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**Bioavailability of copper in contaminated sediments  
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Keywords:	bioavailability, copper, ecological risk assessment, plant uptake, sediment toxicity
Abstract:	<p><b>Abstract</b></p> <p>The assessment of the potentially harmful effects of metals on biota depends on the speciation and bioavailability of the metals. In this study, we investigated copper accumulation and toxicity in the aquatic plant <i>Myriophyllum aquaticum</i> after exposure to artificial sediments varying in peat and/or ferric hydroxide content and spiked with Cu (5 to 200 mg.kg<sup>-1</sup>). Modeling of the kinetic DGT (diffusive gradient in thin film) measurements revealed fast and slow copper resupply from the solid phase for sediment formulated with and without peat, respectively. <i>M. aquaticum</i> proved to be sensitive to copper, as the copper accumulation and growth differed depending on the sediment composition and copper concentration. Comparing the copper accumulation in <i>M. aquaticum</i> to total dissolved, free and CDGT (concentration in solution derived from DGT measurements) copper concentrations revealed that CDGT concentrations were a better predictor of the accumulation than the others. However, the relatively weak correlation observed (<math>r^2=0.6</math>) and the fact that plant uptake does not increase proportionally to fluxes to DGT suggest that copper uptake in plants was not diffusion limited. Thus, the free copper concentrations near the root surface were sufficient to meet the plant's demand during the experiment. Furthermore, labile complexes that continuously resupply the Cu<sup>2+</sup> pool may also contribute to the</p>

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	concentrations available for plant uptake. In the range of copper concentrations investigated here, saturation of uptake processes as well as toxicity are considered responsible for the poor DGT prediction of plant uptake.

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3 **1 Bioavailability of copper in contaminated sediments assessed by a DGT approach and the**  
4 **2 uptake of copper by the aquatic plant *Myriophyllum aquaticum***  
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13

14 **Abstract**

15 The assessment of the potentially harmful effects of metals on biota depends on the speciation and  
16 bioavailability of the metals. In this study, we investigated copper accumulation and toxicity in the  
17 aquatic plant *Myriophyllum aquaticum* after exposure to artificial sediments varying in peat and/or  
18 ferric hydroxide content and spiked with Cu (5 to 200 mg.kg<sup>-1</sup>). Modeling of the kinetic DGT  
19 (diffusive gradient in thin film) measurements revealed fast and slow copper resupply from the solid  
20 phase for sediment formulated with and without peat, respectively.

21 *M. aquaticum* proved to be sensitive to copper, as the copper accumulation and growth differed  
22 depending on the sediment composition and copper concentration. Comparing the copper  
23 accumulation in *M. aquaticum* to total dissolved, free and C<sub>DGT</sub> (concentration in solution derived  
24 from DGT measurements) copper concentrations revealed that C<sub>DGT</sub> concentrations were a better  
25 predictor of the accumulation than the others. However, the relatively weak correlation observed  
26 ( $r^2=0.6$ ) and the fact that plant uptake does not increase proportionally to fluxes to DGT suggest that  
27 copper uptake in plants was not diffusion limited. Thus, the free copper concentrations near the root  
28 surface were sufficient to meet the plant's demand during the experiment. Furthermore, labile  
29 complexes that continuously resupply the Cu<sup>2+</sup> pool may also contribute to the concentrations available  
30 for plant uptake. In the range of copper concentrations investigated here, saturation of uptake  
31 processes as well as toxicity are considered responsible for the poor DGT prediction of plant uptake.

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33 Keywords: Bioavailability, *Myriophyllum aquaticum*, Diffusive gradient in thin film, Copper,  
34 Artificial sediment.

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## 36 Introduction

37 Sediments have been recognized as a major sink of metals in aquatic systems, and the ultimate  
38 concern of such metal contamination in sediments is the impact of these metals on aquatic organisms.

39 It is now well known that the total content of contaminants is not a good predictor for risk assessment  
40 as not all fractions are available to organisms [1]. Speciation and bioavailability are necessary  
41 conditions for assessing and predicting the food chain transfer and phytotoxicity of contaminants and  
42 their potentially harmful effects. The bioavailability of a compound can be assessed by biological  
43 methods (bioassay, bioaccumulation) and/or chemical measurements.

44 Different procedures for biological tests on sediment can be used. Tests can be performed on the  
45 elutriate phase, the interstitial water or the whole sediment; the latter represents a more realistic type of  
46 exposure that can be simulated in the laboratory [2]. Among aquatic organisms, the widely used for  
47 bioassays on the whole sediment were nematodes (*Caenorhabditis elegans*), bacteria (*Arthrobacter*  
48 *globiformis*), fish embryos (*Danio rerio*), oligochaetes (*Tubifex tubifex*) and midge (*Chironomus*  
49 *tentans*) [3, 4]. Because macrophytes are considered indicators of water pollution at the same level as  
50 fish and macrobenthos, the integration of a primary producer (plant) that is rooted in the sediment in a  
51 test battery is desirable (European Water Framework Directive; EU-WRRL 2000). Therefore, a new  
52 sediment contact test with the *Myriophyllum aquaticum* (water milfoil) has been developed and will be  
53 normalized [5]. *M. aquaticum* is a submerging plant that extracts nutrients from sediment. The growth  
54 of the *Myriophyllum aquaticum* is affected by the origin of the sediment and the presence of toxicants  
55 [5]. *M. aquaticum* has also been used for studies of the bioaccumulation of heavy metals [6, 7] on  
56 plants collected in situ and plants grown hydroponically. Recently, *M. aquaticum* has been used in  
57 standardized sediment contact tests, and a strong correlation was found between toxic effects and  
58 metal sediment concentration [8].

59 Metal bioavailability can be estimated by various chemical and physico-chemical methods. In  
60 particular, Zhang and coworkers developed the so-called “diffusive gradient in thin films” (DGT)  
61 technique that is expected to mimic bioaccumulation processes and has been adapted for assessing  
62 metal bioavailability in soil and sediments [9-11]. DGT is a priori well adapted for non-equilibrium  
63 conditions where the bioavailability of metals is mainly controlled by the kinetic resupply of free  
64 metals in a solution from solid phase labile pools. Indeed, similar to a plant root, DGT induces a local  
65 decrease in the concentration of metal at the sediment/DGT interface, and metals bound to the  
66 sediment particles may be released in the interstitial water and become available [12]. To improve the  
67 interpretation of DGT experiments, the use of a conceptual model with sorption/desorption and  
68 diffusion reactions in the sediment is required. Two dynamic models have been developed; these  
69 models are known as DGT-Induced Fluxes in Soils and Sediments (1D-DIFS) [13] and DGT-  
70 PRObabilistic Fitting of parameters for Soils and Sediments (DGT-PROFS) [14]. The DGT-PROFS  
71 model has been developed to enhance the DIFS model for interpreting DGT kinetics experiments

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3 72 performed on formulated sediments [14, 15]. Previously, the link between DGT measurements and the  
4 73 bioaccumulation in plants showed contrasting results [9]. A strong correlation has been found between  
5 74 DGT-labile copper in various soils and copper accumulation in a terrestrial plant (*Lepidium*  
6 75 *heterophyllum*)[1]. However, DGT accurately predicted the bioavailability of Zn and Cu in spinach  
7 76 and ryegrass only at nontoxic concentrations [16]. Indeed, in cases of toxicity, plant absorption will be  
8 77 disturbed, whereas the DGT measurement will not be affected.

9 78 The aim of our study was to assess the relationships between speciation (given by different  
10 79 approaches, including DGT probes), bioaccumulation and toxic effects on *M. aquaticum*. Copper,  
11 80 considered a potentially extremely toxic threat to aquatic biota [17], was selected for this study  
12 81 because few studies have been performed comparing plant accumulation of copper and DGT  
13 82 measurements [9]. Therefore, we performed a set of experiments in parallel coupling bioassay and  
14 83 DGT approaches to compare the "real bioavailability" measured by the plant and the "potential  
15 84 bioavailability" estimated by the DGT tool.

16 85 Considering that the speciation of elements and therefore their bioavailability are linked to the solution  
17 86 and particulate phase compositions, we investigated the behavior of copper on formulated sediments  
18 87 of different compositions to reduce the complexity of the system. The sediment composition varied by  
19 88 (i) the addition of different components having more or less strong affinities toward copper (peat, iron  
20 89 oxides...) and (ii) the sediment copper spiking concentrations. Furthermore, the EDTA that is used in  
21 90 the standard solution for the bioassay (*M. aquaticum*) was investigated at different concentrations to  
22 91 assess its impact on copper bioavailability.  
23 92

## 24 93 **1. Material and Methods**

### 25 94 ***1.1 Sediment and solution composition***

26 95 Solid phase composition: Four different artificial sediments were prepared according to a slightly  
27 96 modified protocol of the standard OECD 207-218 protocol. The first sediment, named the "basic  
28 97 sediment" (SB), is formulated as follows: 77% acid-washed sand, 22% kaolinite clay, and 1% calcite.  
29 98 The other formulated sediments differed from the SB composition by the presence or absence of iron  
30 99 oxides or by the presence or absence of peat. The second sediment called SG corresponds to the basic  
31 100 sediment coated with 2 g.kg<sup>-1</sup> goethite. The third sediment named SP corresponds to the basic  
32 101 sediment amended with 5% peat (equivalent to the so-called standard OECD 207-218). The fourth  
33 102 sediment named SPG and is composed of both peat and goethite.

34 103 The quartz and clay were obtained from VWR-France, and the crushed calcite was purchased from La  
35 104 Marchande de Couleurs, (Lyon-France). The peat is composed of blond sphagnum and was purchased  
36 105 from ASB Grünland Helmut Aurenz GmbH (Porschestr.4, D-71634 Ludwigsburg, Germany). The iron  
37 106 oxides were synthesized according to the procedure described in [18].

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3 107 Solution composition: The solution used for the humidification of the sediment is the nutrient solution  
4 108 recommended for the sediment contact test in the standard for the *M. aquaticum* bioassay (Steinberg  
5 109 revised medium, ISO 20079). This nutrient solution is composed of the following ( $\mu\text{M}$ ):  $\text{KNO}_3$  (3462),  
6 110  $\text{Ca}(\text{NO}_3)_2$  (1866),  $\text{KH}_2\text{PO}_4$  (662),  $\text{K}_2\text{HPO}_4$  (75),  $\text{MgSO}_4$  (67),  $\text{H}_3\text{BO}_3$  (2),  $\text{FeCl}_3$  (5),  $\text{MnCl}_2$  (2),  $\text{ZnSO}_4$   
7 111 (1),  $\text{Na}_2\text{MoO}_4$  (0.2) and EDTA (4.5). Because EDTA is known to be a complexing agent for metals,  
8 112 we evaluated the impact of this compound on copper availability by varying the EDTA concentration  
9 113 (0 or 0.45  $\mu\text{M}$ ). A “(E)” was added to the name of the sediment to denote the addition of EDTA.

10 114 Preparation of the sediments: The dry components of the sediment were mixed and humidified with  
11 115 the nutrient solution for 4 hours. The sediments were then spiked with copper by the addition of a  
12 116  $\text{Cu}(\text{NO}_3)_2$  solution to obtain four different copper-sediment concentrations: 5, 15, 50 and 200  $\text{mg}\cdot\text{kg}^{-1}$   
13 117 (values added to the name of the sediment; see below). After the metal addition, the sediments were  
14 118 agitated for 24 hours, and the pH was regularly checked and adjusted if necessary. The sediments were  
15 119 distributed into 15 ml beakers for the bioassay and into 110 ml beakers for the DGT experiments; the  
16 120 sediments were then equilibrated for 7 days at a controlled temperature ( $24 \pm 0.5$   $^\circ\text{C}$ ). Only the  
17 121 combinations of some of the different components of the sediment were tested: SB5, SB15, SB50,  
18 122 SB50(E); SP5, SP15, SP50, SP50(E), SP200(E); SG50, SG50(E), SG200(E); SPG50, SPG50(E) and  
19 123 SPG200(E).

## 20 124 *1.2 Myriophyllum aquaticum experimental protocol*

### 21 125 Plant material and bioassay experiment

22 126 The stock culture of *Myriophyllum aquaticum* was obtained from the UFZ institute (Hemholtz  
23 127 Zentrum Für Umweltforschung). During the culture period before the bioassay, the plants were grown  
24 128 in an artificial sediment formulated following the OECD 207-218 protocol and saturated with the  
25 129 Steinberg medium (DIN EN ISO 20079).

26 130 Prior to the start the bioassay experiment, plants whorls were selected on the basis of their fresh  
27 131 biomass ( $25 \pm 6$  mg) by cutting whorls from 21-day-old plants. Each whorl was then placed into a  
28 132 beaker containing 15 ml of sediments. For each test, 25 whorls were exposed to copper-contaminated  
29 133 sediments, and 10 whorls were exposed to the control sediment (without copper). All tests were  
30 134 performed in a growth chamber (exposure light/dark cycle, 16/8 h at  $24 \pm 1$   $^\circ\text{C}$  at a light intensity of  
31 135  $60\text{-}75$   $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ). The pots were irrigated every 48 h with the semi-concentrated Steinberg medium  
32 136 ISO 20079 (draft ISO/DIS 16191). After 14 days of exposure, the plants were harvested and washed in  
33 137 ultrapure water, washed with an EDTA solution (1 mM) and finally rinsed three times with ultrapure  
34 138 water [19]. The final fresh weight of each plant was recorded. The pH of the sediments was measured  
35 139 at the beginning and the end of the experiment. After pooling several beakers, the interstitial water was  
36 140 recovered by centrifugation of the sediments (3500 rpm, 20 min), filtered at 0.45  $\mu\text{m}$  and acidified  
37 141 with 1%  $\text{HNO}_3$  (suprapur).

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5 143 **Effect (toxicity) assessment:**

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7 144 The growth rate ( $GR_C$  for control and  $GR_T$  for test) during the exposure period was calculated from the  
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9 145 initial and final weight ( $M_0$  and  $M_{final}$ , respectively) of each fresh whorl (eq. 1). This parameter has  
10 146 been shown to be the most suitable for the test due to the low coefficient of variation of this parameter  
11 147 [5]. The criterion of toxicity was expressed as the percentage of inhibition and is given by (eq. 2).

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$$GR_C \text{ or } GR_T = (\ln M_{final} - \ln M_0) / \Delta t \quad (\text{eq. 1})$$

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$$I (\%) = (GR_C - GR_T) / GR_C * 100 \quad (\text{eq. 2})$$

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21 153 **Copper accumulation:**

22 154 After harvesting, the plants (i.e., roots and leaves) were dried at 60 °C until a constant weight was  
23 155 reached. To have sufficient biomass for analysis, the plants were pooled (eight per pool) to obtain  
24 156 three replicates per experiment. Samples were digested in a 5:1 ratio of  $HNO_3$  and  $H_2O_2$  in a  
25 157 microwave oven according to a similar protocol to this of Bervoets and co-workers [20] and the  
26 158 copper concentration in the digests were measured by ICP-OES or SAA furnace.

27 159 In addition, blanks were used, and a reference material (BCR60) was used to control the efficiency of  
28 160 the mineralization.

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3435 162 ***1.3 DGT kinetic experimental protocol***

36 163 DGT kinetic experiments were performed using DGT units (piston-type with a 2-cm-diameter window  
37 164 loaded with a Chelex-100 resin and with an open pore gel (0.76 mm) purchased from DGT Research  
38 165 (Lancaster, UK; <http://www.dgtresearch.com>)). The DGT units were gently pressed into plastic  
39 166 beakers containing 120 ml of sediment until the shoulder of the piston was in contact with the surface  
40 167 of the sediment housing. The devices were retrieved from the sediments at different contact times: 4,  
41 168 8, 24, 96 hours and 14 days. Three replicates were performed for each time. After retrieval, the DGT  
42 169 units were thoroughly rinsed with ultrapure water and stored at 4 °C before the elution of the DGT  
43 170 resins with 5 mL of 1 M  $HNO_3$  (Merck, suprapur). For each contact, the pore waters of each beaker  
44 171 were extracted following the same procedure as that for bioassay experiments. pH and Eh  
45 172 measurements in the sediment were performed each time.

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173 ***1.4 Modeling***174 **DGT-PROFS modeling**

175 The accumulation kinetics of metals to DGT were interpreted using the DGT-PROFS model, which is  
176 described in detail in [14]. Only the main features of this model are provided here. Briefly, the DGT-  
177 PROFS model assumes that trace metals in sediments can be distributed between three separate  
178 phases: pore water (where speciation influences the diffusion coefficient value) and weak and strong  
179 sorption sites on the particulate phase. The accumulation of metals on DGT is governed by the  
180 adsorption-desorption kinetics and the diffusion of metals within the pore water of the sediment and  
181 the gel. Only labile metals (free metals and small inorganic complexes able to dissociate in the gel) are  
182 assumed to diffuse in the DGT gel. Thus, diffusion of the different metal species through the sediment  
183 and gel layer (i.e., free, inorganic and humic species) generates ‘apparent’ diffusion coefficients in the  
184 sediment and in the diffusive gel lower than those of the free metals [21]. The diffusion coefficient can  
185 range between a minimum value, corresponding to humic complexes, and a maximum value,  
186 corresponding to free ions. For DGT-experimented sediment, the DGT-PROFS model was allowed to  
187 fit the following parameters:  $\alpha_{\text{weak}}$ , the proportion of particulate metal associated to weak sites before  
188 DGT deployment;  $k'_{\text{des1}}$ , the desorption rate from weak particulate sites;  $k'_{\text{des2}}$ , the desorption rate from  
189 strong particulate sites; and  $D_{\text{sed}}$ , the diffusion coefficient of metals in sediment. These parameters can  
190 be obtained by fitting the DGT experimental measurements using a probabilistic approach, which  
191 represents parameters by probability density functions (PDFs) (i.e., with indications of their  
192 uncertainty) instead of single values [14].

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3 196 **2. Results**  
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5 197 **2.1 Physico-chemical characteristics of the different sediments:**  
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7 198 The variation of the total pore-water copper concentration ( $C_{(iw)}$ ) over the duration of a given test  
8 199 ranged between 3% to 10% except in the cases of SG50, SG50(E) and SG200(E), which had variations  
9 200 of 13, 14 and 19%, respectively. These features allow us to consider the equilibration with the solid  
10 201 phase to be complete after a sediment incubation time of 7 days (Table 1).

11 202 The average values of the ( $C_{(iw)}$ ) varied from 14.4  $\mu\text{g.l}^{-1}$  to 725  $\mu\text{g.l}^{-1}$  for the DGT experiments and  
12 203 from 24 to 1510  $\mu\text{g.l}^{-1}$  for the bioassays (Table 1). Generally, for the same composition of sediment,  
13 204 copper pore-water concentrations are higher in pots containing plants than in pots containing DGT.  
14 205 This result can mainly be explained by the higher evaporation due to the smaller and less hermetic  
15 206 pots used for the plant exposure. Indeed, a decrease in the supernatant water thickness was observed in  
16 207 the bioassay pots, which was partly compensated by using irrigation as recommended by the protocol  
17 208 (draft ISO/DIS 16191). According to the sediment composition, the lowest  $Cu_{iw}$  concentrations were  
18 209 found in sediments coated with goethite, and higher concentrations were found in sediments  
19 210 formulated with peat. Sediments formulated with both goethite and peat show intermediate values.  
20 211 The presence of EDTA in the sediment results in much higher  $Cu_{iw}$  concentrations compared to tests  
21 212 without EDTA.

22 213 The pH values ranged from 5.76 to 7.17 and from 5.17 to 7.22 in the different sediments tested in  
23 214 beakers with DGT probes and *M. aquaticum*, respectively (Table S1 Supporting Information). The  
24 215 lowest pH values were observed in the sediments formulated with peat; these values are in agreement  
25 216 with the naturally low pH of peat and its good buffering capacity against pH changes, which makes it  
26 217 difficult to obtain a pH around neutrality. The pH did not vary significantly during a given experiment  
27 218 ( $\leq 0.1$  unit pH). The redox potential shows that all the experiments were performed under oxic  
28 219 conditions (Table S1 Supporting Information). The DOC concentrations in the "SP" sediments are  
29 220 very high and show that a part of the peat was dissolved and solubilized in pore water. In the case of  
30 221 the sediment composed of organic matter and goethite, the DOC concentration is slightly lower than in  
31 222 "SP"; we can suppose that a part of the dissolved carbon was fixed on oxides. Finally, in the bioassay  
32 223 experiment, there is an increase in the DOC concentrations, which is certainly related to the  
33 224 evaporation process. The copper distribution between the solid phase and the pore water can be  
34 225 assessed by a partition coefficient ( $K_d$ ). For the DGT experiment, the  $K_d$  values (Table 1) are on the  
35 226 same order of magnitude in the different sediments, with values ranging from 100 to 500 except for  
36 227 GS-50, for which there is a significant increase in the  $K_d$  value up to around 2900. In the presence of  
37 228 EDTA,  $K_d$  exhibited no marked differences.

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## 2.2 *Sediment contact test with *Myriophyllum aquaticum**

The growth rates of *Myriophyllum aquaticum* (Figure 1) exposed to control sediments agree with the standard for this bioassay (growth rate  $\geq 0.09 \text{ day}^{-1}$ ), which demonstrates the applicability of the bioassay in sediments with different physico-chemical characteristics. For the different control sediments, lower growth rates are observed in the presence of iron oxide (SG and SPG) when compared to the basic sediment (SB). Conversely, the highest growth rate was observed for the sediment formulated with peat (SP - OECD reference sediment), which is in agreement with the results obtained by [5]. Furthermore, the growth rates for the control sediments containing EDTA were systematically higher than those in the control sediments without EDTA. This variation in growth rates leads to a strong variation in the increase of plant biomass over the 14 days of exposure. For example, with the control plants exposed to the sediments SPG and SP, the biomass increased by approximately 250% and 600%, respectively. It was observed that growth (rate) inhibition (I (%)) depends on the copper concentration for a given sediment and on the sediment composition (Figure 1). The inhibition range from 6 to 20% for the sediment containing peat (OECD reference sediment) spiked from 5 to 50  $\text{mgCu.kg}^{-1}$  (SP5, SP15, SP50), and that from 23 to 34% for the basic sediment spiked from 5 to 50  $\text{mgCu.kg}^{-1}$  (SB5, SB15, SB50). The presence of EDTA induced an inhibition ranging from 16 to 100%. For the different sediments formulated without EDTA and spiked at 50  $\text{mg.kg}^{-1}$ , there is a higher toxicity in the sediments formulated without peat, SB and SG (I=34 and 32%, respectively), than in the peat-containing sediments, SP and SPG (I=25 and 15%, respectively). The increase in plant biomass for the spiked sediments SG200E and SP15 was 4 and 570%, respectively, which demonstrated the wide range of toxic effects in the sediments tested.

## 2.3 *Copper internalized concentration in *M. aquaticum**

Similar to the growth inhibition, the copper bioaccumulation in *M. aquaticum* depends on the copper concentration and the sediment composition (Table 1). For example, when comparing the basic sediment spiked with either 5 or 50  $\text{mg Cu.kg}^{-1}$  (SB5 and SB50), the Cu accumulation in plants was observed to increase from 44 to 124  $\mu\text{g.g}^{-1}$ . For sediments formulated with iron oxide or peat, the bioaccumulated copper concentrations varied from 72, 32 and 29  $\mu\text{g.g}^{-1}$  for SP50, SG50 and SPG50, respectively.

The presence of EDTA homogenizes the copper bioaccumulation among sediments with concentrations of approximately 60  $\mu\text{g.g}^{-1}$ .

## 2.4 *Copper Flux to DGT*

Sediment composition has a strong influence on copper fluxes as indicated by the comparison of the measured fluxes of copper to the DGT resin (expressed as  $\text{mg.cm}^{-2}.\text{s}^{-1}$ ) over the duration of the test for four of the different formulated sediments tested (Figure 2). For the four experiments performed without EDTA, the highest fluxes to DGT were obtained in the basic sediment spiked with 50 mg

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3 266 (Cu).kg<sup>-1</sup> (SB50), and significantly lower fluxes were observed in the other sediments formulated with  
4 267 peat and goethite and spiked with 50 mg (Cu).kg<sup>-1</sup> (SG50, SP50 and SPG50). In addition, two curves  
5 268 in the fluxes are observed depending on the composition of the sediment: (i) a rapid decrease of the  
6 269 flux during the first 96 hours of deployment followed by a continuous decrease with a lower slope  
7 270 during the rest of the test and (ii) a decrease of the flux during the initial hours followed by a  
8 271 stabilization of the flux from 24 h to the end of the experiment. The mean and the 5<sup>th</sup> and 95<sup>th</sup>  
9 272 percentile simulated fluxes using the DGT-PROFS model are also given in the Figure 2. The good  
10 273 agreement between the simulated and measured fluxes enhances the relevance of the modeling and  
11 274 fitting procedure.

12 275 The fluxes of copper to DGT for the other sediments are available in the supporting information  
13 276 (Figure S1). Briefly, the shape of the fluxes remains the same for each sediment composition  
14 277 regardless of the variation of total copper concentrations or the addition of EDTA. However, an  
15 278 increase in the spiking concentration as well as the addition of EDTA leads to an increase in the flux  
16 279 values. The SB50 (E) and SG50 (E) experiments were not simulated because too much uncertainty  
17 280 exists for certain data.

### 281 3. Discussion

282 One aim of the study was the comparison of the copper accumulation in plants and in the passive  
283 sampler DGT. However, due to the different constraints of the exposure conditions between the  
284 bioassay and DGT experiments, the physico-chemical conditions diverged for a given type of  
285 sediment. It was therefore necessary to correct the DGT data to better mimic the conditions of the  
286 bioassay. For this purpose, assuming that the key parameters  $\alpha_{\text{weak}}$ ,  $k_{\text{des1}}$ ,  $k_{\text{des2}}$ , and  $D_{\text{sed}}$  obtained from  
287 the DGT-PROFS modeling were constant for a given test, the copper pore-water concentration for a  
288 given bioassay was used to simulate the corresponding accumulation in DGT under this slightly  
289 modified condition (designated as corrected values hereafter).

#### 290 *3-1 Copper lability and resupply from solid phase*

291  
292 Figure 3 presents the theoretical DGT fluxes relative to the solution concentration (using DIFS  
293 modeling) as a function of the  $K_d$  for fast (full line,  $T_c$  10 s) and slow desorptions ( $T_c$  1000 s)  
294 obtained from Degryse and co-workers [9] for DGT sampling at 24 h, in which the data of the present  
295 study were added. Three different trends can be observed according to the kinetic characteristics: slow,  
296 intermediate and fast kinetics of desorption. Fast desorption kinetics were obtained for sediments  
297 composed without peat, and slow desorption kinetics were obtained for sediments containing peat.  
298 However, as the DIFS model considers all complexes in the solution to be fully labile, the resupply  
299 from the solid phase may be underestimated in the presence of complexes in the solution that are not  
300 fully labile as copper binds to organic matter. Therefore, the desorption kinetics from sediments

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3 301 containing peat may be underestimated. Indeed, this representation gives information on the solid  
4 302 phase behavior for a given time (24 h) and does not take into account its evolution over the time of the  
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6 303 experiment. Therefore, the description of the fluxes' evolution over time gives supplementary  
7  
8 304 information.

9 305 Two different trends of the fluxes of copper to the DGT were observed for the different sediments  
10 306 tested: constant and decreasing fluxes (Figure 2 and Figure S1 in Supporting Information). Harper and  
11 307 co-workers [13] proposed a classification of fluxes to quantify the "copper lability" according to four  
12 308 typical cases. In our study, we observed the "partially sustained case" for all sediments containing peat  
13 309 (SP and SPG) and the "non-steady state case" for all basic sediments and for the sediments coated  
14 310 with goethite (SB and SG). For sediments belonging to the partially sustained case, the desorption  
15 311 capacity of the metal from the solid phase is sufficient to maintain a constant flux of copper to the  
16 312 DGT device (at least over the time experiment). For sediments belonging to the non-steady state case,  
17 313 the copper flux to the DGT decreases over time, indicating that the resupply from the solid phase is  
18 314 slow. Considering the intensity of the fluxes, significantly high fluxes during the 24 first hours were  
19 315 obtained in the basic sediments. The basic sediments are principally composed of quartz and kaolinite.  
20 316 We can therefore assume that the high initial flux is due to a high initial concentration of the available  
21 317 copper in solution ( $C_{iw}$ ). The following decline of the fluxes may be related to a slow resupply due to  
22 318 copper adsorption to strong bonding-energy sites (kaolinite) [22]. In the case of the sediments coated  
23 319 with goethite, similar curves were observed but with significantly lower fluxes. Indeed, it is well  
24 320 known that iron oxides have a high affinity for metals and that the addition of iron increases the  
25 321 fraction of copper associated to strong sites. As the pH of the sediment is lower than the zero point  
26 322 charge of goethite (7.9), goethite is positively charged [18]. Under these conditions, copper adsorption  
27 323 on goethite results from the formation of inner-sphere complexes (chemisorptions), leading to a strong  
28 324 fixation. The sediments containing peat also lead to significantly lower fluxes than in the basic  
29 325 sediment. The lower values of fluxes can be related to a lower available pool of copper in the system.  
30 326 Moreover, these lower values are also due to a lower velocity of organic-copper complexes in the pore  
31 327 water ( $D_{sed}$ ) and the fact that organo-metallic complexes composed of fulvic or humic acids are only  
32 328 partially labile [23]. The slight decrease of the fluxes followed by a rapid stabilization over time  
33 329 suggests that the peat have the ability to resupply copper easily to the pore water. Concerning the other  
34 330 set of experiments in the supporting Information, the same behavior is observed for the same sediment  
35 331 composition with higher fluxes in the presence of EDTA and for increasing copper spiking  
36 332 concentrations related to higher available pools of dissolved copper in the sediments.

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52 333 All of these qualitative features are confirmed by the values of the DGT-PROFS model parameters  
53 334 that were fitted for each experiment (Table S2 in Supporting Information). The  $\alpha_{weak}$  parameter, which  
54 335 represents the proportion of particulate metal associated to weak sites, is much lower for the SG50  
55 336 sediment (0.06) than for the SP50 or SPG50 sediments (0.37 and 0.2), which confirms that the ability  
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58 337 to resupply copper to the water phase is lower with iron oxide addition. The lowest value of the  $D_{sed}$   
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3 338 parameter, which represents the diffusion coefficient of metal in sediment, was obtained for the SP50  
4 339 sediment ( $2.2 \cdot 10^{-6} \text{ cm}^2 \cdot \text{s}^{-1}$  compared to  $6.2 \cdot 10^{-6} \text{ cm}^2 \cdot \text{s}^{-1}$  for the SB50 sediment), which confirms that the  
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6 340 presence of organic matter in the pore water significantly decreases the diffusion velocity and the  
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8 341 resupply potential at the water-DGT interface.

### 9 10 342 ***3-2 Comparison of different tools to assess copper bioavailability***

11 343 To relate the "actual bioavailability" measured by the plant *M. aquaticum* to the "potential  
12 344 bioavailability" estimated by different chemical measurements in the sediment, including DGT  
13 345 measurements, we first investigated whether a significant linear correlation could be observed between  
14 346 the copper concentration in plant and (i) the total dissolved copper concentration, (ii) the free copper  
15 347 concentration (iii) and the mean labile concentration derived from DGT ( $C_{\text{DGT}}$ ) in the sediment (Figure  
16 348 5). The comparison between the total dissolved copper concentrations with the bioaccumulation in the  
17 349 *M. aquaticum* results in a poor correlation, with a  $R^2$  of 0.05 indicating that the total dissolved  
18 350 concentration does not represent the readily bioavailable fraction for plants. The free copper  
19 351 concentration in the pore water was obtained for each sediment by using the speciation program  
20 352 WHAM VI [24] (details in Table S3 in Supporting Information). The comparison between the free  
21 353 copper concentrations and the copper concentration in plant also showed a poor correlation, with a  $R^2$   
22 354 of 0.08. Finally, the comparison between the DGT measurement and the copper concentration in  
23 355 plants resulted in an improved, but still poor, correlation, with a  $R^2$  of 0.6 (Figure 3).

24 356 One reason for the above poor correlations might be the presence of growth inhibition in the sediments  
25 357 tested depending on the composition and copper content of the sediments (Figures 1 and 4). Indeed, in  
26 358 the literature, good correlations between the DGT-measured labile copper and the accumulation in  
27 359 some plants were obtained only in the cases of plants exposed to non-toxic concentrations [9, 16, 25].  
28 360 Assuming that the growth rate is maximal for low copper concentrations until a threshold where toxic  
29 361 effects arise and that a zero growth rate is reached at high copper concentrations, a theoretical relation  
30 362 between the DGT labile concentrations and the growth rate can be drawn (Figure 4; mathematical  
31 363 relation (Eq. S1) in supporting information). This predicted relation between the DGT and the growth  
32 364 rate agrees with our experimental results (Figure 4) and shows that all the sediments tested are in the  
33 365 range of concentrations inducing a toxic effect on the plant, with high growth rate variations. Indeed,  
34 366 *M. aquaticum* growth began to decrease when the DGT was above  $92 \mu\text{M}$ . However, there is a high  
35 367 variation in the DGT and growth rate measurements between the different sediments tested, which is  
36 368 certainly related to the difference in the copper uptake by the plants according to the toxicity of the  
37 369 sediment compositions.

38 370 The relationship between the plant uptake and the fluxes recorded by the DGT can help to determine  
39 371 the ability of DGT to mimic the plant uptake. Degryse and co-workers [9], reported such relationships  
40 372 for the uptake of Zn and Cd by watercress and spinach, respectively (Figure 5). Plotting our results for  
41 373 copper in the case of *M. aquaticum* on Figure 5 shows that the plant uptake fluxes do not increase in a

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3 374 directly proportional manner with DGT fluxes, i.e., a 10-fold increase in DGT flux does not result in a  
4 375 10-fold increase in plant uptake fluxes or plant concentrations. Considering that DGT mimics the  
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6 376 action of the plant only under diffusion-limited conditions, the absence of correlation suggests that Cu  
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8 377 uptake is not diffusion-limited in our case. If there is no diffusion limitation, labile complexes (that are  
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10 378 not taken up) will not contribute to the plant uptake, but the uptake will be governed by the free metal  
11 379 ion activity and the activity of competing ions (e.g.,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{H}^{+}$ ; cf. BLM concept) [9]. In the  
12 380 present study, the free Cu ( $\text{Cu}^{2+}$ ) was low, especially in the presence of peat (Table 1). The apparently  
13 381 low  $\text{Cu}^{2+}$  should promote conditions for a diffusion limitation that are not observed. In fact, there is  
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15 382 most likely a large contribution of labile complexes leading to a continuous availability of sufficient  
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17 383  $\text{Cu}^{2+}$  for the plant uptake, which results in no diffusion limitation conditions.  
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19 384 Moreover, this study showed that in the wide range of sediments tested, the normalized bioassay was  
20 385 sensitive to copper and was able to accumulate it. These data provide support for other studies that  
21 386 have shown a strong sensitivity of *M. aquaticum* toward sediments contaminated with metals [8]. Until  
22 387 now, the *M. aquaticum* was commonly used in toxicity tests on pesticides [26, 27]; thus, these results  
23 388 show the interest of this new bioassay for the assessment of risks associated with metal contamination  
24 389 and the need to go further in the knowledge of its sensitivity towards metals. The recently normalized  
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26 390 sediment biotest using *M. aquaticum* appears to be a sensitive tool for the assessment of copper-  
27 391 contaminated sediments; however, further research is needed to assess this test's sensitivity toward  
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29 392 other metals.  
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### 32 393 **Supporting Information.**

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35 394 Details on physico-chemical characteristics of pore water, flux of copper to DGT versus time and  
36 395 modeling using the DGT PROFS model, DGT PROFS model parameters, WHAM VI modeling of the  
37 396 different sediments investigated and on the plant growth rate modeling.  
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Sediment	C <sub>(iw)</sub> "DGT" ( $\mu\text{g}\cdot\text{L}^{-1}$ )	C <sub>(iw)</sub> "bioassay" ( $\mu\text{g}\cdot\text{L}^{-1}$ )	Cu <sup>2+</sup> "DGT" ( $\mu\text{g}\cdot\text{L}^{-1}$ )	Cu bioaccumulated ( $\mu\text{g}\cdot\text{g}^{-1}$ )
SB- 5	44 ± 2	58 ± 4.6	3,7E-02	44 ± 4
SB- 15	40 ± 3	60 ± 4	2,5E-02	49 ± 3
SB- 50	96.4 ± 10.1	157*	2,6E+00	124 ± 8
SP-5	14,4 ± 1	34*	4,1E-06	50 ± 8
SP-15	38 ± 0,6	50 ± 1	1,4E-04	71 ± 3
SP-50	104.1 ± 6.8	144.2*	2,2E-03	72 ± 6
SG-50	17.3 ± 2.3	23.9*	1,9E-01	32 ± 5
SPG-50	83.5 ± 2.9	84.9*	7,2E-04	29 ± 5
SB-50(E)	236 ± 18	275 ± 5	5,5E-02	61.3 ± 11
SP-50(E)	295 ± 8.1	560 ± 9	2,8E-03	54.7 ± 5
SG-50 (E)	148 ± 21.4	190*	4,1E-02	63.9 ± 22
SPG-50 (E)	234 ± 14.2	402*	4,8E-03	52 ± 8
SP-200(E)	620 ± 25.7	1510 ± 61	1,5E-01	88 ± 11
SG-200 (E)	725 ± 141	299 ± 2	8,3E+01	163 ± 14
SPG-200 (E)	478 ± 30	510 ± 7	8,5E-02	60 ± 12

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489 Table 1: Copper pore-water concentrations (C<sub>(iw)</sub>;  $\mu\text{g}\cdot\text{L}^{-1}$ ) in DGT and bioassay experiment, free copper  
490 concentration (Cu<sup>2+</sup>;  $\mu\text{g}\cdot\text{L}^{-1}$ ) and copper accumulated in *M. aquaticum* in the different sediments ( $\mu\text{g}\cdot\text{g}^{-1}$ )  
491 for the different sediments studied (see text for nomenclature). "DGT" stands for DGT experiment,  
492 and "Bioassay": stands for bioassay experiment. Cu<sup>2+</sup> was calculated with WHAM VI (Tipping (24),  
493 see details in the Table S2 in Supporting Information). The values of experimental data represent  
494 average values: for all the DGT n=15; for the bioassay experiment, there are less replicates because  
495 the measurements was only performed at the end of the experiment; for Cu<sub>(iw)</sub>, n=2 or n=1 (\*) and, for  
496 bioaccumulation, n=3.

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### Figure Captions

**Figure 1** : Growth rate ( $\text{days}^{-1}$ ) and growth inhibition (%) of *Myriophyllum aquaticum* after exposure to control and spiked sediments. “SB” corresponds to the basic sediment, “SG” to the sediment coated with goethite, “SP” to the sediment formulated with peat and “SPG” to the sediment formulated with peat and goethite. “E” represents sediments formulated with EDTA. The errors were calculated from  $n=10$  for controls and  $n=25$  for contaminated samples.

**Figure 2** : Copper fluxes resupply ( $\text{mg.cm}^{-2}.\text{s}^{-1}$ ) from particulate phases in sediments SB, SP, SG, SPG spiked at  $50 \text{ mg.kg}^{-1}$  (see text and Figure 1 for nomenclature) as a function of time. The replicates of the measured fluxes are represented by the three different open symbols. The full line represents mean flux calculated by the DGT PROFS model. The upper and lower dotted lines represent the 5<sup>th</sup> and 95<sup>th</sup> percentiles respectively.

**Figure 3** : Flux to DGT relative to copper pore-water concentration ( $F_{\text{DGT}}/C_{\text{iw}}$ ) as a function of  $K_d$  ( $\text{L.kg}^{-1}$ ) for all the sediments studied in the presence of peat (full triangle) or absence of peat (empty diamond); (see text and Figure.1 for nomenclature). The full and dotted lines represents theoretical data for slow (dotted line) and fast desorption kinetics obtained from<sup>8</sup>.

**Figure 4** : Relationship between growth rate and DGT copper concentrations ( $\mu\text{M}$ ) for all the sediments studied in the presence of peat (full triangle) or absence of peat (empty diamond); (see text and Figure.1 for nomenclature). The full line represents the theoretical relation between the corrected DGT concentration (see text) and the growth rate (mathematical equation (Eq. S1) is presented in Supporting Information) based on several assumptions developed in the text.

**Figure 5** : Relationship between fluxes to DGT ( $\text{pmol.cm}^{-2}.\text{s}^{-1}$ ; 14 days, corrected values; see text) and copper accumulation in plants ( $\text{mmol.kg}^{-1}$ ) for all the sediments studied in the presence of peat (full triangle) or absence of peat (empty diamond); (see text and Figure.1 for nomenclature). The full line represents the theoretical limiting diffusive flux. The dotted line and the black circles represent the theoretical plant demand and the theoretical plant uptake respectively<sup>8</sup>. The circles represent zinc uptake by watercress (*Lepidium sativum*) and DGT measurements determined for a range of soils sampled near galvanized structures<sup>26</sup>. The crosses represent cadmium uptake by spinach and DGT measurements at varying NaCl doses at background cadmium concentration or soils amended by Cd-salt<sup>27</sup>.

Figure 1

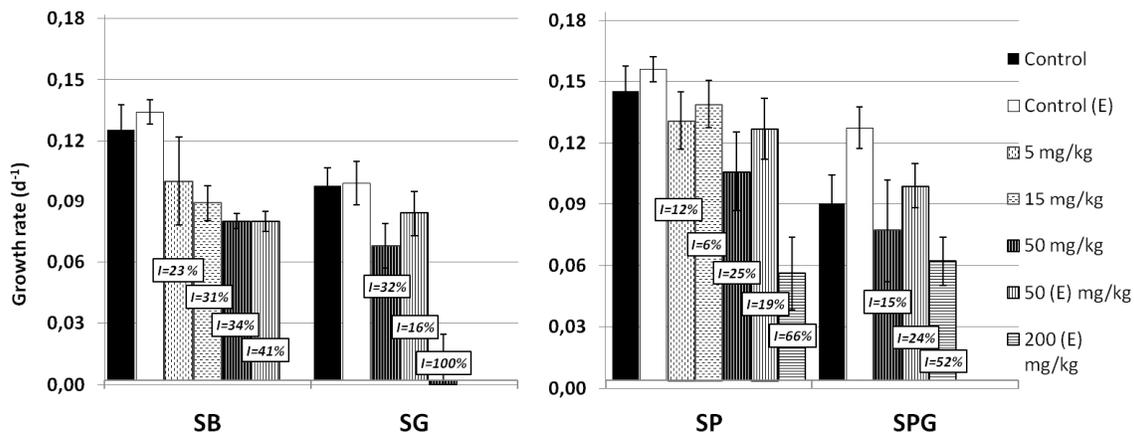
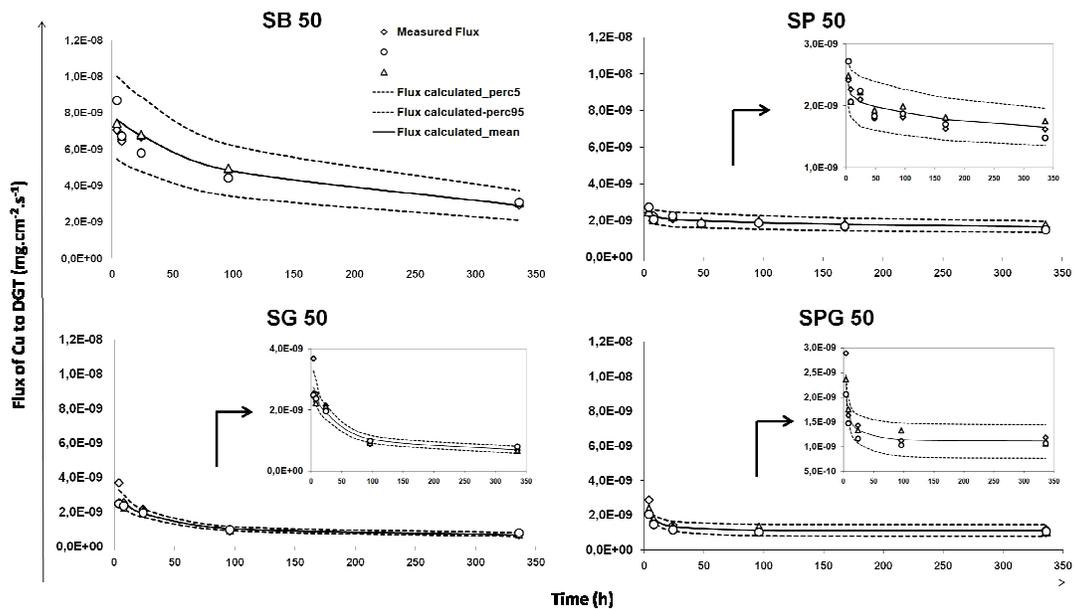


Figure 2



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Figure 5

