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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.

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

52

53 1. Introduction

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

70 The challenge is the determination of the bioavailability which depends on a complex
71 interaction of biological and physico-chemical parameters that affect the interaction between
72 the metal and the biological receptor. Such parameters may include pH, ionic strength,
73 complexing agent, reactivity (association/dissociation kinetics) of the complex as well as the
74 affinity between the metal and the biological receptor and the biological uptake flux ([Sunda,](#)
75 [2012](#)). For assessment of water quality in freshwaters, the Biotic Ligand Model (BLM) is the
76 only one that directly links trace metals bioavailability to ecotoxicological impact ([Ardestani et](#)
77 [al., 2014](#); [Balistrieri and Blank, 2008](#); [Di Toro et al., 2001](#); [Rudel et al., 2015](#)) but such model
78 does not yet exist for coastal or transitional (estuarine) waters. Estuarine waters are
79 physically and chemically dynamic areas with high temporal and spatial variability, making
80 TM cycling (and TM bioavailability) more complex to predict in comparison with other
81 aquatic systems ([Biati and Karbassi, 2010](#); [Oursel et al., 2013](#); [Waeles et al., 2008](#)).

82 As mentioned above, not all TM species are bioavailable, but only the free hydrated ion, the
83 species that can release their metal to bind with the biological receptors (reactive/labile metal
84 inorganic/organic complexes) and those that can cross the biological membrane (e.g.
85 lipophilic metal complexes) ([Gao et al., 2019](#)). Several techniques can be used to determine
86 TM speciation and assess the bioavailability of TM ([Feldmann et al., 2009](#)). Amongst those,

87 electrochemical stripping techniques such as Anodic Stripping Voltammetry (ASV) ([Garnier et](#)
88 [al., 2004](#); [Gibbon-Walsh et al., 2012](#); [Omanović et al., 2015a](#); [Omanović et al., 1996](#)) or
89 Competitive Ligand Exchange Adsorptive Cathodic Stripping Voltammetry (CLE-AdCSV)
90 ([Buck et al., 2012](#); [Pižeta et al., 2015](#)) are widely used to measure the concentrations of
91 reactive (electrochemically labile) and free metal ion, respectively, as well getting insights
92 into the presence and strength of complexes/ligands. *In-situ* passive sampling techniques such
93 as Diffusive Gradients in Thin-films (DGT) are also very popular because of their perceived
94 simplicity, multi-elemental capabilities, and *in-situ* application, i.e. directly in the water
95 column ([Amato et al., 2014](#); [Baeyens et al., 2018](#); [Cindrić et al., 2017](#); [Davison and Zhang,](#)
96 [1994](#); [Davison and Zhang, 2012](#); [Degryse et al., 2009](#); [Menegário et al., 2017](#); [Omanović et al.,](#)
97 [2015b](#); [Peijnenburg et al., 2014](#); [Shiva et al., 2016](#); [Warnken et al., 2009](#); [Zhang and Davison,](#)
98 [2015](#)). More importantly, concentrations of metals determined by DGT (defined here as DGT-
99 labile) have been found in many cases to closely relate to the concentrations of bioavailable
100 metals ([Baeyens et al., 2018](#); [Degryse et al., 2009](#); [Ferreira et al., 2013](#); [Koppel et al., 2019](#);
101 [Philipps et al., 2019](#); [Zhang and Davison, 2015](#)) making the DGT technique one of the most
102 preferred method for monitoring purposes. In addition, the ability to measure time-weighted
103 average (TWA) concentrations overcomes the problem of episodic contamination, which could
104 not be spotted by the classical discrete sampling methodology ([Altier et al., 2019](#); [Gao et al.,](#)
105 [2019](#); [Unsworth et al., 2006](#)). DGTs can be tuned for the monitoring of specific metals by
106 choosing various suitable solid or liquid binding agents (Table 1 in ([Li et al., 2019](#))). Chelex-
107 100 is by far the most frequently used in the marine environment since it can determine 24
108 elements, including environmentally important elements such as Pb, Zn, Co, Ni, Cu, Cd, Al,
109 Mn, Fe, Cr and U ([Gao et al., 2019](#); [Garmo et al., 2003](#); [Gimpel et al., 2003](#); [Li et al., 2019](#)). In
110 addition, using hydrogels with different pore size (open pore (OP) with size of ~10 nm and
111 restrictive pore (RP) with size of ~1 nm), it is also possible to distinguish labile complexes
112 based on their molecular size and lability ([Gao et al., 2019](#)). Although relatively simple to
113 implement experimentally, the metal-uptake flux at this complexing interface is by no means
114 straightforward and can be influenced by many parameters that have been critically discussed
115 in details in several papers ([Galceran and Puy, 2015](#); [Mongin et al., 2011](#); [Town et al., 2009](#);
116 [Uribe et al., 2011](#); [van Leeuwen, 2016](#)). Applications and recent progresses of the DGT
117 technique for *in-situ* analysis have been reviewed recently ([Li et al., 2019](#); [Menegário et al.,](#)
118 [2017](#)).

119 While the Diffusive Gradients in thin Films (DGT) technique has widely been used for trace
120 metals (TM) speciation in freshwater environments, there are much fewer studies in estuarine

121 and marine waters ([Dabrin et al., 2016](#); [Schintu et al., 2008](#)). The aim of this work here was to
122 evaluate the DGT technique as a passive sampling methodology for *in-situ* determination of
123 potentially bioavailable fractions of TM in a highly dynamic estuarine environment. For this
124 purpose, a multimethodological approach was used: time-averaged concentrations of DGT-
125 labile metals were measured in the highly stratified Krka River estuary (Croatia) and
126 compared to the dissolved concentrations, ASV-labile Cu concentrations obtained in
127 representative discrete samples as well as to those calculated by Visual MINTEQ, a speciation
128 modelling software.

129

130 **2. Working methodology and analytical techniques**

131 **2.1. Study site and sampling strategy**

132 This study was performed in the highly stratified Krka River estuary (Croatia, Fig. 1). The
133 main characteristic of this salt-wedge type estuary is the presence of a sharp halocline within a
134 vertical salinity gradient characterized by three separate layers: the upper fresh/brackish water
135 layer (FWL), the middle freshwater-seawater interface layer (FSI) and the bottom seawater
136 layer (SWL). The FSI is a sharp halocline that is usually formed at a depth of between 1.5 and
137 3.5 m. Its width ranges from only a few centimetres to ~1 m, depending on whether (wind,
138 precipitation) and hydrological conditions (the Krka River flow). As a result of seawater
139 entrainment, the salinity range in the surface layer increases from $S = 0$ at the head of the
140 estuary to $S = 39$ towards its mouth. A wider map and additional description of the estuary is
141 provided in supplementary materials (Fig. S1). *In-situ* measurements were made at two sites
142 within the Šibenik Bay (Fig. 1): M1, located in front of the marine station Martinska, and M2
143 in the nautical marina D-Marin. M1 is representative of the major part of the estuary which is
144 relatively “clean”; it was sampled during 7 campaigns covering 4 summer (2009, 2010, 2011
145 and 2017) and 3 winter periods (2010, 2012 and 2017). M2 is representative of the most
146 “polluted” part of the estuary, according to previously measured TM concentrations ([Cindrić
147 et al., 2015](#)). M2 was sampled only in summer 2009.

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

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

165

166 **2.2. DGT deployment**

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

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

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

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

193

194 **2.3. Preparation of samples**

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

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

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

216

217 **2.4. Analytical methods**

218 **2.4.1. Voltammetric analysis of dissolved trace metal concentrations**

219 Dissolved TM concentrations in composite samples were determined by differential pulse
220 stripping voltammetry: anodic (DPASV) for Zn, Cd, Pb, and Cu ([Omanović et al., 2006](#)) and

221 adsorptive cathodic (DPAdCSV) with 10^{-5} M nioxime as complexing ligand for Ni and Co
222 ([Vega and van den Berg, 1997](#)). Measurements were carried out using an Autolab
223 (EcoChemie) potentiostat (μ Autolab2 or PGSTAT128N) controlled by GPES 4.9 software in
224 a three-electrode cell (663 VA Stand, Metrohm). TM concentrations were determined by
225 means of standard addition method. A certified “Nearshore seawater reference material for
226 trace metals” – CASS-5 (NRC CNRC) was used for validation of the analysis. All determined
227 metal concentrations were within the certified limits. The precision of analytical
228 measurements was within 10% of measured concentrations (Table S1).

229

230 **2.4.2. Determination of Cu ASV-labile concentrations**

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

245

246 **2.4.3. Carbon analysis**

247 A TOC-VCSH analyser (Shimadzu) was used for determination of DOC and DIC
248 concentrations. Calibration was done with hydrogen-phthalate (Shimadzu) and
249 $\text{NaHCO}_3/\text{Na}_2\text{CO}_3$ standard solutions, respectively, with an accuracy of 0.02 mgC L^{-1} ([Louis et](#)
250 [al., 2009](#); [Oursel et al., 2013](#)). The analytical validity of the method was confirmed by
251 measuring the certified reference material MISSIPPI-03 (Environment Canada).

252

253 **2.4.4. Analysis and calculation of DGT-labile metal concentrations**

254 A multi-elemental analysis of DGT-extracts (at 3 times dilution) was performed by high
255 resolution inductively coupled plasma mass spectrometer (HR ICP-MS, Element 2, Thermo,
256 Bremen, Germany). From a total of 1.5 mL acidified DGT extract, 0.7 mL was collected and
257 diluted using 2% HNO₃ (*suprapur*). Indium (In) was used as an internal standard (IS) for
258 analysis. The quantification of metal concentrations was performed using external
259 calibrations. The validity of HR ICP-MS measurements was confirmed by measuring a river
260 water reference material (SLRS-4 and SLRS-5, National Research Council Canada); a good
261 agreement with the certified values (within 10% of certified) was always obtained.

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

269 Relatively high blank values for Zn were found in our study, ranging from 8% to 74% of the
270 corresponding dissolved Zn concentration. Thus, the majority of DGT results for Zn were
271 considered as unreliable and only indicative values are presented for cases where the blank
272 was relatively low (<40%) and/or where good repeatability between 3 DGT devices
273 (including blanks) was within 15%. For other metals, DGT blanks were consistent and
274 acceptable. The highest percentages of blanks, for each campaign and all metals, are provided
275 in Table S2. Procedural blanks of used milli-Q and acids for all measured metals were up to
276 ~2%.

277

278 **2.4.5. Determination of effective diffusion coefficients for metals**

279 Although DGT diffusion coefficients (D_M) for metals were supplied by the manufacturer
280 (DGT Research Ltd), they are experimentally obtained in 0.01 M NaNO₃ solution at a pH of
281 ~5 (which is more representative of freshwater) using diffusive cell methodology. To correct
282 for potential differences in D_M in brackish/seawater, due to a different matrix (especially in
283 terms of ionic strength) compared to common D_M ([Garmo et al., 2003](#); [Scally et al., 2006](#)), an
284 effective D_{eff} of TM were estimated using the “direct uptake” method for each metal by
285 deploying the DGT devices in spiked solutions of different salinities for a fixed amount of
286 time and constant temperature. Samples with different salinities ($S = 0.1, 19$ and 38) were

287 prepared with organic-free water from end-members (filtered and UV irradiated). The
288 concentration of each added metal was selected to be ~50-100 times higher than the ambient
289 metal concentration, in order to have well defined metal concentrations needed for
290 calculations (100 nM of Cd, Pb, Co, 500 nM of Cu, Ni and 1000 nM of Zn). The pH of these
291 solutions was adjusted to ~7.5 by addition of borate buffer. Under these conditions, model
292 calculations using Visual MINTEQ 3.0 showed that precipitation of any metal is not likely to
293 occur. Three 7-L PET bottles (canisters) were positioned upside-down above a magnetic
294 stirrer. The bottom of the bottle (facing up) was cut, 5 L of sample were poured in, stirred
295 with a teflon magnetic stirrer and DGT devices were placed inside. The full setup is presented
296 in Fig. S4. Five OP and five RP DGT devices were mounted on previously cleaned plastic
297 holders and deployed in each of the stirred solutions for 6 hours. Before deployment, DGTs
298 were rinsed by immersing them in the washing solution (water with the same salinity as the
299 working sample, unspiked with metals). The temperature was kept stable at 25.0 ± 0.5 °C (air-
300 conditioned room) and was controlled in a separate canister with the same sample volume and
301 under the same stirring conditions. After 6 hours of deployment, the DGTs were taken out,
302 treated and analysed as described in previous sections. The concentrations of metals in all
303 three samples were monitored throughout the experiment (before deployment and after 15
304 min, 1 h, 2 h, 4 h and 6 h) and were found within 5% of the expected concentrations (recovery
305 was $100 \pm 5\%$). Note that the DBL is also in this way indirectly incorporated into the
306 estimated effective D_{eff} . A more thorough approach to determine the extent of the DBL would
307 have consisted in using DGTs with different diffusion layer thickness ([Warnken et al., 2006](#))
308 but this was beyond the scope of this field study.

309

310 **3. Results and discussion**

311 **3.1. Major physico-chemical parameters during the deployment period**

312 Horizontal and vertical salinity gradients along the Krka River estuary mainly depend on the
313 river flow and weather conditions (primarily the wind). Amongst all DGT-campaigns, only
314 two campaigns (winter 2010 and winter 2017) were conducted in a period of relatively high
315 Krka River flow (Fig. S5). Vertical profiles of average salinity at the M1 site for all sampling
316 events are plotted in Fig. 2, whereas representative examples of the salinity variation during
317 the deployment period for two contrasting seasons (summer 2009 and winter 2012) are
318 presented in Fig. S6. A well-developed halocline was formed for all campaigns at a depth
319 between 1.5 and 3 m. In winter 2012, the depth of the halocline was moving upward over the

320 deployment period and thus the depths of the DGT devices were adapted after the second day
321 (moved upward by 0.5 m). Salinities measured in the surface layer during the period of DGT-
322 deployments showed a “typical” summer/winter pattern, exemplifying contrasting conditions
323 that occur in the estuary, with exception of unusually low surface salinity in the summer
324 2009, and relatively high salinity in the winter of 2012. In the summer 2009, the vertical
325 salinity profiles obtained at M1 and M2 were similar.

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

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

335 The DOC concentrations at the M1 site were in agreement with the results from a previous
336 study covering the same period ([Cindrić et al., 2015](#)). DOC concentrations in FWL were
337 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
338 summer of 2009 displayed a similar behaviour to winter presumably due to very calm
339 conditions in the estuary preceding the sampling campaigns (no strong wind). Higher summer
340 concentrations of DOC at the FSI were related to enhanced biological activity. Note that the
341 DOC profile at the M2 site (summer 2009) was practically the same as for the M1 site
342 (differences at each depth were lower than 0.05 mgC L⁻¹).

343

344 **3.2. "Effective diffusion coefficients" of metals in diffusive gel (D_{eff})**

345 The DGT-technique provides an operationally defined concentration of the DGT-labile metal
346 fraction. It is generally assumed that diffusion coefficients of different inorganic complexes
347 are the same; however some metal-organic complexes have diffusion coefficients much lower
348 than those of inorganic species ([Han et al., 2014](#); [Zhang and Davison, 2001](#)). Consequently,
349 DGT-labile concentrations of trace metals measured in natural samples are normalised to
350 inorganic species, and thus can be considered only as operational/conditional values, i.e.
351 technique dependent and matrix dependent. Two types of DGT devices used in our study (OP
352 and RP) were expected to provide slightly different DGT-labile concentrations, with higher

353 values from the OP DGT. However, for the first set of measurements, the concentrations were
354 almost the same. In order to check the validity of these surprising results, whether there were
355 related to non-suited diffusion coefficients or any other experimental/mechanistic problem, it
356 was decided to determine the effective diffusion coefficients (D_{eff}) of inorganic metal species
357 at three different salinities. Any additional influence of the sample composition (e.g. ionic
358 strength, inorganic speciation change) or mechanical/structural imperfections on the
359 production of DGT-devices were implicitly taken into account via D_{eff} .

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

374 In regards to Pb whose D_{eff} value is 23% lower than that provided by the manufacturer,
375 Garmo et al. (2003) reported a value ~20% higher ([Garmo et al., 2003](#)), obtained in 0.01 M
376 NaNO_3 at pH of ~5-6. As different values of diffusion coefficients have been reported in the
377 literature, it was decided to use the value of Pb obtained in our experiment. To summarise, we
378 used the following effective diffusion coefficients in our calculations: 6.18 (Cd), 6.22 (Pb),
379 6.23 (Cu), 5.68 (Zn), 5.84 (Ni) and $6.17 \text{ cm}^2 \cdot \text{s}^{-1}$ for Co.

380 One of the important parameters in DGT technique calculations is the DBL, especially in
381 calm waters (such as lakes or seawater) where it can significantly influence the calculated
382 concentrations ([Levy et al., 2012](#); [Warnken et al., 2006](#); [Zhang and Davison, 1995](#)). Within
383 our experimental set up (Fig. S4), although the solution is stirred, slightly different mixing
384 hydrodynamic conditions are expected to exist between the DGTs placed at the surface and
385 those placed at the bottom. However, based on 30 separate results (15 OP and 15 RP), no

386 clear differences with the position of the DGT probes were found ([Warnken et al., 2006](#)),
387 suggesting that under these experimental conditions, the effect of any variations of the DBL
388 size on the final results was insignificant.

389

390

391 **3.3. Vertical distributions of dissolved and DGT-labile trace metals**

392 Typical profiles of dissolved and OP/RP DGT-labile metal concentrations of Cu are presented
393 in Fig. 3. Vertical profiles of other metals are presented in Figs. S9 to Fig. S14. Except for
394 two winter campaigns (2010 and 2017), higher concentrations of Cu were measured in the
395 FWL and FSI layers than in the SWL. Increased Cu concentrations in the surface layer
396 measured in the summer were attributed to higher nautical traffic within the bay, with Cu
397 being released from antifouling paints used as a biocide on boats ([Cindrić et al., 2015](#)), similar
398 to previous reports in other studies ([Dunn et al., 2007](#)). Although the Cu concentration in the
399 FWL varied by a factor of 5 (ranging from ~4 nM in February 2010 to almost 20 nM in July
400 2017), only a small variability was observed in the SWL (within ~40%) indicating a minor
401 vertical transport of Cu, even in winter conditions.

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

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

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

420 DGT-labile ones (except for Zn) but the ratio DGT-labile/dissolved varied significantly for
421 different metals with an average ratio of 0.92 ± 0.03 , 0.64 ± 0.02 , 0.23 ± 0.05 , 0.61 ± 0.03 and
422 0.57 ± 0.06 for Cd, Pb, Cu, Ni and Co, respectively. Lower DGT-labile concentrations over
423 dissolved ones can be due to various reasons such as incomplete exchange of strong metal
424 complexes at the DGT complexing gel, presence of DGT-labile organic complexes that have a
425 low diffusion coefficient or presence of colloidal material with low diffusion coefficients
426 and/or a size that is $> \sim 10$ nm ([Liu et al., 2013](#); [Shiva et al., 2016](#)). These metal species exist
427 as non-dynamic forms (i.e. non-labile and/or non-mobile) that cannot be measured by DGT
428 ([Galceran and Puy, 2015](#); [Puy et al., 2014](#); [Warnken et al., 2008](#)).

429

430 **3.4. Variability of DGT-labile fraction**

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

439 Vertical profiles for Cu, Ni and Co each show characteristic trends. While higher DGT-labile
440 Cu percentages are generally found in the FWL than in the SWL, similar to dissolved
441 concentrations, percentages are relatively low along the all water column, in agreement with
442 strong organic complexation ([Warnken et al., 2009](#)). Higher levels of DOC in the FWL in
443 summer were not sufficient to fully buffer the increase of dissolved Cu concentrations (due to
444 nautical traffic), allowing higher percentage of inorganic and weak Cu organic complexes to
445 be accumulated by the DGT in the surface layer. The increase of Cu-labile fraction with
446 dissolved Cu was obvious for the "polluted" site (M2) where DGT-fraction increased to $\sim 50\%$
447 (Fig. S14). This was also confirmed by voltammetric measurements (see section 3.7), in
448 agreement with previous studies ([Louis et al., 2009](#)). For Ni and Co profiles, a small
449 increasing trend with depth was observed. Nickel followed the trend of the corresponding
450 dissolved concentrations, being generally lower in surface than in the seawater layer,
451 following basically the conservative dilution line (see Fig. 2 and Fig. S12) ([Cindrić et al.,](#)
452 [2015](#)). This was however not the case for Co, for which dissolved concentrations were

453 generally higher in the surface layer than in the bottom seawater layer (Fig. S13). Co
454 percentages varied significantly amongst various campaigns and no consistent trend was
455 found (Fig. 4). For Zn, due to the high Zn blank levels, no reliable data was obtained.

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

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

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

486 known and easy solving issues would not allow easy comparison of DGT results from
487 different studies.

488

489 **3.5. Comparison of open pore (OP) and restricted pore (RP) DGT-labile** 490 **concentrations**

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

506 The percentages of RP DGT-labile metal fractions were very close to that obtained by OP
507 DGT, with RP/OP ratios around 1 (numbers given above the bars in Fig. 6). A lower ratio was
508 obtained only for Pb (0.88 ± 0.44), but with relatively high uncertainty. Relatively high ratio
509 between these two DGT types has also been reported previously ([Osterlund et al., 2012](#); [Shiva
510 et al., 2016](#)). The observed similarities in DGT-labile fractions could be related to the
511 mechanism of accumulation, which depends not only on diffusion of metal complexes, but
512 also on their lability (kinetics of complexes dissociation) and molecular size ([Mongin et al.,
513 2011](#); [Osterlund et al., 2012](#); [Uribe et al., 2011](#)). The negligible difference obtained here
514 between OP and RP suggests that DGT-labile complexes are small molecules that diffuse
515 freely through both the RP and OP diffusive gels ([Forsberg et al., 2006](#); [Osterlund et al.,
516 2012](#)). The differences among the heterogeneous metal-organic ligand complexes existing at
517 ambient conditions are indistinguishable by the two different DGT types applied here.
518 Although results obtained indicate that it is unnecessary to use both types of devices ([Liu et](#)

519 [al., 2013](#); [Osterlund et al., 2012](#)), other studies have shown larger differences between these
520 two DGT types for various metals ([Baeyens et al., 2011](#); [Shiva et al., 2016](#); [Zhang and](#)
521 [Davison, 2000](#)).

522

523 **3.6. Heterogeneity of the binding ligands**

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

533 The value of Γ can be obtained from the slope of metal binding affinity versus the fraction of
534 occupied sites, i.e. by plotting DGT-labile concentrations versus dissolved metal
535 concentrations normalized to DOC (Fig. 7). Literature values of Γ for metal binding by
536 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](#)
537 [al., 2009](#)). Low Γ values represent more heterogeneous sites, while $\Gamma = 1$ assumes a fully
538 homogeneous binding site distribution. Heterogeneity factors calculated from this study are
539 0.60, 0.91 and 0.94 for Cu, Pb and Cd, respectively.

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

549

550 **3.7. Comparison of DGT-labile and modelled dynamic metal concentration**

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

$$566 \quad C_{\text{dyn}} = C_{\text{inorg}} + \frac{C_{\text{FA}}D_{\text{FA}}}{D_{\text{eff}}} + \frac{C_{\text{HA}}D_{\text{HA}}}{D_{\text{eff}}} \quad (\text{eq. 1})$$

567 where C_{FA} , C_{HA} and D_{FA} , D_{HA} are concentrations of metal complexes with fulvic (FA) or
 568 humic (HA) acid and corresponding diffusion coefficients, respectively, while C_{inorg} and D_{eff}
 569 represent the concentration of inorganic complexes and effective diffusion coefficients,
 570 respectively. It should be noted that diffusion coefficients for metal complexes with FA and
 571 HA are assumed to be the same as for FA and HA alone (without the metal) and are taken
 572 from the literature ([Zhang, 2004](#)). For modelling of chemical speciation, a free program
 573 Visual MINTEQ ver. 3.0 was used ([Gustafsson, 2011](#)). The major anion and cation
 574 concentrations were calculated according to the "Dittmar's law". For each sample, a
 575 corresponding DOC concentration and dissolved metal concentrations were used. Modelling
 576 of interactions of metals with organic ligands was performed using the Stockholm Humic
 577 Model (SHM). Different ratio of FA and HA (e.g. FA from 50% to 100%) could be assumed
 578 to represent natural organic matter in marine and freshwater systems ([Allan et al., 2008](#);
 579 [Gledhill and Gerringa, 2017](#); [Han et al., 2014](#); [Unsworth et al., 2006](#)). As the FA/HA ratio is
 580 an unknown parameter, it was assumed that 90% of organic matter is represented by FA, and
 581 10% by HA. No other adaptation of default parameters was undertaken.

582 Fig. 8 presents the comparisons between measured and predicted (eq. 1) DGT-labile
 583 concentrations for all metals along with the obtained correlation coefficients and the slopes of

584 the linear regression. The best agreement, both relative (correlation) and absolute (slope
585 around 1), was obtained for Cd as a result of the relatively weak complexation of Cd with
586 organic matter, i.e. Cd is primarily present as inorganic complexes with chloride.

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

598 Equation 1 applied in this study assumes that all metal complexes with FA and HA are labile
599 and accessible to DGT. The only difference compared to inorganic complexes was the applied
600 diffusion coefficient (values taken from Zhang (2004) are $1.15 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ for FA and
601 $0.6 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ for HA metal complexes). However, [Balch and Guéguen \(2015\)](#) showed that
602 diffusion coefficients for “bulk” dissolved organic matter and HS are 2-3 times higher than
603 values used for calculations in this study ([Balch and Guéguen, 2015](#)). However, application of
604 higher diffusion coefficients (by factor 3) led to an overestimation of the predicted metal
605 fractions for Cu and Pb.

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

611 For both Ni and Co, the predicted dynamic metal concentrations were higher than those
612 measured by the DGT, but the data was highly correlated only for Ni. Significantly higher
613 predicted Ni concentrations point to the underestimation of organic complexation of Ni with
614 HA and FA by the model used. Similar behaviour was found by [Warken et al. \(2009\)](#) which
615 concluded that differences between WHAM predictions and the measured Ni-DGT indicated
616 that WHAM used with the default binding parameters underestimates Ni binding to natural

617 organic matter ([Warnken et al., 2009](#)). A better agreement between DGT-measured and
618 predicted dynamic metal concentrations were found in studies performed in rivers ([Han et al.,](#)
619 [2014](#); [Meylan et al., 2004](#); [Omanović et al., 2015b](#); [Unsworth et al., 2006](#); [Warnken et al.,](#)
620 [2008](#)). This was rather expected, because the modelling of metal-organic ligand interactions
621 incorporated in programs were developed on the basis of data collected primarily in studies
622 performed in freshwater environments. Thus, the prediction of metal speciation in estuarine or
623 seawater system is still a challenging task, demanding extensive and more focused studies.
624 For example, separation and accumulation of representative organic matter from estuarine and
625 seawater environment would be needed to get a reliable set of acidity, complexation and
626 kinetic constants. Along with the DGT experiments in such model solutions an assessment of
627 the potential contribution of metal-organic complexes to DGT-labile concentration, i.e. to
628 potential bioavailable fraction would be achievable.

629

630 **3.8. Comparison of ASV-labile and DGT-labile Cu**

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

648 Indeed, the effective time of the measurement for the ASV is ~ 0.1 s, with a diffusion layer
649 thickness of ~ 10 μm , and a diffusion coefficient of 5×10^{-6} $\text{cm}^2 \text{s}^{-1}$, whereas in the DGT device

650 the diffusion layer thickness is ~0.9 mm and the effective measurement time is ~13.5 minutes
651 ([Zhang and Davison, 2000](#)). Both techniques are sensitive to inorganic Cu species, but also to
652 kinetically labile weak Cu organic complexes, although some labile species for the DGT
653 might be inert to ASV which is having a stronger discrimination towards accumulation of
654 organic complexes ([Ferreira et al., 2008](#); [Mongin et al., 2011](#); [Omanović et al., 1996](#); [Scally et al., 2003](#);
655 [Scally et al., 2006](#); [Uribe et al., 2011](#)). This is consistent with complexation titration
656 experiments for which curvature relationship of ASV-labile with the total Cu is usually
657 obtained if compared to DGT-labile titration curve for which curvature trend was not
658 observed ([Apte et al., 2005](#); [Louis et al., 2008](#)).

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

666

667 **4. Conclusions**

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

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

691

692

693

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705

706

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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 ($D_{\text{eff}} / \text{cm}^2 \text{ s}^{-1}$), “official” diffusion coefficient recommended by DGT-Research Company ($D_{\text{DGT-Research}}$), 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 $D_{\text{eff}} \times 10^6$	6.18	6.22	5.77	5.68	5.84	6.17
^a $D_{\text{DGT-Res}} \times 10^6$	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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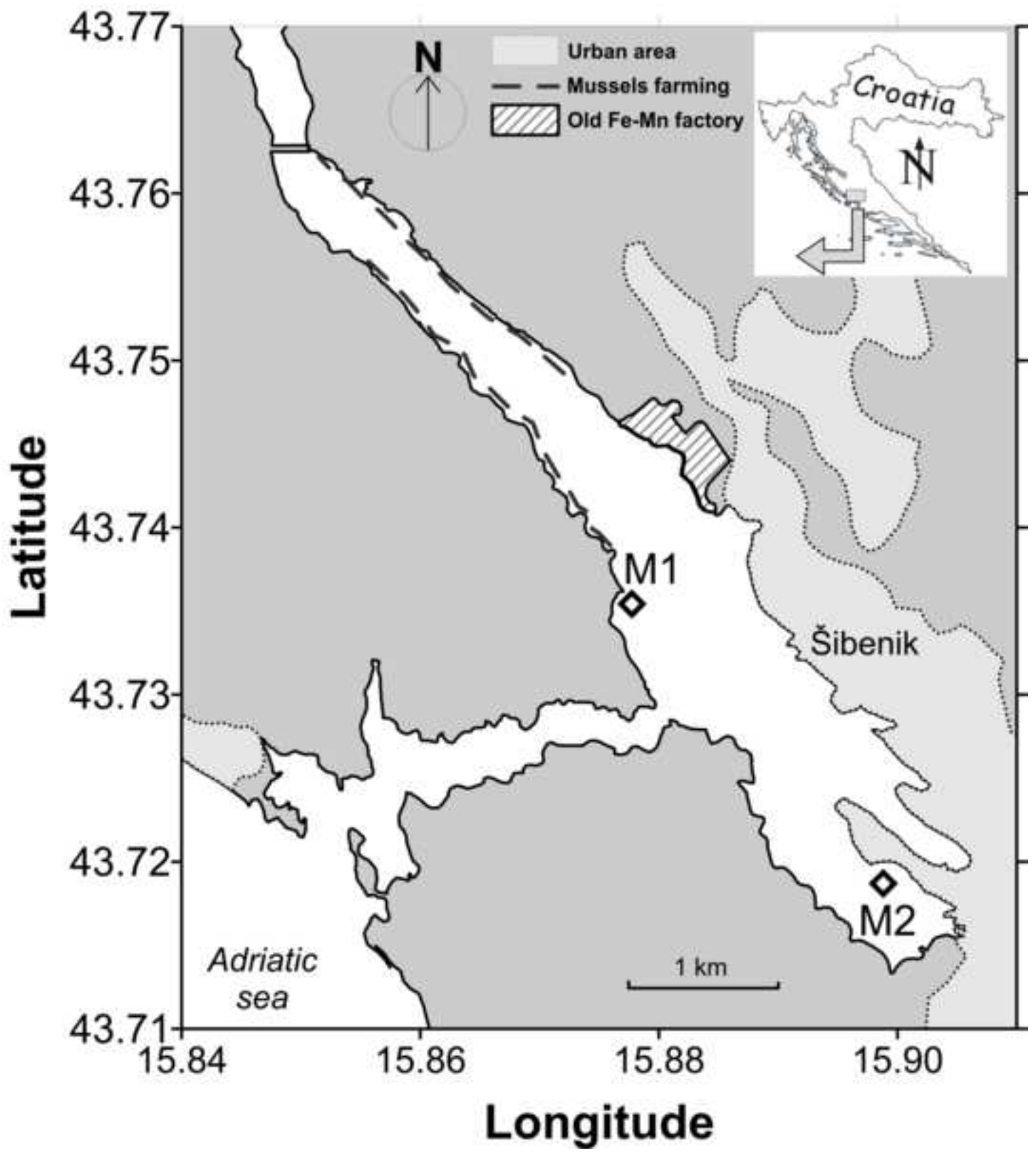


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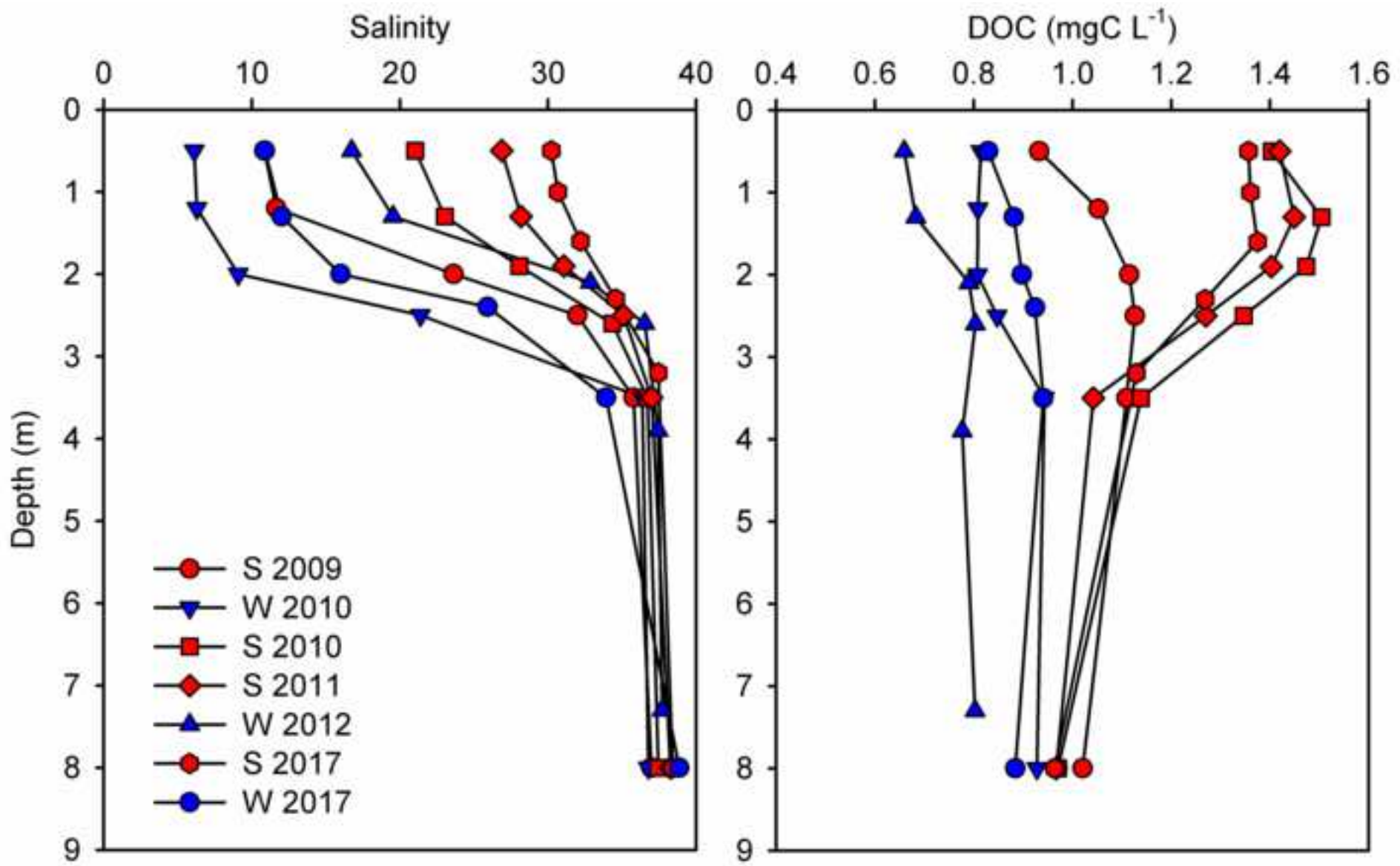


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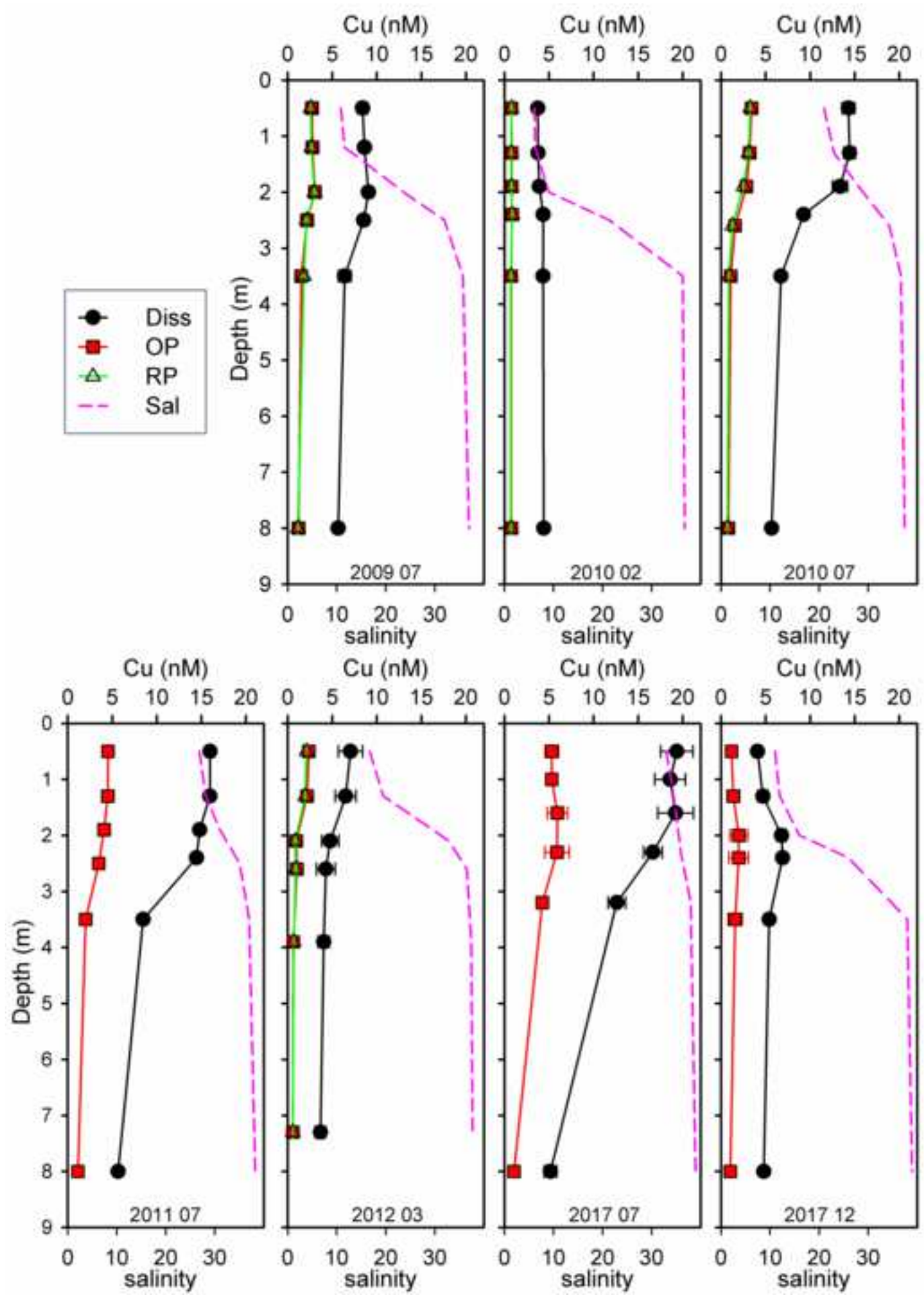


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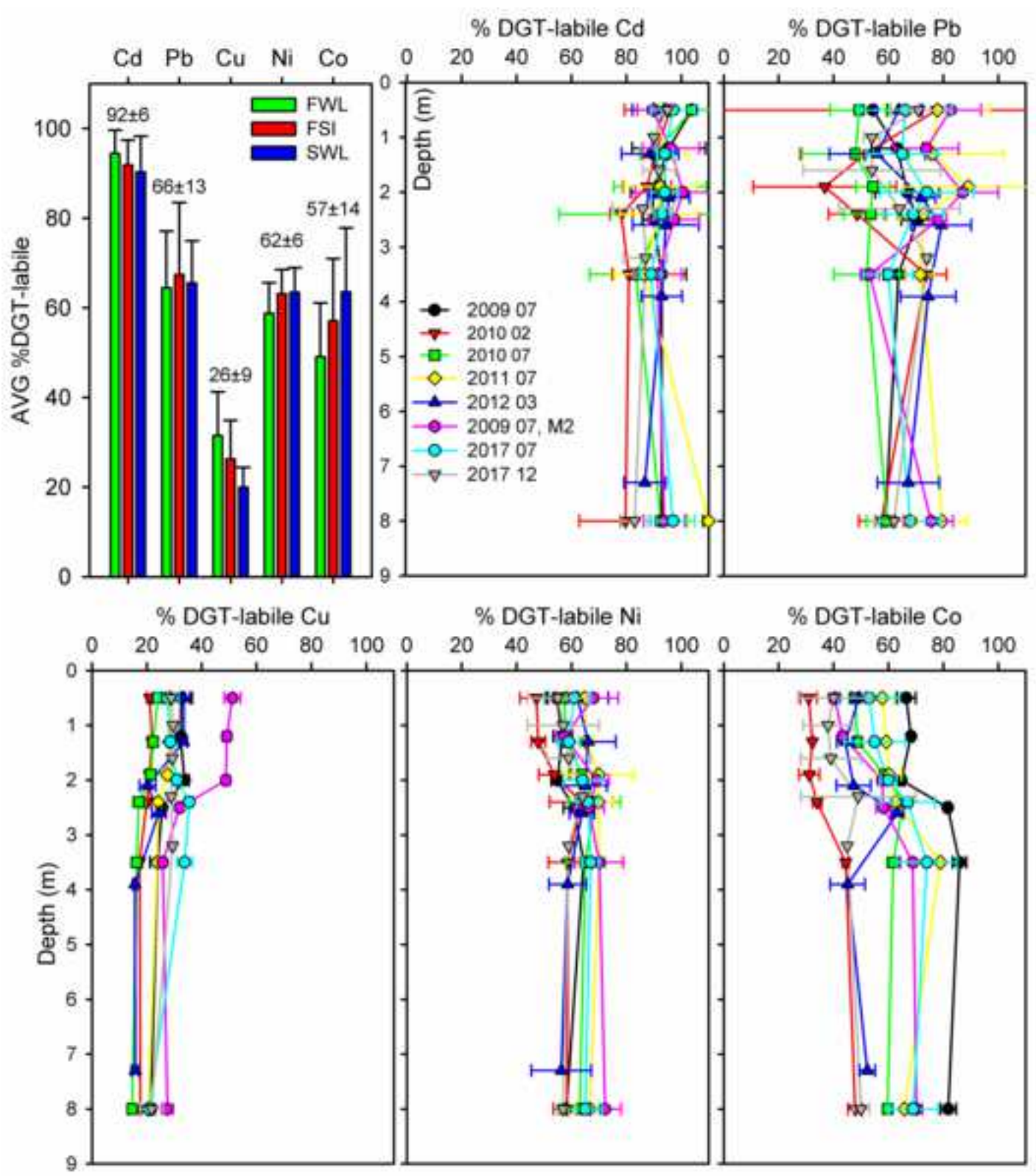


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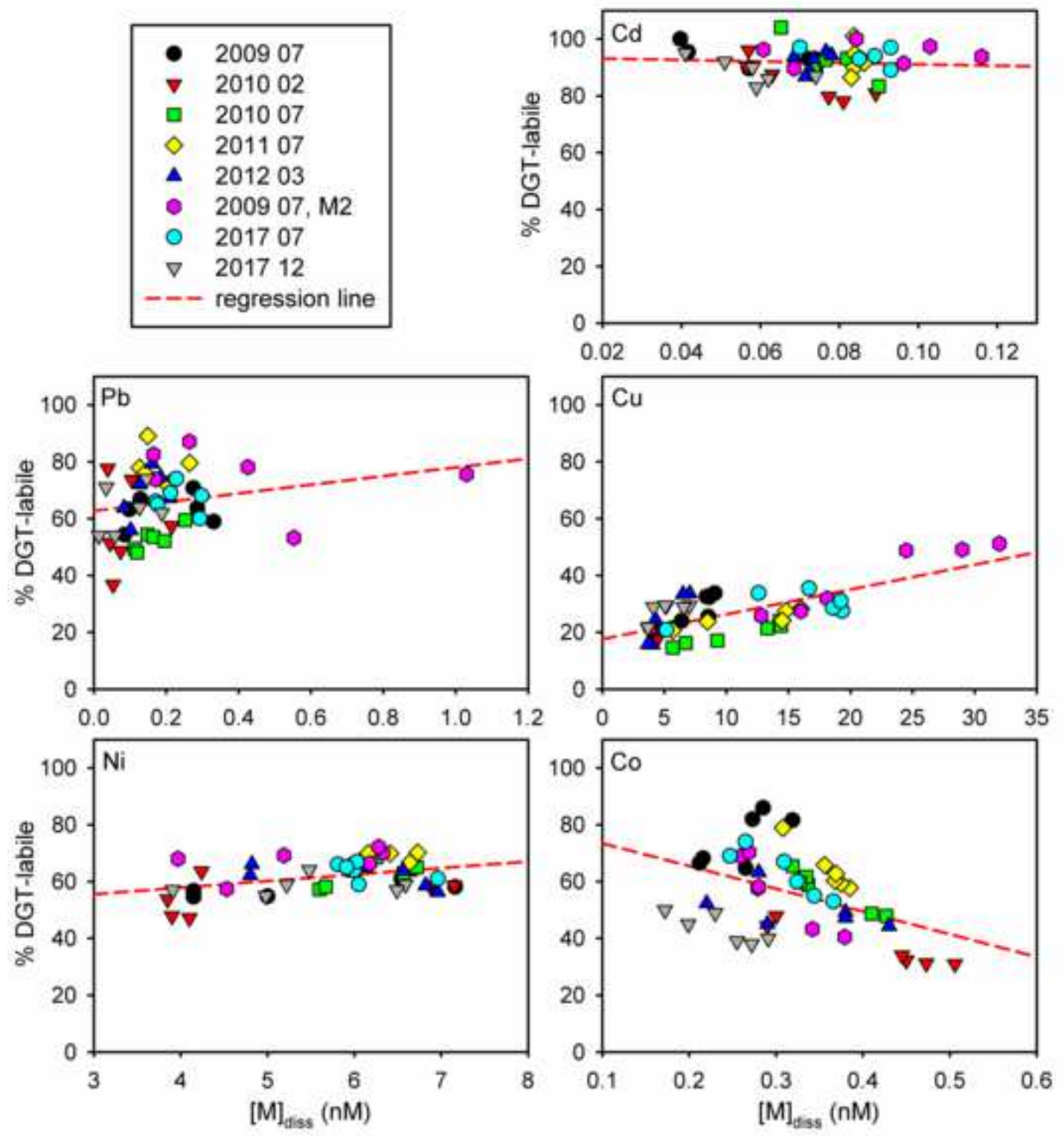


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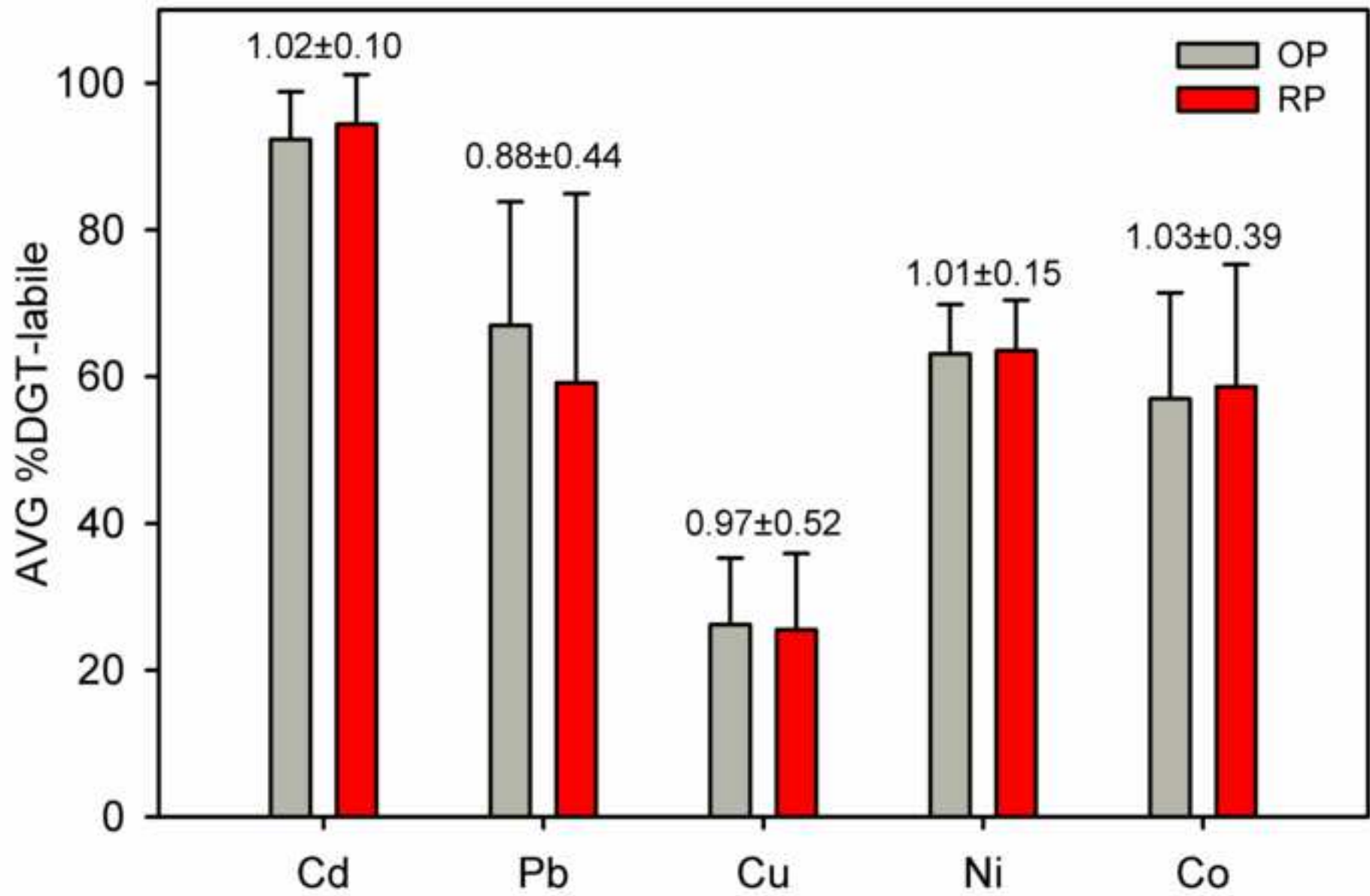


Figure 7

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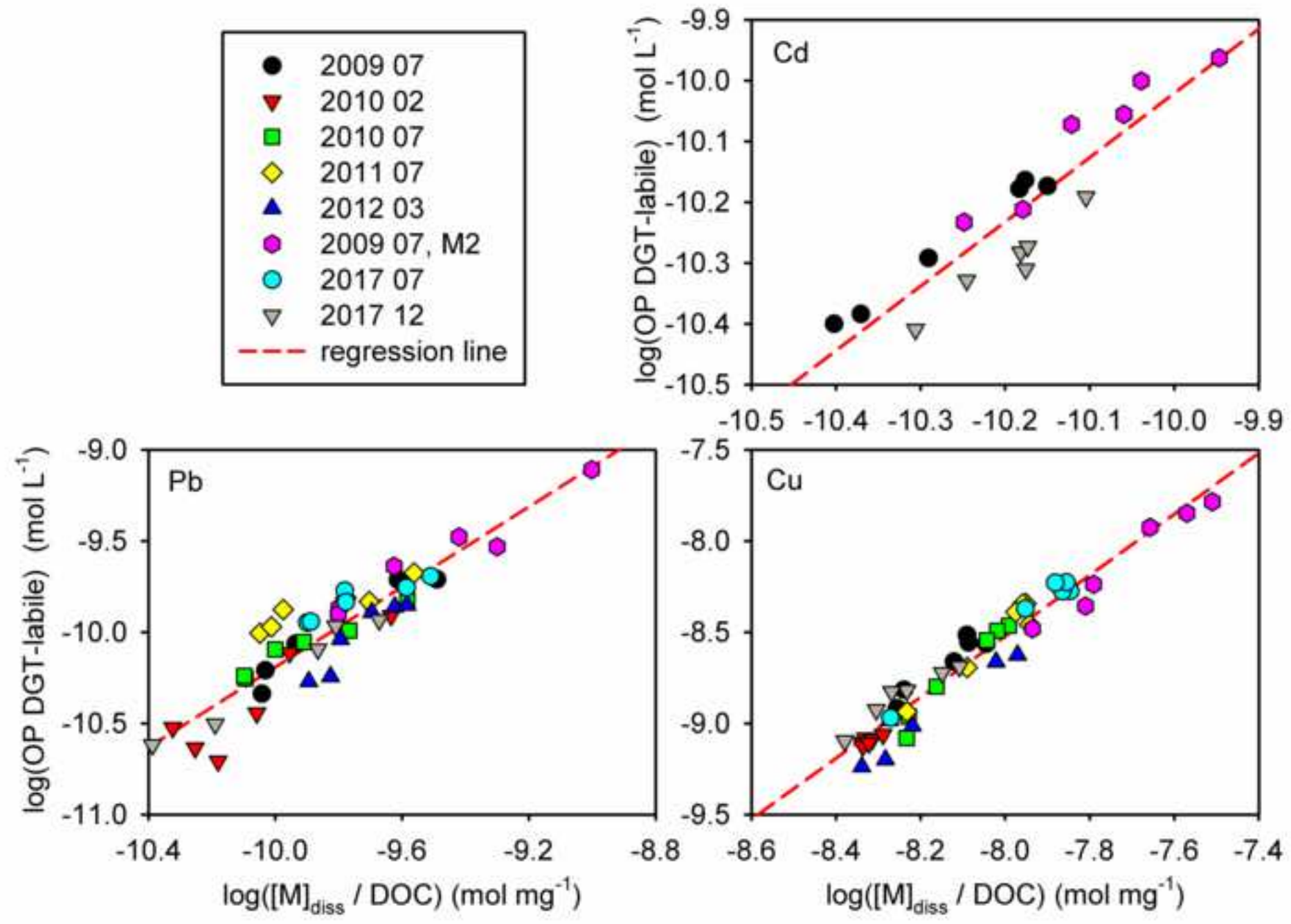
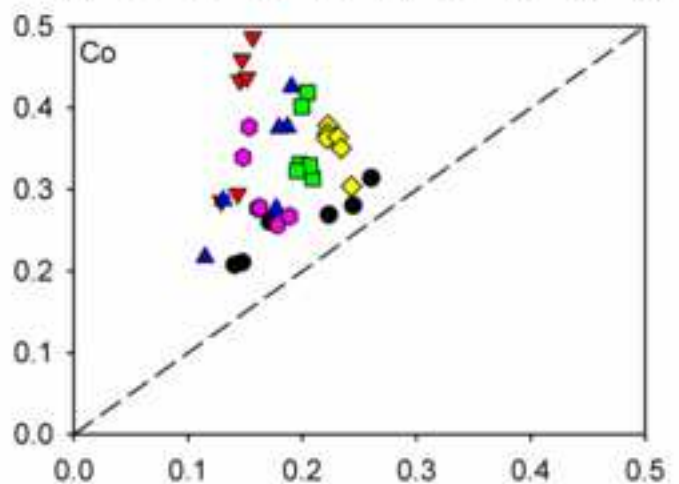
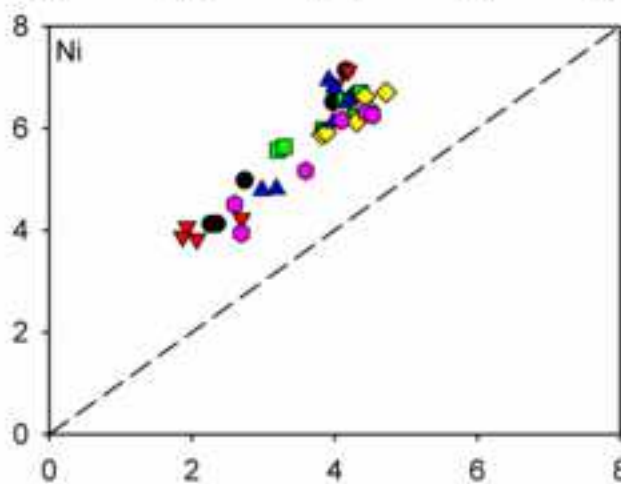
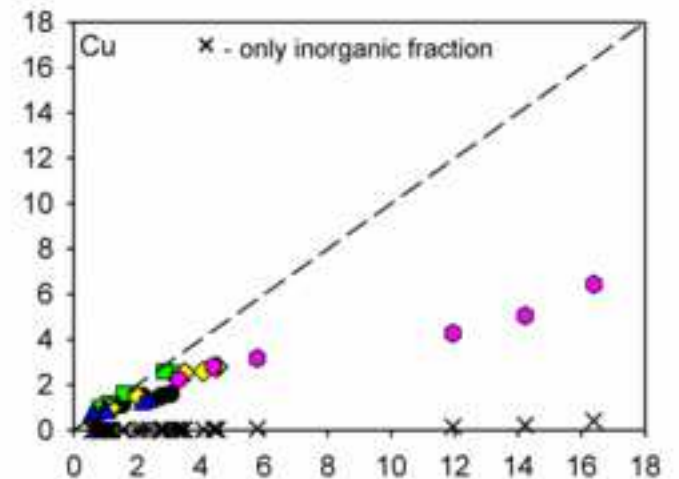
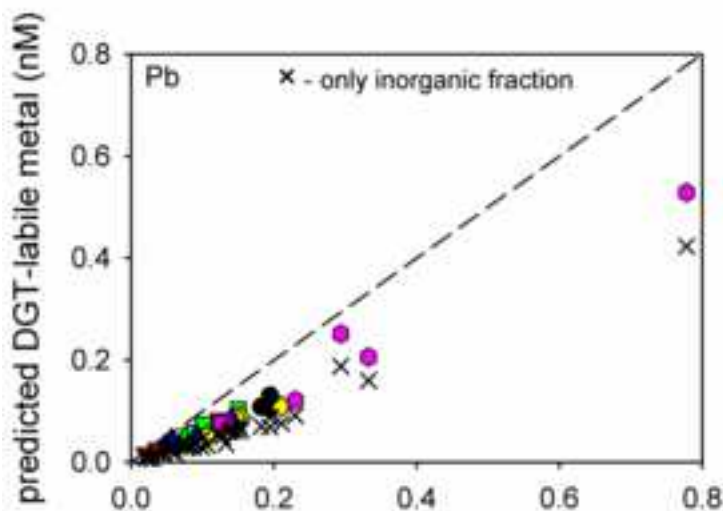
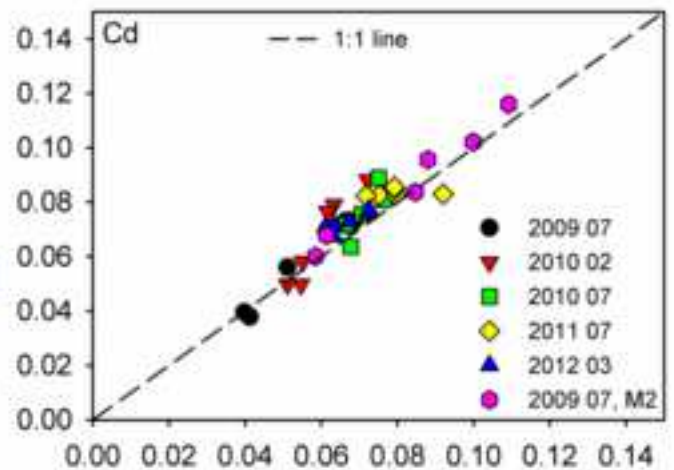


Figure 8
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Pearson's Correlation coefficients and the slopes of the linear regression

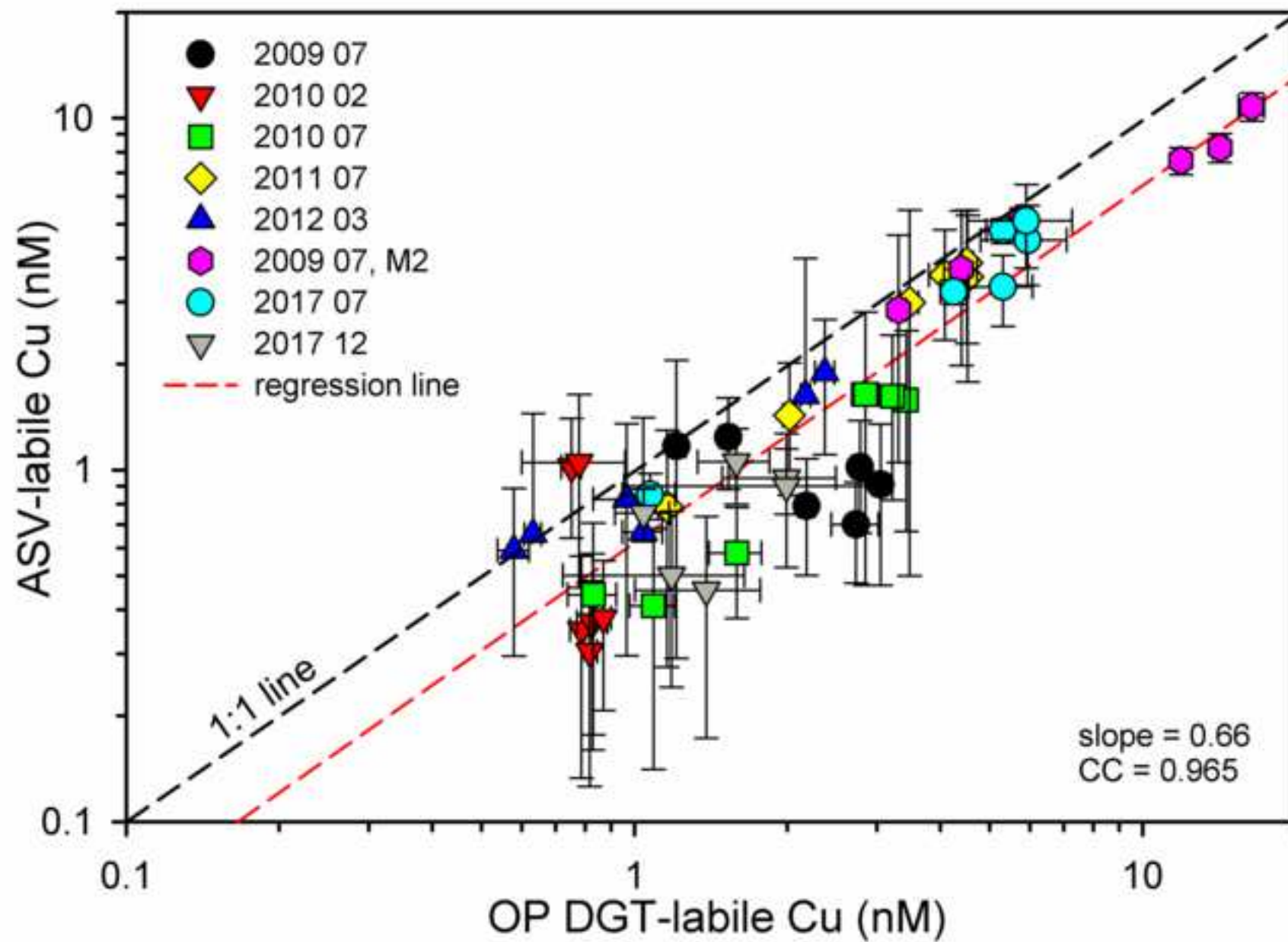
	Cd	Pb	Cu	Ni	Co
correlation	0.94	0.99	0.95	0.93	0.12
slope	1.03	0.68	0.35	1.19	0.22



measured DGT-labile metal (nM)

Figure 9

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Supplementary material for on-line publication only

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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