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**Chromium bioavailability in aquatic systems impacted by tannery wastewaters. Part 2: New insights from laboratory and in situ testing with *Chironomus riparius* Meigen (Diptera, Chironomidae)**

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2 **Chromium bioavailability in aquatic systems impacted by tannery wastewaters. Part 2:**  
3 **new insights from laboratory and *in situ* testing with *Chironomus riparius* Meigen**  
4 **(Diptera, Chironomidae)**

5

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51 **Abstract**

52 Chromium is widely used as a tanning agent and can become a contaminant of concern in  
53 aquatic ecosystems receiving discharges from industrial or artisanal tanning activities. In a  
54 companion study, we showed that Cr discharged by tanneries was bioavailable to indigenous  
55 chironomids with accumulation via sediment ingestion likely to represent the predominant  
56 exposure route. However, Cr accumulation by chironomids did not directly reflect the degree  
57 of sediment contamination and the potential adverse effects of Cr accumulation on  
58 chironomids were not evaluated. In the present study, chironomids were exposed to  
59 homogenised, field-collected sediments in the laboratory and to intact sediments in situ using  
60 a customised caging system. Chromium concentrations were assessed in sediments, exposed  
61 larvae of laboratory-reared *Chironomus riparius* and overlying waters of in situ cages.  
62 Experimental results of Cr bioaccumulation were compared with expected Cr body burden in  
63 chironomids calculated using biodynamic modelling. Our data provided strong support to the  
64 hypothesis that Cr bioaccumulation in the field is specifically controlled by the deposition of  
65 contaminated suspended particulate matter (SPM) containing a pool of Cr readily bioavailable  
66 to surface deposit feeders. Considering freshly deposited SPM as an additional route of  
67 exposure for surface deposit feeders leads to a good agreement between the modelling and  
68 experimental results. Additionally, a Cr body burden of about  $77 \mu\text{g g}^{-1}$  d.w was identified as  
69 a tentative threshold above which effects on the growth of *C. riparius* may appear. While both  
70 laboratory and in situ experiments provide evidence for the availability of Cr in aquatic  
71 system impacted by tannery wastewaters, standard laboratory exposure conditions may miss  
72 additional exposure routes in the field and underestimate possible adverse effects on benthic  
73 organisms.

74 **Keywords.** Chironomids; Bioaccumulation; Suspended matter; Body residue; Sediment;  
75 Reservoir

## 76 **1. Introduction**

77 Bioaccumulation and toxicity of trace elements strongly depend on their speciation and on the  
78 actual exposure conditions experienced by target organisms; e.g. continuous vs. discontinuous  
79 inputs, contaminant uptake via different exposure routes (water vs. food), biological traits  
80 (e.g. feeding habit, respiration, growth, reproduction) of the species of interest (Fairbrother et  
81 al., 2007). Standardized laboratory experiments cannot account for all these factors although  
82 their importance is well-recognized and their predictive ability can be improved by a careful  
83 characterization of the actual exposure conditions in the selected laboratory settings (Simpson  
84 and Batley, 2007). Similarly, pulse and chase experiments using radiolabelled elements allow  
85 the study of the relative importance of different exposure routes, notably water exposure vs.  
86 food ingestion, in a variety of organisms (Baumann and Fisher, 2011; Wang et al., 1997), but  
87 cannot reproduce the complex array of environmental factors that influences bioavailability  
88 and toxicity in situ. To address these problems, the scientific community has engaged in  
89 actively developing tools to narrow the gap between laboratory and field studies. At present,  
90 microcosm and in situ techniques are sufficiently mature and versatile to be of use as  
91 supporting studies in tiered risk assessment procedures (Burton Jr et al., 2012; Crane et al.,  
92 2007; Ferrari and Faburé, 2017; Ferrari et al., 2014). An appropriate integration between  
93 laboratory and in situ approaches can help to better understand the bioavailability and toxicity  
94 of specific contaminants in ecosystems impacted by complex mixtures of contaminants such  
95 as tannery effluents (Vignati et al., 2007).

96 Adverse biological effects on aquatic organism in tannery contaminated systems are well  
97 documented (Khwaja et al., 2001; Koukal et al., 2004; Leghouchi et al., 2009). However,  
98 tannery effluents have a very complex composition and specifically ascribing the observed  
99 effects to Cr, typically used as a tanning agent in its trivalent form, remains difficult.  
100 Understanding of Cr bioavailability and toxicity in the field is further complicated by the

101 possible simultaneous presence of trivalent and hexavalent Cr forms that are characterized by  
102 different environmental mobility and toxicity (Vignati et al., this issue). On the other hand,  
103 Michailova et al. (2011) showed a genotoxic response in chironomids growing in a tannery-  
104 impacted system (the Dunajec river, southern Poland), where Cr and Cd were the only trace  
105 elements of concern. Although genotoxic and cancerogenic effects are mostly attributed to  
106 Cr(VI) exposure ((De Flora et al., 1990), genotoxic effects of Cr(III) organic compounds were  
107 also observed, along with changes in DNA and protein levels, in the yeast *Saccharomyces*  
108 *cerevisiae* (Chatterjee and Luo, 2010). The number of studies documenting a high toxicity of  
109 Cr(III) in standard laboratory settings is also increasing (Aharchaou et al., 2018; Bencheikh-  
110 Latmani et al., 2007; Kováčik et al., 2015; Ponti et al., 2014; Vignati et al., 2010), suggesting  
111 that Cr(III) is less harmless than admitted by the current scientific consensus. Significant  
112 polytene chromosome aberrations were found in *Chironomus riparius* exposed to Cr(III)  
113 contaminated sediments in laboratory conditions (Michailova et al., 2001).

114 In part 1 of the present study (Vignati et al., this issue), we showed that Cr discharged by  
115 tanneries is bioavailable to chironomids, but that the accumulation patterns do not simply  
116 reflect the contamination in abiotic matrices. By combining laboratory tests and in situ studies  
117 with caged, transplanted larvae of the model species *Chironomus riparius* Meigen (Diptera,  
118 Chironomidae), we specifically tested: a) if bioaccumulation in the field can be controlled by  
119 the ingestion of freshly-deposited contaminated SPM containing a pool of Cr readily  
120 bioavailable to surface deposit feeders such as chironomids and, b) at which level Cr  
121 accumulation may have adverse effects on the growth of *C. riparius*.

122

## 123 **2. Material and Methods**

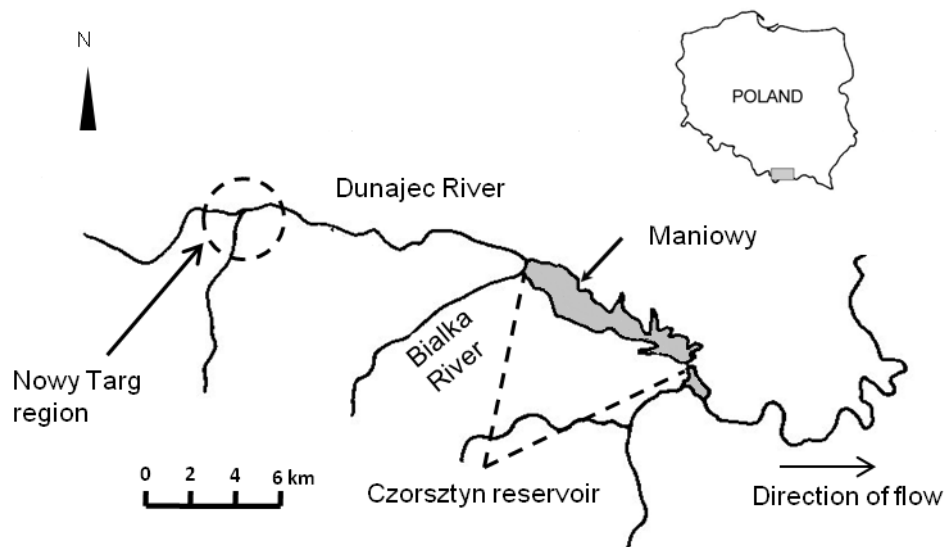
### 124 *2.1 Model organisms and study area*

125 The non-biting midge larvae *Chironomus riparius* were used as model species for all  
126 experiments. This species is commonly used for ecotoxicological purposes, can be reared in  
127 laboratory conditions and has easily measurable and well modelled life-cycle traits.  
128 *Chironomus riparius* has been considered as a model organism in several internationally  
129 validated guidelines for assessing the toxicity of chemicals and for evaluating the quality of  
130 natural sediments (see Ferrari et al. 2017 and references therein).

131 Larvae used in the tests originated from a culture maintained in our laboratory. The culture  
132 was started from egg masses kindly provided by Dr. J. Garric (Irstea, formerly Cemagref,  
133 Lyon, France). The methods for maintaining the culture and preparing the organisms for  
134 experiments are detailed in Ferrari et al. (2014). For this study, two kinds of experiments were  
135 conducted, either by starting with young second instar larvae (7-day laboratory tests) or by  
136 starting with young fourth instar larvae (4-day laboratory and in situ tests). The larval stages  
137 were determined by checking the length of the larvae and by controlling on few specimens the  
138 head capsule width. Each set of experiment was designed to allow assessment of  
139 bioaccumulation of Cr, but the toxicity of the whole sediment on chironomids (growth and  
140 survival) was recorded at the same time. The chronological steps for implementing the tests  
141 are depicted in Figure S1.

142 In situ exposure and sample collection for the concomitant laboratory experiments were  
143 carried out in the Czorsztyn reservoir (Southern Poland, Fig. 1) which receives the tannery  
144 contaminated wastewaters of the Dunajec river (about 300 small tanneries in the Nowy Targ  
145 region) and of other smaller creeks. The in situ exposure/sampling site was located in  
146 Maniowy Bay (Fig. 1) which is locally fed by a small creek collecting the effluent from a  
147 rural wastewater treatment plant (Fig. 1). Tanneries are also present in the region of Maniowy,  
148 although only some of them confer their wastes to the wastewater treatment plant. More

149 details on Cr contamination in the Czorsztyn reservoir can be found elsewhere (Dominik et  
150 al., 2007; Szalinska et al., 2010) and in part 1 of this study (Vignati et al., this issue).  
151



152  
153 Figure 1. Study area (Czorsztyn reservoir; southern Poland) and location of the Maniowy Bay area where in situ  
154 exposures performed and samples collected for laboratory experiments. The Nowy Targ region, where the many  
155 artisanal tanneries are active, is also indicated.

156  
157 *2.2 In situ bioaccumulation*

158 In situ exposure of *C. riparius* was carried out with a customized Sediment Water Interface  
159 Study System (S-WISS1.1) suitable for exposing surface deposit feeders to intact sediments  
160 for periods up to 4 days and allowing for food delivery to caged organisms during this period.  
161 Construction details and functioning were described in detail in Ferrari et al. (2014). Briefly,  
162 each exposure chamber consisted in a transparent polyvinyl chloride tube surmounted by a  
163 customized corer-head to deliver the chironomids, ensure water exchange between the



164 exposure chambers and the surrounding waters and suspensions (150  $\mu\text{m}$  Nitex mesh) and  
165 deliver the required food doses.

166 Twelve S-WISS1.1 were deployed at Maniowy (water depth approx. 3 m) by scuba divers.  
167 In addition, twelve control cages consisting in 1-L high-density polyethylene (HDPE), large-  
168 mouth bottles containing clean sand and partly covered with 150  $\mu\text{m}$  Nitex mesh (Ferrari et  
169 al., 2014) to ensure water exchange were deployed in the vicinity of the S-WISS1.1 devices.  
170 Food delivery in the control cages was also ensured by adopting the design described in  
171 Ferrari et al. (2014). At  $t=0$ , 20 individuals of *C. riparius* (4<sup>th</sup> instar larvae) were introduced  
172 into each exposure system (i.e. S-WISS1.1 and control cages). Food doses (1 mg Tetramin®  
173 per individual) were delivered immediately after deployment and then every 24 hours until the  
174 end of the exposure period (4 days). At  $t=24, 48, 72,$  and 96 hours, three exposure chambers  
175 and three control cages were randomly recovered.

176 Upon arrival at the laboratory, each S-WISS1.1 was opened and an aliquot of the overlying  
177 water was sampled, filtered at 1.2  $\mu\text{m}$ , acidified to 1% v/v with concentrated (65 %) Suprapur  
178  $\text{HNO}_3$  and stored in a polypropylene (PP) container at 4°C pending Cr analysis. After  
179 siphoning-off the remaining water, an aliquot of the top layer of sediments (0–0.5 cm) was  
180 gently scraped with a plastic spatula, passed through a 1 mm sieve to remove debris, and  
181 possibly chironomids, and stored in a PP container at –22 °C pending Cr analysis.

182 The sediment from the surface layer was chosen because it represents the most relevant  
183 exposure zone for surface deposit-feeders such as *C. riparius* (Rasmussen, 1984). The whole  
184 sediment core was then sieved (500  $\mu\text{m}$ ) to recover surviving chironomids. No indigenous  
185 chironomids were found in the cores. Recovered organisms were rinsed with site water and  
186 placed for 5 min in trays filled with ultrapure water (MilliQ water, Millipore), followed by 10  
187 min in 1 mM EDTA, and additional 5 min in ultrapure water. After gently blotting with paper  
188 towels, each larva was measured using a digital image analysis system and organisms were

189 pooled and conditioned for Cr analysis (see Ferrari et al., 2014 for more details). Individual  
190 length (n=20) and initial Cr body residue of unexposed larvae (2 pools of 30 individuals) were  
191 determined at the start of each experiment.

192

### 193 *2.3 Laboratory bioaccumulation studies*

194 Laboratory experiments were set up using both field-collected sediments from Maniowy  
195 and clean sand. Maniowy sediments were collected with an Ekman grab during the  
196 deployment of the in situ test systems and stored at 4 °C. Upon return to the laboratory, the  
197 overlying water was decanted and sediment were sieved at 1mm and homogenised on the day  
198 before the start of the tests. Sediments (clean sand for controls) were transferred into 1-L  
199 HDPE bottles analogous to those used for the control cages in the field experiment and  
200 covered with filtered (1.2 µm) Lake Geneva water at a minimum ratio of 1:4. The following  
201 day, 20 individuals of *C. riparius* were introduced into each exposure bottle and fed daily as  
202 the chironomids exposed in situ. Young second instar larvae were used for performing a 7-day  
203 bioaccumulation tests, while young fourth instar larvae were used for a 4-day tests.

204 All exposures were performed in triplicate at 21 °C under a 16:8-h light-dark photoperiod  
205 and light intensity of 500 lux. Air was continuously bubbled into each beaker during the test  
206 taking care to avoid sediment resuspension. The water level was controlled daily in each  
207 beaker and adjusted with deionised water if necessary. Larvae were fed daily ad libitum with  
208 0.6 mg of commercial food (Tetramin®) per individual (Péry et al., 2002).

209 For the 4-day bioaccumulation tests, organisms were retrieved and handled for length  
210 measurement (i.e. each individual) and Cr content (i.e. pool of 20 individuals) as in situ  
211 exposed ones at day 1, 2, 3 and 4. For the 7-day exposure tests, organisms were retrieved and  
212 handled in the same way only at the end of the test. For each exposure condition, the surface  
213 layer sediments (or sand) were sampled for total Cr analysis.

214

## 215 *2.4 Chemical analyses*

216 Chromium concentration in sediments and chironomids were assayed using the same  
217 methodologies described in Vignati et al. (this issue). Total Cr concentrations in overlying  
218 waters (filtered at 1.2 µm) in S-WISS1.1 cages were determined by ICP-MS, while Cr(VI)  
219 levels were determined by catalytic adsorptive stripping voltammetry (Bobrowski et al.,  
220 2012). Accumulation by chironomids was corrected for gut sediment content according to  
221 (Hare et al., 1989) assuming that ingested sediments represented 6% of the total weight of  
222 individual organisms (see Ferrari et al., 2014). For quality control procedures, reference  
223 materials LKSD-4 (Lynch, 1990) and WQB3 (NRC, Canada) were used for sediments,  
224 TORT-2 (NRC, Canada) for biological material and SLRS3 (NRC, Canada) and 1643e  
225 (NIST) for waters. Reference material 1643e was analyzed following a 10-fold dilution.

226

## 227 *2.5 Modelling of bioaccumulation*

228 According to the biodynamic bioaccumulation modelling — DYMBAM (Baumann and  
229 Fisher, 2011; Wang et al., 1997), accumulation of a contaminant by living organism can be  
230 described by the following formula:

231

$$232 \quad C_{biota} = \frac{k_u \cdot C_w}{k_{ew} + g} + \frac{AE \cdot IR \cdot C_s}{k_{es} + g} \quad (1)$$

233

234 where  $C_{biota}$  is the concentration accumulated by organisms (in µg g<sup>-1</sup>),  $k_u$  is the uptake rate  
235 constant from the dissolved phase (in L g<sup>-1</sup> d<sup>-1</sup>),  $C_w$  is the concentration in the water phase  
236 (filtered water, in µg L<sup>-1</sup>),  $k_{ew}$  and  $k_{es}$  are the efflux rate constants following uptake from the  
237 dissolved phase and from food (sediments in the case of deposit feeders; both in d<sup>-1</sup>),  $AE$  is the

238 assimilation efficiency (in % total content) from food (i.e., sediment),  $IR$  is the food ingestion  
239 rate (in  $\text{g g}^{-1} \text{d}^{-1}$ ),  $C_s$  is the concentration in sediments (in  $\mu\text{g g}^{-1}$ ) and  $g$  is the growth rate  
240 constant (in  $\text{d}^{-1}$ ).

241 Although model parameters were not available for *C. riparius*, first-order estimates of  
242 expected Cr accumulation were obtained based on the following considerations.  
243 Accumulation from the aqueous phase was neglected considering that sediments should be the  
244 dominant route of contaminant uptake for chironomids (Dabrin et al., 2012; De Jonge et al.,  
245 2010; Martin et al., 2008). An ingestion rate of  $0.325 \text{ g g}^{-1} \text{ d}^{-1}$  estimated for *C. riparius* at 10  
246 °C (Bervoets et al., 2003) was adopted and corrected for the field/laboratory temperature of  
247 this study (21 °C) as described in the supporting information. A value of 0.1%, determined  
248 using the biomimetic approach described in the companion paper (Vignati et al., this issue),  
249 was used for the AE of Cr from Maniowy sediments. Estimation of the organisms' growth  
250 rate constant ( $g$ ) was performed by fitting an exponential curve to the plot of weight of  
251 individual chironomids' larvae vs. time (Fig. S2). Finally, the  $k_{es}$  constant was neglected  
252 considering that literature values for Cr egestion by various organisms (Baumann and Fisher,  
253 2011; Roditi and Fisher, 1999; Wang et al., 1997) did not exceed  $0.019 \text{ d}^{-1}$  and thus were 4 to  
254 8-fold lower than the growth rates calculated from our experimental data (Fig. S2). The  
255 conclusions of the present study would not have been altered by considering  $k_{es}$  in model  
256 calculations (see section 4.1) and this approach was preferred to the arbitrary choice of  
257 parameters derived for organisms other than *C. riparius*.

258

## 259 2.6 Statistics

260 Data were analyzed using Student's t-test, analysis of variance (one-way ANOVA  
261 followed by post-hoc Tukey's HSD test) and linear regression. All data were checked for  
262 normality and homogeneity of variance by the Shapiro-Wilk test and the Bartlett test,

263 respectively. If conditions for ANOVA were not fulfilled, the data were log-transformed and  
264 reanalysed. For all statistical tests, the significance level was set at 0.05 and calculations were  
265 performed using the software package R (v 2.9.0).

266

### 267 **3. Results**

#### 268 *3.1. Quality control*

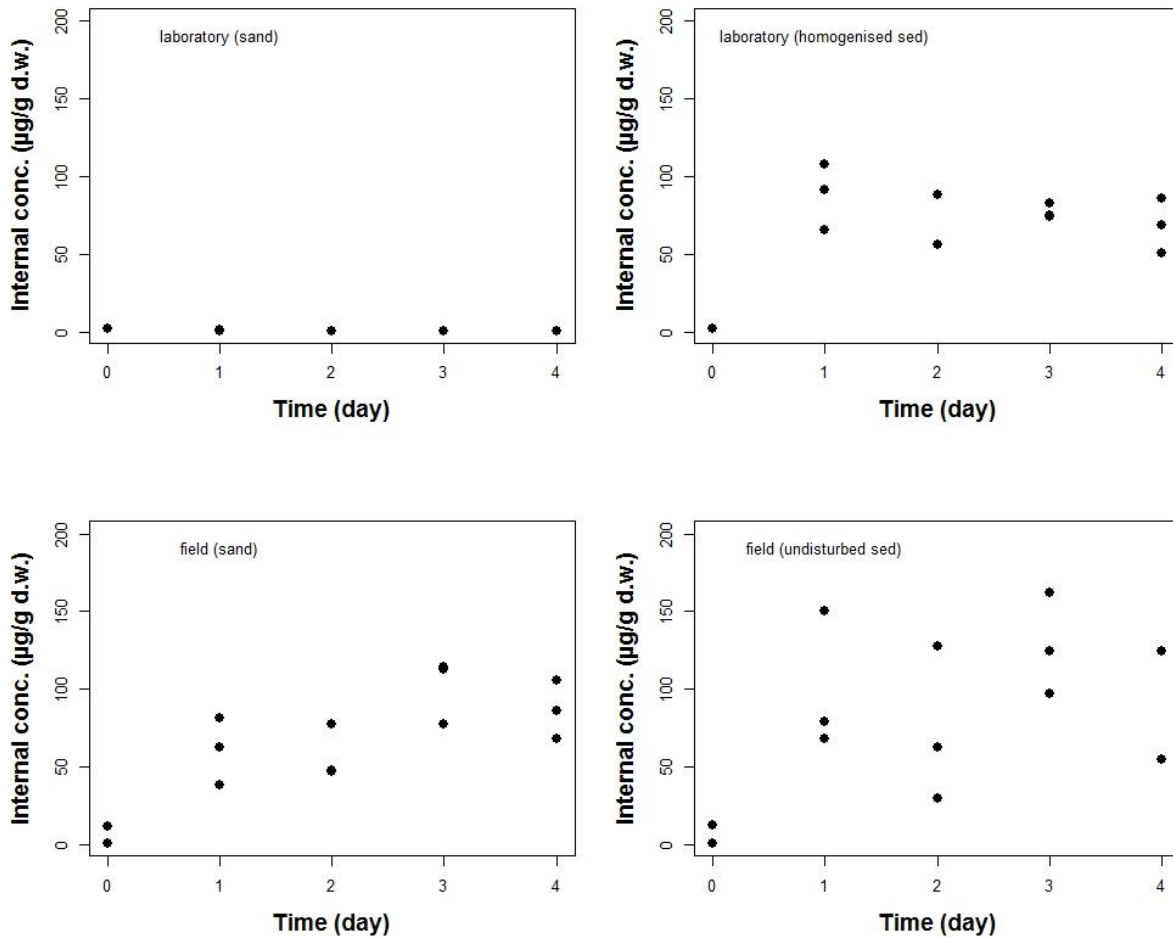
269 Figures of merit and overall accuracy of Cr analysis in aqueous matrices were analogous to  
270 those reported in Vignati et al. (this issue). Percentage Cr recovery from LKSD-4 was about  
271 120% when based on the concentrated HNO<sub>3</sub>/concentrated HCl extractable fraction (21±2 µg  
272 g<sup>-1</sup>) and 75% when based on total Cr content determined by X-ray diffraction (33±6 µg g<sup>-1</sup>;  
273 n=2 in both cases). For WQB3, measured Cr concentrations were 96±8 % (n=5) of the  
274 indicative value of 119 µg g<sup>-1</sup>. Triplicate analyses of sediment subsamples from selected cages  
275 agreed to 10% or better. Chromium recovery from TORT-2 (0.77±0.15 µg g<sup>-1</sup>) was 96±3 %  
276 (n=3). Due to the insufficient biological material, no replicate analyses of exposed  
277 chironomids were possible. However, the small standard deviation on the three replicates of  
278 TORT-2 material suggests an acceptable precision in the digestion of the biological material.

279

#### 280 *3.2 Bioaccumulation under in situ and laboratory conditions*

281 Chromium concentrations in sediments recovered inside the S-WISS1.1 units were  
282 homogeneous among the cages and across the four days of exposure (325±35 µg g<sup>-1</sup>, n=11).  
283 The Cr content of homogenised sediments from Maniowy used for the laboratory exposures  
284 was uniform (150±14 µg g<sup>-1</sup> d.w., n=12), albeit about half of that measured in the sediment  
285 recovered from in situ cages. Chromium levels in sand used for control exposures were  
286 1.2±0.5 µg g<sup>-1</sup> (n=12) in the field and 0.8±0.1 µg g<sup>-1</sup> (n=12) in the laboratory (Fig. S3).

287 Bioaccumulation kinetic patterns in chironomids exposed to Maniowy sediments were  
288 similar for laboratory and in situ exposures, with a marked increase over the first day  
289 followed by a plateau in accumulated Cr levels. Daily and overall variability were however  
290 higher for organisms exposed in situ (Fig. 2).



291  
292 Figure 2. Cr accumulation vs. time in *Chironomus riparius* larvae exposed to sand and sediment in experiments  
293 performed in situ (Maniowy Bay, June 2007) and in the laboratory using sand and field collected sediment. In all  
294 experiments, each point represents the measure of Cr internal concentration obtained for the pooled organisms  
295 retrieved in each exposure system. Concentrations in chironomids were gut corrected according to Hare et al.  
296 (1989).  
297

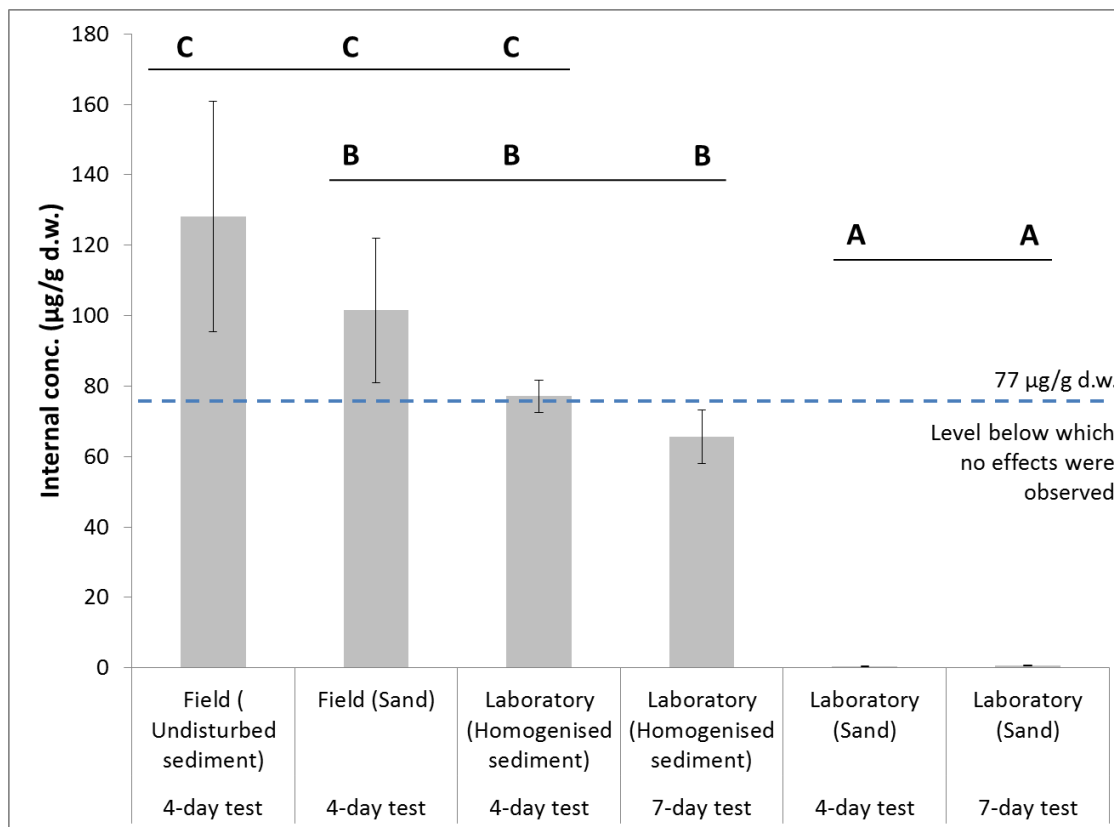
298 At plateau (days 2–4), chironomids exposed in the laboratory to homogenised sediments  
299 from Maniowy accumulated an average Cr concentration of  $78 \pm 16 \mu\text{g g}^{-1}$  (n=12), comparable  
300 to the concentration of  $98 \pm 43 \mu\text{g g}^{-1}$  (n=11) measured in chironomids exposed in situ to  
301 undisturbed field sediments using the S-WISS1.1 system. At the opposite, bioaccumulation  
302 kinetic patterns in chironomids exposed to clean sand showed a marked difference between  
303 the laboratory and in situ exposures. Indeed, no increase in Cr concentration was observed  
304 during laboratory exposures with control chironomids accumulating less than  $1 \mu\text{g g}^{-1}$  (Fig.  
305 2). On the contrary, chironomids exposed in cages filled with clean sand but placed on the  
306 reservoir bottom close to the S-WISS1.1 devices accumulated an average of  $77 \pm 25 \mu\text{g Cr g}^{-1}$   
307 (n=12). This value is comparable to the concentrations obtained for chironomids exposed to  
308 the Maniowy sediments, whether in laboratory experiments or after in situ exposures (Fig. 2).

309 Results obtained by exposing 4<sup>th</sup> instar larvae in situ or in the laboratory also compared  
310 favourably with those of 2<sup>nd</sup> instar larvae exposed for 7 days to homogenised sediments in the  
311 laboratory (Fig. 3). Indeed, three days after the start of any type of exposure protocol using  
312 young 4<sup>th</sup> instar larvae (see sections 2.2 and 2.3), all larvae were 9 days old after hatching  
313 (Fig. S1). Thus, 4<sup>th</sup> instar larvae exposed over 3 days to sediments in situ or in the laboratory  
314 were as old as 4<sup>th</sup> instar larvae remaining at the end of the 7-day bioaccumulation test in the  
315 laboratory which was started with young 2<sup>nd</sup> instar larvae (Fig. S1).

316 For 3 days exposures of 4<sup>th</sup> instar larvae, Cr concentrations in organisms exposed to  
317 sediment and sand (i.e., controls) were  $128 \pm 33 \mu\text{g g}^{-1}$  vs.  $101 \pm 21 \mu\text{g g}^{-1}$  for in situ  
318 experiments and  $77 \pm 5 \mu\text{g g}^{-1}$  vs.  $0.42 \pm 0.07 \mu\text{g g}^{-1}$  for laboratory experiments (Fig. 3). A  
319 statistically significant difference in Cr accumulation (Tukey HDS test on log-transformed  
320 data,  $p < 0.05$ ) existed between controls and exposed chironomids only in laboratory  
321 experiments. Despite the variability in the Cr content of sediment substrates (Fig. S3), Cr  
322 accumulation was not significantly different between organisms caged in the field (control

323 and exposed) and organisms exposed to homogenised Manioway sediments in the laboratory  
 324 (Tukey HDS test on log-transformed data,  $p > 0.05$ ). For 7 days experiments started using 2<sup>nd</sup>  
 325 instar larvae, measured Cr accumulation at day 7 was  $66 \pm 8 \mu\text{g g}^{-1}$  and  $0.67 \pm 0.17 \mu\text{g g}^{-1}$  in  
 326 organisms exposed to sediments and control sand, respectively. Chromium accumulation in  
 327 2<sup>nd</sup> instar larvae exposed to Manioway sediment for 7 days was significantly lower than in 4<sup>th</sup>  
 328 instar larvae exposed for 3 days to undisturbed field sediments (Tukey HDS test on log-  
 329 transformed data,  $p < 0.05$ ), but did not show significant differences with other experimental  
 330 conditions (Fig. 3).

331



332

333

334 Figure 3. Cr accumulation at day 3 in *Chironomus riparius* larvae exposed to sand and sediment in experiments  
 335 performed in situ (Manioway Bay, June 2007) and in the laboratory using field collected material. The 4-day tests  
 336 were initiated with young fourth instar larvae, while the 7-day tests were initiated with young second instar  
 337 larvae. Error bars represent 1 standard deviation (n=3). Internal concentrations that do not share the same letter



338 are significantly different (ANOVA followed by post-hoc Tukey's HSD test,  $p < 0.05$ ). The dotted line  
339 symbolizes the level below which no effects were observed by considering the results obtained for growth rates  
340 (see Fig. S2).

341

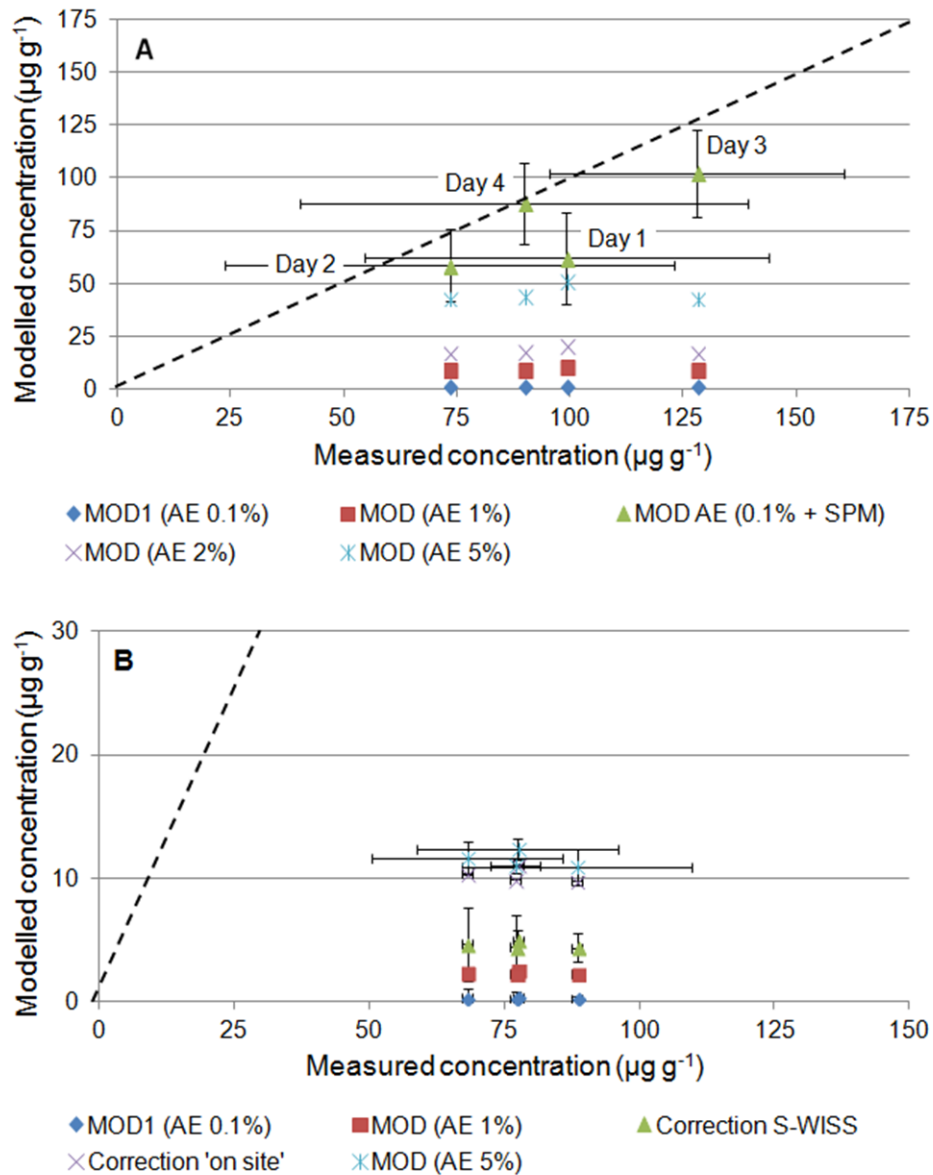
### 342 3.3. Modelling chromium bioaccumulation via sediment ingestion

343 The modelled Cr accumulation via sediment ingestion by *C. riparius* exposed to Maniowy  
344 sediments did not exceed  $1 \mu\text{g g}^{-1}$  for neither in situ nor laboratory exposures. These values  
345 were 1% (or less) of measured ones and strongly underestimated the actual Cr burden in both  
346 scenarios (Fig. 4a, 4b). Because the biomimetic approach used to derive the AE of 0.1%  
347 employed in model calculation (see section 2.5) may overlook other factors resulting in higher  
348 assimilation efficiencies (Vignati et al., this issue), additional model calculations were  
349 performed using AE values of 1, 2 and 5%. These AEs were reported in studies on Cr uptake  
350 by various organisms and from different substrates including sediments and algae (Baumann  
351 and Fisher, 2011; Roditi and Fisher, 1999; Wang et al., 1997). Even for the maximum AE  
352 value of 5%, modelled concentrations were 40–60% lower than the measured ones (Figure 4a,  
353 b).

354 Chironomids exposed to clean sand in laboratory experiments did not show any  
355 appreciable Cr bioaccumulation suggesting that neither the ingestion of sand substrate (in  
356 field controls) nor the added Tetramin® food (all experimental conditions) contributed to the  
357 observed Cr burdens. Measured Cr concentration in laboratory control chironomids actually  
358 decreased from  $0.9 \pm 0.5 \mu\text{g g}^{-1}$  at day 1 to  $0.4 \pm 0.06 \mu\text{g g}^{-1}$  at day 4 (data not shown). At  $t=0$ ,  
359 control chironomids had a Cr content of about  $2 \mu\text{g g}^{-1}$ , which was diluted by the ingestion of  
360 'clean' food and by growth. On the other hand, measured concentration in field control  
361 organisms were comparable to those of organisms exposed to Maniowy sediments (Fig. 3). As  
362 it will be discussed in detail in section 4.1, we surmise that accumulation from freshly

363 deposited SPM was responsible for the Cr burden measured in the field control chironomids  
 364 and also contributed the majority of accumulated Cr to organisms exposed in situ to  
 365 unperturbed sediments.

366



367

368 Figure 4. Comparison of measured vs. modelled Cr concentrations in *Chironomus riparius* exposed to  
 369 undisturbed Maniowy sediments in situ (S-WISS1.1 cages – panel A) and to homogenised Maniowy sediments  
 370 in the laboratory (panel B). All values are in µg g<sup>-1</sup>. MOD, modelled accumulation of Cr obtained using different  
 371 values for assimilation efficiency (AE) in formula 1; SPM, Cr accumulation in field control organisms (assumed  
 372 to originate from ingestion of freshly deposited Suspended Particulate Matter — see text for details); Correction

373 'on site' and 'S-WISS', modelled accumulation using formula 1 and corrected for changes in bioavailability due  
374 to sediment manipulation according to Ferrari et al. (2014). The dashed lines show the theoretical 1:1  
375 correspondence between modelled and measured concentrations.

376

### 377 *3.4 Effects on chironomids*

378 Survival of chironomids in laboratory controls was almost complete ( $19.6 \pm 0.7$  individuals;  
379  $n=12$ ) over the 4 days of exposure and only slightly reduced ( $18.1 \pm 2.2$  individuals,  $n=12$ ) for  
380 exposure to Maniowy sediments in the laboratory. Average survival in field controls was  
381  $16.3 \pm 3.7$  individuals ( $n=12$ ); significantly higher than in organisms directly caged on  
382 sediments in situ ( $9.8 \pm 2.7$  individuals,  $n=11$ ). In this last case, no temporal trend in survival  
383 was observed.

384 The length of alive chironomids increased linearly and significantly ( $R^2 > 0.84$ ,  $p < 0.0001$ )  
385 over time during the 4-day exposure both in the laboratory and in the field (Fig S2). In the  
386 laboratory, the growth rate of the chironomids exposed to sand was  $0.1499 \pm 0.0054$  cm d<sup>-1</sup>; not  
387 significantly different from the growth rate of  $0.1421 \pm 0.0096$  cm d<sup>-1</sup> for the chironomids  
388 exposed to homogenised Maniowy sediments (ANOVA,  $p > 0.05$ ; Fig. S2). In the field, the  
389 growth rate of the chironomids exposed to sand ( $0.1273 \pm 0.0065$  cm d<sup>-1</sup>) was significantly  
390 lower than the value obtained for control chironomids in the laboratory (ANOVA,  $p = 0.016$ ;  
391 Fig. S2). Growth rate further and significantly decreased to  $0.092 \pm 0.0114$  cm d<sup>-1</sup> when the  
392 chironomids were exposed to the undisturbed sediments in situ using S-WISS1.1 (ANOVA,  
393  $p = 0.011$ ; Fig. S2).

394 Concerning the 7-day bioaccumulation test, no significant effect was observed between  
395 controls and exposed chironomids. The lowest number of alive chironomids per beaker ( $n=3$ )  
396 was 19 and 18 in controls and exposed organisms, respectively. The mean length at the end of

397 exposure period was  $1.01 \pm 0.02$  cm in controls and  $0.99 \pm 0.05$  cm in chironomids exposed to  
398 the homogenised Maniowy sediment (t-test,  $p > 0.05$ ).

399

#### 400 **4. Discussion**

##### 401 *4.1. Importance of SPM as an additional exposure route to contaminants in the field*

402 The comparable Cr body burden in chironomids exposed in situ to undisturbed field  
403 sediments and to clean sand (Figs. 2 and 3) suggests the presence of an additional source of  
404 Cr for the organisms exposed in the field. The lower accumulation of Cr in chironomids  
405 exposed in the laboratory to homogenised sediments from Maniowy also supports the  
406 hypothesis that one (or more) additional source of Cr exists in the field. A possible  
407 contribution of Tetramin® to Cr bioaccumulation can be ruled out by the absence of Cr  
408 accumulation in control laboratory chironomids. We surmise that freshly deposited SPM  
409 entering the exposure cages can act as an important source of Cr to surface deposit feeders  
410 exposed in the field at the site of Maniowy. Besides the strong Cr accumulation by control  
411 chironomids exposed in situ, several observations collectively form a basis for evidence to  
412 support this hypothesis as detailed in the next paragraphs.

413 The lower accumulation by laboratory-exposed chironomids likely reflects the lower Cr  
414 concentration in homogenised sediments used for laboratory experiments compared with  
415 undisturbed field sediments (Fig. S3). The differences between homogenised and undisturbed  
416 (surface) sediments may be explained by the vertical decrease of Cr contamination in  
417 Maniowy sediments (Vignati et al., this issue). However, Ferrari et al. (2014) showed that, for  
418 sediments collected from the same site in Lake Geneva, Cr bioaccumulation was about 2- to  
419 3-fold higher for chironomids exposed to homogenised sediments in the laboratory (sediment  
420 Cr content  $25\text{--}30 \mu\text{g g}^{-1}$ ) than to undisturbed field sediments using S-WISS1.1 type cages  
421 ( $28\text{--}32 \mu\text{g g}^{-1}$  of Cr) or to homogenised sediments placed back in the field ( $27\text{--}32 \mu\text{g g}^{-1}$  of

422 Cr). In the study of Ferrari et al. (2014), temperature differences between laboratory (21 °C)  
423 and field (15 °C) alone could explain the observed differences. Sediment manipulation is also  
424 known to trigger a series of reactions that increase the bioavailability of sediment-bound  
425 contaminants (Vandegheuchte et al., 2013); in disagreement with our observations. Overall,  
426 these previous observations suggest that the higher accumulation of Cr in chironomids  
427 exposed in the field to undisturbed Maniowy sediments requires a supplementary source of  
428 bioavailable Cr.

429 As observed in previous research on metal accumulation by chironomids (Dabrin et al.,  
430 2012; De Jonge et al., 2010; Martin et al., 2008), uptake of aqueous Cr species from overlying  
431 water and pore water is unlikely to be the main responsible for the observed bioaccumulation  
432 patterns in chironomids. In laboratory experiments, homogenized Maniowy sediments were  
433 covered with filtered water from Lake Geneva; which has a low Cr content (Edder et al.,  
434 2008; Kottelat, 2008) and should not contribute much to Cr accumulation. The occurrence of  
435 higher Cr body burdens in field control chironomids compared with organisms exposed to  
436 Maniowy sediments in the laboratory can therefore be considered indicative of Cr uptake  
437 from the overlying waters of Maniowy. This pattern was actually observed after 3 and 4 days  
438 of exposure (Figs. 2 and 3). However, differences in accumulated Cr concentrations were only  
439 30% of the total body burdens, confirming the need for another source of Cr to explain the  
440 observed bioaccumulation in field controls. Furthermore, field control chironomids were  
441 exposed on a substrate of (initially) clean sand so that Cr uptake from pore water is unlikely to  
442 have contributed markedly to the elemental burden of these organisms.

443 Similar conclusions can be obtained by examining the total filterable Cr concentrations  
444 measured in overlying waters inside S-WISS1.1 cages (Table S1). These concentrations  
445 showed some variability between days 1+2 ( $3.9 \pm 1.4 \mu\text{g L}^{-1}$ ; n=6) and 3+4 ( $35 \pm 29 \mu\text{g L}^{-1}$ ;  
446 n=6), but Cr accumulation by chironomids was not directly proportional to filterable Cr

447 concentration inside the cages (Table S1). DGT measurements over 89 hours (slightly shorter  
448 than the 96 hours of chironomid exposure for logistic reasons) did not show any marked  
449 change in labile Cr in overlying or interstitial waters either (Fig. S4). The concentration of  
450 Cr(VI) measured in overlying waters of cages recovered on days 3 and 4 (Table S1) was  
451  $0.59 \pm 0.321 \mu\text{g L}^{-1}$  (n=6, range: 0.076–1.04  $\mu\text{g L}^{-1}$ ) and comparable to the levels measured in  
452 pore waters recovered from sediment cores sampled in Maniowy (Vignati et al., this issue).  
453 Otherwise stated, caging did not markedly change Cr(VI) concentrations (which could have  
454 had consequences on Cr bioavailability) and DGT-labile Cr, a proxy for bioavailable trivalent  
455 Cr, remained constant during chironomids' exposure. Changes in pH, conductivity and  
456 oxygen content within the S-WISS1.1 cages are also too limited (Ferrari et al., 2014) to result  
457 in significant increases of Cr oxidation from field sediments. Considering the egestion rate of  
458 Cr assimilated via the dietary pathway (i.e., the  $k_{es}$  parameter in formula 1) would have further  
459 reduced the modelled Cr body burden. Overall, Cr uptake from freshly deposited SPM  
460 remains the hypothesis best supported by the available data to explain a) the observed Cr  
461 accumulation in field controls and b) the higher Cr accumulation by chironomids exposed to  
462 Maniowy sediment in situ compared with laboratory exposures to homogenised sediment.

463

#### 464 *4.2. A unifying framework for modelling, experimental results and field observations*

465 The hypothesis that ingestion of freshly deposited SPM (section 4.1) represents a  
466 significant route of Cr accumulation for chironomids exposed in situ allows to reconcile  
467 modelling calculations and experimental results to a great extent. Summing accumulation via  
468 SPM (i.e., the experimental values measured for field control cages) to the quantity of Cr  
469 accumulated from sediment ingestion (using an AE of 0.1% as determined by the biomimetic  
470 approach – see section 2.5) markedly improves the agreement between modelled and  
471 measured bioaccumulation for organisms exposed to Maniowy sediments in situ (Fig. 4a).

472 With this procedure, modelled concentrations remain within 20% of measured ones for 2, 3  
473 and 4 days of exposure, again supporting the hypothesis that SPM is a major route of  
474 exposure in field conditions. The  $\pm 20\%$  differences are within the possible contribution of Cr  
475 uptake via the aqueous route; as indicated by the comparison between laboratory exposure  
476 to Maniowy sediments and in situ control cages (see section 4.1).

477 On the other hand, a contribution of freshly deposited SPM to Cr accumulation cannot be  
478 invoked to reduce the discrepancies between modelled and experimental Cr concentrations for  
479 chironomids exposed to Maniowy sediments in the laboratory (Fig. 4b). Accumulation via  
480 aqueous exposure, if any, would not lead to a better agreement between modelled and  
481 experimental results either (see sections 3.3 and 4.1). It is however possible that sediment  
482 manipulation during the setup of laboratory exposure may increase Cr bioavailability for  
483 chironomids. In the case of the Vidy Bay (a polluted bay in Lake Geneva,  
484 Switzerland/France), Ferrari et al. (2014) reported that Cr bioaccumulation by *C. riparius* in  
485 the laboratory was about twice than in specimen of the same species exposed in the field (S-  
486 WISS1.1 cages). Chromium accumulation in the laboratory was even 4–5 times higher than in  
487 organisms exposed on site in cages containing the same homogenised field sediments used in  
488 the laboratory. However, including a correction factor of 2 (in situ vs. laboratory) or 4.5 (on  
489 site vs. laboratory) into modelled bioaccumulation values for chironomids exposed to  
490 Maniowy sediment in the laboratory does not improve the agreement between observed and  
491 predicted results to any appreciable extent (Fig. 4b).

492 Another factor that may increase Cr bioavailability in laboratory exposure following  
493 sediment manipulation is the association of Cr with Fe/Mn oxides in Maniowy sediments  
494 (Vignati et al., this issue). However, even assuming a Cr assimilation efficiency of 5% for  
495 chironomids exposed to Maniowy sediments in the laboratory, modelled results still remain  
496 about 15% of the measured accumulation at day 4 (Fig. 4b). One last option is that the

497 development of an active Mn redox cycle at the sediment-water interface might have led to  
498 the formation of highly bioavailable Cr(VI) via oxidation of Cr(III) in the laboratory exposure  
499 beakers (Landrot et al., 2012). While the available experimental data do not allow to test this  
500 hypothesis, it appears that chromium bioaccumulation in the laboratory occurred to a different  
501 extent, and possibly via different routes, compared with real-field situations. Otherwise stated,  
502 the use of homogenised sediment to test for contaminant bioavailability may change the  
503 relative importance of the various exposure routes and lead to results not directly applicable in  
504 the field (Ferrari et al., 2014; Simpson and Batley, 2007; Wang et al., 2004).

505 It is also instructive to examine how well Cr accumulation by caged specimen of *C.*  
506 *riparius* can mimic Cr accumulation by indigenous chironomids. As already seen,  
507 consideration of Cr uptake via ingestion of freshly deposit SPM is necessary to reconcile  
508 DYMBAM calculations and experimentally measured accumulation for specimen of *C.*  
509 *riparius* exposed in situ to Maniowy sediment (Fig. 4a). However, even after accounting for  
510 the SPM route, the average accumulation of Cr by *C. riparius* inside S-WISS1.1 cages ( $98 \pm 23$   
511  $\mu\text{g g}^{-1}$ ; arithmetic mean  $\pm$  one standard deviation for 4 days of exposure,  $n=11$ ) remains about  
512 3 times lower than accumulation by indigenous chironomids recovered from Maniowy  
513 sediments during the same period ( $315 \mu\text{g g}^{-1}$  for the pool of chironomids sampling in June  
514 2007; see Vignati et al., this issue). This difference may originate from individual indigenous  
515 chironomids weighing, on average, about 3.5 times as much as S-WISS1.1 exposed specimen  
516 on day 4 (1.43 vs. 0.388 mg). Larger individuals would ingest larger sediment quantities to  
517 meet their nutritional needs, while simultaneously accumulating more Cr from contaminated  
518 sediments. Furthermore, ingestion rate is inversely related to the organic matter content of a  
519 given sediment. Contrary to caged *C. riparius*, indigenous chironomids could not rely on  
520 Tetramin® as an additional source of Cr-free carbon; which could again have led to higher  
521 ingestion rates and, eventually, higher Cr body burdens. These considerations equally apply to



522 the ingestion of freshly deposited SPM and do not invalidate the hypothesis of SPM as the  
523 main Cr source to chironomids. Even if not performed in the same experimental conditions, it  
524 is interesting to note that Bervoets et al. (2004) found a very good agreement in Cr internal  
525 concentrations between caged and resident larvae, especially in the most contaminated sites.

526

#### 527 *4.3. Effect of chromium on the growth of chironomids*

528 Individuals of *C. riparius* exposed to homogenised Maniowy sediments in the laboratory  
529 for 4 or 7 days did not exhibit higher mortality or reduced growth rate compared with control  
530 chironomids (section 3.4 and Fig. S2), indicating that Maniowy sediments were not toxic in  
531 standardized conditions. The absence of effects in laboratory exposures may be linked with  
532 sediment homogenisation. The Cr content of Maniowy sediments rapidly decreases below 5–6  
533 cm (Vignati et al., this issue) because the Czorsztyn reservoir was flooded in 1997 and a thin  
534 layer of Cr-contaminated sediments overlies a relatively compact layer of former soil with  
535 low Cr content.

536 On the other hand, exposure to undisturbed sediments in the field resulted in reduced  
537 survival and growth rate compared with laboratory and field controls as well as with  
538 homogenised Maniowy sediments tested in the laboratory (section 3.4 and Fig. S2). During  
539 the in situ exposure period, water temperature in the field was around 21 °C and hence similar  
540 to the optimal temperature used for laboratory experiments. Moreover, chironomids have been  
541 daily fed ad libitum in all laboratory and in situ tests, thus preventing the impact of limiting  
542 food availability during in situ exposure. The differences between field controls and field  
543 exposed chironomids are therefore indicative of some kind of adverse effect of Maniowy  
544 sediments. Furthermore, the difference between field and laboratory controls also points to  
545 adverse effect linked to the presence of freshly deposited SPM which appears as an important  
546 route of exposure to Cr for chironomids during in situ experiments.

547 When considering the quantity of Cr accumulated by specimen of *C. riparius* in relation to  
548 the growth results, it can be assumed that no effect on growth can be observed up to a total  
549 concentration of  $77 \mu\text{g g}^{-1}$  (Fig. 3 and Fig. S2). This concentration could correspond to a  
550 Critical Body Residue (CBR). The CBR is the concentration of a chemical bioaccumulated in  
551 an organism above which ecotoxicity effects appear (McCarty and MacKay, 1993). The  
552 tentative CBR value of  $77 \mu\text{g g}^{-1}$  agrees with the data of Méndez-Fernández et al. (2013) who  
553 determined a CBR of approx.  $34 \mu\text{g g}^{-1}$  for *Tubifex tubifex* in tests with natural sediments  
554 spiked with  $\text{K}_2\text{Cr}_2\text{O}_7$ . Upon entering living cells, Cr(VI) is rapidly converted to Cr(III) (Viti et  
555 al., 2014), so that a comparison of CBR for the two redox forms of Cr remains meaningful.

556 Recent studies have demonstrated that metal body burdens in relative resistant  
557 invertebrates such as chironomids can be used as predictors of ecological effects of metals on  
558 aquatic ecosystems. Bervoets et al. (2016) have linked threshold body burdens of metals in  
559 fourth instar larvae of indigenous *Chironomus* sp. to effects on resident macroinvertebrate  
560 communities. Depending on the descriptor chosen for the structure of the macroinvertebrate  
561 community, the threshold values ranged from 10 to  $69 \mu\text{g g}^{-1}$  d.w. of Cr accumulated in  
562 chironomids. It is therefore possible that Cr contamination at Maniowy adversely affects  
563 sensitive taxa of macroinvertebrates. Furthermore, the tentative CBR value obtained for *C.*  
564 *riparius* in the present study suggests that Cr levels above  $77 \mu\text{g g}^{-1}$  d.w. can also affect  
565 relatively pollution-tolerant organisms such as chironomids themselves. These findings are  
566 not at odds with the thresholds proposed by Bervoets et al. (2016) because laboratory-reared  
567 organisms are likely more sensitive than adapted local indigenous species.

568 However, the bioaccumulation results obtained in this study showed a large variability  
569 (Fig. 2) which hampers a clear definition of the actual CBR for Cr in *C. riparius*. Other  
570 studies also suggest that metals have toxic effects only when their uptake rate exceeds their  
571 combined rates of efflux and detoxification (Casado-Martinez et al., 2010) or the fraction of

572 metabolically available metal increases (Rainbow, 2007). Knowledge of the subcellular fate  
573 of metals is therefore required to better understand the physiological processes underlying  
574 their bioaccumulation and toxicity. Such methods have been developed for larvae of  
575 chironomids (Gimbert et al., 2016; Péry et al., 2008) and will have to be considered to further  
576 clarify the metabolically available concentration of Cr that can affect the development of  
577 chironomids at different stages in real-field situations.

578

## 579 **5. Conclusions**

580       Comparable levels of Cr accumulation by specimen of *Chironomus riparius* exposed to  
581 undisturbed field sediments in situ and to clean sand on-site suggest that ingestion of freshly  
582 deposited SPM can be the main route of Cr accumulation by surface deposit feeders in real  
583 field conditions. This additional route of exposure is not accounted for in standardized  
584 laboratory experiments or, as yet, by biodynamic bioaccumulation modelling, so that both  
585 approaches will lead to an underestimation of Cr accumulation compared with field  
586 conditions. Changes in the bioavailability of sediment-bound Cr also seem to occur during  
587 sediment sampling and handling, with the correction of such experimental artefacts being far  
588 from straightforward. While both laboratory and in situ experiments provide evidence for the  
589 availability of Cr in aquatic system impacted by tannery wastewaters, in situ investigations  
590 appear necessary to obtain an adequate understanding of Cr bioavailability under real  
591 environmental conditions.

592       A tentative threshold of Cr accumulation for effects on the growth of *C. riparius* is  
593 proposed at  $77 \mu\text{g g}^{-1}$  d.w. Further studies using toxicokinetics approaches and subcellular  
594 fractionation methods are however required to refine the actual concentration of Cr linked to  
595 the biologically active sites and leading to adverse effects in these benthic organisms.  
596 Extrapolation from the model organism *C. riparius* to other species of indigenous

597 chironomids also represents a future challenge to better link laboratory and in situ results with  
598 real field situations.

599

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608

609

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769 **Supporting information**

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771 **Chromium bioavailability in aquatic systems impacted by tannery wastewaters. Part 2:**

772 **new insights from laboratory and *in situ* testing with *Chironomus riparius* Meigen**

773 **(Diptera, Chironomidae)**

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809 **Correction of sediment ingestion rate (IR) for temperature**

810 Temperature correction for sediment ingestion rates was calculated according to the formula:

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$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\left(\frac{10}{T_2-T_1}\right)} \quad (\text{A})$$

812 where:  $Q_{10}$  (dimensionless) is the rate of increase of a reaction for a 10 degrees temperature  
813 increase (in °C or K),  $R_1$  and  $R_2$  are the reaction rates at  $T_1$  and  $T_2$ , respectively (with  $T_1 <$   
814  $T_2$ ). Web-based calculator ([http://www.physiologyweb.com/calculators/q10\\_calculator.html](http://www.physiologyweb.com/calculators/q10_calculator.html) )  
815 was used to estimated temperature-adjusted sediment ingestion rates entering an arbitrary  $Q_{10}$   
816 value of 2 in formula (A) (see below for the rationale behind this choice). Other input values  
817 for formula A were the reference temperature of 10°C used by Bervoets et al. (2003), the  
818 corresponding IR value of 0.325 g g<sup>-1</sup> d<sup>-1</sup> for *C. riparius* and the measured field and  
819 laboratory temperature of 21°C for our study. A temperature corrected value of IR equal to  
820 0.697 g g<sup>-1</sup> day<sup>-1</sup> resulted from this calculations.

821 The  $Q_{10}$  factor of 2 was selected based on (Croteau et al., 2002; Gresens, 2001) who studied  
822 the effect of temperature on Cd accumulation and sediment ingestion and digestion. It is  
823 important to note that uncertainties exist as to the influence of temperature on sediment  
824 ingestion rates and contaminant accumulation by chironomids In particular, the relationship  
825 between temperature and sediment ingestion rate is not necessarily a monotonous one  
826 (Gresens, 2001) and its determination would require experimental approaches outside the  
827 scope of the present work.

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829 **Table S1.** Concentration of total Cr measured in overlying waters recovered inside each of the  
 830 three S-WISS cages on days 1, 2, 3, and 4; concentrations of hexavalent Cr in the same  
 831 waters; and concentration of total Cr in the chironomids exposed within each cage.

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Day	Replicate	Water		Chironomids
		Total Cr ( $\mu\text{g L}^{-1}$ )	Cr(VI) ( $\mu\text{g L}^{-1}$ )	Total Cr <sup>a</sup> ( $\mu\text{g g}^{-1}$ ) d.w.
1	1	3.42	n.a.	68.1
1	2	6.31	n.a.	150
1	3	4.31	n.a.	79.2
2	1	2.48	n.a.	29.9
2	2	2.87	n.a.	63.0
2	3	3.93	n.a.	127
3	1	93.5	0.57	125
3	2	26.1	0.61	162
3	3	21.8	0.46	97.3
4	1	23.8	1.04	54.9
4	2	29.3	0.08	n.a.
4	3	18.7	0.76	125

833 a) Concentrations corrected for sediment content in the gut (see main text for details)

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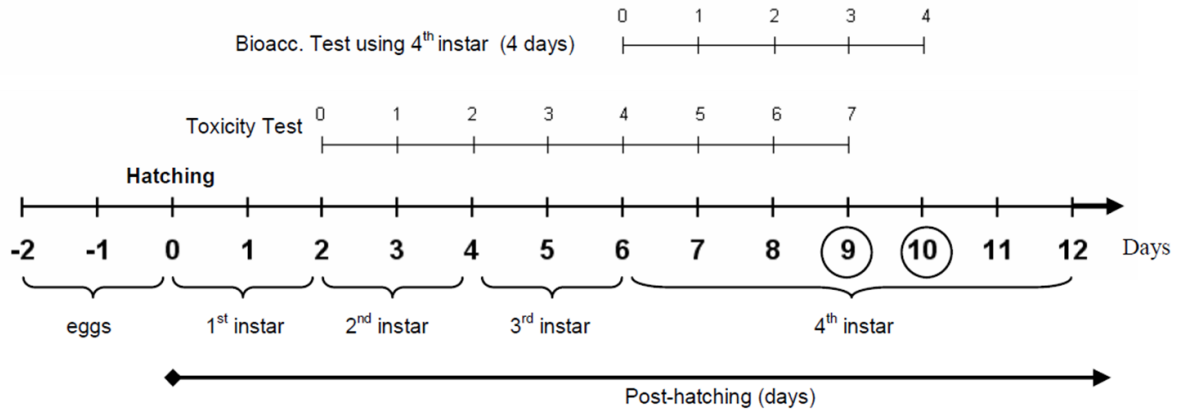
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836 **Figure S1.**

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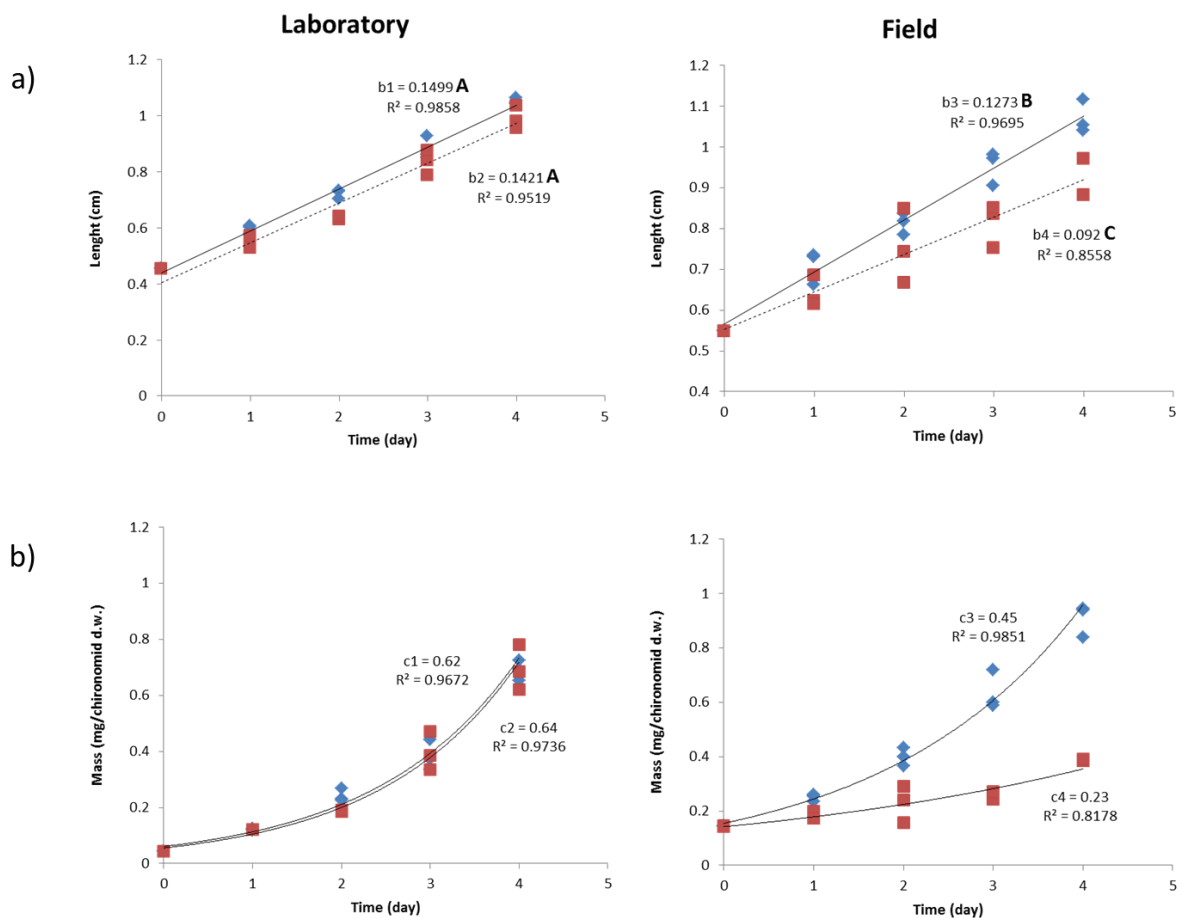
842 **Figure S1.** Chronological step for implementing the bioaccumulation and toxicity tests with  
843 the non-biting midge larvae *Chironomus riparius*. For the present study, experiments were  
844 started either with young second instar larvae (7 day laboratory tests) or with young fourth  
845 instar larvae (4 day laboratory and in situ tests). Numbers correspond to the post-hatching  
846 days needed to reach the end of the corresponding test. Note that 2<sup>nd</sup> instar larvae used in 7-  
847 day toxicity test and 4<sup>th</sup> instar larvae used in bioaccumulation tests will have the same post-  
848 hatching age at day 9.

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851 **Figure S2.**

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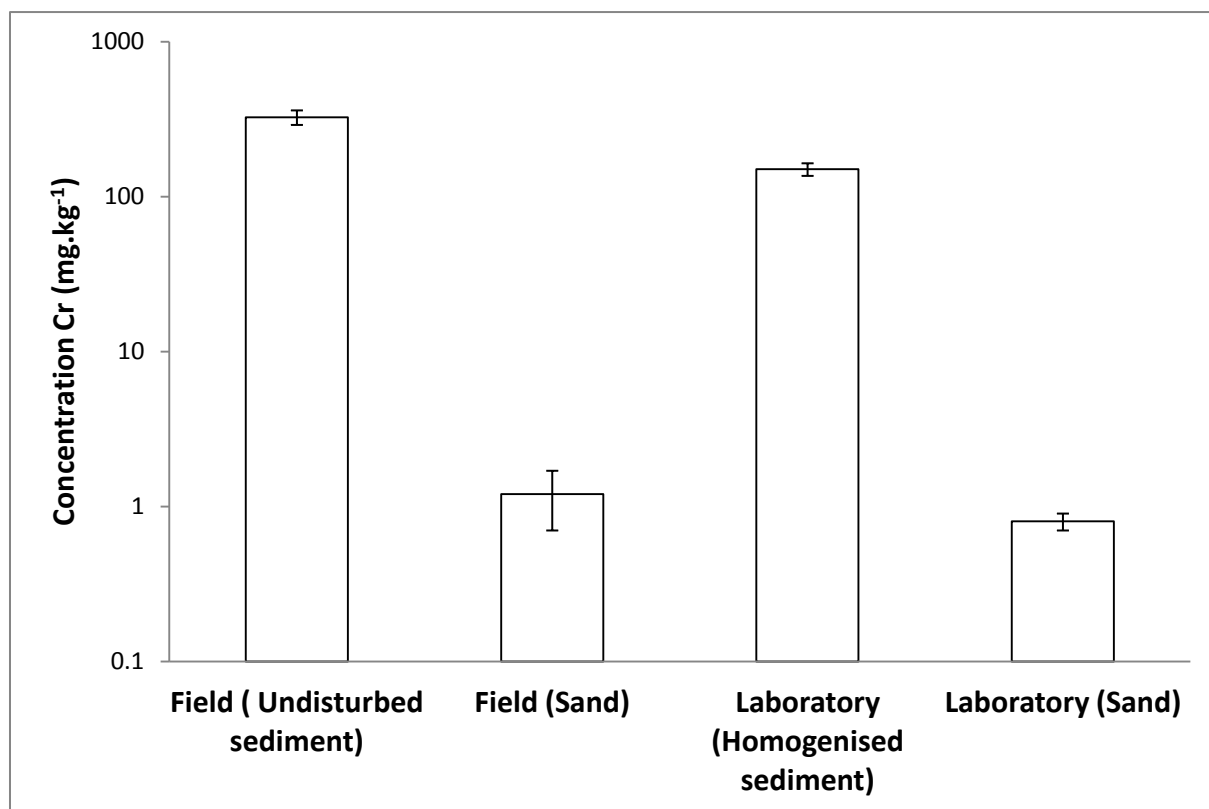
855 **Figure S2.** Evolution of the length (a) and weight (b) of 4<sup>th</sup> instar *Chironomus riparius* larvae  
856 as a function of time in bioaccumulation experiments performed in the field (Manioway Bay,  
857 June 2007) and in the laboratory using field collected material. Blue diamonds correspond to  
858 the measured responses of the chironomids exposed to the sand, while the red squares to the  
859 measured responses of those exposed to Manioway sediments. In figures (a), each point  
860 represents the average of the lengths obtained by individually measuring live specimen  
861 recovered daily from each exposure batch. The lines represent linear regressions and  
862 parameters b1, b2, b3 and b4 their slopes (means  $\pm$  1 s.e., in cm d<sup>-1</sup>) defined as the  
863 representative growth rates (g) for the different conditions. Slopes that do not share the same  
864 capital letter are significantly different (ANOVA,  $p < 0.05$ ). In the figures (b), each point  
865 represents the weight of the pooled live chironomids. The lines represent exponential  
866 regressions and parameters c1, c2, c3 and c4 their slopes (means  $\pm$  1 s.e., in d<sup>-1</sup>). R<sup>2</sup> and p  
867 indicate the goodness-of-fit and the statistical significance of each fitted regression.

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869 **Figure S3.**

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874 **Figure S3.** Total Cr concentrations (mg kg<sup>-1</sup> d.w.) in sediments used in bioaccumulation  
875 experiments performed in situ (Maniowy Bay, June 2007) and in the laboratory using field  
876 collected material. Data are the arithmetic mean  $\pm$  one standard deviation (n=12) of Cr  
877 concentration in each system of exposure retrieved during the 4 days of exposure *in situ* or in  
878 the laboratory. Note the log scale on the vertical axis.

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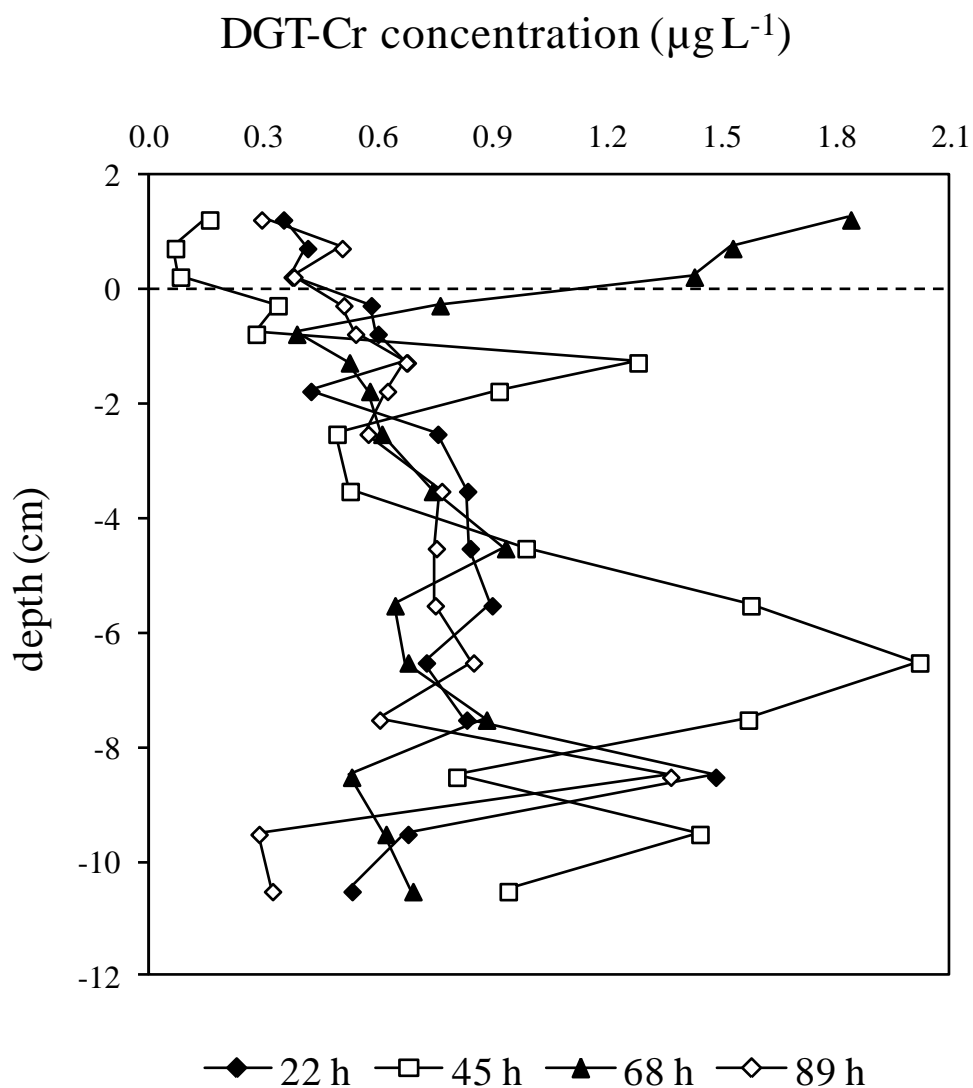
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881 **Figure S4**

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886 **Figure S4.** Temporal and vertical variability of DGT-labile Cr concentrations in interstitial  
887 (negative depth values; in cm) and overlying (positive depth values; in cm) at Maniowy bay in  
888 June 2007. Four different DGT probes were exposed at the same time and recovered after 22,  
889 45, 68 and 89 hours of exposures. Exposure location was close to the cages for chironomids  
890 and is assumed to be representative of Cr behaviour within the cages.