Environ Sci Pollut Res
DOI 10.1007/s11356-016-8261-1

RESEARCH ARTICLE

Encaged *Chironomus riparius* larvae in assessment of trace metal bioavailability and transfer in a landfill leachate collection pond

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Received: 8 September 2016 / Accepted: 13 December 2016

Abstract Household wastes may constitute a vector of environmental contamination when buried, in particular through degradation and production of leachates containing significant trace metal (TM) concentrations that may constitute a serious risk to biota. The objectives of this study were to assess the bioavailability and transfer potential of various TMs present in water and sediments in a reservoir receiving landfill leachates. An active biomonitoring approach was adopted consisting of exposing naive laboratory organisms in cages deployed in the field. Aquatic insects such as *Chironomus riparius* larvae are good candidates since they represent key organisms in the trophic functioning of aquatic ecosystems. The results show that water, suspended particles, and sediments were significantly contaminated by various TMs (As, Cd, Cu, Ni, Pb, and Zn). Their contribution to the transfer of TMs depends, however, on the specific element considered, e.g., Cd in sediments or Pb in both suspended particles and sediments. The internal fate of TMs was investigated according to their fractionation between an insoluble and a cytosolic fraction. This approach revealed different detoxification strategies capable of preventing the induction of deleterious effects at the individual scale. However, the accumulation of several TMs in *C. riparius* larvae tissues may also represent a significant load potentially transferable to higher trophic levels.

Responsible editor: Philippe Garrigues

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Keywords Active biomonitoring · Wetland · Toxicokinetics · Fractionation · Contamination sources

Introduction

In 2010, the earth's population went over the bar of seven billion. In France, between 2004 and 2010, the population increased by 4% (United Nations 2015). However, waste production increased by about 20% (Haeusler et al. 2014) during the same period. In spite of the European framework directive that regulates waste production and encourages its valorization (Directive 2008/98/EC), waste quantities continue to increase by 1% per year (Haeusler et al. 2014). In France, for example, landfill disposal of household waste was authorized until 2002. However, this policy led to certain environmental impacts as waste landfill sites may be important sources of chronic and diffuse contamination (Urase et al. 1997; Butt et al. 2014; Grisey and Aleya 2016a).

According to the level of microbial activity and water seepage, leachate production results from waste degradation and constitutes a vector of pollution through contact with the surrounding soils and surface or ground waters (Matejczyk et al. 2011; Bichet et al. 2016). The composition of these effluents may vary according to the age and the type of landfilled wastes, but they are often heavily loaded with the most commonly found trace metals (TMs) such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn), but also with organic pollutants such as polycyclic aromatic hydrocarbons (PAHs), chloride aliphatic compounds, and pesticides (Kulikowska and Klimiuk 2008; Hernández et al. 2012; Ben Salem et al. 2016). As the release of such leachates into the environment may have ecotoxicological consequences (Senese et al. 2010; Ben Salem et al. 2014a), treatment and monitoring is necessary for the

Published online: 09 January 2017

prevention and management of potential environmental risks (Matejczyk et al. 2011). In addition to classical physicochemical characterizations, active biomonitoring figures among existing risk assessment procedures as a well-known and relevant approach, involving exposure of naive organisms (i.e., from laboratory cultures) in the field. In aquatic ecosystems, chironomid larvae, developing at the water-sediment interface, are recognized as good bioindicators of sediment and water quality with respect to contamination by TMs (Meregalli et al. 2000; Bervoets et al. 2004; Faria et al. 2008; De Jonge et al. 2014; Bervoets et al. 2016). Moreover, due to their position at the bottom of food webs, they may also be used as early indicators of TM trophic transfer. However, for this purpose, the internal fate of TMs must be known since it conditions their trophic availability to predators (Wallace and Luoma 2003).

Located in northeastern France, the Etueffont landfill functioned for nearly 30 years with a non-conventional mode of operation. Shredded waste from each of the landfill's two operating cells was successively deposited without compaction in meter-thick layers with a 2 or 3-month period of biostabilization between deposits. The resulting leachate was drained into a shared leachate collection system and then discharged into four interconnected ponds (Khattabi et al. 2006; Ben Salem et al. 2014a, b) (Fig. 1). We therefore hypothesized that using encaged *Chironomus riparius* larvae kept in the first pond which directly receives the gross leachate containing various TMs and other inorganic and organic compounds (Grisey and Aleya 2016b) might contribute to a better understanding of the bioavailability and transfer potential of various TMs present in water and sediments.

The aim of this study was thus to (i) determine the TM accumulation kinetics in *C. riparius* larvae, (ii) evaluate the contribution of water and sediments in TM bioavailability to larvae, and (iii) investigate the fate of TMs in two internal fractions of different ecotoxicological significance.

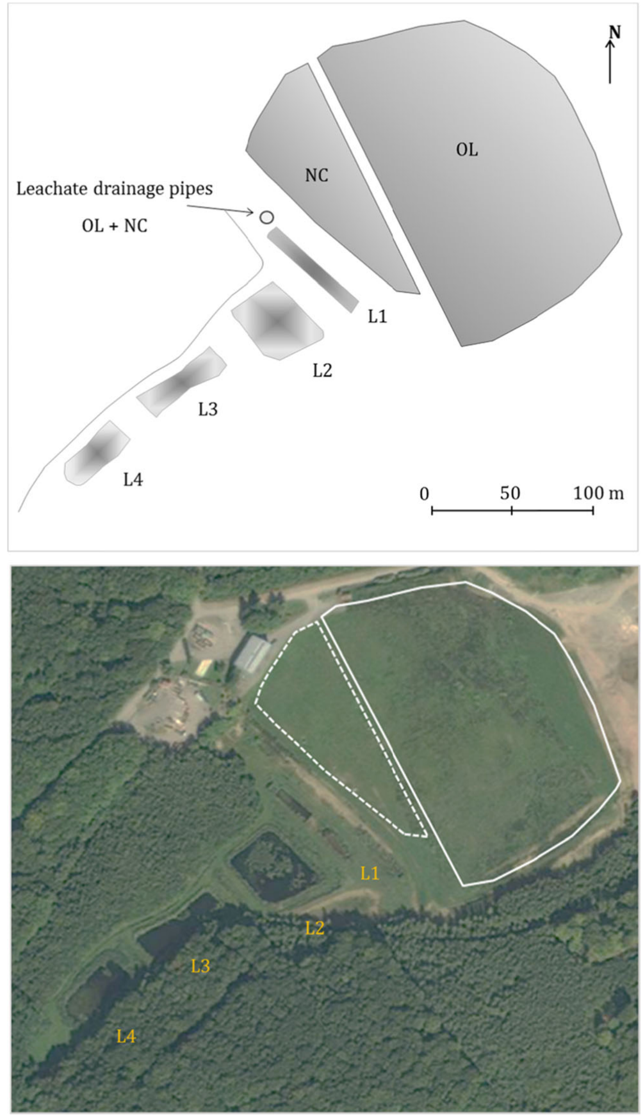

Fig. 1 The Etueffont landfill with lagooning ponds (L1: sampling pond, L2, L3 and L4) and location of old landfill (OL) and new cell leachates (NC) (*top*). Aerial view of the landfill (*bottom*)

Materials and methods

Study site

This study was carried out in the Franche-Comté region (northeastern France), in the leachate treatment wetland of a municipal solid waste landfill located at Etueffont (47° 43′ 19″ N/6° 56′ 57″ E). In operation from 1976 to 2002, the landfill received and shredded household waste from 66 communities (totaling 47,650 inhabitants), representing about 200,000 t of waste which were deposited over an area of 28,000 m^2 until the site was closed (Grisey and Aleya 2016a). The landfill also received municipal solid waste, bulky waste and construction and demolition waste, deposited into the two cells, with the waste depth ranging from 6 to 15 m. The first cell, known as the old landfill (OL), was in operation from 1976 to 1998 and was located directly on impermeable schist (Fig. 1). The new cell (NC) was in operation from 1998 to 2002 and was equipped with a watertight polyethylene bottom liner, surrounded on either side by a geotextile mat.

Organisms

The studied pond is the first in a four-basin lagooning system and that received leachates directly from the landfill. We used the fourth-instar larvae of *Chironomus riparius* (Hexapoda; Diptera) cultured in the laboratory prior to the experiments according to standard methods (OECD 218 2004). Briefly, egg masses were removed from the culture 10 days before the experiment and left to hatch at room temperature for 2 days in 20 mL pillboxes filled with 50% dechlorinated tap water and 50% ultrapure (UP) water (20 °C, 280 µS cm^{-1}, pH = 7.2, 16-h photoperiod). Synchronized new born organisms (first instar larvae) were then transferred to 3-L containers filled with 1 L of water and a thin layer of silica sand, and were fed ad libitum each day with 1 mg of organic oat flour per individual (Péry et al. 2002). After 2 to 3 days, second-instar larvae were individually sorted and 20 individuals were placed in 0.6-L beakers, filled with 0.1 L of clean sand and 0.4 L water, and fed as previously. After 4 to 5 days, larvae reached the fourth instar and were ready for experimentation.

Exposure modalities

The biomonitoring procedure used in this study was implemented according to Ferrari et al. (2014). The larvae were exposed in two different types of cage allowing estimation of the water and sediment contributions to TM bioavailability and transfer (Fig. 2). In the water and sediment (WS) cages, larvae were exposed to both water (including suspended particles) and sediments, while W cages were filled with uncontaminated sand, and therefore, larvae were exposed to water (and suspended particles) only. In each type of cage, 20 fourth-instar larvae were introduced using to a delivery system attached to the cage head (see Ferrari et al. 2014 for technical details). Altogether, 30 cages were randomly deployed in the field (March 2015) over a surface of about 30 m^2 and for a period of 5 days. Each day, a triplicate of each cage was randomly removed (with intact sediments in the WS cage) and brought back to the lab where they were disassembled. Living larvae were recovered (by gently sieving the sediment), rinsed in UP water, dried on an absorbent paper sheet, individually measured for their length, then pooled per replicate in cryogenic tubes, weighed and finally sacrificed at −80 °C. The same preparation procedure was applied to larvae ($n = 20$, triplicate) from the culture, i.e., at t0. Tubes were kept frozen (−20 °C) until fractionation and TM analysis. At each sampling date (including t0), overlying water and sediments were also sampled for TM analysis. Suspended particle traps were concomitantly deployed in the pond and collected after 1, 3, and 5 days of exposure, pooled and analyzed for TMs.

Internal fractionation

Partially thawed larvae ($n = 17$ and 24 mg, on average) were then homogenized (Ultra-Turrax®) in 3 mL of ice-cold 20 mM Tris-HCl buffer (Sigma-Aldrich, purity 99%) adjusted to a pH of 7.5 using 1 M NaOH (Sigma-Aldrich, purity 99.99%). After aliquot sampling (1 mL), the homogenate (H) was fractionated by centrifugation at 25,000*g* for 1 h at 4 °C (Péry et al. 2008; Gimbert et al. 2016) separating an insoluble fraction (called P for pellet and containing the exoskeleton, gut content, granules, and cellular debris) from a soluble fraction (called S for supernatant and corresponding to the cytosolic compartment).

Trace metal analysis

All samples (filtered water, freeze-dried sediments, suspended particles, and larval fractions) were analyzed by inductively coupled plasma mass spectrometry (ICP-MS, iCAP 6000 Series, Thermo Scientific) after hot acidic mineralization in *aqua regia* (HNO_3/HCl, 2:5 *v*/*v*, Sigma-Aldrich, purity 99.9%) using a Digiprep block digestion system. The reliability of the analyses was assessed with matrix-specific certified reference materials (water: NIST 1643e, sediment: CRM052 loamy clay, and animal tissue: TORT-2). Average TM recoveries for the six metals studied (As, Cd, Cu, Ni, Pb, and Zn) were 98 ± 8, 109 ± 16, and 97 ± 12% for the three reference materials, respectively.

Statistics

Trace metal concentrations in water, sediments, and *C. riparius* fractions were compared between sampling dates and cage type using an analysis of variance (ANOVA) or a

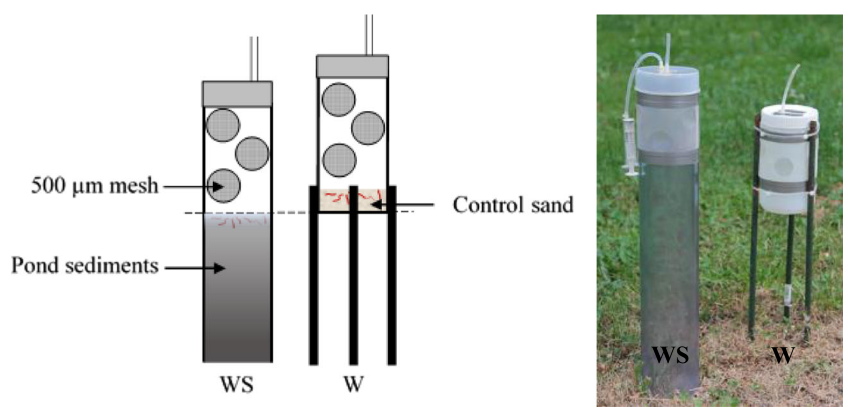

Fig. 2 Cage design for the in situ biomonitoring using *Chironomus riparius* larvae. "WS" allowed the larvae to be exposed to sediments and water; "W" cages to water only

Kruskal-Wallis test when normality or homoscedasticity were not reached. Post hoc pairwise comparisons were then checked using Tukey's honest significant difference (HSD) or a multiple comparison test (kruskalmc) using the package "pgirmess," respectively.

Regression on Order Statistics (ROS, package "NADA") were used to extrapolate TM concentrations in larvae fractions below the quantification limit (QL). Briefly, this semi-parametric method performs a regression on the measured data assuming lognormal quantiles. The intercept and the slope then allow prediction of the mean and standard deviations of unknown observations (Helsel 2012). In comparison with other substitution methods (such as the classical 1/2 QL), this procedure is considered as the more reliable and robust (Chowdhury et al. 2015).

The bioaccumulation kinetics were described using a second-order polynomial model fitted to the replicate data. Trace metal uptake rates were estimated by the initial slope of the curve (derivative at time zero) and are recognized as good indicators of TM bioavailability for invertebrates (van Straalen et al. 2005; Gimbert et al. 2006). Differences in TM uptake rates between the two types of cage were determined using a generalized likelihood ratio test (GLRT).

All statistics were performed with the free statistical software R (version 3.1.0).

Results

Trace metal concentrations in exposure compartments

The concentrations measured in water samples from the cages exceeded the European Quality Standards (EQS) and the Predicted No Effect Concentrations (PNEC) for numerous TMs such as As and Zn for which concentrations were ten times higher than PNEC and 15 to 40 times greater than EQS, respectively. For Pb, however, measured concentrations were threefold lower than PNEC and 1.5 times below EQS (Table 1). Moreover, regarding the standard deviations, TM concentrations varied only slightly over the 5-day exposure duration.

Except for Cd, TM concentrations in pond sediment were higher than those representing a good ecological sediment status, with, for instance, values two to ten times higher in the following order Pb < Ni < Zn < Cu < As (Table 1). More specifically, according to the consensus values defined by Macdonald et al. (2000), the studied TMs could be fitted into three categories. The first concerns Cd where concentrations are below both threshold effect concentrations (TECs) and probable effect concentrations (PECs) and hence, where detrimental effects are unlikely to be observed. The second category includes Cu, Ni, and Pb whose concentrations reached values between TECs and PECs and where adverse impacts were observed. The last group is composed of Zn and As and presents concentrations exceeding both TECs and

Table 1 Average trace metal concentrations over the 5-day exposure duration (mean ± standard deviation (SD)) in water, suspended particles, and sediments from the pond and related quality guidelines

	As	Cd	Cu	Ni	Pb	Zn
Water (μg L^{-1})	39.6	0.24	100	65.8	0.82	114
SD	*11.7*	*0.01*	*19*	*4.9*	*0.42*	*19*
Guidelines[a]	**4.2**	**0.08**	**1.4**	**3.8**	**2.3**	**10.8**
Guidelines[b]	**0.83**[g]	**0.08–0.25**[f]	**1.0**[g]	**4.0**	**1.2**	**7.8**[g]
Pond sediments (μg g^{-1})	71.0	0.91	62.6	24.2	42.5	476
SD	*12.1*	*0.14*	*13.9*	*6.0*	*14.6*	*52*
Control sediments (μg g^{-1})	11.0	0.10	7.48	2.50	4.74	55.4
Guidelines[c]	**7.9**	**0.93**	**14.0**	**11.0**	**25.0**	**146**
Guidelines[d]	**9.79–33.0**	**0.99–4.98**	**31.6–149**	**22.7–48.6**	**35.8–128**	**121–459**
Suspended particles (μg g^{-1})	4.42	0.59	5.60	3.50	161	662
Guidelines[e]	**8.00**	**0.30**	**50.0**	**80.0**	**40**	**110**

[a] Predicted No Effect Concentrations in water (James et al. 2009)

[b] European quality guidelines expressed as an annual average value (AA-EQS) and ensuring protection against long-term exposure to pollutants in the aquatic environment (DIRECTIVE 2013/39/EU)

[c] Consensus values for a good ecological sediment status (de Deckere et al. 2011)

[d] Consensus-based values, TECs (i.e., below which harmful effects are unlikely to be observed)–PECs (i.e., above which harmful effects are likely to be observed) (MacDonald et al. 2000)

[e] Average values for world rivers (Thomas and Meybeck 1992)

[f] For cadmium and its compounds, the EQS values vary depending on the hardness of the water (see DIRECTIVE 2013/39/EU)

[g] These standards have been in force in France since December 2015 (JORF, 2015: Decree of July 27, 2015)

PECs, which, consequently are most likely to lead to harmful effects. In addition, the weak standard deviations also testified to the low spatial heterogeneity of sediment contamination in the studied pond. Obviously, according to the sediment quality guidelines, the control sediments sampled in the W cages were not contaminated.

Trace metal concentrations in suspended particles did not exceed average values encountered in world rivers except for Cd, Pb, and Zn for which they were two, four, and six times higher, respectively (Table 1).

Recovery, survival, and growth of larvae

Over the exposure duration, the average recovery rates of *C. riparius* larvae (number of larvae retrieved divided by the number of larvae introduced) reached 98 ± 4% in the W cages and 70 ± 16% in the WS cages.

A low mortality rate (number of retrieved larvae found dead divided by the number of larvae introduced) was observed in both types of cage, i.e., 0 and 7 ± 8% in the W and WS cages, respectively.

Over the exposure duration, *C. riparius* larval growth rates were on average 0.044 and 0.25 mm day^{-1}, with no significant difference identified between the two types of cage. Linear relationships were determined between individual length and fresh mass, highlighting the isomorphic development of larvae whatever the exposure modality (Fig. 3).

Trace metal bioaccumulation kinetics

The accumulation kinetics in whole tissues (H fraction) of *C. riparius* larvae showed five contrasting patterns (Fig. 4). For Zn, no accumulation was registered and internal concentrations kept values close to 60 μg_{Zn} g^{-1} larvae (fresh weight), whatever the exposure modality. For Cd, a slow but gradual

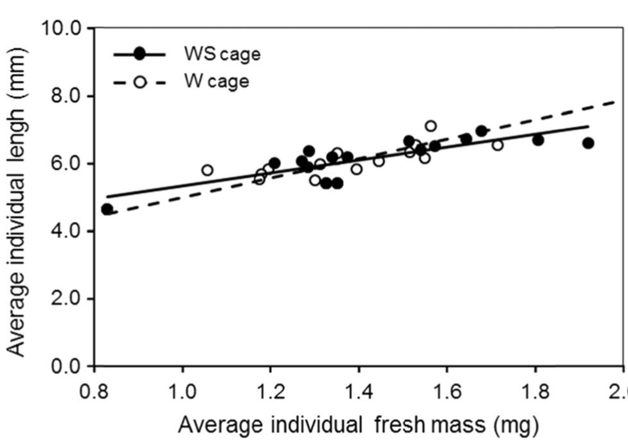

Fig. 3 Developmental growth (length vs fresh mass) of *Chironomus riparius* larvae over the 5-day exposure period in each type of cage. Each point represents a replicate of 10–20 pooled larvae

accumulation was observed, especially in larvae exposed to both water and sediments (WS cages) where internal concentrations increased up to 0.2 μg_{Cd} g^{-1} at the end of exposure. Lead presented a biphasic accumulation pattern with increasing internal concentrations during the first half of exposure before they reached a steady state around 0.5 and 1.0 μg_{Pb} g^{-1} larvae in WS and W cages, respectively. For As, after an initially rapid accumulation phase, internal concentrations reached a threshold (around 1.2 and 1.5 μg_{As} g^{-1} larvae in WS and W cages, respectively) before they decreased to values close to the first concentration (0.5 μg_{As} g^{-1}). Finally, internal concentrations of Cu and Ni showed a surprising decreasing pattern, reaching a plateau during the second half of exposure. Although cage type did not affect Ni bioaccumulation, a significant difference was determined for Cu in larvae exposed to water and sediment (WS cage) where internal concentrations decreased linearly (Fig. 4).

Uptake rates for each TM and exposure modality were obtained from these accumulation patterns (Table 2). They were negative for Ni and Cu, non-significantly different from zero for Zn, low for Cd and higher for Pb and As. The influence of the exposure modality was identified for As and Pb, with higher values estimated for organisms exposed in the W cages, while the combined exposure to water and sediments led to a higher Cu uptake rate in the WS cages (Table 2).

Trace metal fractionation in larvae tissues

The efficiency of the fractionation protocol to determine TM compartmentalization between insoluble (P) and cytosolic (S) fractions was evaluated using TM recoveries (differences between TM concentrations measured in the homogenate (H) and the sum of TM concentrations in P and S) (Table 3). Recoveries were 108 ± 12% on average.

Trace metal distribution between insoluble and cytosolic fractions of *C. riparius* larvae is presented in Fig. 5. First, it is noteworthy that the affinity for either fraction was clearly dependent on the TM considered. Indeed, whereas Cd and Zn were allocated in the same way between both fractions, As, Ni, and Pb were largely (about 80%) contained in the insoluble fraction. The various TMs studied can be ranked according to their affinity for the cytosolic fraction as follows: Cd > Zn > Cu > Pb > Ni > As. Secondly, the temporal trend of TM fractionation in the S and P compartments was assessed using the kinetic approach (Fig. 5). The influence of the exposure compartment in the internal TM distribution was assessed by comparing the fractionation patterns between the two types of cage; the cytosolic contents of As, Cd, and Ni did not vary in water-exposed larvae, while they progressively and significantly increased in larvae exposed to both water and sediments. This increasing trend was also observed for Pb in the WS cages while its cytosolic proportion slightly decreased in the W cages during the first 2 days of exposure. Concerning

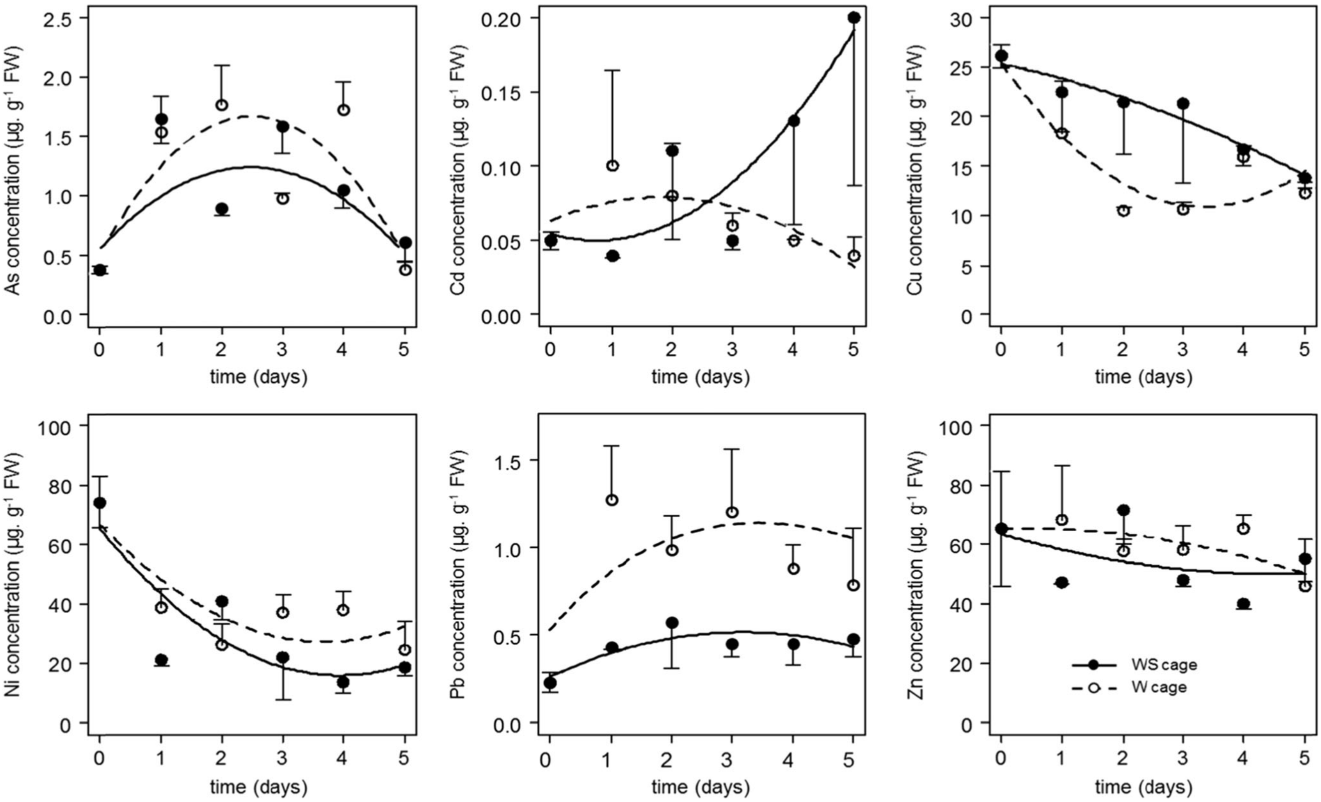

Fig. 4 Trace metal bioaccumulation kinetics in the whole tissues (H fraction) of *Chironomus riparius* larvae exposed in each type of cage. *Each point* represents the mean of three replicates of 10–20 pooled larvae; *error bars* indicate standard deviations. *FW* fresh weight

Cu, a similar fractionation pattern was observed, whatever the type of cage, with decreasing values during the first day of exposure followed by a stabilization of cytosolic proportions of around 30% (Fig. 5). However, Cu proportions in the insoluble fraction were significantly lower (−10% after 3-day exposure) in water-exposed larvae than in the WS cages. For Zn, cytosolic proportions varied neither with time nor modality of exposure.

Discussion

The Etueffont site is in the methanogenic phase as demonstrated by Grisey and Aleya (2016a), illustrating that the site has already achieved advanced stable conditions, i.e., with pH reaching neutral values, methane production, and a decreased release of organic and inorganic compounds. However, our results indicated TM contamination and potential toxicity along with a significant load potentially transferable to higher trophic levels.

Trace metal contamination and toxicity

The studied pond is the first in a four-pond lagooning system and directly received leachates from the landfill. The TM concentrations measured in water and sediments are in agreement with other studies conducted on the same site (Guigue et al. 2013; Ben Salem et al. 2014b) but also on other sites receiving landfill leachates (Ettler et al. 2006, 2008; Gibbons et al. 2014). Trace metals and the aquatic compartments at risk were identified through comparison of concentrations measured in water, suspended particles, and sediments using several

Table 2 Trace metal uptake rates (mean ± standard deviation; $\mu g_{TM}\ g_{larvae}\ day^{-1}$) in *Chironomus riparius* larvae exposed in each type of cage

	As	Cd	Cu	Ni	Pb	Zn
W	0.93 ± 0.34	0.02 ± 0.01	−8.67 ± 3.83	−21.4 ± 10.7	0.57 ± 0.24	0.62 ± 6.11
WS	0.48 ± 0.28 *	−0.01 ± 0.03	−1.29 ± 1.12 *	−25.0 ± 13.6	0.16 ± 0.07*	−5.90 ± 11.67

*Significant difference between the two types of cage (GLRT)

Table 3 Trace metal concentrations (mean ± standard deviation; μg g^{-1}) measured in whole tissues (homogenate, $H_{measured}$) or computed ($H_{computed}$ = S + P), and recoveries (percentage relative to $H_{measured}$) in *Chironomus riparius* larvae exposed in each type of cage

Cage type	As		Cd		Cu		Ni		Pb		Zn	
	WS	W	WS	W	WS	W	WS	W	WS	W	WS	W
$H_{measured}$	1.02	1.29	0.095	0.07	20.5	15.6	33.1	39.7	0.45	0.93	54.9	59.9
SD	*0.5*	*0.8*	*0.08*	*0.04*	*6.1*	*6.1*	*23.8*	*18.6*	*0.18*	*0.42*	*15.7*	*15.7*
$H_{computed}$ = S + P	1.05	1.16	0.10	0.08	19.9	14.3	37.3	44.7	0.47	0.97	71.2	76.1
SD	*0.5*	*0.7*	*0.06*	*0.04*	*6.3*	*5.6*	*26.8*	*22.47*	*0.13*	*0.40*	*26.1*	*21.7*
Recovery (%)	103	90	105	114	97	92	113	113	104	104	130	127

quality guidelines. Hence, TM concentrations in the surface water of the leachate collecting pond greatly exceed the PNECs (James et al. 2009) and the annual average values of EQS (AA-EQS) which are intended to ensure protection of the aquatic environment against long-term exposure to pollutants (DIRECTIVE 2013/39/EU). Among the studied TMs, this is the case for As, Cu, Ni, Zn, and, to a lesser extent, Cd. Only Pb appears to pose no risk for aquatic organisms. Concerning sediments, concentrations are above the consensus values for a good ecological sediment status (de Deckere et al. 2011) and the TECs. Consequently, numerous TMs are ranked in the categories for which the prospect of adverse impacts are likely or widely to be expected, except Cd whose concentrations are just below the guidelines defined by Macdonald et al. (2000) and de Deckere et al. (2011). To our knowledge, no guideline values exist for TM concentrations in suspended particles and therefore data from uncontaminated rivers are used for comparison (Thomas and Meybeck 1992). It appears that this aquatic compartment presents a relatively moderate environmental risk since only Cd, Pb, and Zn present anomalous concentrations. It may, however, represent a significant source of contamination for macroinvertebrates and thus, when combined with contaminated water and sediments, may lead to harmful effects. This could explain the alteration of the macroinvertebrate and especially the chironomid assemblages in the pond. Indeed, Khattabi and Aleya (2007)

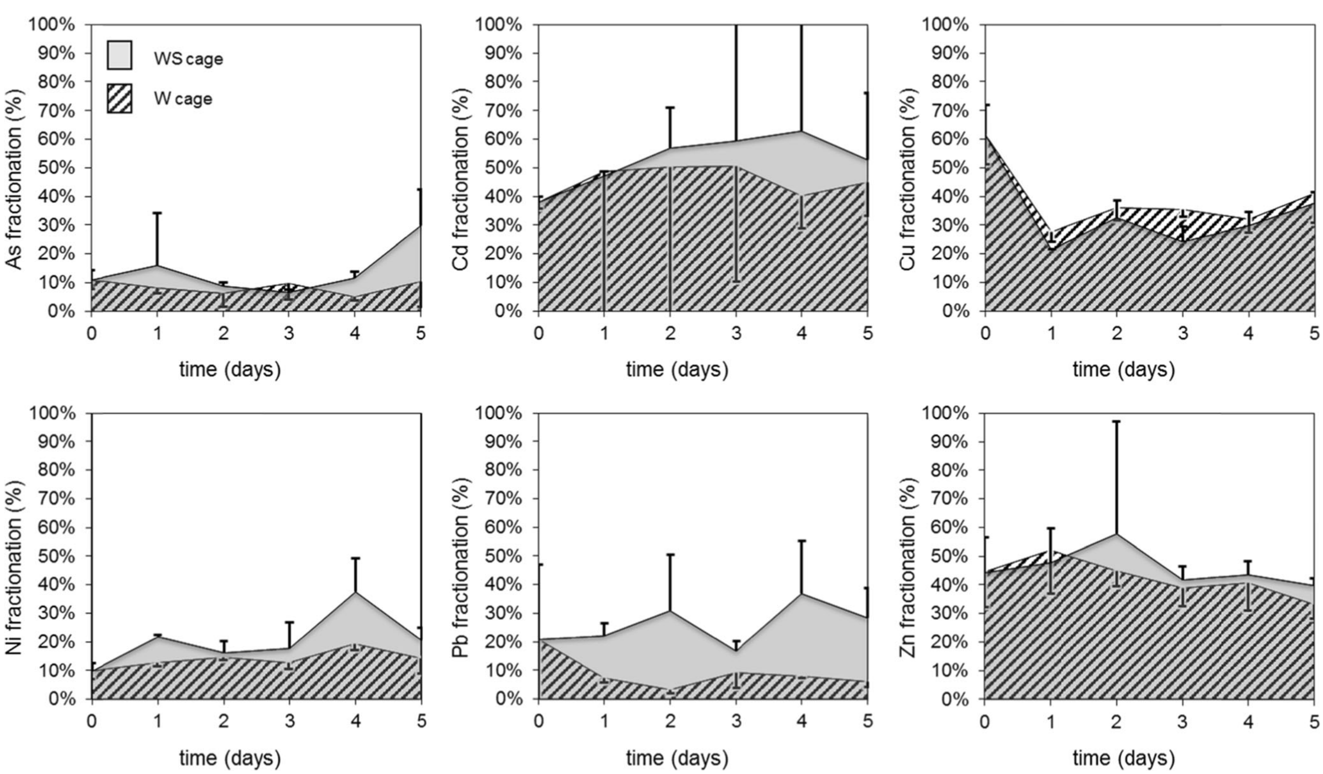

Fig. 5 Time course of trace metal distribution in the cytosolic fraction (S) of *Chironomus riparius* larvae exposed in each type of cage (*shaded area*: WS cage; *striped area*: W cage). The difference to 100% corresponds to the contribution of the insoluble fraction (P)

found that the chironomid larvae density was significantly decreased in this first pond in comparison with the downstream lagooning basins. At the individual scale, our results indicate no mortality related to environmental contamination. Moreover, a similar isomorphic development of larvae was observed, regardless of cage type. However, larval growth was quite slow (0.25 mm day^{-1}) in comparison to values that can be reached under field conditions (for instance, 0.7 to 1 mm day^{-1} in Ferrari et al. 2014). This may be explained either by the exposure conditions, such as food availability, of course, but also water temperature (Péry and Garric 2006) (8.8 ± 0.5 °C in the present study), or, referring to the dynamic energy budget applied to toxicology (DEBtox, Kooijman et al. 2009) theory, by a change in the energetic allocation between somatic maintenance (excretion and/or detoxification processes) and growth (and/or reproduction). This latter hypothesis implies proof that the *C. riparius* larvae have truly absorbed the TMs.

Bioavailability and internal fate of trace metals

C. riparius larvae were used as biomonitors of the sediment and water quality related to their contamination by TMs. Accumulation kinetics testify to the bioavailability of several TMs present in the water and sediments of the leachate collecting pond. This is particularly the case of As whose uptake rates reach 0.93 and 0.48 $\mu g_{As}\ g_{larvae}\ day^{-1}$ in the W and WS cages, respectively. This high bioavailability leads to a quick increase in As internal concentrations during the first phase of exposure. During this phase, As is mainly found in the insoluble compartment, likely bound to cellular debris. Indeed, after 3 days of exposure, total internal concentrations of As drastically decrease and, at the same time, As fractionation changes in favor of the cytosolic compartment. This shift in As management strategy may correspond to a physiological mechanism of elimination, as already observed in *Arenicola marina* (Casado-Martinez et al. 2012), or biotransformation to facilitate its excretion or detoxification. For example, Mogren et al. (2013) showed that the reduction of arsenate to arsenite may be a detoxification and elimination pathway in the midgut of the *C. riparius* larvae. Total Pb concentrations in larvae tissues increase during the first 2 days of exposure and were three times higher in larvae exposed to water and suspended particles (the W cage). This may be related to the consumption of deposited particles, heavily contaminated by Pb and representing, in the W cages, the larvae's main source of food. This is confirmed by the fractionation results showing that Pb is predominantly retrieved in the insoluble fraction (around 90%). In contrast, and though only slightly bioavailable, Pb from the sediments in the WS cages progressively accumulates in the cytosolic fraction, where it could be associated with metallothioneins (Arambourou et al. 2013). Cadmium bioavailability is low, but two different patterns of accumulation were observed according to exposure modality, highlighting the main sediment contribution in the transfer of Cd to *C. riparius*. Initially equally distributed between insoluble and soluble fractions, the Cd assimilated from sediments in the WS cages is mainly stored within the cytosolic compartment. This could be interpreted as a progressive association with some soluble proteins, such as metallothioneins implicated in the detoxification of Cd and its sequestration in cytosol (Amiard et al. 2006; Toušová et al. 2016). Weakly mobile in the aquatic environment (Ingvertsen et al. 2013), Ni is not bioavailable to *C. riparius* larvae, neither from water nor sediments and, in agreement with Dumas and Hare (2008), more than 80% is observed in the insoluble fraction. Bioaccumulation patterns even decrease over the exposure duration. This must be related with already significant Ni concentrations in larvae tissues at the beginning of the experiment and is probably explained by food intake (oats) during the breeding of organisms. Regarding kinetic fractionation over the exposure duration, the elimination of Ni from *C. riparius* tissues resembles that of As, with a remobilization of Ni from the insoluble to the cytosolic fraction. Copper accumulation kinetics in the whole tissues also decrease, though through the draining of the cytosolic fraction which loses half of its Cu content during the first day of exposure. Nevertheless, sediments appear as a significant source of Cu for *C. riparius* as illustrated by the higher uptake rates calculated for larvae exposed in the WS cages. However, Cu cannot be considered as truly bioavailable since it is mainly retrieved in the insoluble fraction, probably bound to sediment particles in the gut. Finally, the absence of Zn accumulation has to be related to its essential status for the metabolic functioning and homeostatic equilibrium in animals. Optimal cytosolic concentrations in larvae are maintained by this physiological regulation (Timmermans et al. 1992) by sequestering Zn within the insoluble fraction, for instance bound to metal-rich granules (MRGs) particularly implied in subcellular storage and excretion pathways of essential TMs in aquatic insects (Hare 1992).

Since chironomid larvae constitute an important food source for various aquatic (macroinvertebrates, fishes) and terrestrial (birds) species, knowledge of the internal distribution of TMs is a valuable tool for interpreting their potential ecotoxicological hazard through trophic transfer. Indeed, Wallace and Luoma (2003) defined a trophically available metal (TAM) fraction according to its transfer potential to a higher trophic level. The composition of the TAM fraction has to be determined in function of the predator, and more precisely, to the strength of its digestive and assimilative processes (Rainbow et al. 2011). Considering the trophic position of chironomid larvae, the cytosol fraction can at least be estimated to be trophically available to most if not all predators. In our case, it represented up to 60, 40, and 30%, respectively, of the total Cd, Pb, and As concentrations in the exposed organisms. These results may thus help to explain the elevated TM concentrations accumulated in the common roach *Rutilus rutilus* sampled in the lagooning basins of the study site and the genotoxic effects that have been detected (Ben Salem et al. 2014a).

Conclusion

The in situ caging of *C. riparius* larvae is thus a relevant tool for the assessment of TM bioavailability and transfer and, using specifically designed exposure devices, allows the main sources of contamination to be identified. This type of active biomonitoring could also be reproduced in the successive basins of this and other constructed wetlands in order to assess the efficiency of the lagooning process in reducing contamination by landfill effluents and environmental releases. Although TM concentrations measured in the various exposure compartments may constitute a risk for the aquatic ecosystem, internal detoxification strategies of *C. riparius* larvae prevent the induction of individual sublethal effects. However, the accumulation of several TMs in larvae tissues may represent a significant load potentially transferable to food chains.

Acknowledgements The authors wish to thank Dr. Hervé Grisey and the SICTOM (Solid Waste Management Service) of Etueffont (Territoire de Belfort, France) for financial support. We also thank C. Amiot and N. Crini for their technical assistance and B. Pauget for fruitful discussions of the results.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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