Use of Diffusive Gradients in Thin Films (DGT) to measure potentially bioavailable metals in southeastern USA blackwater streams

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Abstract

We used Diffusive Gradients in Thin Films (DGT) to measure potentially bioavailable metals in coastal plain streams in the southeastern USA that exhibited strong to moderate blackwater characteristics. Metals were partitioned into particulate metals, DGT inert metals (i.e., colloidal and refractory organic complexes not accumulated by DGT), and DGT labile metals (i.e., free metal ions, small inorganic complexes, and labile organic complexes). We also examined the influence of different DGT deployment times using data collected from the field and a follow-up laboratory study. The DGT measured fraction of dissolved metals in the streams was 15% for Cd, 21% for Zn, 33% for Cu, 37% for Pb, and 98% for Mn. Metals bound to particulates predominated only for Pb. Most of the Cd, Pb, Zn, and Cu was associated with colloids, refractory organic complexes, or particles. Relatively small amounts were in free ion or labile complexes likely to be bioavailable through respiratory surfaces. Modeled concentrations of free and inorganically bound Cu and Pb were lower than the DGT fraction indicating that DGT accumulated some organically bound Cu and Pb that might not have been bioavailable. DGT exposure times in excess of five days may have contributed to the accumulation of partly labile organic-metal complexes and were associated with substantial biofouling that caused metal uptake by DGT to depart from linearity.

Keywords: Diffusive gradients in thin films, bioavailability, metals, blackwater streams, biofouling

Declarations

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Conflicts of interest/Competing interests

No conflicts of interest or competing interests.

Availability of data and material

Data will be made available upon request.

Code availability

Not applicable.

Authors' contributions

M.H. Paller contributed to the text and analysis. A.S. Knox contributed to the text and analysis. S.M. Harmon provided analytical support and review. S.A. Blas provided funding and administrative support, assisted with study design, and provided review.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable

Introduction

Monitoring surface waters for trace metals is complicated by concentration fluctuations that necessitate repeated sampling for adequate characterization and sometimes by low analyte levels that are difficult to measure. In addition, water samples may require post processing and may not represent potential toxicity. For example, dissolved metals obtained by filtration may include metals bound to organic molecules that are unavailable for biological uptake. Some of these issues can be avoided by using passive sampling methods that differentially exclude inert metal fractions resulting in a time integrated sample of potentially bioavailable metals. Also, by accumulating metals over time, passive samplers facilitate the measurement of low concentrations that would not be readily detectable in grab samples (INAP 2002, Davison and Zhang 2012).

Diffusive Gradients in Thin Films (DGT) is a passive, diffusion-based sampling technology that employs a collection resin layer that selectively binds to metals, an overlying diffusion gel layer that regulates metal entry, and an outer layer of filter paper. These components are incorporated into a plastic probe that is deployed in water for days to months depending upon expected metal concentrations (DGT Research 2020). The diffusion gel admits metals at a rate determined by the diffusion coefficient for the metal but selectively exclude metals bound to particles and some ligands. The establishment of a constant concentration gradient in the diffusion gel provides a basis for quantifying metal concentrations based on the metal diffusion rate. The time-averaged, mean concentration of metals during the DGT deployment period is calculated based on the measured concentration in the collection resin, device configuration, analyte diffusion coefficients, and deployment time (Davison and Zhang 1994; Van der Veeken and Van Leeuwen 2010).

DGT has been used to measure free metal ions, small inorganic complexes, and labile organic complexes that more accurately represent the bioavailable metal fraction than measurements of total or dissolved metals (Zhang and Davison 2000; Van Leewen et al. 2005). Total metals encompass all metals including those bound to particles. Dissolved metals, usually defined as the fraction that passes through a 0.45 μm filter, exclude particle-bound metals, but may include nonbioavailable metals in filterable colloids or bound to ligands like large organic molecules. Because DGT excludes some or most of these, it provides a comparatively simple method of fractionating potentially bioavailable from nonbioavailable metals (Buzier et al. 2006; Senila et al. 2015).

The coastal plain of the southeastern United States includes low-gradient, slow-flowing, "blackwater" streams fed by water seeping through sandy soils that underlie floodplains and swamps. Such streams are also common in the Amazon and Orinoco basins as well as parts of Africa and Australia. Blackwater streams are typically acidic, low in conductivity, carry relatively little sediment, and have low concentrations of cations including calcium, magnesium, sodium, and potassium. They are darkly stained by organic matter consisting largely of fulvic acids leached from allochthonous decaying organic material in the watershed (Sabater et al. 1993). Organic matter produced by the decay of lignin containing plants may be more effective than other types of organic matter at binding metals and reducing their bioavailability (Luider et al. 2004). Trace metal speciation, hence, bioavailability and toxicity, has not been extensively investigated in these streams where it may be influenced by low pH and high dissolved organic matter content. Exceptions include Yabuki et al. (2014) and Tonello et al. (2007), who used DGT and other techniques to study trace metal speciation in the Amazon and Rio Negro Rivers, the latter of which exhibits strong blackwater characteristics.

In this study, we used DGT to measure potentially bioavailable trace metals in warm-water coastal plain streams in the southeastern USA that exhibited strong to moderate blackwater characteristics. Metals were partitioned into particulate metals, DGT inert metals (i.e., colloidal and refractory organic complexes not accumulated by DGT), and DGT labile metals (i.e., free metal ions, small inorganic complexes, and labile organic complexes accumulated by DGT). We also examined the influence of different DGT deployment times using data collected from the field and a follow-up laboratory study. Deployment times of 3 - 21 days are typical for unpolluted waters, but long deployment times can result in erroneous results if biofouling is extensive (DGT Research 2020). Preferred deployment times may also be affected by organic ligands that bind with metals if the kinetics of metal-ligand dissociation rather than rate of diffusion through the diffusive gel layer limit metal assimilation by DGT probes (Davison and Zhang 2012).

Materials and methods

Description of sampling locations

Data were collected from three third to fourth order (Strahler stream order, Strahler 1952) tributaries of the Savannah River located on the Savannah River Site (SRS), an 800 km^2 USA Department of Energy reservation in the Sand Hills ecoregion near Aiken, SC (Figure 1). Upper Three Runs (UTR) is a blackwater stream that flows through the northwestern portion of the SRS before joining the Savannah River. Conductivity, pH, alkalinity, and cation concentrations are relatively low in UTR, which exhibits strong blackwater characteristics (Table 1). Lower Three Runs (LTR) and Steel Creek (SC), two other tributaries of the Savannah River are characterized by somewhat higher pH, conductivity, alkalinity, and cations and anions concentrations than UTR (Table 1). The watersheds of all three streams are largely forested, and LTR and SC have impoundments in their upper reaches. There were three sample sites in UTR (Tyler Bridge, Road A.2, and Road C), two in LTR (Road B and Donora Station), and two in SC (below L Lake dam and near Rd A) (Figure 1).

Field methods

DGT water probes were obtained from DGT Research Ltd; each consisted of a plastic housing with a 2 cm diameter window covered with a polyethersulphone filter membrane backed by an agarose crosslinked polyacrylamide diffusive gel (pore size about 5 nm) and an ion-exchange resin gel (Chelex 100) for analyte collection. Eight DGT probes were deployed in shallow water (15 – 40 cm) at each sample site in LTR, SC, and UTR between 6/25/2019 and 8/6/2019. The probes were attached by monofilament to a 225 cm^2 piece of plastic hardware cloth (13 mm diagonal openings). The hardware cloth with attached DGT probes was suspended in an area of moderate flow at each sample site with the probe exposure windows facing the current.

Two or three probes were retrieved from each sample site after 2.5, 5.5, 14.5 and 35 days of exposure. Exposure times of 2.5-14.5 days were employed because previously collected water samples (M. Paller, unpublished data) indicated that dissolved metal concentrations were comparatively low (i.e., typical of unpolluted streams), which could necessitate relatively long exposures to ensure sufficient metal uptake for accurate analysis. The longest exposure period, 35 days, was included to evaluate the effects of prolonged deployment on measurement accuracy. Upon retrieval, the probes were rinsed with deionized water, sealed in a clean plastic bag, and stored under refrigeration. DGT blanks (approximately 5% of all samples) consisted of DGT probes that were not deployed in the field or laboratory.

Water temperature was measured to the nearest 0.1 °C with a hand-held thermometer when the DGT probes were deployed and retrieved. Conductivity and pH were measured with an Accumet XL600 electronic meter. Water samples were collected in clean Nalgene bottles from about 12 cm beneath the surface including a 1 L grab sample for measurement of pH, conductivity, and suspended solids; a 30 ml filtered grab sample for measurement of dissolved organic carbon (DOC), a 15 ml grab sample for measurement of total metals, and a 15 ml filtered grab sample for measurement of dissolved metals. Samples for dissolved metal analysis and DOC were filtered in the field through 0.45 μm filter paper. Samples for metal analysis were acidified to pH 2.0 by adding 0.2 mL of trace metal grade nitric acid. All samples were placed on ice for transfer to the laboratory.

Suspended solids were measured by drying 0.45 μm filter paper in a desiccator, weighing the paper, suction filtering approximately 500 ml of water from the shaken 1000 ml sample through the filter paper, and again drying and weighing the filter paper with residue. Suspended solids (mg L^{-1}) were calculated from the residue mass and filtrate volume. Organic carbon was measured with a GE 5310 C Laboratory TOC analyzer.

A composite UV-visible light spectrophotometer was used to measure light absorbance of stream water samples placed in 40 mm path-length optical cuvettes. Scans were performed from 190-1100 nm, but absorbance at 440 nm was selected for comparison among streams because this wavelength is correlated with the humic and fulvic substances that constitute the principal organic matter in most natural surface waters (Davies-Colley and Vant 1987; Fuentes et al. 2006).

Laboratory Methods

The laboratory study evaluated the potential effects on DGT measurements of biofouling associated with prolonged deployment in stream water. Ten DGT water probes were placed in a 5 L beaker filled with untreated water from Tims Branch, a blackwater tributary of UTR, and ten probes were placed in a second 5 L beaker filled with municipal water from a laboratory faucet (pH near 7.0 and conductivity of 100 umhos cm^{-1}). Although microbial analyses were not conducted, we assumed that stream water supported microorganisms capable of biofouling, whereas chlorinated municipal water did not. The probes were suspended face-up at mid depth on a plastic hardware cloth screen. Water in the beakers was continually circulated by stirring and was usually renewed daily with fresh tap or Tims Branch water by gently removing the probes, discarding the old water, adding new water, and replacing the probes. Two probes were retrieved from each beaker at 2, 5, 14, and 26 days, rinsed with deionized water, and stored under refrigeration before processing. Filtered (0.45 µm) water samples (20 ml) were taken from each beaker in conjunction with DGT removal, at the start and end of the study, and at other times for a total of 10 samples. Untreated Tims Branch water was collected directly from the stream on two occasions and stored in 25 l Nalgene bottles before use. The tap water was collected directly from a laboratory faucet.

Processing DGT Probes

At the conclusion of the field and laboratory work, the DGT probes were removed from refrigeration, split open, and the filter and diffusive gel layers peeled away to expose the bottom resin-gel layer. The resin-gel was removed and placed in a small, trace metal free sample tube with 1 ml of trace metal grade 1 M HNO_3. After 24 h, an aliquot of the solution was removed and diluted with deionized water prior to analysis.

DGT concentrations in the streams were calculated for Cd, Cu, Mn, Pb, and Zn, selected because of their possible presence as legacy contaminants (Paller and Dyer 2010). Cu, Pb, and Zn were measured in the laboratory study. Metal concentrations in DGT extracts and in filtered and unfiltered water samples were analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) using a NexION 300x mass spectrometer and standard QA/QC protocols including internal standards, duplicate samples, blanks, and certified reference materials (TORT-3: Lobster Hepatopancreas Reference Material for Trace Metals) following USEPA Method 6020B (USEPA 2014).

DGT metal concentrations were adjusted for dilution to determine the concentration of metals in the 1M HNO_3 elution, C_e. The mass of metal accumulated in the resin gel layer (M) was calculated with equation 1:

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

where V_{HNO3} equals the amount of nitric acid required to submerge the resin-gel layer (1000 µL for water probes), V_{gel} is the volume of the resin gel (or 150 µL), and f_e is the elution factor of 0.8 (Zhang and Davison 2000).

The concentration of metal measured by the DGT unit (C_{DGT}) was calculated with Equation 2.

$$C_{DGT} = \frac{M * \Delta g}{D * t * A} \qquad \text{Eq. 2,}$$

Where Δg is the thickness of the diffusive layer and filter layer (0.096 cm), D is the diffusion coefficient for each metal at the deployment temperature, t is the deployment time (24 h = 86400 s), and A is the exposed area of the DGT unit (3.14 cm^2) (DGT Research 2020).

Data analysis

One-way analysis of variance (ANOVA) was used to assess the significance ($P \leq 0.05$) of differences in water chemistry variables among LTR, SC, and UTR. ANOVAs were conducted for pH, conductivity, suspended solids, temperature, total organic carbon, and light absorption at 440 nm. Data points were the individual measurements from each sample site in each stream producing total sample sizes of 31-32 for each variable.

Factorial two-way ANOVA was used to assess the significance of differences between metal concentrations measured by DGT probes deployed for 5.5 days and dissolved metal concentrations (i.e., metal concentrations in filtered water samples) collected during the 5.5-day DGT deployment. DGT concentrations were compared with dissolved rather than total metals because the latter includes particle bound metals that cannot pass through the DGT filter paper and are not measured by DGT. Main effects included measurement method (DGT vs filtered water) and stream (LTR vs SC vs UTR). The statistical interaction between main effects was also tested. Holm-Sidak pair-wise multiple comparison tests were used to assess the significance (overall $P \leq 0.05$) of differences among individual means when main effects or interactions were significant. There were four to six replicates for each combination of measurement method and stream.

Linear regression was used to assess the relationship between metal concentrations measured by DGT and metal concentrations in filtered water during the deployment period. Separate regressions were conducted for each metal and each DGT deployment period (2.5, 5.5, 14.5, and 35 days). Linear regression was also used to assess relationships between DGT and dissolved metals in the laboratory study.

Most data in the preceding analyses (including the ANOVAs) satisfied tests for normality (Shapiro-Wilk) and equal variance (Brown-Forsythe) making transformations unnecessary. All statistical analyses were conducted with SigmaPlot 13 (Systat Software 2007).

Metals were partitioned into DGT labile metals (i.e., dissolved metals accumulated by DGT), DGT inert metals (i.e., dissolved metals not accumulated by DGT), and particulate metals (i.e., total metals minus dissolved metals). Cd, Cu, Pb, and Zn in the streams were also divided into free ionic, inorganically bound, and organically bound species based on output from the Biotic Ligand Model (BLM) (WindWard Environmental^{LLC} 2017) run in speciation mode. This capability was not available for Mn. BLM inputs included measurements of temperature, pH, metal concentrations, dissolved organic carbon, cation (Ca, Mg, Na, and K) concentrations, anion (SO_4^{-2} and Cl^-) concentrations, and alkalinity (Table 1). Sulfide concentrations were assumed to be zero. Output included estimates of free metals, total organic metals, and inorganic metals. Inorganic metals were calculated by subtracting the sum of free and organic metals from total metal.

Results

Stream water chemistry

Conductivity and pH during the study were significantly ($P \leq 0.05$) lower in UTR than SC and LTR, averaging 22 compared with 51-67(µS/cm) and about 6.0 compared with 6.7-6.8, respectively (Table 1). In contrast, suspended solids and light absorbance at 440 nm were significantly ($P \leq 0.05$) higher in UTR than SC and LTR. Temperature was nearly 5°C lower in UTR than the other streams as a result of groundwater inputs, but the difference was not significant, nor were differences in DOC. These results were similar to those recorded in the 1980s (Table 1). They indicate that UTR exhibits stronger “blackwater stream” characteristics than LTR and SC (i.e., lower pH, conductivity, and cation concentrations and stronger color) and that water chemistry has changed little over time.

<u>Relationships between DGT deployment time and dissolved metal concentrations in streams</u>

The relationships between DGT and dissolved metal concentrations differed among metals and deployment times (Figure 2). DGT Zn and Cd concentrations were lower than dissolved Zn and Cd concentrations regardless of deployment time, although the difference increased with time. DGT Cu, Mn, and Pb concentrations were comparable to or greater than dissolved concentrations at short deployment times (2.5 days) but decreased with longer deployment times, finally reaching a small fraction of the dissolved concentration at 35 days (Figure 2). A progressive reduction in the DGT metal concentration relative to the dissolved metal concentration with deployment time was observed in all streams, a partial exception being DGT Cd, which showed smaller decreases with deployment time in UTR than SC and LTR.

DGT concentrations were not significantly related to dissolved concentrations of Cu, Cd, Zn, and Pb at the sample sites, regardless of deployment time (Figure 2). In contrast, the relationships between DGT and dissolved Mn concentrations was significant ($p<0.05$) for all deployment times except 14.5 days. Coefficients of determination (R^2s) for the significant relationships ranged from 0.82-0.99.

Biofouling of the DGT probe windows steadily increased with time and was extensive by the end of the 35-day deployment. The biofouling material was not analyzed; however, it consisted of a brownish film sometimes accompanied by other material including attached insect pupal cases. Also, aquatic plant debris sometimes collected around the DGT probes, which likely reduced water renewal at the probe surface.

Relationships between DGT deployment time and dissolved metal concentrations in the laboratory

Biofouling did not occur on DGT probes held in municipal tap water in the laboratory, likely because the biocidal effects of chlorine prevented biological growth. However, fouling occurred on DGT probes in Tims Branch water in the laboratory, although not to the degree observed on DGT probes in the streams. The fouling material was brownish, some of which could be dislodged by agitation and may have been settled particles but some of which was firmly attached to the probe face (Picture 1).

Metal accumulation by DGT probes in tap water was consistent and linear with time (mean R^2 =0.99) (Figure 3). In contrast, the accumulation of Cu, Pb, and Zn varied with time in Tims Branch water with the greatest departure from linearity observed for Pb (mean R^2 = 0.71) (Figure 3).

Differences in metal accumulation between tap and Tims Branch water affected the calculation of DGT metal concentrations. DGT concentrations in tap water were consistent over time for Cu and Pb and about the same as the average concentrations in filtered tap water (Figure 3). DGT concentrations of Zn in tap water decreased between days 2 and 5 and then stabilized at a level below the average concentration in filtered water. In contrast, DGT concentrations of all metals in Tims Branch water initially decreased strongly with time before stabilizing well below filtered water concentrations after about 10 days. Time-related decreases in DGT concentrations in Tims Branch water (which paralleled the temporally declining DGT concentrations observed in the streams) were likely the result of biofouling, which did not occur in tap water because of the biocidal effects of residual chlorine.

Metal partitioning in streams

The concentrations of most metals in filtered and unfiltered stream water exceeded detection limits at all sample sites. An exception was Pb, for which 7% and 9% of filtered and unfiltered water samples, respectively, were below detection limits. There was usually little difference between concentrations in filtered and unfiltered water for Mn, Zn, Cu, Cd, and Ni indicating these metals were mostly dissolved (Table 2). In contrast, unfiltered water concentrations were usually higher than filtered water concentrations for Pb (Table 2). Dissolved concentrations of several elements occasionally exceeded total concentrations, likely due to inaccuracies associated with low-level measurements. Metal concentrations fluctuated over time but did not show consistent trends.

The DGT deployment time of 5.5 days was selected for the two-way factorial ANOVAs of differences between streams and measurement method. Five and a half days was long enough to accumulate sufficient metal for accurate analysis but not long enough for extensive biofouling of the probes. Metal concentrations were generally similar at different sites within the same stream so sites within streams were pooled (Table 2). Dissolved Mn and Cu differed significantly ($P \leq 0.05$) among streams and were significantly higher in LTR than SC and UTR. Dissolved Cu was significantly higher in UTR than SC, and dissolved Pb (but not DGT PB) was higher in UTR than the other streams resulting in a significant interaction between measurement method and stream. Cd and Zn concentrations differed little among streams (Table 2). The DGT measured fraction was 15-37% of the dissolved Cd, Pb, Zn, and Cu, with Cd, Pb, and Zn showing the largest differences between measurement methods (Figure 4). These differences were statistically significant ($P \leq 0.05$) for all four metals. In contrast, DGT Mn concentrations were comparable to dissolved Mn concentrations. Relationships between DGT and dissolved metal concentrations differed little among streams (Figure 4).

DGT samples (5.5-day deployment), filtered water samples, and unfiltered water samples were analyzed to determine the DGT labile dissolved fraction, DGT inert dissolved fraction, and particulate fraction for Cd, Cu, Mn, Pb, and Zn (Figure 5). Average DGT labile fractions were under 25% for Cd and Zn in all streams and for Pb in SC and UTR. DGT labile Pb was higher (about 40%) in LTR than in SC and UTR (7-8%). DGT labile fractions were higher for Cu and Mn than for the other metals, ranging from 37-53% for Cu and 68-91% for Mn. The DGT inert fraction constituted most of the total Cd and Zn (56-96%) and about half of the total Cu (47-63%) but relatively little of the total Mn (6-13%) and Pb (15-42%). Except for Pb in SC and UTR, the particulate fraction of all metals was relatively small ranging from 0-30%.

Free plus inorganically bound metals calculated with the BLM constituted <2% of the dissolved Cu and Pb in the streams with the rest of these metals complexed with organic compounds (Figure 4). DGT concentrations greatly exceeded free plus inorganically bound concentrations of both metals. In contrast, free plus inorganically bound metals constituted 36-74% of the dissolved Cd and 24-48% of the dissolved Zn. These amounts were about equal to the DGT concentrations for Zn but exceeded the DGT concentrations for Cd. Generally, there was little difference in proportions of free plus inorganically bound metals among streams, although proportions of free and inorganically bound Pb and, to a lesser extent, Cd were higher in LTR than in the other streams.

Discussion

Evaluation of metal partitioning using DGT probes

With a few exceptions, metal concentrations differed little among the streams under study and were below levels considered indicative of pollution (U.S. EPA 2020). Relatively high concentrations of Mn in LTR were likely the result of water released from outlets located near the bottom of Par Pond, a reservoir located upstream of the sample sites. Dissolved Mn accumulates in the deeper water of this reservoir when it becomes thermally stratified during the summer and reducing conditions are established (Zaw and Chiswell 1999; Paller et al. 2006). Dissolved Cu and Pb levels were higher in UTR than in the other streams, possibly because UTR's lower pH contributed to the mobility of these elements. Except for Pb, little metal was bound to particulates in any stream. These results differ from those in rivers with high levels of suspended solids in which large fractions of metals may be bound to particles (Senila et al. 2015).

With a few exceptions, the partitioning of metals among DGT labile, DGT inert, and particulate phases was similar among streams despite significant differences in water chemistry (Figure 5). Most of the aqueous Cd, Pb, and Zn and about half the aqueous Cu in the streams was DGT inert. The DGT labile fraction predominated only for Mn, the only element that exhibited significant relationships between dissolved and DGT metal measurements. These results suggest that most of the metals in the study streams (and possibly other southeastern streams with similar water quality) are associated with colloids, refractory organic compounds, or particles (in the case of Pb) and not bioavailable to aquatic organisms through interactions with respiratory surfaces, although they may be available to some organisms through ingestion (Luoma 1983). Furthermore, it is possible that the DGT labile metal fraction (which presumably represents more bioavailable metals) in Upper Three Runs was overestimated due to the low concentrations of cations in this stream (Table 1). Under such conditions, the flux of cations from the sample water into the diffusion gel can be accelerated by charge imbalances resulting from the reverse flow of sodium in the polyacrylamide gel to the sample water due to a negative concentration gradient (INAP 2002). This issue, together with alternative DGT methods for blackwaters in the Amazon basin, are discussed in Tonello et al. (2007) and Yabuki et al (2014).

The DGT labile fraction generally correlates with bioavailability; however, not all DGT labile metals are bioavailable (Philips et al. 2018; Tusseau-Vuillemin 2004; Kim and Choi 2016; Paller et al. 2019). Metal species that enter the diffusive gel and accumulate in the collection resin include free metal ions plus inorganic and organic metal species that readily dissociate within the gel to release free metals (Zhang and Davison 2000; Luider et al. 2004; van Leewen et al. 2005; Warnken et al. 2008). They can also include organically complexed metals that diffuse slowly in the gel or that are only partly labile and dissociate slowly (Luider et al. 2004; Davison and Zhang 2012). The latter can result in an overestimate of the bioavailable metal fraction by DGT because only free metal ions and, in some cases (e.g., copper), hydroxyl–metal complexes are generally bioavailable and toxic (Luider et al. 2004). Tusseau-Vuillemin et al. (2004) found that different types of organic matter formed copper complexes that were nontoxic to *Daphnia magna* but measured in part by DGT with open pore gels. Similarly, Buzier et al. (2006) found that DGT accumulated some Cu complexed with organic matter, even when used with restricted pore gels that minimized the accumulation of organic-metal complexes.

The proportion of DGT labile organically complexed metals is strongly dependent on organic matter type and ranges from very little to nearly all the total organically complexed metals (Tusseau-Vuillemin 2004; Buzier 2006). Generally, highly colored natural organic matter derived from the breakdown of lignin containing plants, which is prevalent in blackwater streams, forms metal complexes that are both less bioavailable and less susceptible to uptake by DGT (Luider et al. 2004). However, in the streams we studied, modeled concentrations of free and inorganically bound Cu and Pb were much lower than the DGT labile concentrations of these metals indicating that the latter included organically bound metal (assuming the accuracy of model predictions) that was likely nontoxic. In contrast to Cu and Pb, DGT Zn in the streams was about equal to the free and inorganically bound Zn predicted by modeling.

Relationships between DGT deployment time and dissolved metal concentrations

Long deployment times were associated with departures from the linear accumulation of metals with time as a likely consequence of biofouling. DGT devices have been deployed for several months to a year in deep or pristine waters where biofouling does not occur or occurs slowly (DGT Research 2020). Under such circumstances long deployment times permit the accumulation of sufficient metal for accurate analysis and provide a time-averaged concentration over the deployment period. However, long deployments were unsuitable in the streams we studied because of conditions that promoted biofouling including shallow, clear, insolated water that encouraged periphyton growth and currents that enabled the transport and deposition of organic matter and organisms on the probes.

Biofouling of the probe window creates several problems that contribute to inaccurate DGT measurements. It increases the thickness of the material diffusion layer thereby affecting Δg in equation 2. Similarly, D (diffusion coefficient) in equation 2, which describes the diffusion rate of metals through the diffusive gel, does not apply to the heterogenous, unknown materials in the biofouling layer. Lastly, the irregular surface of the biofilm likely increases the thickness of the diffusive boundary layer (i.e., the thin layer of quiescent solution adjacent to the probe surface), thereby increasing the distance metals travel by diffusion before reaching the probe surface and delaying the approach to steady state conditions. Our results suggest that deployment times exceeding five days should be avoided during the summer in streams and rivers that support the rapid growth of biofouling organisms. Longer deployment times may be permissible in winter when the growth of biofouling organisms is slowed by low temperatures or in deep water where periphyton growth is not encouraged by sunlight.

In addition to influencing the potential for biofouling, deployment time can affect the uptake of organic-metal complexes by DGT. Organically complexed metals can extend the time required to attain a steady state flow of metals from solution to the DGT collection gel by moving slowly through the diffusive gel or binding to the diffusive gel and gradually dissociating to release free metals (Luider et al. 2004; Davison and Zhang 2012). Binding of complexes of Cu and Pb to materials of the diffusive layer may substantially increase the time required to reach a steady-state metal flux, especially when the metals in solution are at low concentrations (Davison and Zhang 2012). When partially labile organic metal complexes are present, metal accumulation by DGT may have two slopes: the first proportional to un-complexed metal concentrations and a second more gradual slope that may require considerable time to reach steady state and fully represent all DGT labile metals (Davison and Zhang 2012). Long deployment times that result in the accumulation of partly labile metal complexes not readily bioavailable may be undesirable if the primary interest is the measurement of free and highly labile metal complexes that are likely toxic to aquatic life.

We observed that DGT concentrations of Cu and Pb exceeded filtered water concentrations at the shortest deployment time of 2.5 days. A possible explanation is that these metals accumulated in the diffusive gel and were gradually stripped from it by the Chelex collection resin during the several week storage period between DGT retrieval and analysis. Both metals may form ligand complexes that accumulate in the gel (Davison and Zhang 2012). This problem is likely greater when metal concentrations in the collection resin are comparatively low due to low concentrations in solution, deployment times are short, and a steady-state flux has not been attained between the diffusive and collection gels. These conditions are more likely to occur in unpolluted streams with low metal concentrations and with organic ligands that form metal complexes.

Conclusions

The largely unpolluted, coastal plain, blackwater streams in this study were characterized by acidic to neutral pHs, low concentrations of most cations, and relatively high concentrations of dissolved organic matter derived from the decomposition of terrestrial vegetation. Most of the dissolved Cd, Pb, and Zn and about half of the aqueous Cu in these streams was DGT inert, hence unlikely to be bioavailable through respiratory surfaces. In addition, modeling suggested that much of the DGT labile Cu and Pb consisted of organically bound metals that also may not have been bioavailable.

Biofouling caused the accumulation of metals by DGT to depart from linearity with prolonged exposure in the streams. Exposure times of two to five days may be optimal for shallow, ecologically productive warm-water streams, with longer times resulting in substantial biofouling and accumulation of labile organic metal complexes. Even shorter deployment times (i.e., 24 hours) may be feasible if the emphasis is on free and inorganically bound metals, and DGT metal uptake is sufficient for accurate determination of metal concentrations. We found DGT to be a convenient and useful tool for evaluating metal partitioning in coastal plain streams and likely in similar ecologically productive, warm-water streams. However, our study indicates that DGT results in such environments can be compromised by biofouling and suggest that the DGT labile fraction may overestimate the bioavailable metal fraction.

References

Buzier, R., Tusseau-Vuillemin, M-H., Mouchel, J-M. (2006). Evaluation of DGT as a metal speciation tool in wastewater. *Sci Total Environ*. 358, 277-85.

Davies-Colley, R.J., Vant, W.N. (1987). Absorption of light by yellow substances in freshwater lakes. *Limnol. Oceanogr*. 32, 416-425.

Davison, W., Zhang, H. (1994). In situ speciation measurements of trace components in natural waters using thin-film gels. *Nature* 367, 546–548.

Davison, W., Zhang, H. (2012). Progress in understanding the use of diffusive gradients in hin films (DGT) - back to basics. *Environ. Chem*. 9, 1–13.

DGT Research. (2020). LSNM-NP Loaded DGT device for metals (A) in solution. https://www.dgtresearch.com/product/lsnm-loaded-dgt-device-for-metals-a-in-solution/.

Fuentes, M., Gonza´lez-Gaitano G., Garci´a-Mina, J.M. (2006). The usefulness of UV–visible and fluorescence spectroscopies to study the chemical nature of humic substances from soils and composts. *Org. Geochem*. 37, 1949–1959.

INAP (International Network for Acid Prevention) (2002). Diffusive gradients in thin-films (DGT): a technique for determining bioavailable metal concentrations. International Network for Acid Prevention, Mitcham, Victoria, Australia.

Kim, M.S., Choi, M.S. (2016). Monitoring tracemetals in seawater using a diffusive gradient in thin film probe in Ulsan Bay, East Sea, Korea: comparison with transplanted mussels. *Ocean Sci. J.* 51, 169–182.

Luider, C.D., Crusius, J., Playle, C., Curtis, P.J. (2004). Influence of natural organic matter source on copper speciation as demonstrated by Cu binding to fish gills, by ion selective electrode, and by DGT gel sampler. *Environ. Sci. Technol.* 83, 2865-2872.

Luoma, S.N. (1983). Bioavailability of trace metals to aquatic organisms—A review. *Sci. Total Environ*. 28, 1-22.

Paller, M.H., Dyer., S.A. (2010). Ecological Effects of Metals in Streams on a Defense Materials Processing Site in South Carolina, USA, *Hum Ecol Risk Assess* 16, 1095-1114.

Paller, M.H., Fletcher, D.E., Standora, M.M., Grabowski, T.B., Jones, T.A., Dyer., S.A. (2011). Emigration of fish from two South Carolina cooling reservoirs. *N. Am .J .Fish. Manage*. 26, 976-982.

Paller, M.H., Harmon S.M., Knox, A.S., Kuhne, W.W., Halverson, N.V. (2019). Assessing effects of dissolved organic carbon and water hardness on metal toxicity to *Ceriodaphnia dubia* using diffusive gradients in thin films (DGT). *Sci. Total Environ*. 697, 134107

Philipps, R.R., Xu, X., Mills, G.L., Bringolf, R.B. (2018). Evaluation of diffusive gradients in thin films for prediction of copper bioaccumulation by yellow lampmussel (*Lampsilis cariosa*) and fathead minnow (*Pimephales promelas*). *Environ. Toxicol. Chem*. 37, 1535-1544.

Sabater, F., Meyer, J. L., Edwards, R. T. (1993). Longitudinal patterns of dissolved organic carbon concentration and suspended bacterial density along a blackwater river. *Biogeochemistry* 21, 73-93.

Senila, M., Levei, E.A., Senila, L.R, Roman, M. (2015). Preliminary investigation concerning metals bioavailability in waters of Aries River catchment by using the Diffusive Gradients in Thin Films Technique. *J Chem-NY*. Article ID 762121, 8 pages.

Strahler, A.N. (1952). Hypsometric (area-altitude) analysis of erosional topology. *Geol. Soc. Am. Bull*. 63, 1117–1142.

SYSTAT Software, Inc. (2007). SigmaPlot 13.0. San Jose, CA, USA, 2007.

Tonello, P. S., Menegário, A. A., & Rosa, A. H. (2007). Use of diffusive gradients in thin films and tangential flow ultrafiltration for fractionation of Al(III) and Cu(II) in organic-rich river waters. *Analytica Chimica Acta*, 558, 162–168.

Tusseau-Vuillemin, M.H., Gilbin, R., Bakkaus, E., Garric, J. (2004). Performance of diffusion gradient in thin films to evaluate the toxic fraction of copper to Daphnia magna. *Environ. Toxicol. Chem*. 23, 2154–2181.

U.S. Environmental Protection Agency (U.S. EPA). (2014). Method 6020B, Rev. 2. Inductively coupled plasma-mass spectrometry. In *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods* (SW-846). Washington, DC: Office of Solid Waste.

U.S. Environmental Protection Agency (U.S.EPA). (2020). National recommended water quality critera – aquatic life criteria table. https://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table.

Van der Veeken, P.L.R., Van Leeuwen, H.P. (2010). DGT/DET gel partition features of humic acid/metal species. *Environ. Sci. Technol.* 44, 4253–4257.

Van Leeuwen, H.P., Town, R.M., Buffle, J., Cleven, R.F., Davison, W., Puy, J., van Riemsdijk, W.H., Sigg, L. (2005). Dynamic speciation analysis and bioavailability of metals in aquatic systems. *Environ. Sci. Technol.* 39, 8545–8556.

Warnken, K.W., Davison, W., Zhang, H. (2008). Interpretation of in situ speciation measurements of inorganic and organically complexed trace metals in freshwater by DGT. *Environ. Sci. Technol.* 42, 6903-6909.

WindWard Environmental^{LLC} (2017). Biotic ligand model Windows interface, research version 3.16.2.41 user's guide and reference manual. Seattle, Washington.

Yabuki, L., Colaco, C.D., Menegario, A.A., Domingos, R.N. (2014). Evaluation of diffusive gradients in thin films technique (DGT) for measuring Al, Cd, Co, Cu, Mn, Ni, and Zn in Amazonian rivers. *Environ Monit Assess* 186, 961-969.

Zhang, H., Davison, W. (2000). Direct in situ measurements of labile inorganic and organically bound metal species in synthetic solutions and natural waters using diffusive gradients in thin films. *Anal. Chem.* 72, 4447–4457.

Zaw, M., Chiswell, B. (1999). Iron and manganese dynamics in lake water. *Water Res.* 33, 1900-1910.

Picture 1. DGT probe after 26 days in laboratory tap water (left) and Tims Branch water (right).

Table 1. Average values for water chemistry variables collected from Savannah River Site streams. Data from 2019 include standard deviations (n=31-32) in parentheses.

Variable	Lower Three Runs	Steel Creek	Upper Three Runs
1980s (historical)			
pH	7.09	7.03	6.19
Alkalinity (mg $CaCO_3$ L^{-1})	30.17	16.70	1.81
Conductivity (umhos cm^{-1})	80.97	64.23	17.30
Calcium (mg L^{-1})	9.59	4.62	1.20
Chloride (mg L^{-1})	4.57	5.16	1.68
Magnesium (mg L^{-1})	0.88	0.96	0.37
Potassium (mg L^{-1})	0.81	0.75	0.18
Sodium (mg L^{-1})	3.69	4.87	1.27
Sulfate (mg L^{-1})	2.05	4.17	0.49
2019 (current study)			
pH	6.66 (0.21)	6.80 (0.31)	6.03 (0.35)
Conductivity (umhos cm^{-1})	51.05 (12.34)	66.93 (6.22)	22.02 (4.29)
Total suspended solids (mg L^{-1})	1.6 (0.7)	2.0 (2.2)	5.2 (2.8)
Temperature (°C)	29.1 (1.5)	29.4 (1.8)	24.8 (0.9)
Light absorption (440 nm)	0.013 (0.002)	0.014(0.011)	0.028(0.010)
Dissolved organic carbon (mg L^{-1})	3.25 (0.80)	3.89 (1.22)	3.80 (1.54)

Table 2. Average total metal (unfiltered water), dissolved metal (0.45 µm filtered water), and DGT metal concentrations (µg L^{-1}) after 5.5 days of exposure in SRS streams (usually 2 or 3 replicates).

	DGT		Water (filtered)*		Water (unfiltered)*	
Site	Avg	Stdev	Avg	Stdev	Avg	Stdev
Lower Three Runs Road B						
Cd	0.01	<0.01	0.16	0.05	0.16	0.05
Cu	0.10	0.02	0.32	0.00	0.31	0.17
Mn	152.24	23.05	174.50	61.05	184.19	57.00
Pb	0.02	0.01	0.03	0.02	0.05	0.01
Zn	8.61	0.19	66.07	6.76	62.52	14.10
Lower Three Runs Donora Station						
Cd	0.08	0.10	0.18	0.02	0.17	0.00
Cu	0.16	0.11	0.43	0.20	0.24	0.06
Mn	114.72	11.20	102.58	30.36	111.23	28.85
Pb	0.02	0.01	0.06	0.00	0.06	0.00
Zn	11.00	2.08	68.70	9.01	46.25	18.87
Steel Creek Road A						
Cd	0.01	<0.01	0.20	0.04	0.15	0.05
Cu	0.18	0.02	0.38	0.04	0.29	0.00
Mn	10.88	3.23	16.79	1.85	22.99	4.64
Pb	0.02	0.00	0.05	0.02	0.12	0.03
Zn	11.10	1.01	68.17	12.68	57.44	5.39
Steel Creek below L Lake dam						
Cd	0.01	0.00	0.17	0.01	0.16	0.00
Cu	0.10	0.03	0.22	0.04	0.21	**
Mn	11.23	0.26	9.14	0.52	12.72	1.32
Pb	0.02	0.01	0.04	0.03	3.78	5.33
Zn	15.83	4.87	62.49	6.61	144.03	91.64
Upper Three Runs Road A2						
Cd	0.02	**	0.18	0.01	0.18	0.04
Cu	0.15	**	0.76	0.29	0.58	0.34
Mn	15.31	**	20.24	22.31	27.92	24.40
Pb	0.03	**	0.11	0.01	0.67	0.71
Zn	22.19	**	59.30	3.73	66.39	3.95
Upper Three Runs Road C						
Cd	0.03	<0.01	0.15	0.01	0.18	0.02
Cu	0.25	0.06	0.43	0.13	0.39	0.06
Mn	23.57	0.33	13.84	10.82	20.52	7.79
Pb	0.03	<0.01	0.09	0.02	0.23	0.01
Zn	17.07	5.60	62.34	22.43	76.87	14.88
Upper Three Runs Tyler Bridge Road						
Cd	0.03	<0.01	0.22	<0.01	0.18	0.02
Cu	0.09	<0.01	0.54	0.28	0.41	0.01
Mn	11.23	0.57	6.92	3.79	10.09	3.86
Pb	0.03	<0.01	0.09	0.02	0.47	0.24
Zn	13.65	2.10	62.81	6.45	57.15	14.71
All sites						
Cd	0.02	0.03	0.18	0.03	0.18	0.04
Cu	0.22	0.14	0.48	0.23	0.45	0.38
Mn	50.62	51.47	48.83	61.06	44.98	48.56
Pb	0.03	0.01	0.07	0.03	0.45	1.32
Zn	13.21	3.97	64.64	9.80	66.14	29.45

* Instrument detection limits for water samples: Cd, 0.025; Cu, 0.028; Mn, 0.069; Pb, 0.018; Zn, 0.266 (µg L^{-1}).

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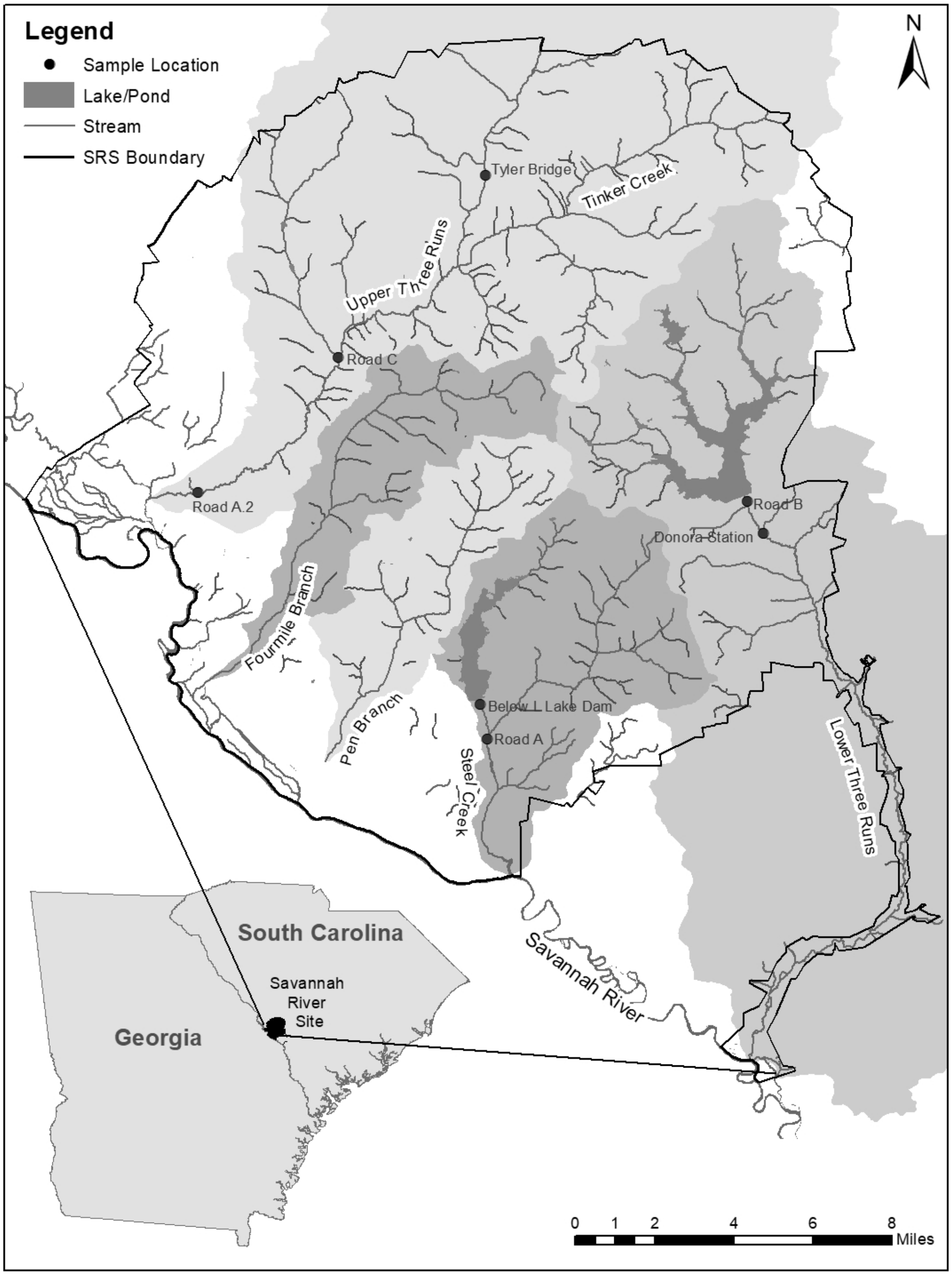

Figure 1. Sample stations for DGT deployment.

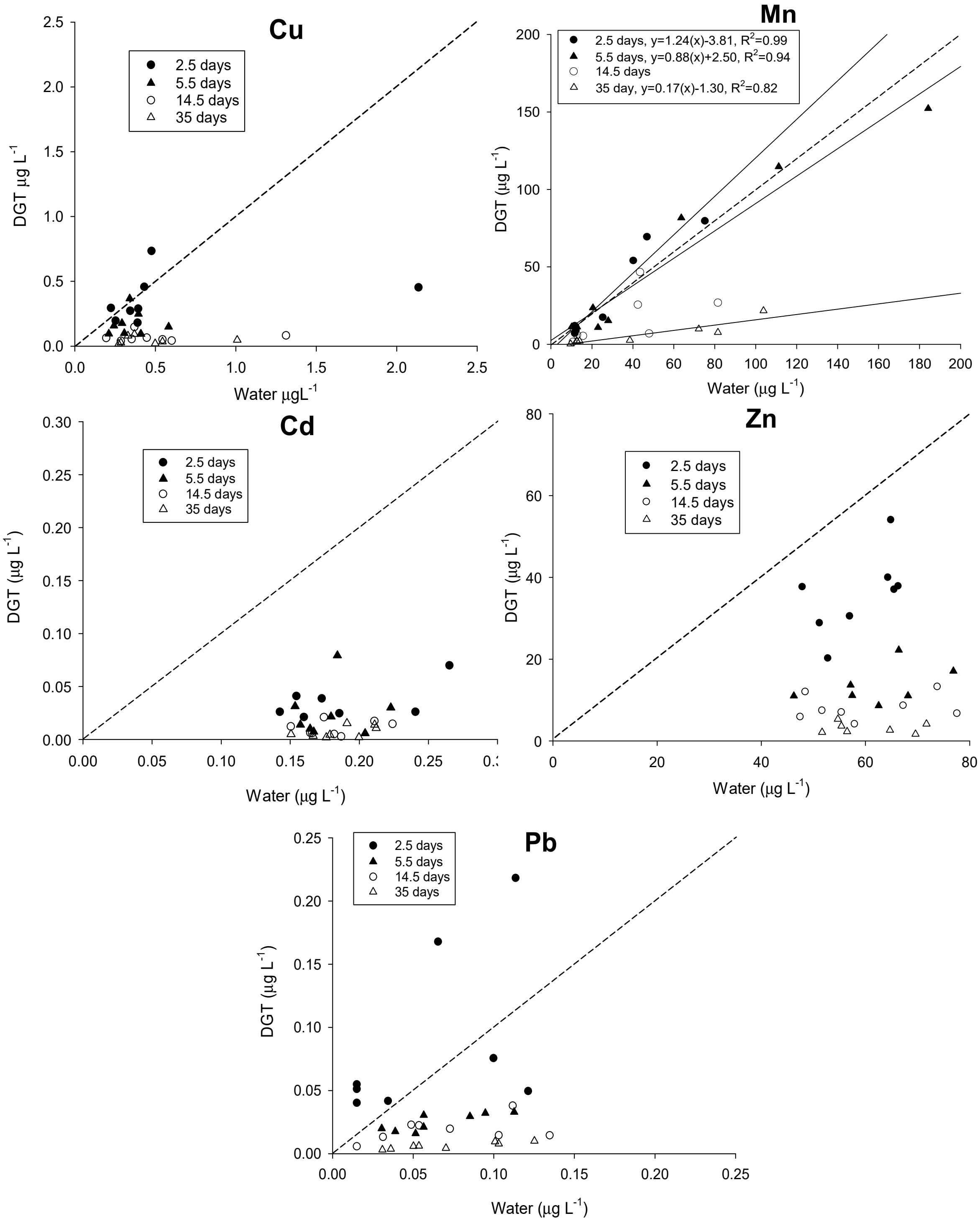

Figure 2. Relationships between metal concentrations measured by DGT and dissolved metal concentrations in filtered water during DGT deployment periods ranging from 2.5 to 35 days. Regression lines are shown when the relationship between water and DGT concentrations was significant ($P \leq 0.05$). Dashed line represents a one-to-one relationship.

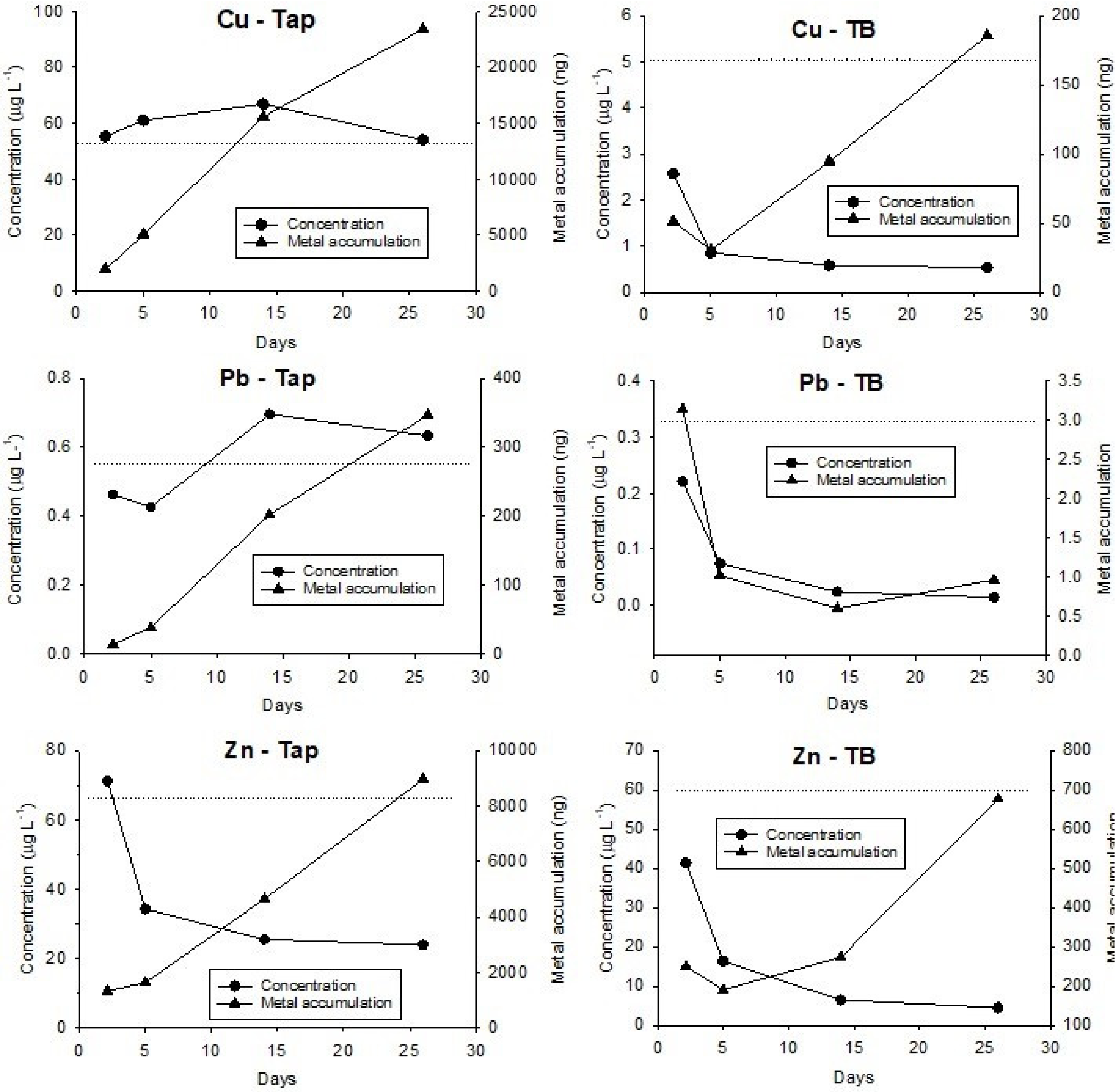

Figure 3. Accumulation of metals in DGT probes and metal concentrations measured by DGT in chlorinated municipal water (Tap) and water from Tims Branch (TB) over 26 days. Dotted lines represent average dissolved metal concentrations in filtered water samples.

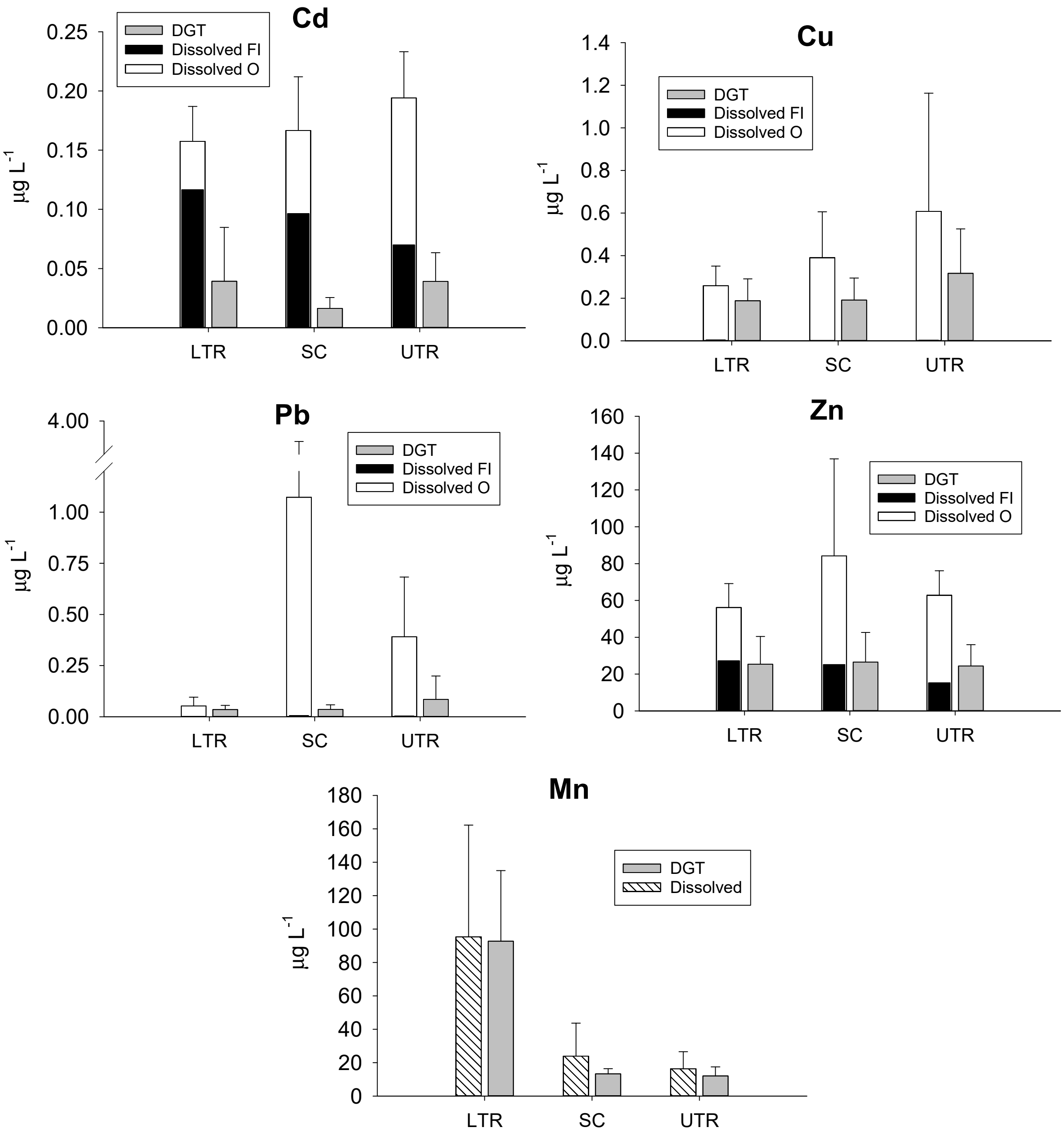

Figure 4. Metal concentrations measured by DGT after 5.5 days of deployment in SRS streams (LTR=Lower Three Runs, SC=Steel Creek, and UTR=Upper Three Runs) compared with dissolved metal concentrations. Dissolved concentrations of Cd, Cu, Pb, and Zn were divided into free ionic and inorganically bound metals (FI) and organically bound metals (O) based on Biotic Ligand Model calculations (details in text). Calculations were unavailable for Mn, which is shown as total dissolved concentrations (Dissolved) and DGT concentrations (DGT). Error bars are standard deviations.

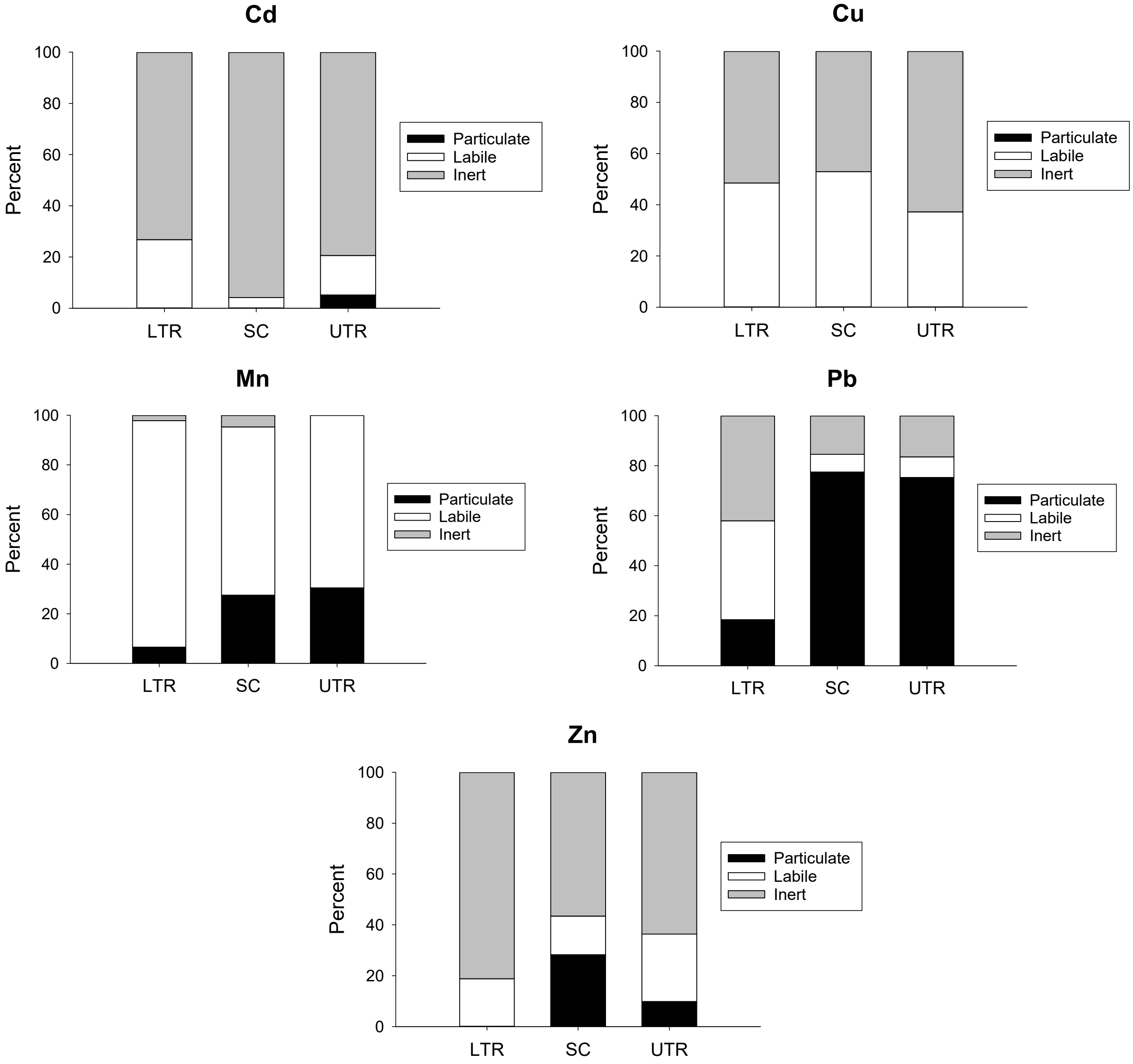

Figure 5. Partitioning of metals in SRS streams (LTR=Lower Three Runs, SC=Steel Creek, and UTR=Upper Three Runs) into particulate fractions, DGT labile fractions (i.e., dissolved metals measured by DGT), and DGT inert fractions (i.e., dissolved metals able to pass through a 0.45 µm filter but not measured by DGT).