}) of Cu concentrations, downstream of a mine-impacted lake (East Lake, ON, Canada). Most of the Cu is present as filterable species (74 – 100 % total concentration). Measurements of labile Cu measured by DGT suggest that most of the Cu is unavailable to aquatic biota (9 to 24 %). Measurements of bioavailability were conducted with *Ceriodaphnia dubia* (7-d incubation) and have showed that variations in the filterable Cu concentration result in 50% mortality (LC50 = 96-203 µg L^{-1}) and inhibition of reproduction by 25% (IC25 = 75 - 156 µg L^{-1}). The studies of Ferreira et al. [97] have investigated the influence of DOC on Cu bioavailability at environmentally relevant concentrations (1 - 5 µg L^{-1} of dissolved Cu, 1 - 4 mg L^{-1} of dissolved organic Cu).

Bioavailability evaluation takes into account two biological endpoints (short-term and steady-state bioaccumulation of Cu by the aquatic moss *Fontinalis antipyretica*). Sampling of labile Cu using DGT in mineral water and various forms of DOC (EDTA, humic acid and natural

Seine River (France) extracts-hydrophobic and transphilic fractions) were also investigated. All types of DOC reduce the bioavailability of Cu to aquatic mosses mainly for short-term bioaccumulation. Labile Cu measured by DGT was in agreement with short-term bioaccumulation in the case of EDTA and natural Seine River extracts. However, with humic acid solutions, labile Cu was lower than bioavailable Cu concentration, suggesting that in certain types of natural DOC, bioavailable Cu might include some inert (non-labile) organic complexes. Concentration of *free metal* ions of Cd, Cu, Ni, and Pb was assessed by hollow fiber permeation liquid membrane (HF-PLM) and by DGT in seawater. The results obtained by DGT were higher than the ones obtained by HF-PLM, which is explained by their different analytical windows, because while HF-PLM provides *free ions* concentration DGT provides the concentration of mobile and labile species. Also, metal bioavailability to microorganisms was successfully assessed by exposing *Chlorella salina* to the analytes and comparing the results to the ones obtained by the other techniques [98]. Results were in good agreement. Bourgeault et al. [99] have reported results for DGT-labile Cd, Co, Cr, Cu, Mn, Ni, and Zn and transplanted zebra mussels in river water. Transplanted zebra mussels indicated difference between sites mainly for Zn, Cr, Cu and Cd (higher concentration for downstream). Similar results were reported for DGT measurements. Frequently, labile metal represented only for 14–35% of total dissolved metal, suggestive of this being due organic ligand binding. Lin et al. [100] have performed the speciation of Cu in an effluent by combining X-ray diffraction (to identify inorganic species), DGT (to identify diffusible Cu^{2+}) and the vMINTEQ software (to identify organic species). Thus, they have identified Cu in the effluent as $Cu(H_2PO_2)_2$ and organic Cu. This paper has also combined these obtained results to the ones from the exposure of zebrafish to evaluate the capacity of these simulation models to assess bioavailability of the species.

There is a possibility that the DGT devices with live organisms will provide bioavailability measurement metals. Dried *Saccharomyces cerevisiae* immobilized in agarose gel has already been successfully used in DGT (DGT-Yeast) as a binding agent for determination of Pb in river and seawater [67], Cd [101] and MeHg in riverwater [85]. As compared with DGT-Chelex, DGT-yeast was selective for measurements of MeHg in river water and cationic Pb species in seawater. Furthermore, an anaerobic iron reducing bacterium, *Shewanella oneidensis*, has been incorporated into a thin layer of agarose to replace the polyacrylamide gel, and named BMDGT. The proposed device was deployed in solution containing Co and Cd (ionic strength 0.01 mol L^{-1} $NaNO_3$). Under stationary conditions, there were no significant differences in measurements between cell free control DGTs (in aerobic and anaerobic conditions). Whereas deployment of BMDGTs (containing cells grown in Luria Broth (LB)) in Cd solution under anaerobic conditions was expressively lower when compared to cell free control DGT devices [102].

3. Conclusions

In this review, several analytical approaches for speciation, fractionation, speciation analysis and bioavailability in water based on DGT technique were systematically split into three groups. Adoption of the relatively simple fractionation procedures provided by DGT (measurement of labile and inert species) can be considered as a significant improvement to traditional grab sampling (to determine the dissolved fraction in waters), mainly when information about bioavailable and toxicity of metals is required. By comparing the papers, frequently the results obtained by DGT (normally lower than the total dissolved concentration) suggest that, in most environmental systems, the labile fraction is lower than the dissolved fraction when *in situ* measurements are performed. However, this assumption needs to be analyzed carefully because the comparison of the data from grab sampling and DGT is complex.

Approaches based on varying the gel thickness will provide *in situ* kinetic information of metal complexes and can be considered an important research field to explore further. While, organic and inorganic speciation analysis has been reported as only being suitable for analytes present in an environment at high concentrations. Despite this limitation, this approach has allowed inorganic and organic complexes (humic and fulvic substances), for the first time, to be evaluated using *in situ* passive sampling measurements and consequently can be compared with measurements performed in the laboratory, once again highlighting DGT capability of promoting progress on information about sample storage and preservation.

The discrimination of colloids and nanoparticles from other species has been studied by DGT in what remains only a very limited number of papers, justifying this field of knowledge to be expanded.

As an *in situ* sampling technique, DGT has great potential to meet the aims of the selective determination of species or speciation analysis. Thus, it is possible to find many DGT papers targeting the development of selective binding layers (especially regarding Cr and As redox speciation analysis and Hg speciation analysis). The use of ion-imprinted binding layers appears to be a new trend on developing selective binding phases.

Although application of DGT for *in situ* speciation analyses is now well established, it still needs to be extended to other species. There is a gap in the DGT literature concerning speciation analyses by systematic comparative studies *in situ* and *in lab.*

The comparison of biomonitoring and DGT techniques is difficult due to the complexity of the uptake of trace elements by living organisms, whereas DGT provides a linear relationship based on Fick's laws and therefore the results from both approaches may not be corroborants for some analytes.

Taking all the content above reported into account, DGT is demonstrated to be a very versatile technique. However, although measurements obtained by DGT are usually not very laborious and time consuming, the interpretation of the results often requires deeper analysis in order to fully understand the extent of its potential.

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Figures caption

Fig. 1. Frequency of main fields of study and of approaches in DGT technique based on self-statement, collected from title and abstract of each article.

Fig. 2. Frequency of approaches in DGT technique based on the concepts exposed herein.

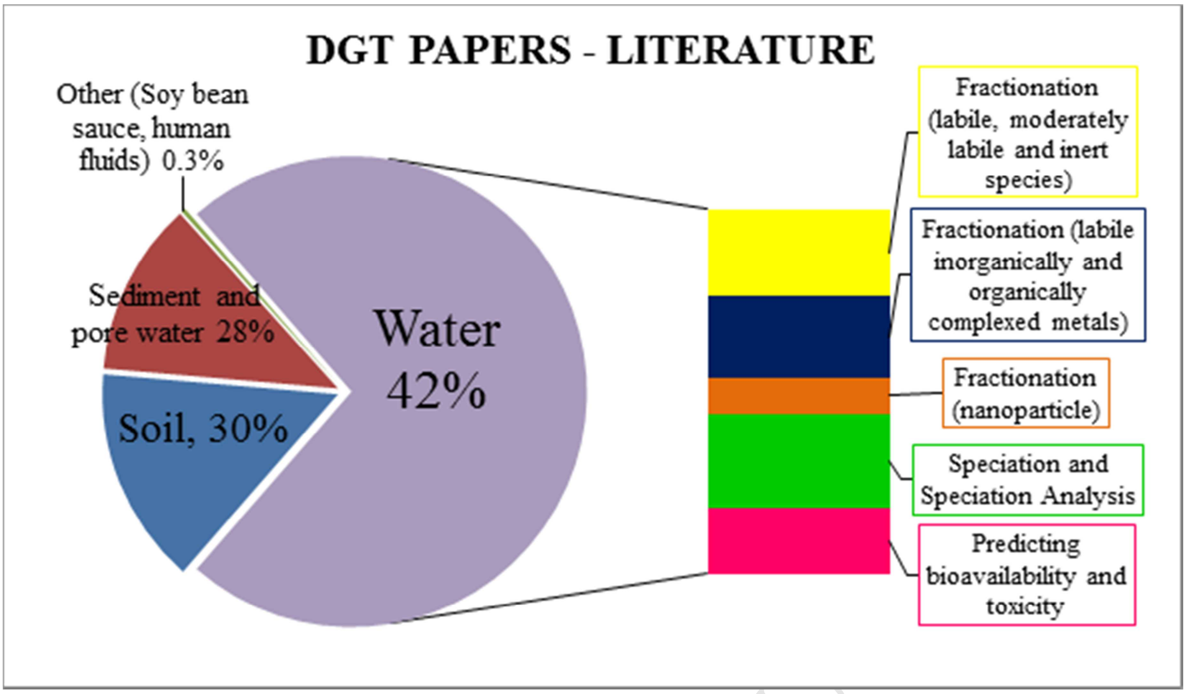
Fig. 3. Diagram of the diverse pools of fractions sampled by DGT.

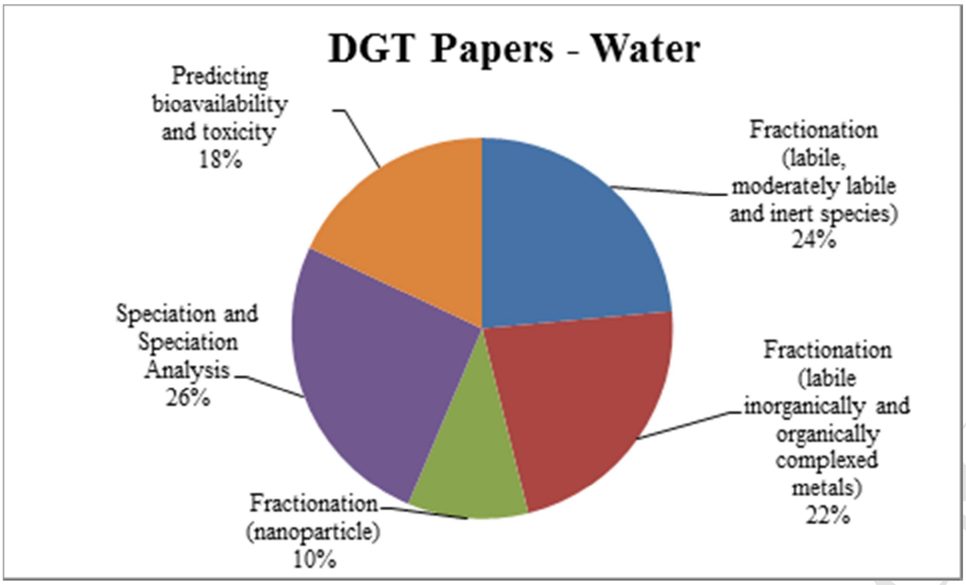
Table 1. New binding agents used in the diffusive gradients in thin films (DGT) technique

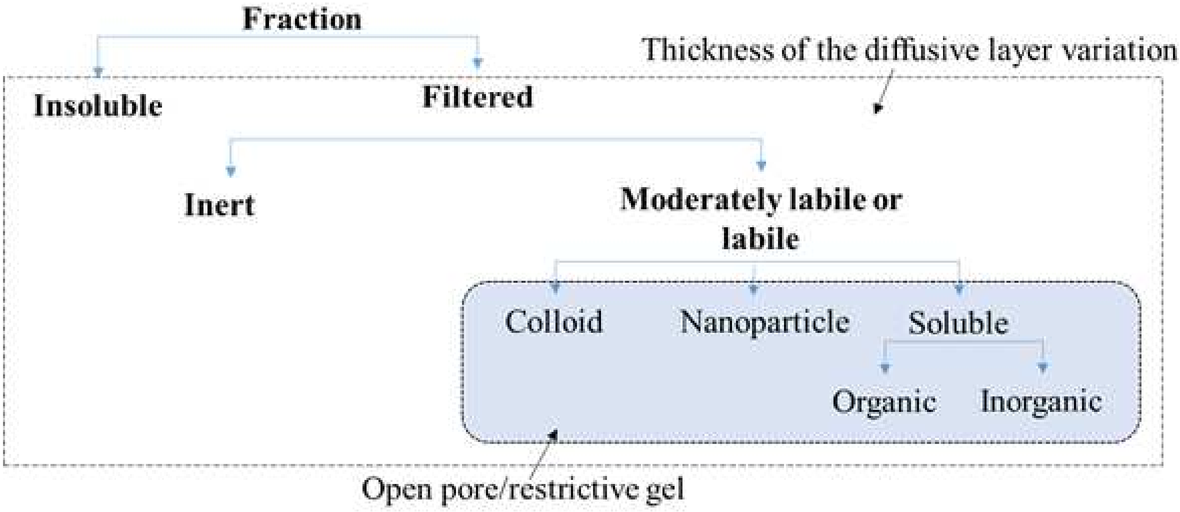
	Analytes	Binding agent	References
Nanoparticles	ZnO	Metsorb (titanium dioxide)	[63]
Species of As	As(III), As(V), MMAA(V), DMAA(V)	3-mercaptopropyl functionalized silica gel (MPS), Amberlite IRA 910, Metsorb (titanium dioxide), Perfluorosulfonated Ionomer	[17, 76, 77, 78]
Species of Cr	Cr(VI) and Cr(III)	Polyquaternary ammonium salt (PQAS), N-Methyl-D-glucamine (NMDG), Precipitated zirconia gel (PZ gel), Sodium Poly(aspartic acid), Whatman® DE81	[71–75]
Species of Hg	Hg^{2+}, CH_3Hg^+, $C_2H_5Hg^+$, $C_6H_5Hg^+$	3-mercaptopropyl functionalized silica gel (MPS), Ambersep GT74, Duolite GT73, *Saccharomyces cerevisiae*	[80, 82, 83, 85]
Alkaline and Alkaline Earth Metals	Ba	Whatman® P81	[19]
	RadioCs	Ammonium molybdophosphate (AMP)	[23]
Transition Metals	Au	Activated Charcoal, Purolite® A100/2412 resin, Dowex® XZ 91419	[29]
	Mn, Cd, Co, Cu, Ni, Pb and Zn	Whatman® DE81, Whatman® P81, ion-imprinted sorbent, Methylthymol blue adsorbed on Dowex 1 X8, Metsorb, Polystyrene sulfonate, *Saccharomyces*	[18, 34, 44, 67, 68, 88, 101]

		cerevisiae, Sodium polyacrylate	
Others	U	Whatman® DE81, Dowex 2×8-400	[64, 65]

1171


DGT PAPERS - LITERATURE
Other (Soy bean sauce, human fluids) 0.3%
Sediment and pore water 28%
Water 42%
Soil, 30%
Fractionation (labile, moderately labile and inert species)
Fractionation (labile inorganically and organically complexed metals)
Fractionation (nanoparticle)
Speciation and Speciation Analysis
Predicting bioavailability and toxicity


DGT Papers - Water
Predicting bioavailability and toxicity 18%
Fractionation (labile, moderately labile and inert species) 24%
Speciation and Speciation Analysis 26%
Fractionation (labile inorganically and organically complexed metals) 22%
Fractionation (nanoparticle) 10%


Fraction
Insoluble
Filtered
Thickness of the diffusive layer variation
Inert
Moderately labile or labile
Colloid
Nanoparticle
Soluble
Organic
Inorganic
Open pore/restrictive gel

Highlights

This review provides an overview of the applications of DGT for speciation.

Approaches were grouped according to IUPAC guideline definitions for speciation.

Knowledge gaps and areas for further DGT research on speciation are highlighted.

First Author: Amauri Antonio Menegário, Doctor

Order of Authors: Amauri Antonio Menegário a*; Lauren N Marques Yabuki a; Karen S Luko a; Paul N Williams b; Daniel M Blackburn c.

*Corresponding Author: Amauri Antonio Menegário amenega@rc.unesp.br

a. Universidade Estadual Paulista (UNESP), Centro de Estudos Ambientais, Rio Claro, Av. 24 A, 1515, 13506-900, São Paulo, Brasil

b. Queen's University Belfast, Institute for Global Security, School of Biological Sciences, Belfast, BT9 5BN, United Kingdom.

c. Lancaster University, Lancaster Environment Centre, LA1 4YQ, Lancaster, United Kingdom

Daniel M Blackburn is a Senior Research Associate at the Environment Centre of Lancaster University (UK), working with plant use of soil organic phosphorus and Diffusive Gradient in Thin films (DGT) methods. Prior to that he was a Humboldt Postdoctoral Fellow at Max Rubner-Institut Karlsruhe (Germany), and his PhD was performed both at the Universidad de la Frontera (Chile) and Università degli Studi di Napoli Federico II (Italy). Daniel is a co-founder and administrator of the Soil Phosphorus Forum and is editor of the Brazilian Journal of Soil Science.

Karen Luko has Ph.D. degree in progress in Geosciences and Environment at Universidade Estadual Paulista, Rio Claro, Brazil, as well as a Geosciences and Environment Master degree and a Biology undergraduate degree at the same university. She works with the Diffusive Gradients in Thin Films technique and speciation analysis of metals in water and soil in Center for Environmental Studies, Rio Claro, Brazil.

Amauri Menegário holds a Ph.D. in Science at the University of São Paulo, a Master's degree in Chemistry (Analytical Chemistry) at the University of São Paulo and a undergraduate degree in Industrial Chemistry at the University of Ribeirão Preto. He works with Analytical and Environmental Chemistry, focusing on the development of methods for Biogeochemistry at the Environmental Studies Center, São Paulo State University (UNESP), Rio Claro, Brazil.

Paul N. Williams has joined IGFS as a Lecturer in Soil & Environmental Biogeochemistry. Paul obtained his Ph.D. degree in Biological Sciences from the University of Aberdeen in 2007. Previous to working at Queen’s he has held Research Fellowships with the Chinese Academy of Sciences, Lancaster University and a Lectureship with the University of Nottingham. Paul, an analytical and environmental chemist, has research interests orientated around the role of soils and rhizospheres in global food security.

Lauren N M Yabuki has a Ph.D. degree in Geosciences and Environment at the Universidade Estadual Paulista (UNESP, Rio Claro, SP, Brazil) working with alternative materials made of residual biomass and their application in Diffusive Gradient in Thin films (DGT) technique at Center for Environmental Studies. She has a Master's degree in Geosciences and Environment at the same university. Before that, I have got an undergraduate degree in Physics at the Universidade Estadual Paulista.