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Chromium bioavailability in aquatic systems impacted by tannery wastewaters. Part 1: Understanding chromium accumulation by indigenous chironomids

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50 **Abstract**

51 The tanning industry uses large quantities of Cr whose contribution to the contaminant burden
52 of aquatic organisms is not yet fully understood. The present study investigated Cr
53 bioaccumulation by indigenous chironomids in a freshwater ecosystem impacted by tannery
54 effluents. Total Cr content in sediments and in chironomids was determined on several
55 occasions. Chromium distribution among sediments and pore waters, and Cr speciation in
56 overlying and pore waters were studied in detail to understand possible factors controlling Cr
57 bioavailability to chironomids. Total chromium concentration ranged from 69 to over 3000 μg
58 g^{-1} dry weight in sediments and from negligible to over 300 μg^{-1} dry weight in chironomids
59 (values corrected for sediment gut content). Filterable ($<0.45 \mu\text{m}$) Cr concentration in
60 overlying waters and pore waters from the surface sediment layers (upper 2 cm) ranged from
61 3 to 120 $\mu\text{g L}^{-1}$, with Cr(VI) representing 0.5–28% of the total filterable Cr. Chromium
62 profiles in pore waters as determined by diffusive equilibration in thin films (DET) and
63 diffusive gradient in thin films (DGT) were comparable. DGT-labile Cr accounted for less
64 than 2% of the total Cr measured by DET. Although Cr concentrations in sedimentary and
65 aqueous matrices were not directly proportional to Cr levels measured in chironomids, the
66 available findings suggested that Cr inputs from tanneries were bioavailable to resident
67 chironomids. These observations are of particular importance considering that Cr(III),
68 putatively of limited bioavailability and ecotoxicological concern, is the predominant redox
69 form of Cr in bed sediments impacted by tannery discharges. The companion paper provides
70 further insight into Cr bioavailability and effects in tannery impacted ecosystems using a
71 combination of in situ and laboratory approaches.

72

73 **Keywords.** Chromium speciation; bioaccumulation; DGT; DET; voltammetry; sediment cores

74 **Introduction**

75 Chromium has a complex biogeochemical cycle which is essentially controlled by the
76 simultaneous environmental occurrence of two redox forms, Cr(III) and Cr(VI), having
77 contrasting (geo)chemical and (eco)toxicological properties (2016). Hexavalent Cr is
78 considered of most concern due to its ecotoxicity and proven carcinogenicity in humans,
79 while trivalent Cr is usually regarded as relatively harmless (WHO, 2009; 2013). Hexavalent
80 Cr also has high environmental mobility, while Cr(III) tends to bind to suspended particulate
81 matter and colloids and eventually accumulates in bed sediments (de Sousa et al., 2016;
82 Dominik et al., 2007; Gunkel-Grillon et al., 2014; Hayzoun et al., 2015). Remobilization of
83 Cr(III) from bed sediments is however possible following re-oxidation to Cr(VI) (Gorny et al.,
84 2016; Gunkel-Grillon et al., 2014). While the concern about Cr(VI) is justified, increasing
85 evidence points to a possible underestimation of the ecotoxicological risks posed by Cr(III)
86 for unicellular organisms (Bencheikh-Latmani et al., 2007; Jasso-Chávez et al., 2010; Lira-
87 Silva et al., 2011; Vignati et al., 2010), crustaceans (Ponti et al., 2014), aquatic plants (Dazy
88 et al., 2008) and terrestrial plants and organisms (Bigorgne et al., 2010; Speranza et al., 2007).
89 The implications of a possible underestimation of Cr(III) ecotoxicity would be particularly
90 relevant in aquatic ecosystems directly impacted by tannery wastewaters.

91 Leather tanning uses large quantities of Cr(III), generally as the basic chromium sulphate
92 $\text{Cr}_2(\text{OH})_2(\text{SO}_4)_2$. Chromium inputs from tanneries can markedly affect Cr concentrations and
93 fluxes in aquatic environments at local scales (Alves and Rietzler, 2015; Dominik et al., 2007;
94 Hayzoun et al., 2015; Sanyal et al., 2015) and the toxicological impact of tannery wastewaters
95 containing numerous potentially harmful compounds was recently reviewed by Saxena et al.
96 (2017). However, understanding the contribution of Cr(III) to the overall biological response
97 is far from straightforward because tannery effluents contain several toxicologically active
98 substances. The difficulties in apportioning the observed effects to the presence of Cr(III) are

99 especially big in the case of bed sediments where Cr preferentially accumulates and Cr(III)
100 tends to be the predominant redox form associated with the solid phase (Pereira et al., 2012;
101 Rosales et al., 2017).

102 Research on the bioavailability and effects of Cr(III) to benthic organism under realistic
103 exposure conditions or field scenarios is actually very limited. Authoritative risk assessment
104 documents (WHO, 2009; European Union, 2005) focus on ecotoxicity data from water-only
105 exposure; neglecting that sediment ingestion can be a major uptake route of Cr(III) for benthic
106 deposit feeders (Baumann and Fisher, 2011). Furthermore, studying the ecotoxicity of Cr(III)
107 to benthic organisms by spiking sediment-water systems with soluble Cr(III) salts is difficult,
108 because the spiked soluble Cr(III) undergoes hydrolysis within seconds and forms scarcely
109 bioavailable Cr(III) oxy-hydroxides that, in turn, undergo polymerization reaction on the time
110 scale of minutes (Pettine et al., 2008). The formation of such precipitates was actually
111 reported in laboratory studies on the relative ecotoxicity of Cr(VI) and Cr(III) on various
112 amphipod species (Berry et al., 2004; Besser et al., 2004); studies that did not highlight
113 measurable effects of Cr(III) on the test organisms. Assessing the possible ecotoxicity of
114 Cr(III) in sediments containing chromite ore processing residues, Becker et al. (2006)
115 concluded that Cr(III) did not impact survival and growth of two amphipods for
116 concentrations as high as $1,310 \mu\text{g Cr g}^{-1}$. However, both Cr oxy-hydroxides formed during
117 spiking with Cr(III) salts and chromite ore processing residues poorly mimic the organic-
118 Cr(III) forms usually discharged from tannery effluents (Outridge and Scheuhammer, 1993;
119 Walsh and O'Halloran, 1996; Wang et al., 2016). Furthermore, Bervoets et al. (2016)
120 determined that Cr body burdens as low as $10 \mu\text{g g}^{-1}$ d.w. (range $10\text{--}69 \mu\text{g g}^{-1}$ d.w.) in
121 specimen of *Chironomus* sp. collected at several field locations in the Flanders (Belgium) can
122 be associated with changes in the macroinvertebrate community composition. Studies
123 simultaneously assessing Cr geochemistry including redox speciation and the corresponding

124 Cr bioavailability to benthic organisms in tannery impacted environments are therefore
125 required to understand the bioavailability and possible biological effects associated with Cr in
126 such specific environmental settings.

127 The present work studies Cr bioaccumulation by indigenous chironomids' assemblages in
128 a river/reservoir system impacted by tannery wastewater discharges (Pawlikowski et al., 2006;
129 Szalinska et al., 2010). Investigations of increasing complexity were carried out during four
130 different sampling periods over four years. A detailed characterization of Cr geochemistry and
131 speciation allows examination of the possible importance of different routes of exposure in
132 contributing to the observed Cr body burden under field conditions. Part two of the present
133 study (Ferrari et al., this issue) compares results from laboratory-based bioaccumulation tests
134 with results obtained using an *in situ* a customized Sediment-Water Interface Study System
135 (Ferrari et al., 2014) to provide further insight in the bioavailability and effects of Cr in
136 aquatic ecosystems receiving tannery discharges.

137

138

139 **2. Methods**

140 *2.1. Study area and sampling*

141 The Dunajec river/Czorsztyn reservoir system (Southern Poland, Fig. 1) is impacted by
142 wastewater discharges from about 300 small tanneries in the Nowy Targ region. Due to
143 inefficient tannery wastewater management, pre-treated or raw wastewaters from these
144 tanneries are discharged into local sewers or directly into nearby watercourses. From late
145 October through February, river sediments in the proximity of tannery effluents are impacted
146 by chromium, while spring and summer floods tend to flush the contaminated sediments into
147 the Czorsztyn reservoir (Pawlikowski et al., 2006; Szalinska et al., 2010). Samples of
148 sediments and indigenous chironomids were collected in June and November 2004,

149 November 2006 and June 2007 at two sites. Site 1 (hereinafter Waksmund) was located in a
150 depositional zone of the Dunajec river and directly impacted by untreated tannery discharges.
151 Site 2 (hereinafter Maniowy) was located in the Czorsztyn reservoir in a small bay, locally fed
152 by a small creek which receives the effluent from the local wastewater treatment plant before
153 flowing into the bay (Fig. 1). Tanneries are also present in the region of Maniowy, however
154 only a few of them discharge their waste to the wastewater treatment plant.

155 For all sampling periods, sediment samples (2–10 kg) were collected using a plastic scoop
156 (Waksmund, wading against the river current along the right bank of the river) or a Van Veen
157 grab (Maniowy, the grab was operated from a small boat) and directly homogenised in the
158 field. Larvae of indigenous chironomids were recovered in the field by sieving additional
159 aliquots through 2 and 1 mm sieves. Fourth instar larvae were recovered from the sieves with
160 plastic tweezers, washed in deionised water (5 min), 1 mM EDTA solution (10 min) and again
161 deionised water (5–10 minutes). After blotting with paper towels, 14 to 20 larvae were pooled
162 and placed in cryogenic vials, snap frozen in liquid nitrogen and freeze-dried upon return to
163 the laboratory. The focus being on Cr accumulation in indigenous chironomids' assemblages,
164 care was taken to select large larvae showing a marked red colour, but no taxonomical
165 identification was performed at this stage. In 2007, samples for a more detailed
166 characterization of the chironomids' communities were collected at the two sites using a
167 20x20 cm (400 cm²) Surber Sampler (Waksmund) and a 15x15 cm (225 cm²) Ekman Grab
168 Sampler (Maniowy). Chironomids were then recovered for identification by further sieving
169 these samples through a 0.3 mm net.

170 In both 2004 and 2007, independent aliquots of homogenised sediments for analytical
171 determinations were sieved (< 1 mm) in the field and collected into preconditioned
172 polypropylene jars that were kept at 4 °C in the dark and freeze-dried upon return to the
173 laboratory. In November 2006 and June 2007, sediment cores were also collected by short (20

174 cm) hand corers either by wading against the river current at 4–5 m from the right bank of the
175 Dunajec River (Waksmund) or by scuba divers in the Czorsztyn reservoir (Maniowy). The
176 corers allowed recovery of sediments (15–25 cm depending on texture and compactness) and
177 associated overlying water (10–15 cm). Further details on core handling are given in section
178 2.2. In 2006, nine cores were collected at each site and analyzed for total Cr content in the
179 uppermost 0.5 cm (see section 2.2). In 2007, one core was collected at each site and sliced for
180 the determination of the following concentration profiles: total Cr in sediments, total Cr in
181 pore waters and Cr(VI) in pore waters (see section 2.2).

182 Diffusive Equilibration in Thin Films (DET) and Diffusive Gradients in Thin Films (DGT)
183 probes were deployed at undisturbed locations close to the sampling sites to obtain
184 information on Cr speciation and lability. The DGT and DET probes (arranged back to back)
185 were recovered after exposures of 46h 25 min (Waksmund) or 45 h (Maniowy). A time-series
186 of Cr-DGT profiles (Maniowy only) was also obtained by exposing DGT probes for 22, 45,
187 68 and 89 h at the same location.

188

189 *2.2. Core slicing and pore waters extraction*

190 Sediments cores were placed under nitrogen atmosphere in a portable glove box. Overlying
191 waters (OW; height of water column = 10–15 cm) were removed using plastic syringes and
192 taking care to avoid sediment resuspension. Aliquots of OW were filtered (1.2 µm syringe
193 filters, Whatman, Puradisc 30, cellulose acetate), collected into acid washed polypropylene
194 ICP tubes and acidified to 1% (v/v) with concentrated HNO₃ (VWR, Suprapur). Prior to use,
195 filters were prewashed with 10 mL of diluted (10% v/v) HCl followed by three rinses with
196 MilliQ water and then kept individually in plastic zip bags. After removing OW, sediment
197 sections from 0.5 to 1 cm thick were transferred to acid washed 100 mL polypropylene
198 centrifuge tubes. Tubes were capped and sealed with Parafilm™ inside the glove box and

199 centrifuged at 4000 rpm for 15 + 15 minutes. Tubes showing visible sediment compaction
200 after the second centrifugation cycle were further centrifuged for an additional 30 minutes.
201 Tubes were then re-introduced into the glove box and the pore waters filtered and handled in
202 the same way as OW. Centrifuge tubes, syringes and filters were purged with N₂ immediately
203 before core slicing.

204

205 *2.3. DET and DGT methodologies*

206 Total dissolved metal profiles were assessed by the DET technique (Davison et al., 1994),
207 which is based on the equilibration between solutes and a hydrogel casted in a sampling
208 device. The DET probes consisted of a base plate with a window of 1.8 cm × 15 cm and a set
209 of 1 mm slits with 1 mm interval between the slits. The slits were filled with 1.5 % agarose
210 gel (Leermakers et al., 2005).

211 Free and labile metal species were determined using the DGT technique (Davison et al.,
212 1994; Zhang and Davison, 1995). The DGT sediment probes consisted of a plastic base (15×5
213 cm) loaded with a “sandwich” of 3 layers: a resin gel (Chelex 100), covered by a
214 polyacrylamide gel (0.8 mm thickness) and a protective 0.45 μm cellulose nitrate filter (0.13
215 mm thickness, Millipore). Materials for the DGT and DET probes were purchased from DGT
216 Research Ltd. (Lancaster, UK).

217 Before deployment, all the probes were deoxygenated, transported to the field in
218 deoxygenated water and rapidly inserted into the sediment to avoid the co-precipitation of
219 iron (Fe) and other metal ions from pore waters within the gel. After retrieving, the DET and
220 DGT probes were processed in a laminar flow hood. Both the DET gel and Chelex resins
221 from DGT were removed from selected slits, transferred to preweighed plastic tubes and
222 eluted with 2.5 mL of 1 M HNO₃ (VWR, Suprapur).

223 Average DGT-labile metal concentrations (C_{DGT} , in $\mu\text{g L}^{-1}$) were calculated according to
224 the formula:

$$225 \quad C_{DGT} = \frac{M \times \Delta g}{D \times t \times A} \quad (1)$$

226
227 where: M is the accumulated mass of metal (ng), D is the diffusive coefficient of the metal
228 ($\text{cm}^2 \text{s}^{-1}$) in the diffusive gel of thickness Δg (cm) for a resin surface A (cm^2) and t is the
229 deployment time (s). Chelex 100-loaded DGT cannot complex anionic Cr(VI) species and
230 therefore allow the selective measurement of labile Cr(III) species, while generally excluding
231 organic complexes and colloidal species (Ernstberger et al., 2002; Giusti and Barakat, 2005).
232 The diffusive coefficient of Cr was obtained from the temperature-dependent values tabulated
233 by DGT Research Ltd (<http://www.dgtresearch.com/diffusion-coefficients/>).

234

235 *2.4. Chemical analyses*

236 Chromium was assayed by ICP-MS (HP4500, Agilent) using external calibration and internal
237 standard correction in overlying waters, pore waters, DET and DGT eluates, sediment and
238 chironomids. Prior to analysis, sediment aliquots (about 60–300 mg d.w.) and chironomids
239 (about 15–40 mg d.w.) were mineralized with a 5:1 (v/v) mixture of concentrated HNO_3
240 (65%) and H_2O_2 (30%), both from VWR, Suprapur grade. Overall accuracy of the digestion
241 procedures was evaluated using reference materials LKSD-1 (lake sediments) and STSD-2
242 (stream sediments) (Lynch, 1990) for sediments and TORT-2 (lobster hepatopancreas, NRC
243 Canada) for chironomids. Reference waters SLRS-3 from NRC and 1643e from NIST were
244 used to verify instrumental accuracy, with material 1643e being analyzed after a 10-fold
245 dilution.

246 The potentially bioavailable fraction of sediment-bound Cr liable to be released during
247 transit of ingested sediments through chironomids' gut was estimated using the biomimetic

248 approach (Chen and Mayer, 1999), consisting in a cold extraction of sediments aliquots with
249 synthetic Bovine Serum Albumin (BSA). Because BSA was supplied in a 3% NaCl matrix,
250 additional aliquots were extracted with a 3% solution of NaCl to account for salt-related
251 matrix effects. The results of 'biomimetic Cr' concentrations are corrected for Cr
252 remobilization due to NaCl. Sediment Loss on Ignition (LOI) at 550°C was performed to
253 estimate the content of total organic matter (OM).

254 In overlying and pore waters (June 2007), Cr(VI) was quantified by Catalytic Adsorptive
255 Stripping Voltammetry (CAdSV) using the differential pulse mode (DPV) and standard
256 addition procedure (Bobrowski et al., 2004). In some cases, pore waters from different
257 sediment layers were combined to obtain enough volume for voltammetric measurements that
258 were performed in triplicate for all samples. To reduce possible interferences due to the
259 presence of large quantities of dissolved organic carbon (DOC) in pore waters, Amberlite
260 XAD-7 resin was added to the (stirred) voltammetric cell five minutes before Cr(VI)
261 determination (Bobrowski et al., 2012).

262

263

264 **3. Results**

265 *3.1. Quality control procedures*

266 For analysis of waters and DGT/DET eluates, typical detection and quantification limits for
267 total chromium (calculated as 3 and 10 times the standard deviation of analytical blanks) were
268 $0.015 \mu\text{g L}^{-1}$ and $0.049 \mu\text{g L}^{-1}$, respectively. Four analytical replicates were measured for each
269 sample and the typical precision (for concentrations above the quantification limit) was 5% or
270 better. Measured values for certified reference waters (arithmetic means \pm 1 standard deviation)
271 were $0.29\pm 0.02 \mu\text{g L}^{-1}$ for SLRS-3 (n=7) and $21.3\pm 0.1 \mu\text{g L}^{-1}$ (n=7) for 1643e; comparing
272 favourably with the corresponding certified values of $0.30\pm 0.04 \mu\text{g L}^{-1}$ (uncertainty expressed

273 as 95% confidence interval) and $20.4 \pm 0.24 \mu\text{g L}^{-1}$ (uncertainty expressed as expanded
274 uncertainty). Triplicate measurements for Cr(VI) agreed to 25% or better. Recovery of Cr(VI)
275 from spiked samples was 93% or higher (Bobrowski et al., 2012).

276 Percentage Cr recovery from certified sediments was about 130% for both materials with
277 measured absolute values of $16 \pm 2.5 \mu\text{g g}^{-1}$ (arithmetic mean \pm [Abs(range/ $\sqrt{2}$)]); n=2) for
278 LKSD-1 and $64 \pm 3.7 \mu\text{g g}^{-1}$ (arithmetic mean \pm 1s.d.; n=3) for STSD-2. Our extraction
279 procedure consisted of concentrated $\text{HNO}_3 + \text{H}_2\text{O}_2$ while certified values ($12 \pm 2 \mu\text{g g}^{-1}$ for
280 LKSD-1 and $50 \pm 9 \mu\text{g g}^{-1}$ for STSD-2, uncertainties expressed as ± 1 s.d.) are given for an
281 extraction with concentrated HNO_3 and concentrated HCl . Sediments LKSD-1 and STSD-2
282 have a total certified Cr content (based on extraction with HF or analysis by XRF or
283 analogous techniques) of 31 ± 3 and $116 \pm 13 \mu\text{g g}^{-1}$, respectively. For biological materials,
284 percentage recoveries from TORT-2 were between 77% and 98% of the certified value
285 ($0.77 \pm 0.15 \mu\text{g g}^{-1}$; uncertainty given as 95% confidence limits) and the averaged measured
286 concentration was $0.69 \pm 0.1 \mu\text{g g}^{-1}$ (arithmetic mean \pm 1s.d.; n=5).

287

288 *3.2. Chromium content in bulk sediments and indigenous chironomids*

289 In the 2004 and 2007 sampling periods, Cr concentrations in chironomids tended to be
290 inversely related to those in sediments (Table 1). Biomimetic extractable Cr levels (2004
291 samples only) were about 0.1–0.2 % of total Cr in sediments at both sites and were
292 comparable between sampling periods and sampling sites (Table 1). In November 2006,
293 surface sediment samples at Waksmund were more contaminated (mean ± 1 s.d. = 1802 ± 744
294 $\mu\text{g g}^{-1}$, n=9) than at Maniowy ($118 \pm 39 \mu\text{g g}^{-1}$; n=9). In this sampling period, concentration in
295 chironomids increased with increasing concentration in sediments; organisms from Maniowy
296 having lower Cr concentrations than those from Waksmund. However, based on average

297 values, Cr concentrations differed by 17-fold in sediments, but less than 3-fold in
298 chironomids.

299

300 *3.3. Chromium concentration, distribution and speciation in sediment cores*

301 Total Cr concentration in sediments from the 2007 cores was higher at Waksmund than at
302 Maniowy (Table 2). The upper 2 cm of the Waksmund core had Cr concentrations in excess
303 of 2500 $\mu\text{g g}^{-1}$ and overlaid three layers (2–4 cm, 4–9 cm, and 9–11 cm) with fluctuating
304 levels of Cr contamination. In the core layer between 5 and 8 cm, we visually noticed the
305 presence of fibers and hairs, which is indicative of material originating from tanneries
306 (Pawlikowski et al., 2006). The alternation of sediment layers with different levels of Cr
307 contamination reflects the seasonality of tanning activities (which are concentrated during
308 winter time — the cold season in the boreal hemisphere) coupled with the deposition of spring
309 and summer flood sediments linked to river hydrology (Szalinska et al., 2010). At Maniowy,
310 Cr concentration was highest (452 $\mu\text{g g}^{-1}$) in the subsurface layer (0.5–1 cm) and then
311 decreased progressively towards the bottom of the core. Considering the upper 2 cm of
312 sediment, which are the most relevant for many chironomids (Stief and de Beer, 2006), Cr
313 concentrations at Waksmund were about 10-fold higher than those at Maniowy.

314 A similar situation was observed in overlying and pore waters. At Waksmund, total Cr
315 concentrations in pore waters were very stable in the first 2 cm ($114\pm 7 \mu\text{g L}^{-1}$, $n=3$) and
316 fluctuated markedly ($30\text{--}300 \mu\text{g L}^{-1}$) in the deeper layers. In the Maniowy core, Cr
317 concentrations in pore waters from the uppermost 2 cm were more variable ($43\pm 15 \mu\text{g L}^{-1}$,
318 $n=3$), but decreased below this depth (Table 2). The proportion of Cr(VI) relative to total Cr
319 could not be measured for all the pore water samples collected from the various layers due to
320 the water volume required for voltammetric measurements (Bobrowski et al., 2012). Based on
321 the available ‘combined values’ (Table 2), the proportion of Cr(VI) relative to the total Cr

322 content of pore waters varied between 0.5% and 15% at Waksmund and between 5% and 15%
323 at Maniowy. At both sites, the proportion of Cr(VI) was higher in overlying waters (20 % and
324 28 % at Waksmund and Maniowy, respectively) than in interstitial waters. In absolute terms,
325 Cr(VI) concentrations were always higher at Waksmund than at Maniowy (Table 2).

326

327 *3.4. Chromium concentration by DET and DGT*

328 In June 2007, the mean dissolved Cr concentration estimated by DET in interstitial waters at
329 Waksmund was $284 \pm 85 \mu\text{g L}^{-1}$ (n=11; range 178–437 $\mu\text{g L}^{-1}$) and the corresponding mean
330 DGT-labile concentration was $4.9 \pm 1.7 \mu\text{g L}^{-1}$ (n=11; range 2.9–9.0) (Fig. 2). Based on these
331 data, DGTs trapped about 1.7% of the total dissolved species (as measured by DETs) present
332 in pore waters. At Maniowy, average Cr concentrations measured by DET and DGT were
333 $74 \pm 21 \mu\text{g L}^{-1}$ (n=12; range 39–113 $\mu\text{g L}^{-1}$) and $1.0 \pm 0.54 \mu\text{g L}^{-1}$ (n=13; range 0.33–2.02 $\mu\text{g L}^{-1}$),
334 respectively; with DGT trapping 1.3% of the total dissolved concentrations. Chromium
335 concentrations measured by DET were higher than the corresponding Cr levels determined in
336 pore waters after centrifugation (Table 2): $130 \pm 86 \mu\text{g L}^{-1}$ (n=10) and $25 \pm 16 \mu\text{g L}^{-1}$ (n=9) for
337 Waksmund and Maniowy, respectively. These difference are linked to the predominance of
338 colloidal Cr(III) (Bobrowski et al., 2004; Dominik et al., 2007) that can be partly removed
339 during centrifugation but is caught by the DET probe. Despite all precautions, precipitation of
340 Fe and Mn oxides may have occurred during core slicing and sediment centrifugation.
341 Regardless of the methodological aspects, Cr concentrations at Maniowy were lower than
342 those at Waksmund for the same period, but the average proportion of dissolved concentration
343 which was labile (DGT with respect to DET) was small (1 to 2 %) and roughly similar for the
344 2 sites.

345 Finally, Cr concentrations in overlying waters were comparable with those in pore waters
346 from the uppermost 2 cm at Waksmund, while they were lower than the values in pore waters

347 at Maniowy. A flux of chromium from sediments to the water column is therefore possible at
348 Maniowy, while the situation at Waksmund is more variable and no definite conclusions can
349 be drawn (Fig. 2).

350

351

352 **4. Discussion**

353 *4.1. Chromium levels in environmental matrices and chironomids*

354 At Waksmund, the bioaccumulation factor (BAF – calculated as the ratio between total Cr
355 content in sediments vs. total Cr content in indigenous chironomids) ranged from less than 0.1
356 in 2004 and 2006 to 0.17 in June 2007. The same calculation for Maniowy gives values one
357 order of magnitude higher with a maximum of 1.3 for June 2007 (Table 1). Assuming that
358 ingested sediments represent 6% of the chironomids' body weight (Ferrari et al. (2014),
359 correction for the sediment contained in the organisms' gut (Hare et al., 1989) decreases Cr
360 concentration in chironomids by 60–100% at Waksmund and by, at most, 30% at Maniowy
361 (Table 1). Calculation of BAFs using Cr gut-corrected concentrations (data not shown)
362 therefore confirms that Cr bioavailability is higher at Maniowy than at Waksmund.
363 Pawlikowski et al. (2006) reported the presence of microscopic pieces of tanned leather in
364 sediments from Waksmund; a situation which increases the total Cr content of sediments, but
365 not its bioavailable fraction. However, the biomimetic analysis performed in 2004 suggests
366 that the quantity of bioavailable Cr (*sensu* Chen and Mayer, 1999) is comparable for the two
367 sites (Table 1). Because no major changes in river/reservoir hydrodynamics or tanning
368 operations occurred between 2004 and 2007, the amount of bioavailable, sediment-bound Cr
369 at the two sites should have remained similar also for the 2006 and 2007 sampling periods.

370 Another possible explanation for the observed discrepancies between sediment
371 contamination and Cr accumulation by chironomids is that pore waters and/or overlying

372 waters contribute most of the bioavailable Cr for uptake by living organisms. However,
373 concentrations of filterable Cr in pore waters (Table 2) and concentrations obtained by
374 DET/DGT (Fig. 2) were systematically higher at Waksmund than at Maniowy; contrary to
375 what was observed in chironomids (Table 1). Even the bioavailable Cr(VI) form was more
376 abundant in pore- and overlying waters from Waksmund than in those from Maniowy.
377 Although these analyses (Table 2) and the results of DET/DGT (Fig. 2) represent a snapshot
378 of the variable aqueous concentrations experienced by chironomids at the sediment-water
379 interface, Cr accumulation by DGT probes exposed for different times at Maniowy was
380 similar (Fig. S1). These additional findings indicate that the sediments' particulate phase was
381 able to constantly resupply Cr to the pore waters (Roulier et al., 2008), thus ensuring a certain
382 stability of DGT-labile Cr concentrations (assumed as a proxy of bioavailable Cr) in this
383 compartment.

384 On the other hand, direct comparisons between Cr concentrations in aqueous/sedimentary
385 matrices vs. indigenous chironomids do not capture the actual dynamic nature of contaminant
386 accumulation by living organisms. Contaminant accumulation is the net result of uptake from
387 different exposure routes, excretion and growth dilution, as formalized by e.g. the biodynamic
388 modelling approach (Baumann and Fisher, 2011; Luoma and Rainbow, 2005; Wang et al.,
389 1997). In the present study, the use of biodynamic modelling to estimate the relative
390 contribution of different routes of exposure to the Cr burden of indigenous chironomids is not
391 straightforward because the model's parameters vary among species, food types and
392 contaminants. To the best of our knowledge, only a handful of studies have examined Cr
393 uptake by aquatic organisms from aqueous and dietary exposure routes (Baumann and Fisher,
394 2011; Roditi and Fisher, 1999; Roditi et al., 2000; Wang et al., 1997). With the exception of
395 Roditi et al. (2000), who estimated that only 9–45% of Cr accumulation by zebra mussel
396 originated from particle ingestion, these studies identified uptake from food ingestion

397 (sediments, suspended particles or algae) as the predominant contributor to the total Cr body
398 burden. None of these studies used chironomids as model organisms, but the predominance of
399 contaminant uptake via food ingestion has been confirmed for chironomids in the case of
400 other trace elements (De Jonge et al., 2010; Martin et al., 2008). It is therefore important to
401 examine more in detail the possible factors that can influence the bioavailability of sediment-
402 bound Cr at the study sites.

403

404 *4.2. Factors affecting the bioavailability of sedimentary Cr*

405 Given the similar concentrations of biomimetic extractable Cr measured at the two sampling
406 sites (Table 1), a similar Cr burden in indigenous chironomids would be expected under the
407 assumption that uptake from sediments determines most of the Cr body burden and that
408 contaminant desorption from sediments is the limiting factor for their subsequent absorption
409 (Wang and Fisher, 1999). These expectations not being confirmed by experimental data, other
410 aspects of Cr geochemistry (not captured by the biomimetic approach) may make Cr more
411 bioavailable at Maniowy than at Waksmund or limit Cr bioavailability in the highly
412 contaminated Waksmund sediments.

413 In the cores collected in June 2007 and in November 2006 (Table 2 and Tables S1a, S1b),
414 a relationship between sedimentary Cr and Fe existed at Maniowy ($r=0.88$, $n=10$, $p<0.01$ in
415 2007 and $r=0.69$, $n=11$, $p<0.05$ in 2006; Spearman Rank Correlation test), but not at
416 Waksmund. The Fe-Cr correlation is indicative of an association between Cr and Fe-
417 oxyhydroxides that is often highlighted using sequential extraction procedures of sedimentary
418 Cr in aquatic systems impacted by tannery wastewaters (Hursthouse et al., 2001; Khwaja et
419 al., 2001; Rodrigues and Formoso, 2006). However, in such procedures, the Fe-Mn oxides
420 extraction step usually employs hydroxylamine hydrochloride at pH values around 2; much
421 lower than those of 6–7 typical of the chironomids' gut (Crozier, 1924). Nonetheless, anoxic

422 conditions in the chironomids' gut could favour reductive dissolution of Fe oxides with
423 subsequent liberation of Cr directly in the gut lumen (Stief and Eller, 2006). The redox
424 potential (E_h) in the gut lumen of chironomids is about -50 mV (Stief and Eller, 2006); low
425 enough to allow for the dissolution of amorphous $Fe(OH)_3$ given the E_h value of 14 mV at pH
426 7 for the redox couple $Fe(OH)_3/Fe(II)$ (Weber et al., 2006). The kinetics of Fe oxy-hydroxides
427 dissolution can also be fast enough for the whole process to become important during feeding.
428 Larval gut-passage time (GPT) for chironomids ingesting natural sediments varies from less
429 than 1 hour for assemblages of indigenous chironomids (Romito et al., 2010) to about 2 to 4
430 hours for *Chironomus riparius* (Wästlund, 1999); the variability in the latter case being linked
431 to sediment characteristics. Under some conditions (pH=7 and partial H_2S pressure of 10^{-4}
432 atm), dissolution of Fe oxy-hydroxides is fast enough to occur during GPT (Dos Santos
433 Afonso and Stumm, 1992). All these physiological factors and kinetics aspects of sediment
434 digestion by chironomids would not be captured by the *in vitro* biomimetic approach. A more
435 effective chromium liberation in the gut lumen following dissolution of Fe oxy-hydroxides
436 could then be responsible for the higher level of Cr bioaccumulation at Maniowy.

437 The presence of heterogeneous levels of Cr contamination in sediments and the possible
438 preference of chironomids for relatively clean sediment patches could also contribute to the
439 observed discrepancies between Cr concentrations in water and sediment vs. accumulation by
440 chironomids. Chromium concentrations in sediments did vary markedly in time and space.
441 Results from November 2006 show that Cr concentrations from surface sediment layers (0–
442 0.5 cm) can vary 3–4-fold inside both sampling zones (Table 1). Surface sediment samples
443 collected at Waksmund in September 2005 from 9 sub-sites arranged in a regular grid
444 covering an area of 1 m^2 had a Cr content ranging between 132 and $1497\text{ }\mu\text{g g}^{-1}$ (arithmetic
445 mean \pm 1 standard deviation = $431\pm 379\text{ }\mu\text{g g}^{-1}$; Table S2). Other samples from Waksmund
446 collected in March, May, August 2005 showed a 38-fold variability of total Cr concentrations

447 (Table S2). However, only a 4-fold change in Cr levels was measured in indigenous
448 chironomids recovered from these sediments at the same time (Table S2) and organisms from
449 the cleanest sediments were those with the highest Cr burden after correction for the sediment
450 gut content (Table S2). Whether this may be indicative of a decreased Cr availability in highly
451 contaminated sediments or avoidance of such sediments by benthic organisms remains to be
452 ascertained.

453 In any case, it is worth noting that the gut-corrected Cr concentrations measured in the
454 pools of indigenous chironomids at Waksmund and Maniowy (Table 1 and Table S2) often
455 exceeded the range of 10–69 $\mu\text{g g}^{-1}$ d.w. associated with a decrease in the presence of other
456 less tolerant taxonomic groups (Bervoets et al., 2016). In June 2004, chironomids were also
457 sampled at two sites not directly impacted by tannery discharges: the Bialka river before its
458 entrance in the Czorsztyn reservoir (Figure 1) and a small sandy beach located east of the
459 mouth of the same river. Chromium levels in sediments from these sites were 44 $\mu\text{g g}^{-1}$ and 32
460 $\mu\text{g g}^{-1}$, respectively, and the corresponding gut-corrected Cr concentrations in chironomids
461 were 2.8 $\mu\text{g g}^{-1}$ and 11.3 $\mu\text{g g}^{-1}$. The latter values compare favourably with Cr levels that
462 should not negatively affect the structure of benthic communities according to Bervoets et al.
463 (2016). Under this assumption, Cr accumulation at Waksmund and Maniowy (Table 1 and S2)
464 could therefore be high enough to cause adverse effects on the benthic community structure.

465 Finally, the actual species composition of the organisms we described under the generic
466 name of ‘indigenous chironomids’ may also contribute to the observed discrepancies between
467 ambient Cr concentrations and Cr body burdens. Because different species of chironomids
468 accumulate contaminants to different extents (Martin et al., 2008), we compared our
469 simplified sampling method with a more inclusive procedure of organism collection to
470 identify the taxonomic groups dwelling at each site in June 2007 (see section 2.1). The
471 chironomids’ community at Waksmund turned out to be more abundant and more

472 heterogeneous than at Maniowy, where the community included only specimen of the genus
473 *Chironomus* (Fig. 3 and Table S3). This situation reflects typical differences between
474 piedmont streams (i.e., Waksmund) where biodiversity is typically high (Kownacki, 1995)
475 and reservoirs (i.e., Rożnów reservoir on the Dunajec River) where larvae of the genus
476 *Chironomus* usually dominate (Dumicka et al., 1986). Practically speaking, our simplified
477 sampling procedure did not alter the community pattern at Maniowy, but selectively sampled
478 individuals of the genera *Chironomus* and *Stictochironomus*, representing only 20 % of the
479 total species, at Waksmund (Fig. 3 and Table S3). *Stictochironomus* can actively migrate
480 along the sediment column (Kiyashko et al., 2004) and feed on less contaminated sediments
481 (see the sediment profile in Table 2). This scenario may then explain why observed Cr
482 concentrations at Waksmund were lower than those expected from the concentration in
483 sediments. The differences in species composition also indicate that chironomids at Maniowy
484 may accumulate higher body burdens of Cr because of their feeding strategy (i.e., surface
485 sediment feeders); a situation similar to that reported by Martin et al. (2008) for two
486 sympatric species of *Chironomus*.

487 Given their ecological traits (McLachlan, 1977; Stief and de Beer, 2006), chironomids may
488 indeed accumulate part of their Cr burden from freshly deposited suspended particulate matter
489 which, in the Dunajec river, is highly variable in both concentration and Cr content (Szalinska
490 et al., 2010; Szalinska et al., 2013). The companion paper (Ferrari et al., this issue) will
491 present evidence supporting the hypothesis that ingestion of freshly deposited suspended
492 matter at the sediment water interface can constitute a prominent route of exposure for surface
493 deposit feeders such as *Chironomus* spp. (i.e. the predominant genus occurring at Maniowy).

494

495

496

497 **5. Conclusions**

498 High Cr concentrations in sedimentary and aqueous matrices impacted by tannery discharges
499 were not systematically accompanied by high Cr body burdens in surface deposit feeders
500 dwelling at the two sampling locations. However, Cr accumulation by indigenous
501 chironomids did occur despite the low proportion of sediment-bound Cr liable to desorption
502 during gut passage (about 0.1% as estimated using a biomimetic approach). Concentrations of
503 DGT-labile Cr, considered as a proxy for bioavailable Cr(III), and Cr(VI) in pore waters were
504 less than 1% of the total Cr levels. On the other hand, Cr(VI) accounted for 20 to 30% of the
505 total in the overlying water confirming that oxidation of Cr(III) used in the tanning process to
506 Cr(VI) occurred and suggesting that sediments can act as a source of Cr(VI) to the overlying
507 water column. While there is a consensus that deposit feeders get most of their contaminant
508 body burden from sediment ingestion, the possible contribution of water born Cr species to
509 the element body burden in chironomids requires further study.

510 Although Cr accumulation by assemblages of indigenous chironomids is potentially
511 affected by numerous factors (from spatial patchiness in sediment contamination to specific
512 traits of various chironomids genera and species), the present work confirms that Cr released
513 by tannery discharges can accumulate in benthic organisms. These findings are particularly
514 important considering that Cr(III), usually regarded as of little environmental concern, is
515 expected to be the predominant redox form of Cr in sediments impacted by tannery
516 discharges. Metal-tolerant organisms such as chironomids were apparently able to cope with
517 total Cr content exceeding $1000 \mu\text{g g}^{-1}$ in the bulk sediment phase. However, such levels of
518 contamination may be high enough to exclude more sensitive taxa, resulting in a simplified
519 community composition and, eventually, non compliance with good ecological quality as
520 required by the Water Framework Directive.

521

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717 **Figure captions**

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719 Figure 1. Study area (Dunajec River and Czorsztyn Reservoir; southern Poland) and location
720 of the two sampling sites of Waksmund and Maniowy. The region of Nowy Targ,
721 characterized by the presence of numerous small tanneries, is also indicated.

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723 Figure 2. Vertical profiles of dissolved and labile Cr concentrations (in $\mu\text{g L}^{-1}$) as estimated
724 by DET and DGT probes exposed in the field at Waksmund (top panel) and Maniowy (bottom
725 panel) for 45 hours in June 2007. Dashed lines represent the sediment-water interface. Note
726 that DET and DGT depth do not exactly match.

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728 Figure 3. Relative percentage abundance of different taxonomic groups in samples at Waksmund
729 (Wak) and Maniowy (Man) following a full procedure (Wak and Man) or a simplified
730 procedure (WakA and ManA). Grey bars, *Chironomus* spp.; White bars, *Stictochironomus*
731 spp.; Black bars, other (see Table S3 for details).

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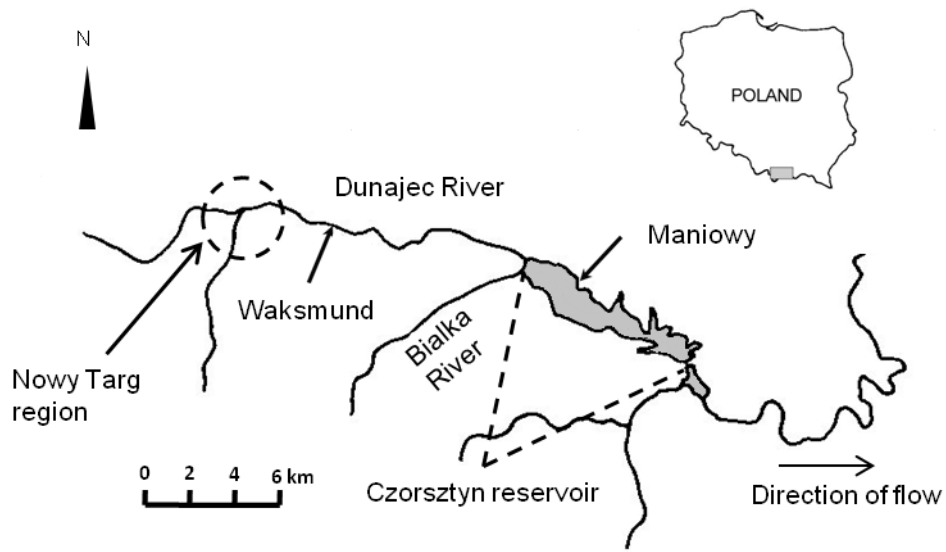
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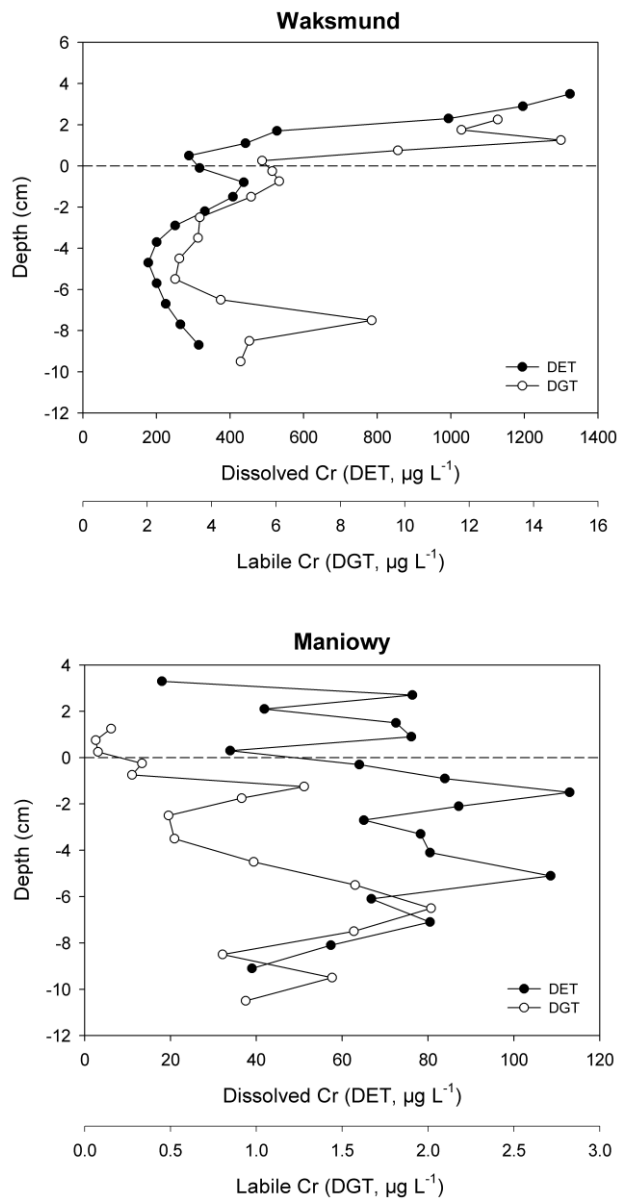
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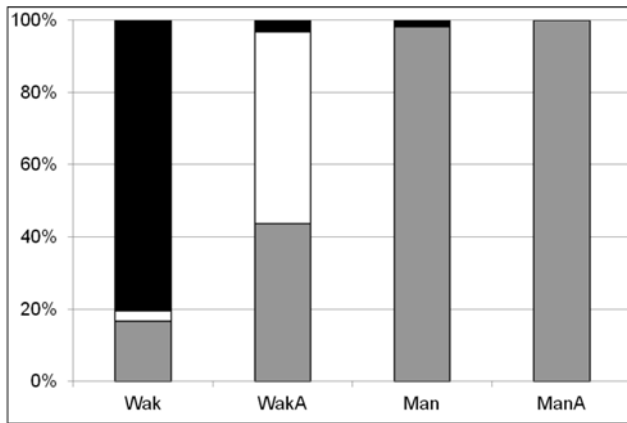
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767 Figure 3



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787 Table 1. Chromium concentrations ($\mu\text{g g}^{-1}$) in field sediment and indigenous chironomids collected at
788 Waksmund and Maniowy during different periods. For samples collected in 2004 and 2007, concentrations in
789 sediments are arithmetic means (± 1 standard deviation; s.d.) of triplicate measures of homogenised sediment
790 aliquots (unless otherwise indicated in the footnotes). For samples collected in 2006, the range and the
791 arithmetic mean ± 1 s.d. of Cr concentration in sediments were obtained from analysis of surface sediment layers
792 (0–0.5 cm) obtained from sediment cores (see footnotes for details). Chromium concentrations extracted from
793 sediments using the biomimetic approach (see section 2.4 for more details) are also reported for the 2004
794 campaigns. Total and gut corrected Cr concentrations are for pooled chironomids samples ($n=1$, except for June
795 2007 where $n=2$). Correction for sediment gut content was done according to Hare et al. (1989) assuming a
796 sediment gut content equal to 6% of the organisms' weight (Ferrari et al. 2014). BAF, Bioaccumulation
797 Factor=total Cr in Chironomids/total Cr in sediments; Wak, Waksmund; Man, Maniowy; OM, Organic Matter;
798 n.d., not determined.

Period	Site	Sediments			Chironomids		
		Total Cr ($\mu\text{g g}^{-1}$)	Biomimetic Cr ($\mu\text{g g}^{-1}$)	OM (%)	Total Cr ($\mu\text{g g}^{-1}$)	Gut corrected Cr ($\mu\text{g g}^{-1}$)	BAF
Jun 04	Wak	695 ^a	0.99 \pm 0.33	13.5	46	4.6	0.07
	Man	427 \pm 17 ^a	0.54 \pm 0.06	4.47	202	188	0.47
Nov 04	Wak	761 \pm 27	0.57 \pm 0.051	9.73	43	0 ^c	0.06
	Man	367 \pm 10	0.51 \pm 0.08	6.52	120	104	0.33
Nov 06	Wak	884–3415 ^b (1802 \pm 744)	n.d.	19.3	81	30–0 ^c 0 ^c	0.10–0.02
	Man	69–174 ^b (118 \pm 39)	n.d.	4.20	30	27–20 24	0.43–0.29
Jun 07	Wak	1300 \pm 21	n.d.	17.6	222	153	0.17
	Man	241 \pm 11	n.d.	5.17	311 \pm 37	315	1.3

799 a) Waksmund, $n=1$; Maniowy, $n=2$. Error calculated as $\text{Abs}[(\text{range})/\sqrt{2}]$

800 b) Range and, in parenthesis, arithmetic mean \pm one standard deviation of Cr concentration measured in surface
801 sediment layers (0–0.5 cm) from 9 independent cores.

802 c) Correction for sediment content in the gut yielded a negative value that was reported as zero in the table.

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804 Table 2. Concentrations of total chromium in sediments, overlying water (OW) and porewaters from cores
 805 collected at Waksmund and Maniowy in June 2007. Cr(VI) concentrations in OW and porewaters are also
 806 reported. For Cr(VI) determinations, porewaters recovered from different sediment layers were sometimes
 807 combined due to analytical constraints (see Material and Methods and Bobrowski et al., 2012). n.a., porewater
 808 could not be recovered from these layers; ---, Maniowy core was sliced down to 9 cm.

Depth (cm)	Waksmund			Maniowy		
	Sediment ($\mu\text{g g}^{-1}$)	Porewater ($\mu\text{g L}^{-1}$)		Sediment ($\mu\text{g g}^{-1}$)	Porewater ($\mu\text{g L}^{-1}$)	
	Total Cr	Total Cr	Cr (VI)	Total Cr	Total Cr	Cr (VI)
OW	n.a.	116	22.8	n.a.	3.16	0.90
0-0.5	5123	115		339	26.8	
0.5-1	2596	120	3.53	452	44.8	1.48
1-2	2488	106		291	56.2	
2-3	356	34.7	5.47	201	20.1	
3-4	161	18.0	0.73	214	26.2	
4-5	2155	74.6	n.a.	213	24.6	
5-6	6198	198	1.00	141	13.7	1.03
6-7	4789	187	1.04	69.9	7.16	
7-8	3642	310	1.16	80.0	n.a	
8-9	982	134		52.6	8.58	
9-10	470	n.a	n.a	---	---	---
10-11	591	n.a	n.a	---	---	---

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810 **Supplementary information for:**
811 **Chromium bioavailability in aquatic systems impacted by tannery wastewaters. Part 1:**
812 **understanding chromium accumulation by indigenous chironomids**

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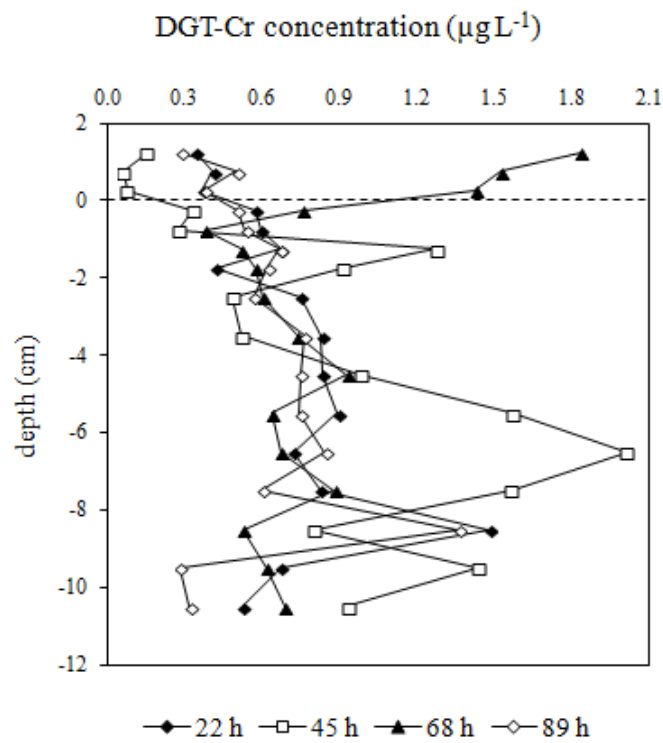
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842 Figure S1. Depth profiles of DGT-labile Cr ($\mu\text{g L}^{-1}$) in pore waters obtained in June 2007 for
843 different exposure periods at Maniowy.

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851 Table S1a. Vertical profiles of total concentrations of Cr, Fe, and Mn (all in $\mu\text{g g}^{-1}$ d.w.) in
 852 two sediment cores collected in June 2007 at Waksmund and Maniowy. Raw data were
 853 centred and reduced before performing the correlation statistics presented in section 4.3 of the
 854 main text.

Depth (cm)	Waksmund				Maniowy		
	Cr	Fe	Mn		Cr	Fe	Mn
0–0.5	5123	37,333	860		339	18,870	219
0.5–1	2596	40,268	943		452	22,209	238
1–2	2488	36,503	974		291	17,111	197
2–3	356	42,301	1371		201	15,487	206
3–4	161	42,321	1513		214	19,146	238
4–5	2155	32,965	1106		213	17,980	214
5–6	6198	29,898	774		141	13,868	186
6–7	4789	22,590	392		69.9	14,847	181
7–8	3642	27,691	405		80.0	15,917	212
8–9	982	15,254	199		52.6	12,879	167
9–10	470	10,247	140		n.a.	n.a.	n.a.
10–11	591	11,983	143		n.a.	n.a.	n.a.

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874 Table S1b. Total concentrations of Cr, Fe, and Mn (all in $\mu\text{g g}^{-1}$ d.w.) in 12 (Waksmund) or
 875 11 (Maniowy) sediment cores collected in November 2006 at Waksmund and Maniowy.
 876 Concentrations are for the uppermost sediment layer (0–0.5 cm). Cores from 1 to 9 are those
 877 considered in compiling Table 1 of the main text; cores from 10 to 12 are additional
 878 measurements. Raw data were centred and reduced before performing the correlation statistics
 879 presented in section 4.3 of the main text.

Core number	Waksmund				Maniowy		
	Cr	Fe	Mn		Cr	Fe	Mn
1	2131	28,740	631		94.5	9,740	115
2	1371	28,273	496		101	10,072	133
3	1959	26,545	470		122	11,776	161
4	2073	29,761	669		144	10,199	144
5	3415	28,287	545		95.8	10,324	117
6	1329	31,776	672		174	16,063	235
7	2017	29,101	575		169	12,193	150
8	884	31,643	640		69.2	10,842	155
9	1853	28,056	536		68.7	8,171	123
10	1325	29,776	528		114	10,210	176
11	2096	26,456	561		103	11,653	113
12	1231	26,617	500		Not available		

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893 Table S2. Chromium concentrations ($\mu\text{g g}^{-1}$) in field sediment and indigenous chironomids
 894 collected at Waksmund during different periods. Total and gut corrected Cr concentrations are
 895 for pooled chironomids samples (n=1). Correction for sediment gut content was done
 896 according to Hare et al. (1989) assuming a sediment gut content equal to 6% of the
 897 organisms' weight (Ferrari et al. 2014).

Sampling date	Sediments		Chironomids	
	Total Cr		Total Cr	Gut-corrected Cr
29/03/2005	4471 \pm 449 ^a		217	0 ^b
27/05/2005	391 \pm 21		101	82.6
15/08/2005	116 \pm 1.2 ^a		104	103
Sept. 2005 ^d	431 \pm 379 ^c		52.9	28.8

898 a) Analytical variability ; n=2 . Error calculated as $\text{Abs}[(\text{range})/\sqrt{2}]$

899 b) Correction for sediment content in the gut yielded a negative value that was reported as
 900 zero in the table.

901 c) Arithmetic mean \pm 1 standard deviation of 9 different samples collected over an area of 1
 902 m^2 . Analytical variability on individual samples was less than 5%.

903 d) Exact day of sampling not in the record

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919 Table S3. Number of individuals belonging to different taxa in samples collected at
 920 Waksmund and Maniowy in June 2007 using different sampling strategies: Wak, collection
 921 with a Surber Sampler (400 cm²); Man, collection with an Ekman grab sampler (225 cm²);
 922 WakA and ManA, simplified collection procedure for determination of Cr content in
 923 assemblages of indigenous chironomids described in section 2.1 of the main text.

	Waksmund		Maniowy	
	Wak	WakA	Man	ManA
TANYPODINAE				
<i>Procladius</i> spp.	2	0	0	0
<i>Macropelopia</i> sp.	4			
<i>Thienemannimyia</i> - complex	2	0	0	0
<i>Tanypodinae</i> (juv.)	4			
DIAMESINAE				
<i>Pothastia longimanus</i> KIEFFER 1922	12.5	0	0	0
PRODIAMESINAE				
<i>Prodiamesa olivacea</i> (MEIGEN 1818)	12	0	0	0
ORTHOCLADIINAE				
<i>Eukiefferiella</i> sp	8	0	0	0
<i>Paracladius conversus</i> (WALKER 1856)	66	0	0	0
<i>Orthoclaadiinae</i> (juv.)	4			
CHIRONOMINAE				
Chironomini				
<i>Chironomus</i> spp. ¹	224	42	53	116
<i>Cryptotendipes</i> sp.	4	0	0	0
<i>Microtendipes pedellus</i> group	394	1	0	0
<i>Stictochironomus</i> sp.	40	51	0	0
<i>Polypedilum (Polypedilum)</i> sp.(cf. pedestre MEIGEN 1830) (p)				
<i>Polypedilum (Polypedilum)</i> spp.	12	2	1	0
<i>Polypodilum (Tripodura)</i> sp	2	0	0	0

Table S4 (continued)

	Waksmund		Maniowy	
	Wak	WakA	Man	ManA
<i>Paratendipes</i> sp.	4	0	0	0
<i>Chironomini</i> (juv)	152			
T a n y t a r s i n i				
<i>Micropsectra atrofasciata</i> (KIEFFER 1911) (p)	1			
<i>Micropsectra</i> sp.	5	0	0	0
<i>Tanytarsus brundini</i> LINDEBERG 1963	98	0	0	0
<i>Tanytarsus pallidicorris</i> (WALKER 1856) (p)				
<i>Tanytarsus pallidicorris</i> group)	66	0	0	0
<i>Tanytarsus</i> spp.	140	0	0	0
<i>Cladotanytarsus</i> sp.				
<i>Tanytarsini</i> (juv.)	88			
TOTAL DENSITY	1 345 (ind/400 cm ²)	96	54 (ind/225 cm ²)	116

924 1,two species of *Chironomus* were found in the examined material on the basis of genetic
925 study: *Chironomus plumosus* and *Chironomus bernensis* (Michailova et al., 2009).
926

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