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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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Abstract

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

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

Introduction

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

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

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

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

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

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

2. Methods

2.1. Study area and sampling

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

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

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

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

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

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

2.2. Core slicing and pore waters extraction

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

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

2.3. DET and DGT methodologies

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

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

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

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

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

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

2.4. Chemical analyses

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

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

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

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

3. Results

3.1. Quality control procedures

For analysis of waters and DGT/DET eluates, typical detection and quantification limits for total chromium (calculated as 3 and 10 times the standard deviation of analytical blanks) were 0.015 $\mu\text{g L}^{-1}$ and 0.049 $\mu\text{g L}^{-1}$, respectively. Four analytical replicates were measured for each sample and the typical precision (for concentrations above the quantification limit) was 5% or better. Measured values for certified reference waters (arithmetic means $\pm$ 1 standard deviation) 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 favourably with the corresponding certified values of 0.30 $\pm$ 0.04 $\mu\text{g L}^{-1}$ (uncertainty expressed

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

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

3.2. Chromium content in bulk sediments and indigenous chironomids

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

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

3.3. Chromium concentration, distribution and speciation in sediment cores

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

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

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

3.4. Chromium concentration by DET and DGT

In June 2007, the mean dissolved Cr concentration estimated by DET in interstitial waters at Waksmund was $284 \pm 85 \mu\text{g L}^{-1}$ ($n=11$; range 178–437 $\mu\text{g L}^{-1}$) and the corresponding mean 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 data, DGTs trapped about 1.7% of the total dissolved species (as measured by DETs) present in pore waters. At Maniowy, average Cr concentrations measured by DET and DGT were $74 \pm 21 \mu\text{g L}^{-1}$ ($n=12$; 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 $\mu\text{g L}^{-1}$), respectively; with DGT trapping 1.3% of the total dissolved concentrations. Chromium concentrations measured by DET were higher than the corresponding Cr levels determined in 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 Waksmund and Maniowy, respectively. These difference are linked to the predominance of colloidal Cr(III) (Bobrowski et al., 2004; Dominik et al., 2007) that can be partly removed during centrifugation but is caught by the DET probe. Despite all precautions, precipitation of Fe and Mn oxides may have occurred during core slicing and sediment centrifugation. Regardless of the methodological aspects, Cr concentrations at Maniowy were lower than those at Waksmund for the same period, but the average proportion of dissolved concentration which was labile (DGT with respect to DET) was small (1 to 2 %) and roughly similar for the 2 sites.

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

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

4. Discussion

4.1. Chromium levels in environmental matrices and chironomids

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

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

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

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

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

4.2. Factors affecting the bioavailability of sedimentary Cr

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

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

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

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

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

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

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

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

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

5. Conclusions

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

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

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Figure captions

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

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

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

742 Figure 1

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758 Figure 2

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Figure 3

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

Period	Site	Sediments			Chironomids		
		Total Cr	Biomimetic Cr	OM	Total Cr	Gut corrected	BAF
		($\mu\text{g g}^{-1}$)	($\mu\text{g g}^{-1}$)	(%)	($\mu\text{g g}^{-1}$)	Cr ($\mu\text{g g}^{-1}$)	
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

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

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

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

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

Depth (cm)	Waksmund			Maniowy		
	Sediment	Porewater		Sediment	Porewater	
	($\mu\text{g g}^{-1}$)	($\mu\text{g L}^{-1}$)		($\mu\text{g g}^{-1}$)	($\mu\text{g L}^{-1}$)	
	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	---	---	---

Supplementary information for:

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

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

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.

	Waksmund				Maniowy		
Depth (cm)	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.

	Waksmund				Maniowy		
Core number	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>Orthocladiinae</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</i> (<i>Polypedilum</i>) sp.(cf. pedestre MEIGEN 1830) (p)				
<i>Polypedilum</i> (<i>Polypedilum</i>) spp.	12	2	1	0
<i>Polypodilum</i> (<i>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			
<i>Tanytarsini</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).
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