Evaluation of Diffusive Gradients in Thin films (DGT) technique for speciation of trace metals in estuarine waters - a multimethodological approach

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Abstract

Understanding the potential bioavailability of trace metals (TM) in marine systems is of prime importance to implement adapted regulations and efficiently protect our coastal and estuarine waters. In this study Diffusive Gradients in Thin films (DGT) technique with two different pore size was used to evaluate the potentially bioavailable fractions (DGT-labile) of Cd, Co, Cu, Ni, Pb and Zn at various depths of a highly stratified estuary (the Krka River estuary, Croatia) both in winter and summer. DGT-labile concentrations were compared to (1) total dissolved concentrations, (2) concentrations of labile species measured by anodic stripping voltammetry (ASV-labile) for Cu and (3) concentrations derived by chemical speciation modelling. High correlation between dissolved and DGT-labile concentrations were found for all metals, except for Zn where contamination problems prevented reliable conclusions. Percentages of DGT-labile fractions over total dissolved concentrations were (AVG±SD): 92±3%, 64±2%, 23±5%, 61±3% and 57±6% for Cd, Pb, Cu, Ni and Co, respectively. No significant difference was found between trace metal concentrations measured with an open pore and restricted pore devices, implying the predominance of kinetically labile metal complexes smaller than 1 nm. For Cu, ASV-labile and DGT labile concentrations were highly correlated (0.97) with ASV-labile concentration being around 35% lower than that of the DGT-labile. Modelling of chemical speciation reliably predicted dynamic (free, inorganic and part of organic complexes) concentration of Cd, whereas dynamic concentrations of Cu and Pb were underestimated by 32% and 65%, respectively. In view of the relative simplicity of DGT devices, they are well suited for the monitoring effort of coastal waters, informing on potentially bioavailable concentrations of TM and thereby, helping to achieve good environmental status of coastal waters, as stipulated within the EU Water Framework Directive.

Keywords: bioavailability; trace metals; stratified estuary; Krka River estuary; diffusive gradients in thin films (DGT); speciation modelling

1. Introduction

Trace metals (TM) are naturally occurring elements in aquatic environments: some of them are bio-essential but most of them can be toxic, depending on their concentrations and the chemical forms under which they are present. Due to growing evidence of their toxicity at environmentally relevant concentrations, certain metals are classified as Priority Substances under the European Union Water Framework Directive (WFD) (Directive 2013/39/EU, 2013). Metals are present in the aquatic environment under various chemical forms, which behave differently in terms of mobility, bioavailability and toxicity: some are not bioavailable while others are or might be (Ferreira et al., 2008). To be effective and non-restrictive, environmental quality standards (EQS) required by the EU Water Framework Directive (WFD) for appropriate monitoring of water quality should take into account the chemical speciation of the element, but they are currently based on total dissolved concentration (Directive 2013/39/EU, 2013). One of the very few examples where this is not the case is for Cu where the amount of dissolved organic carbon (DOC) is used to derive site-specific appropriate Cu EQS (SEPA, 2018), based on the fact that DOC complexes Cu ion, decreasing its overall bioavailability and thus toxicity. Ideally, all elements should have EQS limits based on their potential bioavailability, not total dissolved concentration.

The challenge is the determination of the bioavailability which depends on a complex interaction of biological and physico-chemical parameters that affect the interaction between the metal and the biological receptor. Such parameters may include pH, ionic strength, complexing agent, reactivity (association/dissociation kinetics) of the complex as well as the affinity between the metal and the biological receptor and the biological uptake flux (Sunda, 2012). For assessment of water quality in freshwaters, the Biotic Ligand Model (BLM) is the only one that directly links trace metals bioavailability to ecotoxicological impact (Ardestani et al., 2014; Balistrieri and Blank, 2008; Di Toro et al., 2001; Rudel et al., 2015) but such model does not yet exists for coastal or transitional (estuarine) waters. Estuarine waters are physically and chemically dynamic areas with high temporal and spatial variability, making TM cycling (and TM bioavailability) more complex to predict in comparison with other aquatic systems (Biati and Karbassi, 2010; Oursel et al., 2013; Waeles et al., 2008).

As mentioned above, not all TM species are bioavailable, but only the free hydrated ion, the species that can release their metal to bind with the biological receptors (reactive/labile metal inorganic/organic complexes) and those that can cross the biological membrane (e.g. lipophilic metal complexes) (Gao et al., 2019). Several techniques can be used to determine TM speciation and assess the bioavailability of TM (Feldmann et al., 2009). Amongst those, electrochemical stripping techniques such as Anodic Stripping Voltammetry (ASV) (Garnier et al., 2004; Gibbon-Walsh et al., 2012; Omanović et al., 2015a; Omanović et al., 1996) or Competitive Ligand Exchange Adsorptive Cathodic Stripping Voltammetry (CLE-AdCSV) (Buck et al., 2012; Pižeta et al., 2015) are widely used to measure the concentrations of reactive (electrochemically labile) and free metal ion, respectively, as well getting insights into the presence and strength of complexes/ligands. *In-situ* passive sampling techniques such as Diffusive Gradients in Thin-films (DGT) are also very popular because of their perceived simplicity, multi-elemental capabilities, and *in-situ* application, i.e. directly in the water column (Amato et al., 2014; Baeyens et al., 2018; Cindrić et al., 2017; Davison and Zhang, 1994; Davison and Zhang, 2012; Degryse et al., 2009; Menegário et al., 2017; Omanović et al., 2015b; Peijnenburg et al., 2014; Shiva et al., 2016; Warnken et al., 2009; Zhang and Davison, 2015). More importantly, concentrations of metals determined by DGT (defined here as DGT-labile) have been found in many cases to closely relate to the concentrations of bioavailable metals (Baeyens et al., 2018; Degryse et al., 2009; Ferreira et al., 2013; Koppel et al., 2019; Philipps et al., 2019; Zhang and Davison, 2015) making the DGT technique one of the most preferred method for monitoring purposes. In addition, the ability to measure time-weighted average (TWA) concentrations overcomes the problem of episodic contamination, which could not be spotted by the classical discrete sampling methodology (Altier et al., 2019; Gao et al., 2019; Unsworth et al., 2006). DGTs can be tuned for the monitoring of specific metals by choosing various suitable solid or liquid binding agents (Table 1 in (Li et al., 2019)). Chelex-100 is by far the most frequently used in the marine environment since it can determine 24 elements, including environmentally important elements such as Pb, Zn, Co, Ni, Cu, Cd, Al, Mn, Fe, Cr and U (Gao et al., 2019; Garmo et al., 2003; Gimpel et al., 2003; Li et al., 2019). In addition, using hydrogels with different pore size (open pore (OP) with size of ~10 nm and restrictive pore (RP) with size of ~1 nm), it is also possible to distinguish labile complexes based on their molecular size and lability (Gao et al., 2019). Although relatively simple to implement experimentally, the metal-uptake flux at this complexing interface is by no means straightforward and can be influenced by many parameters that have been critically discussed in details in several papers (Galceran and Puy, 2015; Mongin et al., 2011; Town et al., 2009; Uribe et al., 2011; van Leeuwen, 2016). Applications and recent progresses of the DGT technique for *in-situ* analysis have been reviewed recently (Li et al., 2019; Menegário et al., 2017).

While the Diffusive Gradients in thin Films (DGT) technique has widely been used for trace metals (TM) speciation in freshwater environments, there are much fewer studies in estuarine and marine waters (Dabrin et al., 2016; Schintu et al., 2008). The aim of this work here was to evaluate the DGT technique as a passive sampling methodology for *in-situ* determination of potentially bioavailable fractions of TM in a highly dynamic estuarine environment. For this purpose, a multimethodological approach was used: time-averaged concentrations of DGT-labile metals were measured in the highly stratified Krka River estuary (Croatia) and compared to the dissolved concentrations, ASV-labile Cu concentrations obtained in representative discrete samples as well as to those calculated by Visual MINTEQ, a speciation modelling software.

2. Working methodology and analytical techniques

2.1. Study site and sampling strategy

This study was performed in the highly stratified Krka River estuary (Croatia, Fig. 1). The main characteristic of this salt-wedge type estuary is the presence of a sharp halocline within a vertical salinity gradient characterized by three separate layers: the upper fresh/brackish water layer (FWL), the middle freshwater-seawater interface layer (FSI) and the bottom seawater layer (SWL). The FSI is a sharp halocline that is usually formed at a depth of between 1.5 and 3.5 m. Its width ranges from only a few centimetres to ~1 m, depending on whether (wind, precipitation) and hydrological conditions (the Krka River flow). As a result of seawater entrainment, the salinity range in the surface layer increases from S = 0 at the head of the estuary to S= 39 towards its mouth. A wider map and additional description of the estuary is provided in supplementary materials (Fig. S1). *In-situ* measurements were made at two sites within the Šibenik Bay (Fig. 1): M1, located in front of the marine station Martinska, and M2 in the nautical marina D-Marin. M1 is representative of the major part of the estuary which is relatively “clean”; it was sampled during 7 campaigns covering 4 summer (2009, 2010, 2011 and 2017) and 3 winter periods (2010, 2012 and 2017). M2 is representative of the most “polluted” part of the estuary, according to previously measured TM concentrations (Cindrić et al., 2015). M2 was sampled only in summer 2009.

For each campaign, DGT devices were deployed *in-situ* at 6 depths for 5 days. Sampling depth were primarily based on the position of the halocline, as determined 2 days before the sampling/DGT deployment. A 5-day DGT deployment time was chosen as a compromise between low TM concentrations, capability of everyday discrete sampling and potential biofouling of the DGT gel. In addition, in samples of high salinity, prolonged deployment might be detrimental to the DGT process due to high levels of competing cations (Ca and Mg) (Dahlqvist et al., 2002; Jimenez-Piedrahita et al., 2017; Tankere-Muller et al., 2012).

Discrete samples (1 L) were taken by a scuba-diver every day, once (in winter) or twice (in summer) at each DGT depth (6 depths). One composite sample for each DGT depth was assembled from equal aliquots of each of these individual samples collected at the corresponding depth during the deployment period. Aliquots for composite samples were taken as soon as possible after collection. The composite samples were kept in the fridge during the deployment period. Both, acidified composite samples for dissolved trace metal analyses, and non-acidified (kept at natural pH) for Cu speciation measurements were prepared. The main physico-chemical parameters (temperature, salinity, oxygen, pH) were measured *in-situ* 6 to 8 times per day at M1 site and 2 times at M2 site using the Hydrolab DS5 or YSI EXO2 multiparameter probes.

2.2. DGT deployment

Six DGT holders were made in high-density polyethylene (HDPE) with grooves at both ends of a channel into which the DGT devices could easily slide in before being maintained in that channel by a side plate (Fig. S2). Each holder was attached and secured to a polyethylene rope by a plastic screw to maintain a horizontal position in the water column. The DGT devices were locked in place but were able to freely slide along the holder channel, moving along with the movement of the waves. This design intends to keep to a minimum the diffusion boundary layer (DBL), especially in "stagnant" water, although there is no evidence of its efficiency.

It was critical for our study to be able to maintain the DGT devices at well-defined depths during deployment period and avoid them moving vertically due to the influence of wind, waves and/or tide (~40 cm). For that, a weight was placed at the bottom of each site with a fixed pulley. An air-filled plastic bottle was attached to one end of a rope while the other end was passed through the ring (by a diver) and attached to a buoy. The air-filled plastic bottle always maintained a tension to the rope, keeping the DGT devices within an estimated ±10 cm of the pre-defined depth, irrespective of the tides or wind conditions. Before the deployment, the precleaned DGT holders and rope were conditioned overnight in ambient water.

In the majority of sampling campaigns, two types of Chelex-DGT devices were used: open-pore (OP) and restricted pore (RP). The DGT were purchased from DGT Research company as already assembled probes. Diffusive gels in OP DGTs were of APA2 type, while RP type was for restricted pore DGTs. Both types have a diffusive gel layer thickness of 0.8 mm. The

DGT devices were always deployed in triplicate (3 OP and 3 RP). A temperature/depth data logger was attached onto each holder during the entire deployment recording data each 15 minutes. An average temperature during deployment period was used for correction of diffusion coefficients. In addition, three DGT devices of each type, not actually deployed *in-situ* but being subjected to the same handling procedure, were used for blanks.

2.3. Preparation of samples

For the determination of dissolved metal concentrations and speciation studies (ASV-labile Cu and Cu complexing capacity; CuCC), samples were filtered under nitrogen pressure (~1 bar) through 0.22 µm cellulose-nitrate membrane filters (Sartorius) or using 0.22 µm cellulose-acetate syringe filters (Minisart, Sartorius). Only composite samples, prepared by weighing 40 g of each freshly collected discrete samples, were analysed. Samples for total trace metal analyses were acidified with trace metal grade nitric acid (*TraceSelect* Fluka) to pH < 2 and UV-irradiated (150 W low pressure mercury lamp, Hanau, Germany) directly in the FEP/PFA bottles for at least 24 h in order to decompose natural organic matter (Omanović et al., 2006). Samples for CuCC were stored in the fridge (+4 °C) at natural pH.

For DOC measurement samples were filtered under vacuum using a glass filtration system (Wheaton) with glass filters (Whatman GFF, 25mm, 0.7µm) (Oursel et al., 2013) preserved with addition of sodium azide (20 uL of 1 M NaN_3 in ~20 mL of sample) and stored in glass tubes (Wheaton, equipped with Teflon/silicone septa) at +4 °C.

Biofouling of the DGT outer membrane (filter paper) can be a serious problem (Devillers et al., 2017b). After 5 days in water, no visible biofouling was observed by visual inspection (Fig. S3). DGT devices were removed from the holders, rinsed with milli-Q water and stored wet in plastic zip bags at +4°C until further processing . The retrieval of DGT resin gels was performed in the laboratory under clean laminar-flow conditions. The Chelex-100 resin gel was transferred into a pre-cleaned plastic tube (2 mL, Eppendorf) and eluted in 1.5 mL of 1 M HNO_3 (*TraceSelect*, Fluka) for at least 24 h to ensure complete extraction of the metals from the resin. The 3 DGT devices that were used for blanks were treated in the same way.

2.4. Analytical methods

2.4.1. Voltammetric analysis of dissolved trace metal concentrations

Dissolved TM concentrations in composite samples were determined by differential pulse stripping voltammetry: anodic (DPASV) for Zn, Cd, Pb, and Cu (Omanović et al., 2006) and adsorptive cathodic (DPAdCSV) with 10^{-5} M nioxime as complexing ligand for Ni and Co (Vega and van den Berg, 1997). Measurements were carried out using an Autolab (EcoChemie) potentiostat (μAutolab2 or PGSTAT128N) controlled by GPES 4.9 software in a three-electrode cell (663 VA Stand, Metrohm). TM concentrations were determined by means of standard addition method. A certified "Nearshore seawater reference material for trace metals" – CASS-5 (NRC CNRC) was used for validation of the analysis. All determined metal concentrations were within the certified limits. The precision of analytical measurements was within 10% of measured concentrations (Table S1).

2.4.2. Determination of Cu ASV-labile concentrations

Differential pulse anodic stripping voltammetry (DPASV) was used for the determination of Cu ASV-labile concentrations. Aliquot of the sample (pH = 8.2±0.1 adjusted with 0.01 M borate buffer) was titrated with 15 Cu additions up to 300 or 500 nM of total Cu concentration ($[Cu]_T$), with similar increments in $\log[Cu]_T$ in order to reach linear response with Cu addition (Garnier et al., 2004; Louis et al., 2008). After each Cu addition, the solution was left to equilibrate for ~1 h and two voltammograms were used for the construction of a complexometric titration curve. The sensitivity estimated from the linear part of the titration curve at high added $[Cu]_T$ was used to convert signal intensities to ASV-labile Cu concentrations. For the purpose of this work, only Cu ASV-labile concentration obtained at ambient Cu concentration (without Cu addition) was used (full titration curves are treated for determination of Cu complexation parameters, but these results would be published separately). Deposition potential and deposition time were -0.55 V and 300 s, respectively. A "desorption step" methodology was used to minimize the influence of the organic matter adsorption on the voltammetric signal (Louis et al., 2008).

2.4.3. Carbon analysis

A TOC-VCSH analyser (Shimadzu) was used for determination of DOC and DIC concentrations. Calibration was done with hydrogen-phtalate (Shimadzu) and $NaHCO_3/Na_2CO_3$ standard solutions, respectively, with an accuracy of 0.02 mgC L^{-1} (Louis et al., 2009; Oursel et al., 2013). The analytical validity of the method was confirmed by measuring the certified reference material MISSIPPI-03 (Environment Canada).

2.4.4. Analysis and calculation of DGT-labile metal concentrations

A multi-elemental analysis of DGT-extracts (at 3 times dilution) was performed by high resolution inductively coupled plasma mass spectrometer (HR ICP-MS, Element 2, Thermo, Bremen, Germany). From a total of 1.5 mL acidified DGT extract, 0.7 mL was collected and diluted using 2% HNO_3 (*suprapur*). Indium (In) was used as an internal standard (IS) for analysis. The quantification of metal concentrations was performed using external calibrations. The validity of HR ICP-MS measurements was confirmed by measuring a river water reference material (SLRS-4 and SLRS-5, National Research Council Canada); a good agreement with the certified values (within 10% of certified) was always obtained.

DGT-labile concentrations in water were calculated according to the standard protocol provided by DGT Research Ltd. (Cindrić et al., 2017; Davison and Zhang, 1994); details are provided in Supporting materials. Effective diffusion coefficients (determined in separate controlled experiments; see next section) operational for brackish/seawater conditions were used for all calculations. According to Devillers et al. (2017a) a common elution factor of 0.8 was found to be slightly underestimated and they recommended a value of 0.85 (Devillers et al., 2017a). However, in order to be comparable to other studies, a value of 0.8 was used.

Relatively high blank values for Zn were found in our study, ranging from 8% to 74% of the corresponding dissolved Zn concentration. Thus, the majority of DGT results for Zn were considered as unreliable and only indicative values are presented for cases where the blank was relatively low (<40%) and/or where good repeatability between 3 DGT devices (including blanks) was within 15%. For other metals, DGT blanks were consistent and acceptable. The highest percentages of blanks, for each campaign and all metals, are provided in Table S2. Procedural blanks of used milli-Q and acids for all measured metals were up to ~2%.

2.4.5. Determination of effective diffusion coefficients for metals

Although DGT diffusion coefficients (D_M) for metals were supplied by the manufacturer (DGT Research Ltd), they are experimentally obtained in 0.01 M $NaNO_3$ solution at a pH of ~5 (which is more representative of freshwater) using diffusive cell methodology. To correct for potential differences in D_M in brackish/seawater, due to a different matrix (especially in terms of ionic strength) compared to common D_M (Garmo et al., 2003; Scally et al., 2006), an effective D_{eff} of TM were estimated using the "direct uptake" method for each metal by deploying the DGT devices in spiked solutions of different salinities for a fixed amount of time and constant temperature. Samples with different salinities (S = 0.1, 19 and 38) were prepared with organic-free water from end-members (filtered and UV irradiated). The concentration of each added metal was selected to be ~50-100 times higher than the ambient metal concentration, in order to have well defined metal concentrations needed for calculations (100 nM of Cd, Pb, Co, 500 nM of Cu, Ni and 1000 nM of Zn). The pH of these solutions was adjusted to ~7.5 by addition of borate buffer. Under these conditions, model calculations using Visual MINTEQ 3.0 showed that precipitation of any metal is not likely to occur. Three 7-L PET bottles (canisters) were positioned upside-down above a magnetic stirrer. The bottom of the bottle (facing up) was cut, 5 L of sample were poured in, stirred with a teflon magnetic stirrer and DGT devices were placed inside. The full setup is presented in Fig. S4. Five OP and five RP DGT devices were mounted on previously cleaned plastic holders and deployed in each of the stirred solutions for 6 hours. Before deployment, DGTs were rinsed by immersing them in the washing solution (water with the same salinity as the working sample, unspiked with metals). The temperature was kept stable at 25.0±0.5 °C (air-conditioned room) and was controlled in a separate canister with the same sample volume and under the same stirring conditions. After 6 hours of deployment, the DGTs were taken out, treated and analysed as described in previous sections. The concentrations of metals in all three samples were monitored throughout the experiment (before deployment and after 15 min, 1 h, 2 h, 4 h and 6 h) and were found within 5% of the expected concentrations (recovery was 100 ± 5%). Note that the DBL is also in this way indirectly incorporated into the estimated effective D_{eff}. A more thorough approach to determine the extent of the DBL would have consisted in using DGTs with different diffusion layer thickness (Warnken et al., 2006) but this was beyond the scope of this field study.

3. Results and discussion

3.1. Major physico-chemical parameters during the deployment period

Horizontal and vertical salinity gradients along the Krka River estuary mainly depend on the river flow and weather conditions (primarily the wind). Amongst all DGT-campaigns, only two campaigns (winter 2010 and winter 2017) were conducted in a period of relatively high Krka River flow (Fig. S5). Vertical profiles of average salinity at the M1 site for all sampling events are plotted in Fig. 2, whereas representative examples of the salinity variation during the deployment period for two contrasting seasons (summer 2009 and winter 2012) are presented in Fig. S6. A well-developed halocline was formed for all campaigns at a depth between 1.5 and 3 m. In winter 2012, the depth of the halocline was moving upward over the deployment period and thus the depths of the DGT devices were adapted after the second day (moved upward by 0.5 m). Salinities measured in the surface layer during the period of DGT-deployments showed a “typical” summer/winter pattern, exemplifying contrasting conditions that occur in the estuary, with exception of unusually low surface salinity in the summer 2009, and relatively high salinity in the winter of 2012. In the summer 2009, the vertical salinity profiles obtained at M1 and M2 were similar.

Vertical temperature profiles were in agreement with the salinity, but with contrasting seasonal pattern; in summer, the temperature in FWL was higher than in SWL, while the opposite was observed in winter (Fig. S7). During the DGT deployment period (~5 days), the temperature was stable for all campaigns (±0.5 °C), with exception of winter 2012 when a high temperature variability was recorded (high variability in salinity was also recorded).

Measured pH values (8.2±0.1) were relatively stable for all campaigns. Oxygen saturation was 100-110% through the whole water column in the winter periods, while in the summer oversaturation (130-140%) was measured in the FWL and FSI, indicating increased biological activities (Marcinek et al., 2020), in agreement with the DOC profiles.

The DOC concentrations at the M1 site were in agreement with the results from a previous study covering the same period (Cindrić et al., 2015). DOC concentrations in FWL were lower in the winter (0.5-1.0 mgC L^{-1}) than in the summer (1.0-1.5 mgC L^{-1}) (Fig. 2). The summer of 2009 displayed a similar behaviour to winter presumably due to very calm conditions in the estuary preceding the sampling campaigns (no strong wind). Higher summer concentrations of DOC at the FSI were related to enhanced biological activity. Note that the DOC profile at the M2 site (summer 2009) was practically the same as for the M1 site (differences at each depth were lower than 0.05 mgC L^{-1}).

3.2. "Effective diffusion coefficients" of metals in diffusive gel (D_{eff})

The DGT-technique provides an operationally defined concentration of the DGT-labile metal fraction. It is generally assumed that diffusion coefficients of different inorganic complexes are the same; however some metal-organic complexes have diffusion coefficients much lower than those of inorganic species (Han et al., 2014; Zhang and Davison, 2001). Consequently, DGT-labile concentrations of trace metals measured in natural samples are normalised to inorganic species, and thus can be considered only as operational/conditional values, i.e. technique dependent and matrix dependent. Two types of DGT devices used in our study (OP and RP) were expected to provide slightly different DGT-labile concentrations, with higher values from the OP DGT. However, for the first set of measurements, the concentrations were almost the same. In order to check the validity of these surprising results, whether there were related to non-suited diffusion coefficients or any other experimental/mechanistic problem, it was decided to determine the effective diffusion coefficients (D_{eff}) of inorganic metal species at three different salinities. Any additional influence of the sample composition (e.g. ionic strength, inorganic speciation change) or mechanical/structural imperfections on the production of DGT-devices were implicitly taken into account via D_{eff}.

Experimentally estimated D_{eff} for Zn, Cd, Pb, Cu, Ni and Co are provided in Table 1 and in Fig. S8. Average D_{eff} values of OP DGTs were within 8% of those provided by the manufacturer with the exception of Pb, which was significantly lower (23%). The ratio of D_{eff} at RP and OP (defined here as the R-factor) was also similar to that reported by the manufacturer (R-factor = 0.7) for all metals except for Cu (R-factor = 0.5). Average D_{eff} values at different salinities did not show any systematic trends (increasing or decreasing) in respect to salinity (Fig. S8) for all trace metals (except for Cu) and for both diffusive gel types. This is in-line with the expectation that all small inorganic complexes (e.g. chloride, hydroxide or carbonate complexes) of the same metal have similar diffusion coefficients. However, unexpectedly, a systematic decrease with salinity was observed for Cu. Since this trend cannot be explained and due to the unusually low R-factor, it was decided to use the D_{eff} value provided by the manufacturer (D_{eff} = 6.23 10^{-6} $\text{cm}^2.\text{s}^{-1}$) as well as the R-factor of 0.7. However, this observation is puzzling and more studies should be done to confirm or disprove this unusual behaviour.

In regards to Pb whose D_{eff} value is 23% lower than that provided by the manufacturer, Garmo et al. (2003) reported a value ~20% higher (Garmo et al., 2003), obtained in 0.01 M $NaNO_3$ at pH of ~5-6. As different values of diffusion coefficients have been reported in the literature, it was decided to use the value of Pb obtained in our experiment. To summarise, we used the following effective diffusion coefficients in our calculations: 6.18 (Cd), 6.22 (Pb), 6.23 (Cu), 5.68 (Zn), 5.84 (Ni) and 6.17 cm^2s^{-1} for Co.

One of the important parameters in DGT technique calculations is the DBL, especially in calm waters (such as lakes or seawater) where it can significantly influence the calculated concentrations (Levy et al., 2012; Warnken et al., 2006; Zhang and Davison, 1995). Within our experimental set up (Fig. S4), although the solution is stirred, slightly different mixing hydrodynamic conditions are expected to exist between the DGTs placed at the surface and those placed at the bottom. However, based on 30 separate results (15 OP and 15 RP), no clear differences with the position of the DGT probes were found (Warnken et al., 2006), suggesting that under these experimental conditions, the effect of any variations of the DBL size on the final results was insignificant.

3.3. Vertical distributions of dissolved and DGT-labile trace metals

Typical profiles of dissolved and OP/RP DGT-labile metal concentrations of Cu are presented in Fig. 3. Vertical profiles of other metals are presented in Figs. S9 to Fig. S14. Except for two winter campaigns (2010 and 2017), higher concentrations of Cu were measured in the FWL and FSI layers than in the SWL. Increased Cu concentrations in the surface layer measured in the summer were attributed to higher nautical traffic within the bay, with Cu being released from antifouling paints used as a biocide on boats (Cindrić et al., 2015), similar to previous reports in other studies (Dunn et al., 2007). Although the Cu concentration in the FWL varied by a factor of 5 (ranging from ~4 nM in February 2010 to almost 20 nM in July 2017), only a small variability was observed in the SWL (within ~40%) indicating a minor vertical transport of Cu, even in winter conditions.

The same vertical trends of dissolved and DGT-labile Cu concentrations were obtained for all campaigns suggesting that employed experimental procedure of collecting samples at regular intervals during the entire deployment period was successful in adequately representing the average dissolved metal concentrations (Shiva et al., 2016). The differences between concentrations of DGT-labile Cu, measured with OP DGT and with RP DGT, were within the range of measurement uncertainty. Dissolved concentrations of Cu from composite samples were much higher than DGT-labile, pointing to the existence of a large Cu fraction (most probably organic), that is not accessible by DGT. This is consistent with other studies made in estuarine conditions that have shown that DGT labile Cu concentrations could be over an order of magnitude lower than those of dissolved Cu (Dunn et al., 2007; Gaabass et al., 2009; Lucas et al., 2015).

Very comparable vertical profiles between dissolved and DGT-labile concentrations were also found for all other trace metals, except for Zn at M1. In this latter case (Fig. S9), contamination (as seen by the relatively high DGT-blanks) prevent any reliable comparison between dissolved and DGT-labile concentration. This is not the case at site M2 due to the higher Zn levels in the water column.

The vertical profiles of Zn, Cd, Pb, Ni and Co at M1 and M2 sites are described in the Supporting document (Fig S9 to S14). As expected, dissolved concentrations were higher than

DGT-labile ones (except for Zn) but the ratio DGT-labile/dissolved varied significantly for different metals with an average ratio of 0.92 ± 0.03, 0.64 ± 0.02, 0.23 ± 0.05, 0.61 ± 0.03 and 0.57 ± 0.06 for Cd, Pb, Cu, Ni and Co, respectively. Lower DGT-labile concentrations over dissolved ones can be due to various reasons such as incomplete exchange of strong metal complexes at the DGT complexing gel, presence of DGT-labile organic complexes that have a low diffusion coefficient or presence of colloidal material with low diffusion coefficients and/or a size that is > ~10 nm (Liu et al., 2013; Shiva et al., 2016). These metal species exist as non-dynamic forms (i.e. non-labile and/or non-mobile) that cannot be measured by DGT (Galceran and Puy, 2015; Puy et al., 2014; Warnken et al., 2008).

3.4. Variability of DGT-labile fraction

The vertical profiles of percentages of OP DGT-labile fractions of measured metals for both sites and all sampling campaigns are presented in Fig 4, along with average values for each layer at M1 site. The vertical profiles for Cd were similar at all times, showing a high percentage (~80-100%) of DGT-labile fraction. This in agreement with the relatively high affinity of Cd to form inorganic complexes (mostly chlorides) rather than organic complexes (Baeyens et al., 2018; Sierra et al., 2017), even though a slightly higher percentage of DGT-labile fraction was noted in the FWL in comparison to the SWL. For Pb, the DGT-labile fraction was lower and variable (~50-80%) without any apparent trend with depth.

Vertical profiles for Cu, Ni and Co each show characteristic trends. While higher DGT-labile Cu percentages are generally found in the FWL than in the SWL, similar to dissolved concentrations, percentages are relatively low along the all water column, in agreement with strong organic complexation (Warnken et al., 2009). Higher levels of DOC in the FWL in summer were not sufficient to fully buffer the increase of dissolved Cu concentrations (due to nautical traffic), allowing higher percentage of inorganic and weak Cu organic complexes to be accumulated by the DGT in the surface layer. The increase of Cu-labile fraction with dissolved Cu was obvious for the "polluted" site (M2) where DGT-fraction increased to ~50% (Fig. S14). This was also confirmed by voltammetric measurements (see section 3.7), in agreement with previous studies (Louis et al., 2009). For Ni and Co profiles, a small increasing trend with depth was observed. Nickel followed the trend of the corresponding dissolved concentrations, being generally lower in surface than in the seawater layer, following basically the conservative dilution line (see Fig. 2 and Fig. S12) (Cindrić et al., 2015). This was however not the case for Co, for which dissolved concentrations were generally higher in the surface layer than in the bottom seawater layer (Fig. S13). Co percentages varied significantly amongst various campaigns and no consistent trend was found (Fig. 4). For Zn, due to the high Zn blank levels, no reliable data was obtained.

The DGT-labile fraction is expected to be inversely correlated with the portion of metal bound to strong organic ligands, i.e. a lower percentage of DGT-labile fraction is expected for metals forming strong organic complexes. Based on such assumption, the following order of binding strength/capacity was obtained: Cu > Co ≈ Ni ≈ Pb > Cd. This order is fully "operational" and reflects the overall chemical speciation and behaviour of these TM in the analysed water environments during the deployment period - this is considered to be "site specific". A low Cu and high Cd average percentage of DGT-labile fractions were expected due to their known chemical speciation: the strong complexation of Cu with organic ligands, and the predominance of inorganic Cd to complex with chloride (Cindrić et al., 2015; Louis et al., 2009).

The obtained percentage of DGT-labile Cu fraction is consistent with results of other authors, for which value around 25% was reported (Ferreira et al., 2013; Shiva et al., 2016). However, for Cd, Pb, Ni and Co the DGT-labile fraction was much higher than those obtained by Shiva et al. (2016) where DGT-labile fractions were less than 50% of the total dissolved metal concentrations (Shiva et al., 2016). If inorganic and organic ligands were in high excess and homogeneous, metal speciation and the percentage of DGT-labile metals would be expected to remain constant, irrespective of the dissolved concentrations. This was however not the case: for Cu, Pb and Ni, an increase in the percentage of DGT-labile metals was observed with higher dissolved concentrations while an opposite trend was obtained for Co and to a lesser extent Cd (Fig 5). The DGT-labile fraction of Cd slightly decreased with an increase in dissolved concentration, suggesting a more pronounced complexation of Cd with organic ligands in SWL than in FWL. Similarly, results suggest that the complexation of Co was stronger in FWL than in SWL layer. For Pb and Ni, no clear conclusions could be drawn.

Several studies have shown that the accumulation at DGT is complex and depends on many parameters, including the lability of complexes (Altier et al., 2018; Mongin et al., 2011; Uribe et al., 2011) and/or non-linear accumulation of some elements due to the equilibrium or competitive effects at longer deployment times (Jimenez-Piedrahita et al., 2017). Several analytical equations have been derived to calculate the DGT-labile bulk concentration (Jimenez-Piedrahita et al., 2017) but the list of issues that can affect the accumulation is large and a unique analytical solution does not exist (Galceran and Puy, 2015). Even accounting for known and easy solving issues would not allow easy comparison of DGT results from different studies.

3.5. Comparison of open pore (OP) and restricted pore (RP) DGT-labile concentrations

The metal species measured by DGT depend on their molecular size, stability/lability and diffusive coefficients assuming that they not interact with the diffusive gel. Two types of DGT probes, with "open" (OP) and "restricted" (RP) pores were used to discriminate between two main classes of the metal complexes based on the pore size of the diffusive DGT gel: the RP is supposed to allow measurement of only free ions and small inorganic complexes, while the OP also allows diffusion of many metal-organic complexes (Baeyens et al., 2011; Scally et al., 2006; Shiva et al., 2016; Shiva et al., 2015; Twiss and Moffett, 2002; Zhang and Davison, 1999; Zhang and Davison, 2000). However, although not initially thought, it was found that permeation of colloidal particles as large as 130 nm is observed in both types of gels (van der Veeken et al., 2008), implying that part of the accumulated metals could arise from colloids as well. In this study, the difference between DGT-labile concentrations determined by OP and RP probes was negligible, closely following each other at all depth and at all sampling campaigns (Fig. 3) suggesting that any difference in uptake of colloids is probably also negligible. The comparison between average DGT-labile fractions determined by both types of gels is presented in Fig. 6.

The percentages of RP DGT-labile metal fractions were very close to that obtained by OP DGT, with RP/OP ratios around 1 (numbers given above the bars in Fig. 6). A lower ratio was obtained only for Pb (0.88±0.44), but with relatively high uncertainty. Relatively high ratio between these two DGT types has also been reported previously (Osterlund et al., 2012; Shiva et al., 2016). The observed similarities in DGT-labile fractions could be related to the mechanism of accumulation, which depends not only on diffusion of metal complexes, but also on their lability (kinetics of complexes dissociation) and molecular size (Mongin et al., 2011; Osterlund et al., 2012; Uribe et al., 2011). The negligible difference obtained here between OP and RP suggests that DGT-labile complexes are small molecules that diffuse freely through both the RP and OP diffusive gels (Forsberg et al., 2006; Osterlund et al., 2012). The differences among the heterogeneous metal-organic ligand complexes existing at ambient conditions are indistinguishable by the two different DGT types applied here. Although results obtained indicate that it is unnecessary to use both types of devices (Liu et al., 2013; Osterlund et al., 2012), other studies have shown larger differences between these two DGT types for various metals (Baeyens et al., 2011; Shiva et al., 2016; Zhang and Davison, 2000).

3.6. Heterogeneity of the binding ligands

The determination of metal speciation in aquatic systems is a very demanding task due to the presence of natural ligands (e.g. humic substances (HS)), with a high heterogeneity, i.e. heterogeneous distribution of binding sites and affinities for metals as a function of the metal-to-ligand ratio (Town et al., 2009). Binding of metals by HS, which is common in estuarine and coastal waters (Sander et al., 2015; Whitby and van den Berg, 2015), can be described by a distribution characterised by the average conditional stability constant (K), as a function of the fraction of complexing sites bound to metals. In order to apprehend the variations in binding sites affinities toward metals, a heterogeneity parameter Γ was introduced (Town et al., 2009; Warnken et al., 2009).

The value of Γ can be obtained from the slope of metal binding affinity versus the faction of occupied sites, i.e. by plotting DGT-labile concentrations versus dissolved metal concentrations normalized to DOC (Fig. 7). Literature values of Γ for metal binding by isolated HS are 0.3-0.5, 0.6-0.8, 0.8-1.0 and 0.6 for Cu, Pb, Cd and Ni, respectively (Town et al., 2009). Low Γ values represent more heterogeneous sites, while $\Gamma = 1$ assumes a fully homogeneous binding site distribution. Heterogeneity factors calculated from this study are 0.60, 0.91 and 0.94 for Cu, Pb and Cd, respectively.

No reliable value for Ni and Co (considerably above 1) could be found due to the narrow range of measured concentrations. The same problem also applied for Cd for which the value of 0.94 was obtained when using the 3 campaigns with the highest range of measured Cd concentrations. The calculated heterogeneity factor reflects not only the distribution of stability constants but also the dissociation rate constants of metal complexes, which was significant here due to the relatively large diffusion layer thickness and consequently longer “kinetic timescale window”. In a study published by Warnken et al. (2009), a slight evidence of heterogeneous binding of Pb and Cu was found, while results for Cd, and Zn were consistent with weak binding and complete lability (Warnken et al., 2009).

3.7. Comparison of DGT-labile and modelled dynamic metal concentration

As discussed above, DGT is a dynamic speciation technique since the uptake (accumulation) of trace metals is dependent on the dissociation kinetics of metal complexes within the diffusion layer/gel (Davison and Zhang, 2012; Han et al., 2014; Scally et al., 2003; van Leeuwen et al., 2005). Thus, the thermodynamic consideration of the speciation calculations does not necessarily reflect the real processes at DGT/water interface which can be partially or fully under kinetic control (Scally et al., 2003). Accordingly, the DGT technique is considered operational because it depends on the actual physico-chemical conditions. DGT-labile concentrations are very often compared to those provided by some modelling tools (Cindrić et al., 2017; Han et al., 2014; Meylan et al., 2004; Omanović et al., 2015b; Unsworth et al., 2006; Warnken et al., 2008; Zhang, 2004). While in most cases a very good correlation between measured and predicted labile concentrations was found (matching of trends), agreement in absolute values was very variable. In this study, a comparison approach similar to previous studies (Balistrieri and Blank, 2008; Han et al., 2014) was carried out. For the calculation of predicted (dynamic) DGT-labile concentration of metals, the following equation was used:

$$C_{\mathrm{dyn}} = C_{\mathrm{inorg}} + \frac{C_{\mathrm{FA}} D_{\mathrm{FA}}}{D_{\mathrm{eff}}} + \frac{C_{\mathrm{HA}} D_{\mathrm{HA}}}{D_{\mathrm{eff}}} \qquad \text{(eq. 1)}$$

where C_{FA}, C_{HA} and D_{FA} , D_{HA} are concentrations of metal complexes with fulvic (FA) or humic (HA) acid and corresponding diffusion coefficients, respectively, while C_{inorg} and D_{eff} represent the concentration of inorganic complexes and effective diffusion coefficients, respectively. It should be noted that diffusion coefficients for metal complexes with FA and HA are assumed to be the same as for FA and HA alone (without the metal) and are taken from the literature (Zhang, 2004). For modelling of chemical speciation, a free program Visual MINTEQ ver. 3.0 was used (Gustafsson, 2011). The major anion and cation concentrations were calculated according to the "Dittmar's law". For each sample, a corresponding DOC concentration and dissolved metal concentrations were used. Modelling of interactions of metals with organic ligands was performed using the Stockholm Humic Model (SHM). Different ratio of FA and HA (e.g. FA from 50% to 100%) could be assumed to represent natural organic matter in marine and freshwater systems (Allan et al., 2008; Gledhill and Gerringa, 2017; Han et al., 2014; Unsworth et al., 2006). As the FA/HA ratio is an unknow parameter, it was assumed that 90% of organic matter is represented by FA, and 10% by HA. No other adaptation of default parameters was undertaken.

Fig. 8 presents the comparisons between measured and predicted (eq. 1) DGT-labile concentrations for all metals along with the obtained correlation coefficients and the slopes of the linear regression. The best agreement, both relative (correlation) and absolute (slope around 1), was obtained for Cd as a result of the relatively weak complexation of Cd with organic matter, i.e. Cd is primarily present as inorganic complexes with chloride.

While a high correlation factors for Pb and Cu were obtained, the predicted concentrations were significantly lower than the DGT-measured (slope < 1). If only inorganic fraction of Pb and Cu is considered as dynamic one (crosses in Fig. 8), the results are even lower. Based on this modelling approach, it is evident that part of the organic complexes is accumulated by the DGT, as previously suggested (Scally et al., 2003; Scally et al., 2006; Shiva et al., 2016; Twiss and Moffett, 2002). However, the major question is about the source of disagreement between DGT-measured and predicted concentrations. Foremost, there is no evidence that DOC can be represented only by 90% as FA and 10% as HA, i.e. all other partitioning could also be possible, especially in an estuarine zone, where other types of organic matter issued from estuarine aquatic organisms are present and most probably differ in complexation characteristics from classical terrestrial humic substances.

Equation 1 applied in this study assumes that all metal complexes with FA and HA are labile and accessible to DGT. The only difference compared to inorganic complexes was the applied diffusion coefficient (values taken from Zhang (2004) are 1.15×10^{-6} cm^2 s^{-1} for FA and 0.6×10^{-6} cm^2 s^{-1} for HA metal complexes). However, Balch and Guéguen (2015) showed that diffusion coefficients for “bulk” dissolved organic matter and HS are 2-3 times higher than values used for calculations in this study (Balch and Guéguen, 2015). However, application of higher diffusion coefficients (by factor 3) led to an overestimation of the predicted metal fractions for Cu and Pb.

It should be pointed out that very high discrepancy between different modelling programs could be obtained for predicted Cu and Pb dynamic concentrations, in contrast to Cd, Co, Ni and Zn for which good agreement between DGT-labile and dynamic concentrations is common, most probably because organic complexation for these 4 metals is much weaker (Baeyens et al., 2018; Sierra et al., 2017).

For both Ni and Co, the predicted dynamic metal concentrations were higher than those measured by the DGT, but the data was highly correlated only for Ni. Significantly higher predicted Ni concentrations point to the underestimation of organic complexation of Ni with HA and FA by the model used. Similar behaviour was found by Warken et al. (2009) which concluded that differences between WHAM predictions and the measured Ni-DGT indicated that WHAM used with the default binding parameters underestimates Ni binding to natural organic matter (Warnken et al., 2009). A better agreement between DGT-measured and predicted dynamic metal concentrations were found in studies performed in rivers (Han et al., 2014; Meylan et al., 2004; Omanović et al., 2015b; Unsworth et al., 2006; Warnken et al., 2008). This was rather expected, because the modelling of metal-organic ligand interactions incorporated in programs were developed on the basis of data collected primarily in studies performed in freshwater environments. Thus, the prediction of metal speciation in estuarine or seawater system is still a challenging task, demanding extensive and more focused studies. For example, separation and accumulation of representative organic matter from estuarine and seawater environment would be needed to get a reliable set of acidity, complexation and kinetic constants. Along with the DGT experiments in such model solutions an assessment of the potential contribution of metal-organic complexes to DGT-labile concentration, i.e. to potential bioavailable fraction would be achievable.

3.8. Comparison of ASV-labile and DGT-labile Cu

The purpose of complexation studies is to determine the chemical speciation of metals, and to estimate the free/labile metal concentration, the forms considered to be the most bioavailable, and in the case of Cu, the most toxic. DGT is easy to handle for monitoring purposes and it provides TWA concentration of these labile forms. Another technique that also provides labile metal concentration (inorganic and weakly bound organic fraction) is voltammetry, particularly, anodic stripping voltammetry (ASV). The accumulation mechanism by ASV is similar to that of DGT, but with a different "kinetic window" due to the much smaller diffusion layer (Town et al., 2009; van Leeuwen et al., 2005). It is thus expected that both DGT and ASV can provide similar information regarding the distribution of labile metal species in a particular water environment. The concentrations measured by these techniques are fully operational. A comparison between ASV-labile and OP DGT-labile Cu concentrations is plotted in Fig. 9. A linear relationship was found between the two operationally labile concentrations with a high correlation coefficient of 0.965. Most of the values are located under the 1:1 line, i.e. the determined ASV-labile concentrations were lower compared to those of DGT, accounting in average for around 66% of those of DGT-labile. The lower labile fraction estimated by ASV was expected due to the shorter kinetic window (time-scale).

Indeed, the effective time of the measurement for the ASV is ~0.1 s, with a diffusion layer thickness of ~10 µm, and a diffusion coefficient of 5×10^{-6} cm^2 s^{-1}, whereas in the DGT device the diffusion layer thickness is ~0.9 mm and the effective measurement time is ~13.5 minutes (Zhang and Davison, 2000). Both techniques are sensitive to inorganic Cu species, but also to kinetically labile weak Cu organic complexes, although some labile species for the DGT might be inert to ASV which is having a stronger discrimination towards accumulation of organic complexes (Ferreira et al., 2008; Mongin et al., 2011; Omanović et al., 1996; Scally et al., 2003; Scally et al., 2006; Uribe et al., 2011). This is consistent with complexation titration experiments for which curvature relationship of ASV-labile with the total Cu is usually obtained if compared to DGT-labile titration curve for which curvature trend was not observed (Apte et al., 2005; Louis et al., 2008).

Both DGT and ASV labile concentrations are the sum of concentrations weighted by the ratios of each complex diffusion coefficient with respect to the free metal ion diffusion coefficient (Galceran and Puy, 2015). A study performed by Twiss and Moffett (2002) showed that at least 10-35% of the organically complexed Cu derived by CLE-ACSV was DGT-labile (Twiss and Moffett, 2002), which is in line with our measurements. Due to the linear relationship between DGT and ASV labile concentrations, both techniques can thus be used to measure the potentially bioavailable metal fraction in estuarine environment.

4. Conclusions

We present here a thorough application of *in-situ* diffusive gradients in thin films (DGT) technique for monitoring Co, Cu, Cd, Pb and Ni in estuarine waters covering the full salinity range. There are several interesting points: (1) DGTs results are highly reproducible; (2) same concentrations were obtained from both open pore (OP) and restricted pore (RP) devices, suggesting that for all those metals, the species detected are small labile species; (3) the following DGT-labile fraction were obtained (AVG±SD): 92±3%, 64±2%, 23±5%, 61±3% and 57±6% of Cd, Pb, Cu, Ni and Co, respectively; (4) ASV-labile Cu concentrations obtained from composite samples are highly correlated to DGT ones but ~35% lower, in general agreement with their measuring kinetic window; (5) chemical speciation modelling (Visual MINTEQ) predicted the "dynamic" (labile) concentration of Cd quite well, probably because of its weak complexation with organic matter. For Cu and Pb, concentrations were underestimated, despite that partial accumulation of organic complexes with fulvic acid and humic acid was accounted for in modelled dynamic concentrations, suggesting the need of upgrading the prediction models which could fit for a range of the natural water compositions.

These results show that DGT-technique could be successfully applied for the determination of DGT-labile concentrations of TM in estuarine waters, reliably reflecting their distribution over the deployment period. Furthermore, this study, as well as the others, showed that DGT is a promising technique that could be used as an advancing method for measurement of TM bioavailability in fresh as well as in saline waters. Based on the recommended determination of bioavailable metal concentrations as the water quality criteria (WQC) anticipated by the EU WFD, DGT technique could be a complementary method to the biotic ligand model (BLM), which is currently under evaluation by US-EPA as WQC for Cu in estuarine/marine environment (US-EPA, 2016).

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Table 1. Concentrations of metals used in experiment, average percentage of OP DGT-blank (non-exposed probes), determined average (all salinities) effective diffusion coefficients (D_{eff} / cm^{2} s^{-1}), “official” diffusion coefficient recommended by DGT-Research Company ($D_{\mathrm{DGT\text{-}Research}}$), difference between the two values and average factor of the diffusion coefficient conversion for restricted pore (RP) gels (*R*-factor).

	Cd	Pb	Cu	Zn	Ni	Co
conc. (nM)	100	100	500	1000	500	100
% blank	0.1	0.3	2.2	34	0.1	0.4
$^{a}D_{\mathrm{eff}} \times 10^{6}$	6.18	6.22	5.77	5.68	5.84	6.17
$^{a}D_{\mathrm{DGT\text{-}Res}} \times 10^{6}$	6.09	8.03	6.23	6.08	5.77	5.94
% difference	1.4	-23	-7.4	-6.5	1.1	3.8
AVG *R*-factor	0.73±0.04	0.75±0.05	0.5±0.05	0.82±0.32	0.71±0.05	0.71±0.04

[a] – diffusion coefficients @ T = 25 °C

Figure Captions

Figure 1. The Krka River estuary with marked sampling sites.

Figure 2. Vertical profiles of salinity and dissolved organic carbon (DOC) at M1 site for all DGT-campaigns.

Figure 3. Vertical profiles of dissolved (Diss), open pore (OP) and restricted pore (RP) DGT-labile concentrations of Cu for 7 sampling campaigns at M1 site.

Figure 4. Vertical profiles of OP DGT-labile metal fractions (in percentage) for all campaigns and average percentage of OP DGT-labile metals for each separate layer (FWL, FSI and SWL) along the salinity gradient (average data from all campaigns). Number above bars represents the overall average percentage (all depths). Uncertainty is expressed as one standard deviation.

Figure 5. Dependence of percentage of open pore (OP) DGT-labile metals on dissolved metal concentrations at M1 site. Red dotted regression line indicates the observed trend.

Figure 6. An overall average percentages of open pore (OP) and restricted pore (RP) DGT-labile metals (all depths, all campaigns). Numbers above bars indicate ratio between RP and OP DGT-labile concentrations. Uncertainty is expressed as one standard deviation.

Figure 7. DGT-labile concentrations in relation to the dissolved metal concentration normalized by DOC (metal-to-ligand ratio).

Figure 8. Comparison between measured DGT-labile and predicted dynamic metal concentrations.

Figure 9. Relationship between ASV-labile and OP DGT-labile Cu for all campaigns.

Figure 1

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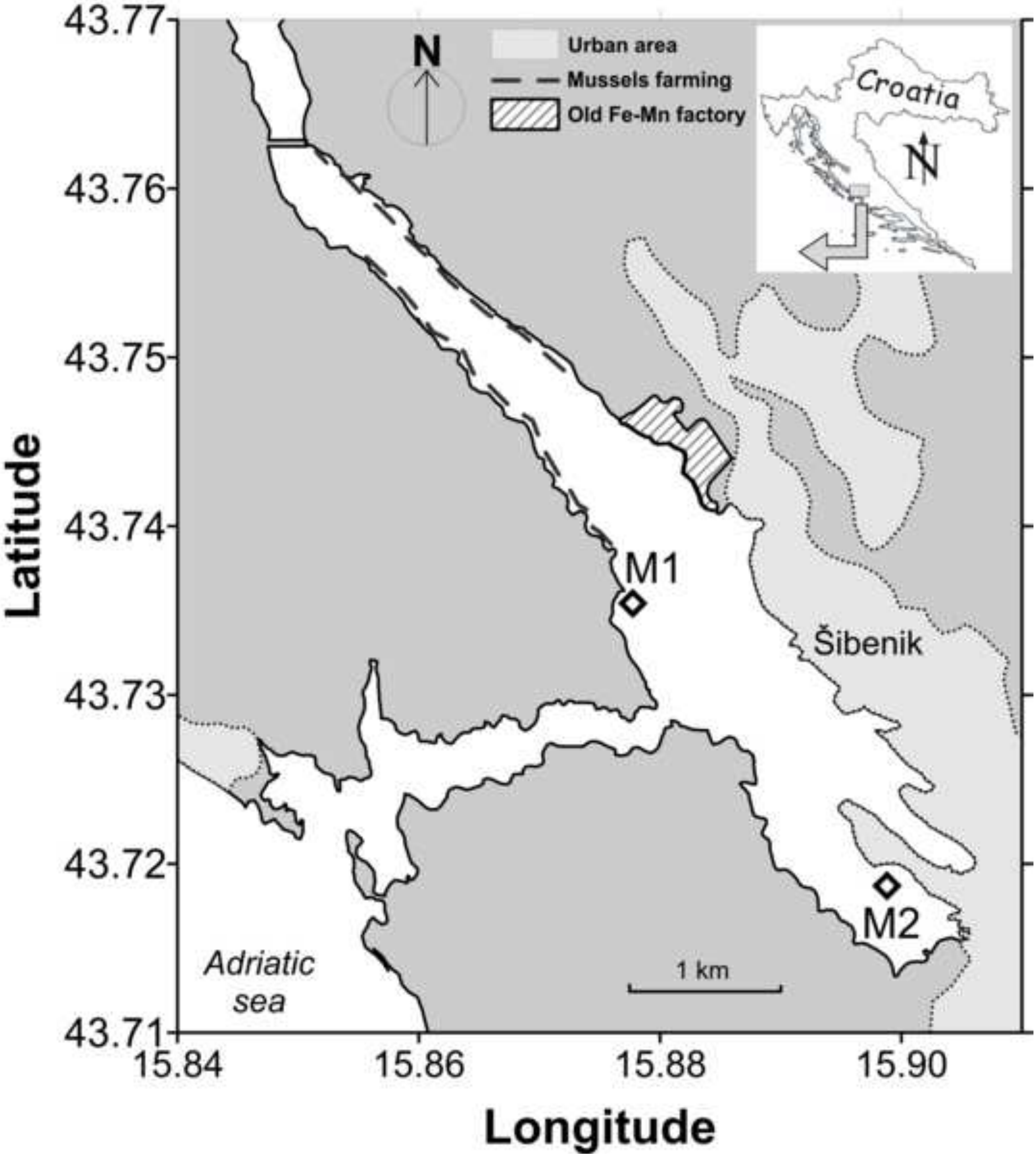


Figure 2

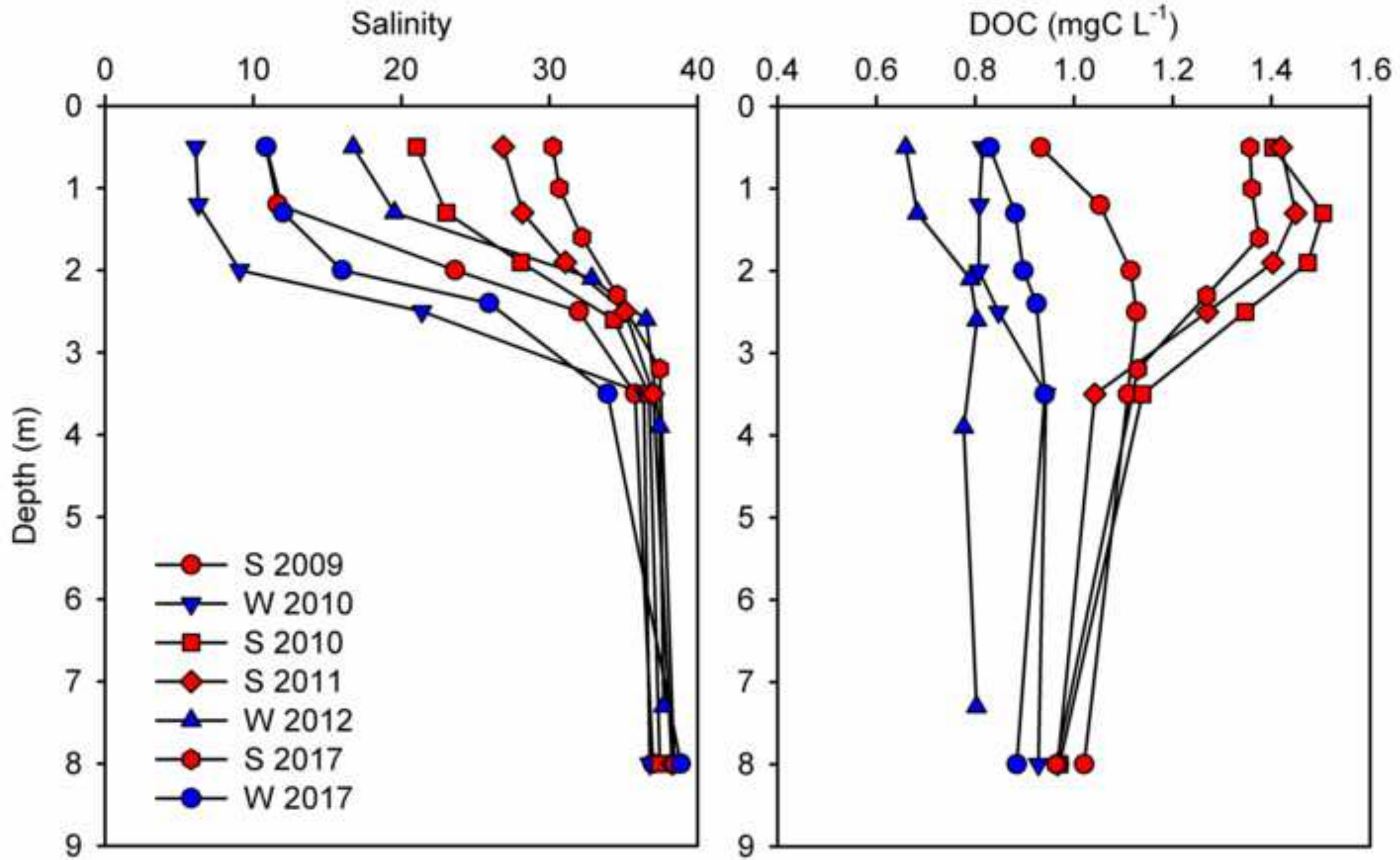

Salinity
0
10
20
30
40
DOC (mgC L^{-1})
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Depth (m)
0
1
2
3
4
5
6
7
8
9
S 2009
W 2010
S 2010
S 2011
W 2012
S 2017
W 2017

Figure 3

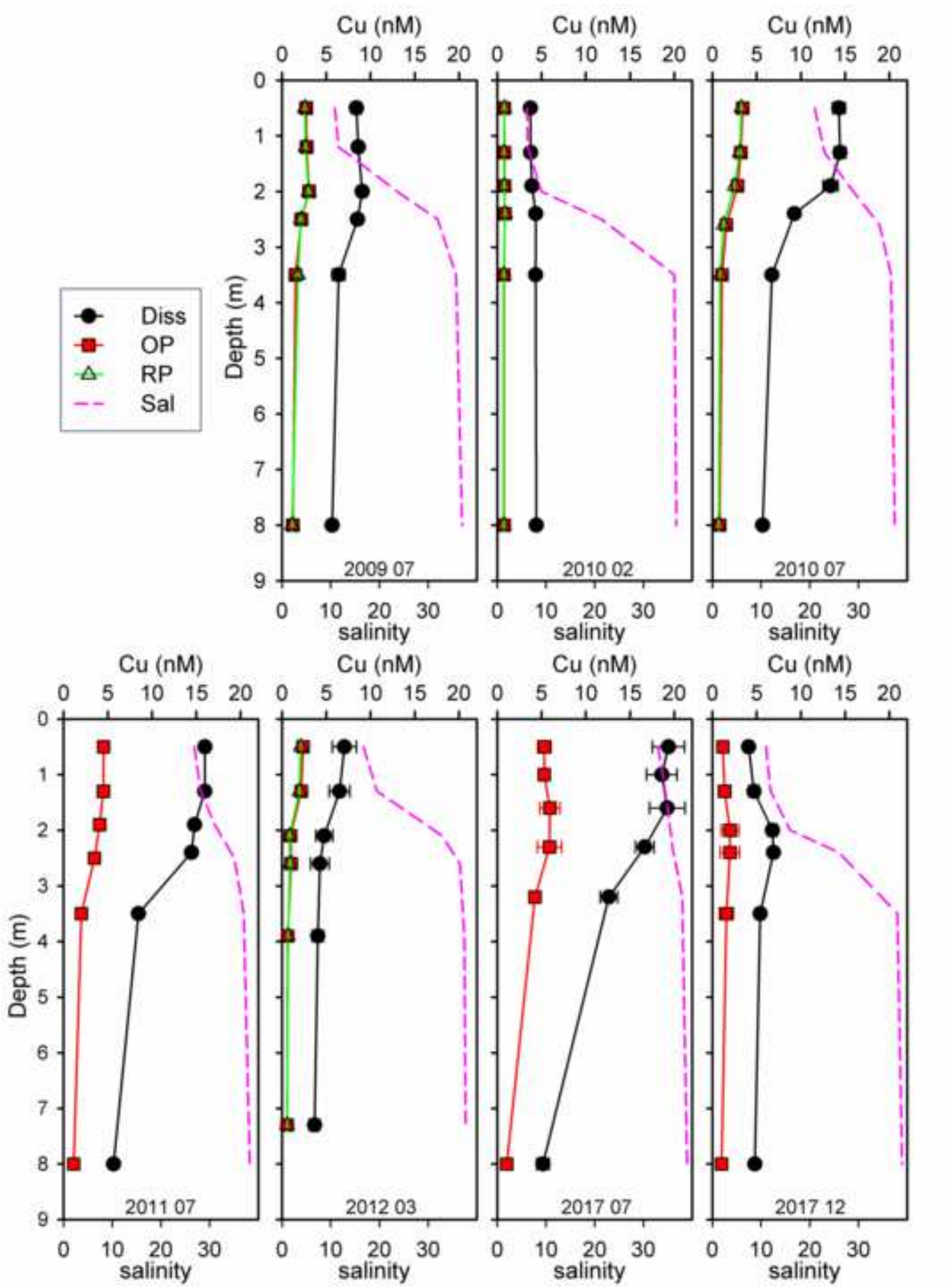

Diss
OP
RP
Sal
Cu (nM)
0
5
10
15
20
Depth (m)
0
1
2
3
4
5
6
7
8
9
salinity
0
10
20
30
2009 07
2010 02
2010 07
2011 07
2012 03
2017 07
2017 12

Figure 4

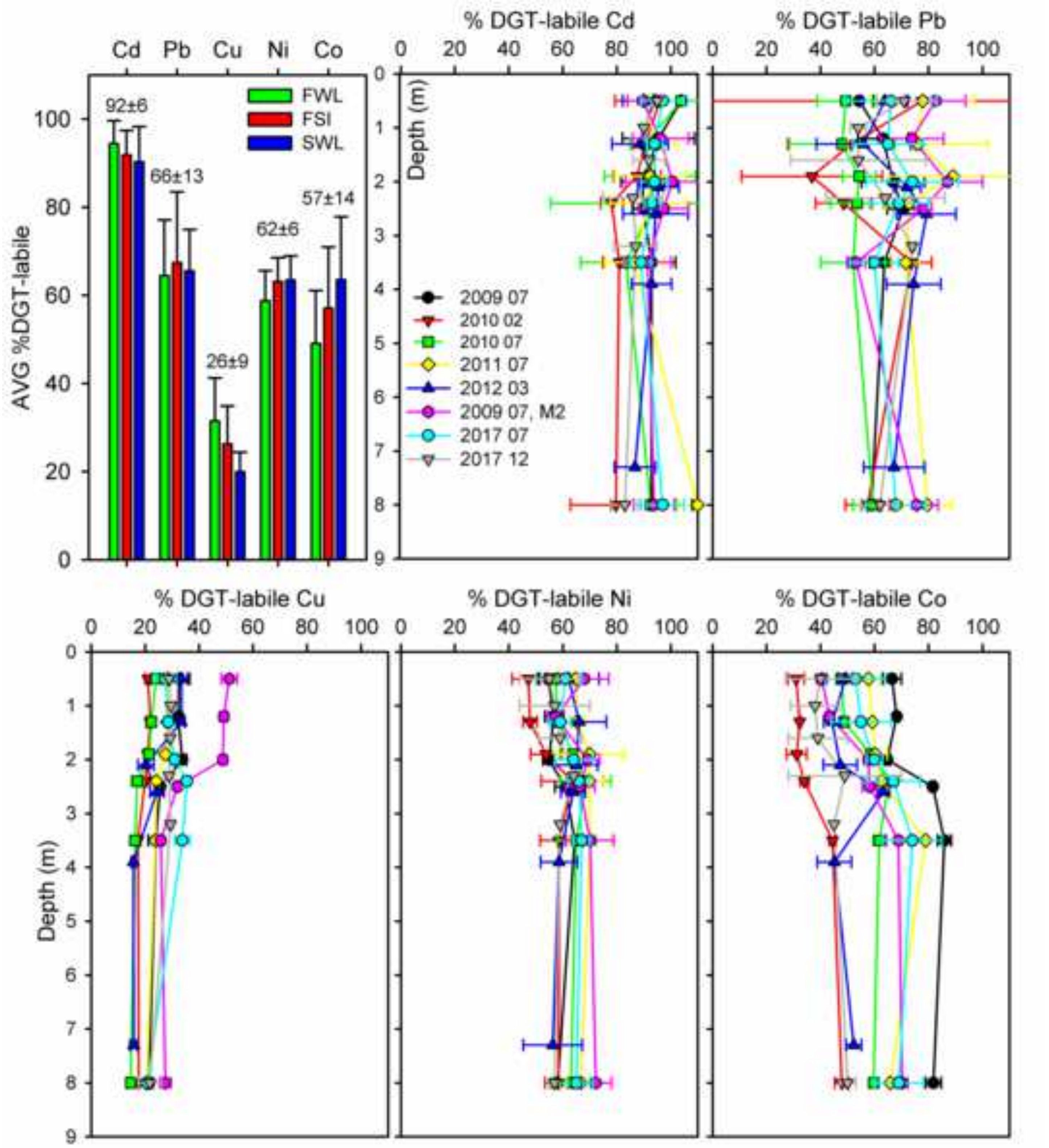

Cd
Pb
Cu
Ni
Co
FWL
FSI
SWL
92±6
66±13
26±9
62±6
57±14
AVG %DGT-labile
0
20
40
60
80
100
% DGT-labile Cd
% DGT-labile Pb
% DGT-labile Cu
% DGT-labile Ni
% DGT-labile Co
Depth (m)
0
1
2
3
4
5
6
7
8
9
2009 07
2010 02
2010 07
2011 07
2012 03
2009 07, M2
2017 07
2017 12

Figure 5

Click here to download high resolution image

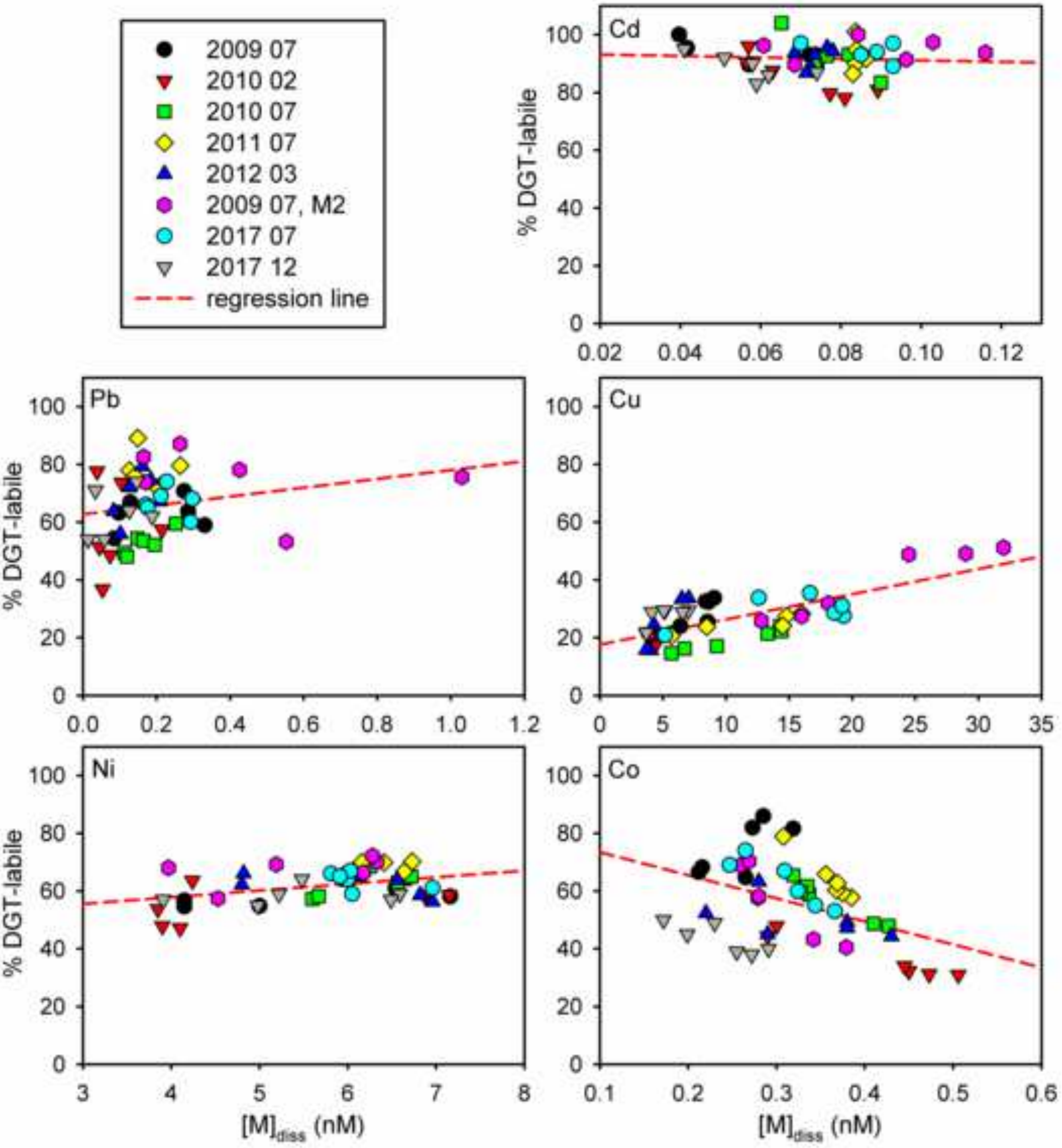


Figure 6

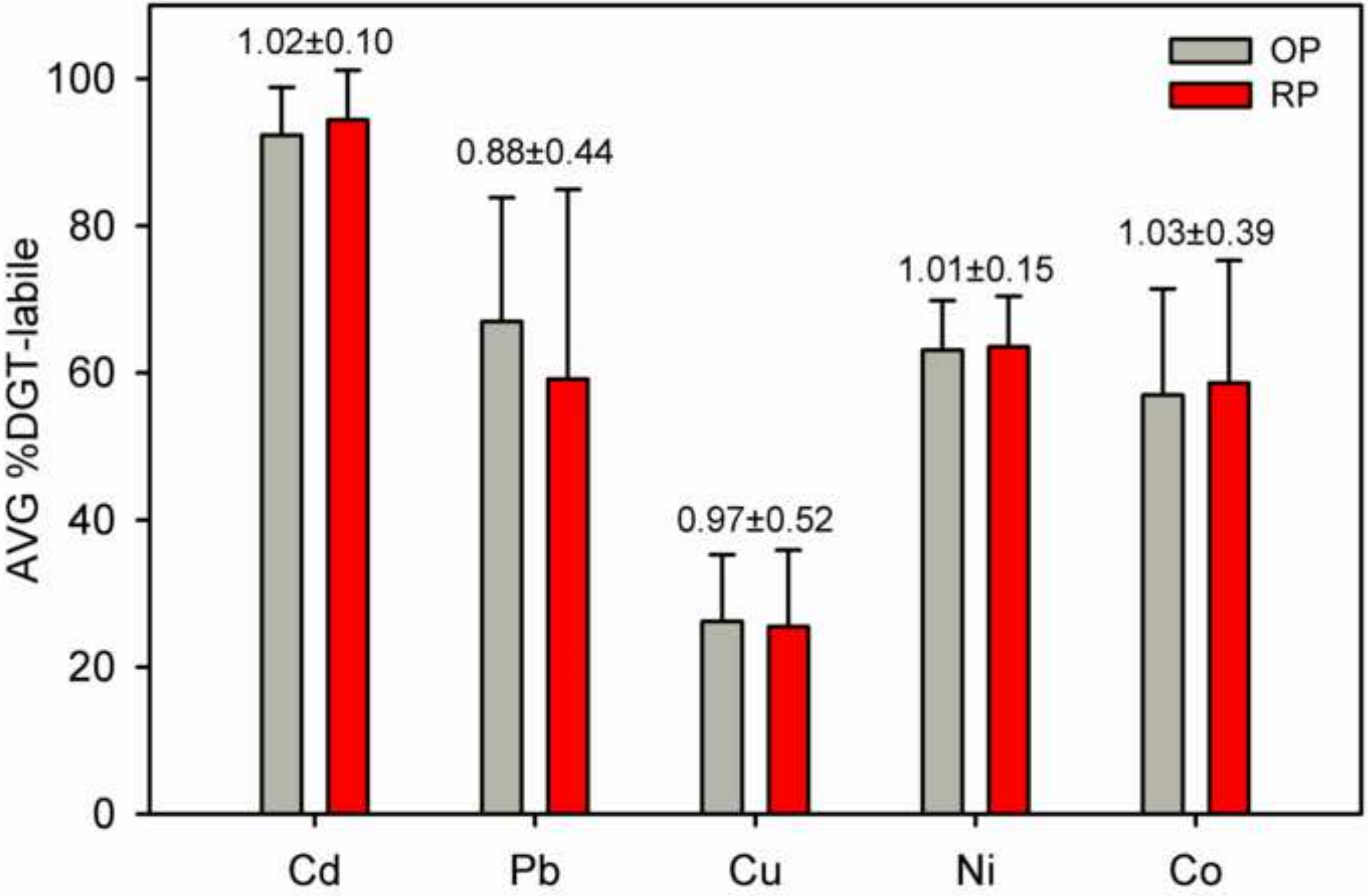

AVG %DGT-labile
0
20
40
60
80
100
Cd
Pb
Cu
Ni
Co
OP
RP
1.02±0.10
0.88±0.44
0.97±0.52
1.01±0.15
1.03±0.39

Figure 7

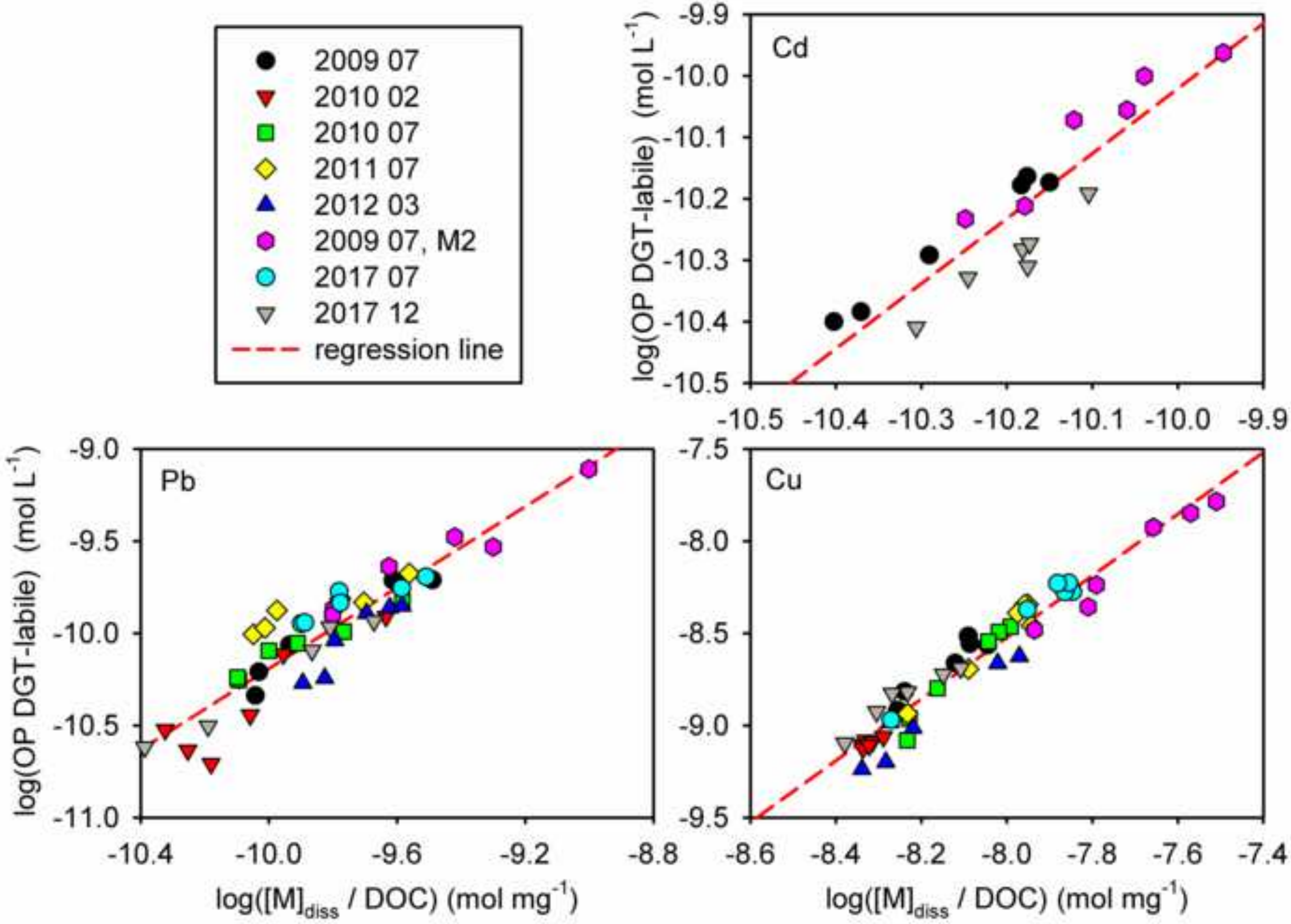
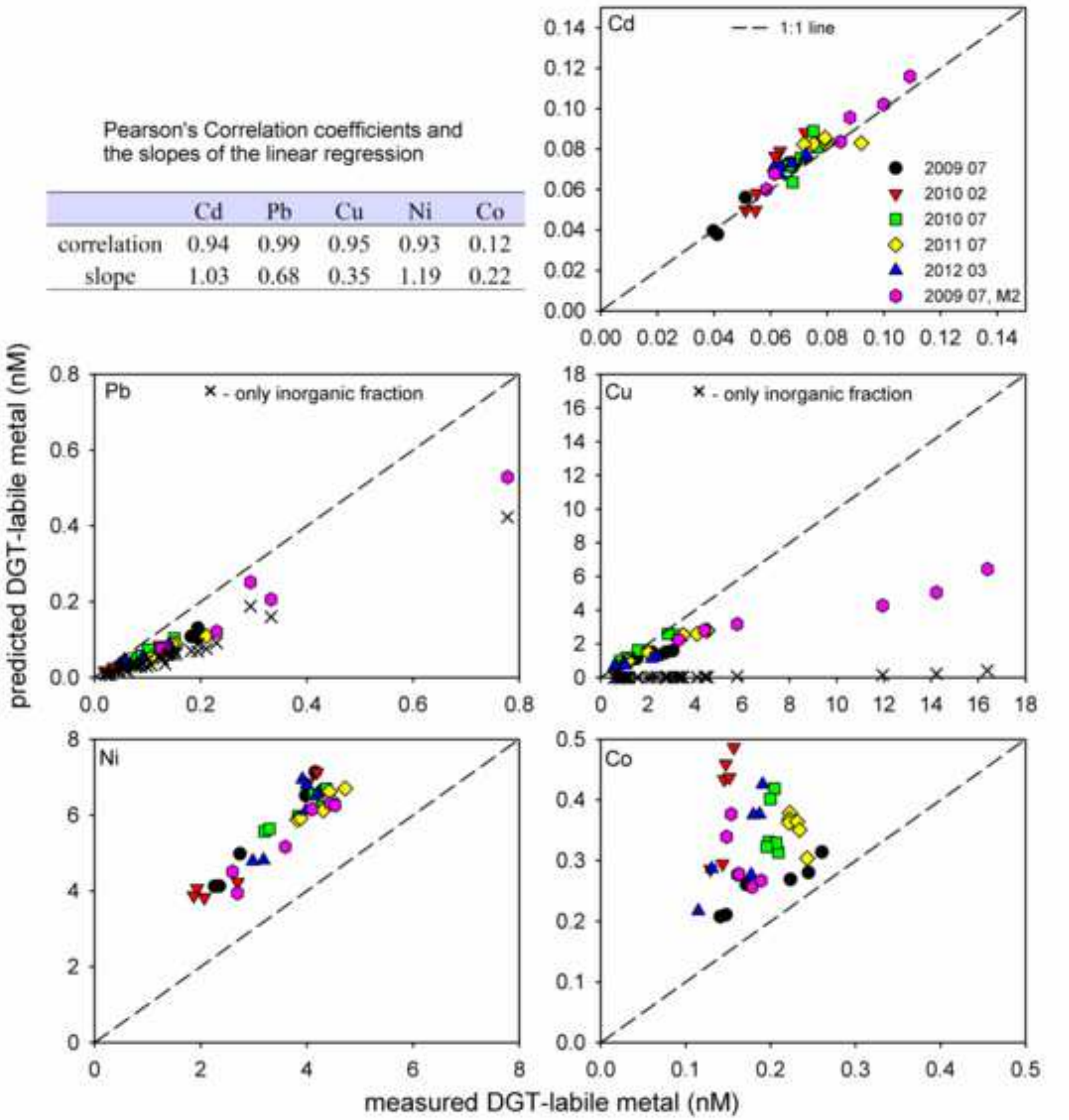

2009 07
2010 02
2010 07
2011 07
2012 03
2009 07, M2
2017 07
2017 12
regression line
Cd
Pb
Cu
log(OP DGT-labile) (mol L^{-1})
log(OP DGT-labile) (mol L^{-1})
-9.9
-10.0
-10.1
-10.2
-10.3
-10.4
-10.5
-10.5
-10.4
-10.3
-10.2
-10.1
-10.0
-9.9
-9.0
-9.5
-10.0
-10.5
-11.0
-10.4
-10.0
-9.6
-9.2
-8.8
-7.5
-8.0
-8.5
-9.0
-9.5
-8.6
-8.4
-8.2
-8.0
-7.8
-7.6
-7.4
log([M]$_{diss}$ / DOC) (mol mg^{-1})
log([M]$_{diss}$ / DOC) (mol mg^{-1})

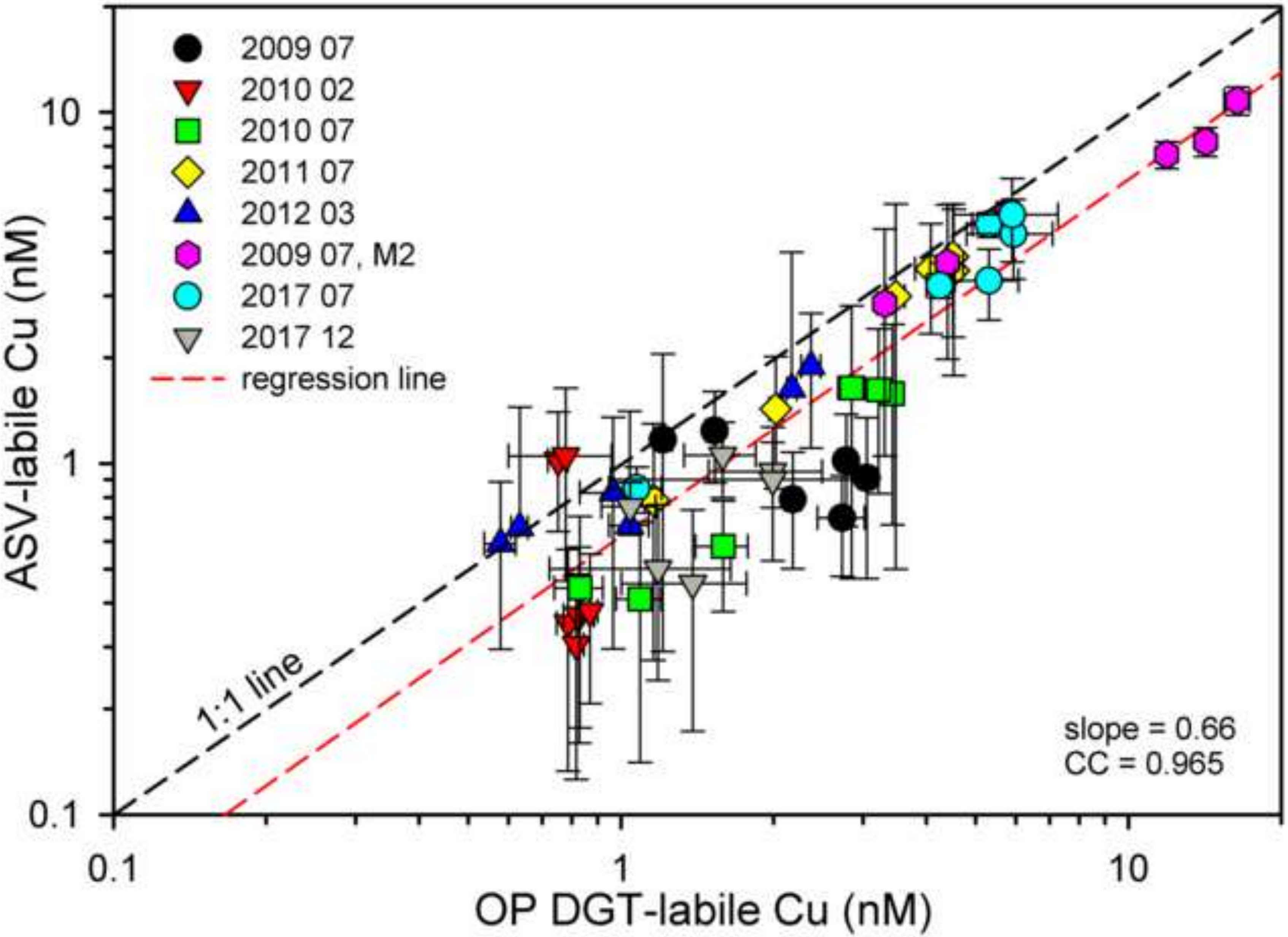
Figure 8



Pearson's Correlation coefficients and the slopes of the linear regression

	Cd	Pb	Cu	Ni	Co
correlation	0.94	0.99	0.95	0.93	0.12
slope	1.03	0.68	0.35	1.19	0.22

Figure 9


2009 07
2010 02
2010 07
2011 07
2012 03
2009 07, M2
2017 07
2017 12
regression line
1:1 line
slope = 0.66
CC = 0.965
ASV-labile Cu (nM)
OP DGT-labile Cu (nM)
0.1
1
10
0.1
1
10

***conflict of Interest Statement**

Declaration of interests

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Credit Author Statement

Ana-Marija Cindrić: Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Visualization; **Saša Marcinek**: Validation, Formal analysis, Investigation, Data Curation, Writing - Review & Editing; **Cédric Garnier**: Conceptualization, Methodology, Formal analysis, Resources, Supervision, Funding acquisition; **Pascal Salaün**: Conceptualization, Methodology, Writing - Review & Editing, Resources, Funding acquisition; **Neven Cukrov**: Investigation, Writing - Review & Editing; **Benjamin Oursel**: Investigation; **Véronique Lenoble**: Conceptualization, Resources, Writing - Review & Editing; **Dario Omanović**: Conceptualization, Methodology, Formal analysis, Resources, Writing - Review & Editing, Supervision, Project administration, Funding acquisition