Abstract

To improve the assessment of metal toxicity in aquatic organisms, it is important to consider the different uptake pathways (i.e. trophic or dissolved). The bioaccumulation of dissolved metals such as Cd and Zn is beginning to be well described in gammarids. But there are very few data concerning the contribution of the diet route, and its related toxicokinetic parameters. Among these, the assimilation efficiency is an essential parameter for the implementation of models considering the trophic pathway. This study aims to estimate the assimilation efficiencies and elimination rates of two type of food, i.e. alder leaves and larvae of chironomids, contaminated by three metals (Ag, Cd and Zn) of major concern for the Water Framework Directive (WFD). The pulse-chase-feeding method was used. Gammarids were fed with alder leaves or chironomid larvae previously contaminated with ^{110m}Ag, ^{109}Cd or ^{65}Zn, for a short period of time (1 to 5 hours), followed by an elimination phase of 14 days. At different time steps, the gammarids were placed alive on the gamma detector to quantify individually the whole-body concentrations of ^{110m}Ag, ^{109}Cd or ^{65}Zn. Our results indicate that: i) Cd has the highest assimilation efficiency (44% for leaves and 34% for larvae), followed by Zn (14% for leaves and 9% for larvae) and Ag (5% for leaves); ii) for Cd and Zn, the AE were higher when gammarids were fed with leaves than with larvae; iii) the elimination rates of metals seem to depend more on the food matrix than the metal assimilated; and thus iv) the biological half-life calculated from the k_{es} is 5.1 days for Ag, between 4.9 and 13 days for Cd and between 3.8 and 13 days for Zn.

Keywords: Trophic transfer, Amphipods, Metals, Alder leaves, Chironomids larvae, Dietary pathway

1. Introduction

In the past decades, a body of works demonstrated the strong capacities of some freshwater organisms to accumulate contaminants from the aquatic environment (Timmermans et al., 1992; Dutton & Fisher, 2011). Consequently, based on chemical concentrations recorded in the whole body, some freshwater invertebrates were used as bioindicators of the good ecological status for the French freshwater systems (Besse et al., 2012; Pellet et al., 2014; Lebrun et al., 2020). The ubiquitous gammarid *Gammarus fossarum* is widely used for this property, considering it accumulate waterborne and diet-borne metals as grazer of leaf-litter and opportunist predator of invertebrate preys (Kunz et al., 2010; Pellet et al., 2014; Filipović Marijić et al., 2016). Although the bioaccumulation processes of dissolved metals were extensively documented (Vellinger et al., 2012; Urien et al., 2017; Gestin et al., 2022, 2023), less is known regarding the metal diet-borne assimilation and elimination in this species. Because the exposure conditions are more difficult to standardize than for dissolved exposures, the diet route in aquatic invertebrates is often poorly understood. However, diet pathway could count for a major metal entry in invertebrates (Borgmann et al., 1989; Pellet et al., 2014), raising the need to consider this contribution to avoid misinterpretation of recorded metal concentrations in this species (Franklin et al., 2005; Besse et al., 2013; Lebrun et al., 2015; Conti et al., 2016; Hadji et al., 2016).

The few studies focusing on freshwater invertebrates demonstrated that the uptake from trophic route predominates for some metals, as for Cd, Cu and Se in *Hyallela azteca* or Cd in *Gammarus pulex* (Borgmann et al., 1989; Pellet et al., 2014). However, for *Daphnia magna*, the uptake from dissolved pathway predominates for Zn (Xu & Pascoe, 1994). These results implies that metal accumulation, detoxification and elimination and thus potential toxicity strongly depend on the uptake pathway. In that sense, Croteau & Luoma (2009) called for a

need to delineate the bioaccumulation mechanisms and toxicokinetic in different organisms, since these processes are metal and species specific.

The assimilation efficiency (AE) is one of the most essential parameters used to quantify trophic transfer. This parameter directly reflect the amount of metals available from food and is a key physiological parameter to understand the trophic transfer mechanisms (Croteau & Luoma, 2009). AE could be partly defined by the proportion of metal in food that cross the intestinal epithelial barrier to accumulate in the body of the consumer (Wang & Fisher, 1999a; Nunez-Nogueira et al., 2006; Croteau & Luoma, 2008). The AE values allow at estimating influences of consumer species, contaminants, temperature, etc. (Wang & Fisher, 1999a; Wang & Wong, 2003). Among them, the food type can greatly influence the AE of metals and thus their bioaccumulation efficiencies (Wang & Fisher, 1999a; Nunez-Nogueira et al., 2006; Dubois & Hare, 2009; Pouil et al., 2016). It has already been established that metals from plant matrices were less assimilated by organisms than elements of animal origin, because the digestion of compounds such as fibers or hemicelluloses is slower and more complex (Xu & Pascoe, 1994; Nunez-Nogueira et al., 2006). It is therefore important to consider dietary diversity in omnivorous organisms, such as gammarids, in trophic pathway studies.

The pulse-chase-feeding approach is commonly used to determine the AE values (Calow & Fletcher, 1972; Warnau et al., 1996; Metian et al., 2007; Pouil et al., 2017) and consists of exposing the organism once to labeled food source and following the elimination of the tracer over time (Wang & Fisher, 1999a; Pellet et al., 2014). A kinetic modelling approach is used on the depuration data to determine the metal specific AE and the depuration rates (k_e). The pulse-chase-feeding technique is widely used and adapted to work with different tools, such as isotopic tracers (stable and radioactive ones) (Wang & Fisher, 1999a; Baines & Fisher, 2002;

Pouil et al., 2017). Regarding metal, the gamma-emitting radiotracers are one of the most powerful tracer to quantify accurately small metal amounts. They also allow individual tracking of metal uptake and loss over time, as gamma detection in the whole body organism is non-destructive (Wang & Fisher, 1999a). Furthermore, it is conventionally accepted that radioactive isotopes of metals possess identical characteristics to their stable counterparts (Warnau & Bustamante, 2007). This confers the ability to evaluate the accumulation and elimination rates of metal by measuring only the concentrations of radioisotopes, and thus avoiding to measure the background of stable metals (Cresswell et al., 2017).

In this framework, the aim of this study was to determine the AE values and the elimination rates of Ag, Cd and Zn in the amphipod *G. fossarum*. A pulse-chase-feeding method was applied, considering two contrasting types of food belonging to the gammarid diet: the alder leaves and the larvae of chironomids. After a short phase of exposure to radiolabeled food, gammarids were placed in depuration conditions and metal loss was followed in tag-identified individuals for 14 days. NLS (Nonlinear Least Squares) models were fitted to the experimental data to determine the AE, k_e and biological half-lives of metals in gammarids. Values were finally compared to discuss the influence of metal and food type on the diet-borne bioaccumulation.

2. Material and methods

2.1 Organisms and alder leaves: origins, collection and maintaining

Adult male gammarids (*Gammarus fossarum*: 27.3 ± 5.71 mg wet weight) were sampled from an uncontaminated watercress pound (Saint-Maurice-de-Rémens, France) (Gestin et al., 2021). They were then transported to the LIENSs laboratory premises (La Rochelle), where they were maintained under constant bubbling in Evian® water (see characteristics in Table S1) with a

dark/light cycle of 8:16h, at a temperature of 12 ± 0.5°C. They were fed with alder (*Alnus glutinosa*) leaves *ad libitum* and kept for a 7-day acclimation period before experiments. Alder leaves (*A. glutinosa*) used for trophic exposure were collected on a clean site (Les Ardillats, France). They were leached (i.e. soaked in well water for several days, with regular renewal of the water) in the ecotoxicology laboratory of INRAE Lyon-Villeurbanne in order to depurate the toxic tannins for gammarid. Then, 6 mm diameter discs were punched out from the wet leaves, avoiding the large veins. Finally, the leaf disks were placed in a container filled with well water (see characteristics in Table S1) and transferred to the LIENSs laboratory.

Chironomid (*Chironomus riparius*) egg masses from INRAE laboratory were transferred to the LIENSs laboratory. The eggs were placed in beakers containing powdered silica and filled with Evian® water and maintained for few days at ambient temperature (ca. 20°C) until hatching. Larvae were then fed with fish pellet until the second larval stage.

2.2 Radiotracers

Throughout the experiment, the rearing and lab materials were decontaminated using 1/10 diluted detergent (Decon® 90 solution), 1/10 diluted HCl solution (Hydrochloric acid S.G. 32%, certified AR for analysis; Fischer Scientific®) and distilled water. The radioisotope carrier-free solutions were purchased from Eckert & Ziegler Isotope Products Inc., Valencia, USA. The ^{109}Cd and ^{65}Zn solutions were in their chloride forms, 0.1M HCl and the ^{110m}Ag solution was in its nitric form, 0.1M HNO_3. These three stock solutions were diluted into intermediate solutions to reach appropriate radiotracers concentrations in the experimental setup. These additions of radiotracers to the media did not change the water pH. For each radioisotope, the detectors were calibrated with two inhouse liquid standards with the appropriate geometry, *i.e.* a 500 µL "gammarid" geometry and a 10 mL “water” geometry. Two NaI detectors coupled to InterWinner 7.0 software (ITECH Instruments®) were used to count radioisotopes activities (in

Bq, $Bq.g^{-1}$ or $Bq.mL^{-1}$) contained in all samples. The results obtained in activity have been then converted into stable metal equivalent (in µg, $\mu g.g^{-1}$ or $\mu g.L^{-1}$).

2.3 Contamination of food

The leaf discs (~ 108) were placed in 10 mL of Evian® water contaminated with 2010, 2030 or 2000 $Bq.mL^{-1}$ of ^{109}Cd, ^{65}Zn and ^{110m}Ag, respectively, for 7 days. They were then placed in 0.1 L beakers filled with clean Evian® water for 5 days to remove the weakly adsorbed radioisotopes on leaf walls. For each metal, 12 discs were randomly counted by gamma spectrometry at the end of these procedures, to follow the radiolabeling of the leaf.

At the end of the third instar (i.e. L3, when they start to turn red), nine pools of 10 chironomids larvae were collected (i.e. 90 larvae in total), placed in 100 mL beakers filled with Evian® water (i.e. n=10 per beaker) and exposed to 201, 203 or 200 $Bq.mL^{-1}$ for ^{109}Cd, ^{65}Zn and ^{110m}Ag (3 pools per radiotracer) during 4 days. This short exposure time was chosen to avoid the emergence of radiolabeled larvae into mosquitoes in the lab premises. At the end of the exposure period, as for leaves, the larvae were gently rinsed with clean water, then individually frozen and all counted by gamma-ray spectrometry. No radiotracer activity was detected in chironomid larvae exposed to ^{110m}Ag.

2.4 Experimental procedure to obtain assimilation efficiencies of gammarids

The Ag AE value and elimination rate was not determined for the chironomid as food source. Before the pulse-chase feeding experiment, gammarids (n = 22 for each alder leaves/metal condition and n = 14 for each chironomids/metal condition) were starved for 2 days and then placed individually in 250 mL beakers filled with Evian® water (Fig. 1). For each metal, they were then either exposed to two radiolabeled leaf discs for 3-5 h, or to one radiolabeled thawed chironomid larva for 1 h (Fig. 1). For the leaves-feeding condition, the 14 gammarids that have

eaten the most were immediately whole body gamma-counted alive after the pulse-chase-feeding period. For the larvae-feeding conditions, all the 14 exposed gammarids were gamma-counted.

Selected gammarids, as explained below in section 3.1, (i.e. 13 ^{109}Cd-leaf fed gammarids, 11 ^{109}Cd-chironomid fed gammarids, 13 ^{65}Zn-leaf fed gammarids, 13 ^{65}Zn-chironomid fed gammarids and 14 ^{110m}Ag-leaf fed gammarids) were then transferred in depuration condition, *i.e.* 200 mL beakers filled with non-radiolabeled with Evian® water (*i.e.* closed circuit, constantly aerated, T = 12 ± 0.5°C). Each beaker contained 7 radiolabeled gammarids individually separated by handmade baskets (i.e., plastic mesh with a height of 11 cm and a diameter of 8.6 cm, with a mesh size of 0.5 cm; Fig. 1). A non-radiolabeled individual was added to each beaker to control potential recycling of radioisotopes due to leaching from gammarid depuration. Gammarids were fed with uncontaminated alder leaves *ad libitum* all along the depuration period (Fig. 1).

The whole-body radiotracer activities in gammarids were individually followed during two weeks of depuration (Tables S2 and S3). Individuals were sampled and counted at days 0.5, 1, 1.5, 2, 4, 7, 9, 11 and 14 (Fig. 1). For this, gammarids were taken from the beakers using a 10 mL pipette, rinsed with clean water and placed in a plastic box (Caubère®, ref 1210) containing 500 µL of clean Evian® water (Fig. 1). The counting times of living gammarids varied between 10 and 20 min in order to minimize stress as much as possible. The counting uncertainties did not exceed 5% for Zn for all samples, whereas uncertainties increased with decreasing activities in gammarids samples without exceeding 20% and 15% for Cd and Ag, respectively. The radiotracers activities in water were daily monitored and the water, at least, changed every two days to minimize tracers waterborne contamination. The mortality was also checked daily.

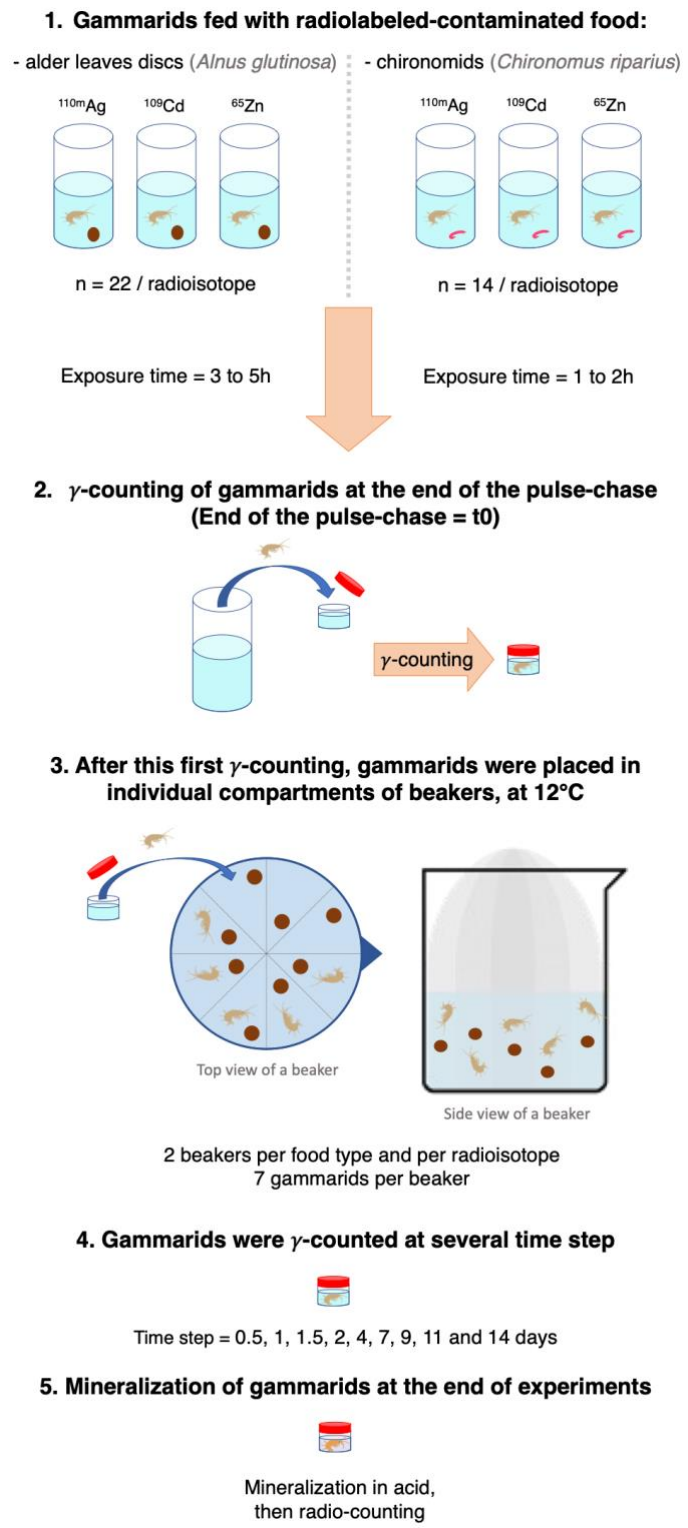

Figure 1. The different steps of the pulse-chase-feeding.

2.5 Estimation of assimilation efficiencies and depuration parameters of gammarids

The depuration of radiotracers by gammarids was expressed as the percentage of remaining activity (i.e. radioactivity at time t divided by the initial radioactivity measured in the organism

at the beginning of the depuration period; Tables S2 and S3; see (Warnau et al., 1996). The kinetics parameters and the assimilation efficiencies with respect to the food type and the metal, were estimated with a non-linear least squares approach modeling following the double component exponential equation:

$$A(t)_{f,r} = \left(100 - AE_{f,r}\right) \times e^{-k_{es,f,r} \times t} + AE_{f,r} \times e^{-k_{el,f,r} \times t} \qquad (1)$$

with $A(t)_{f,r}$ is the remaining activity (%) at time t, with respect to the type of food f and the radioisotope r, $AE_{f,r}$ is the assimilation efficiency, $k_{es,f,r}$ and $k_{el,f,r}$ the elimination rates of the first short-term phase (i.e. elimination of the non-assimilated metal fraction quickly depurated) and of the second long-term phase (i.e. elimination of the assimilated metal fraction depurates slowly), respectively. Indexes f correspond to the type of food ingest by gammarids, with f =1 for alder leaves and f =2 for chironomids larvae; and indexes r are for the radioisotope tested, with r =1 for ^{109}Cd, r =2 for ^{65}Zn and r =3 for ^{110m}Ag.

The biological half-life $T_{b\frac{1}{2},f,r}$ for each metal with respect to the food source were calculated from elimination rate constant (k_{es} or k_{el}), based on the equation:

$$Tb_{\frac{1}{2},f,r} = \frac{ln2}{k_e} \qquad (2)$$

The elimination kinetic parameters and the assimilation efficiencies were estimated using R software.

3. Results and discussion

In order to improve knowledge on the bioaccumulation and fate of metals in organisms, the role of diet should be considered with equal importance to the aquatic route (Xu & Pascoe, 1994; Hadji et al., 2016). Estimating the accumulation efficiencies of metals is an essential first step in understanding bioaccumulation mechanisms. Moreover, AE values are key inputs to the implementation of toxicokinetic models to study these mechanisms (Van Campenhout et al.,

2009). For gammarids *G. fossarum*, this study provides with estimates of the assimilation efficiency and the two rates of elimination of biphasic digestion after the ingestion of food (i.e. of animal or vegetable origin) contaminated by one of the three metals studied here (Ag, Cd and Zn).

3.1 Experimental conditions

The ^{109}Cd is a low-energy photon emitters with a gamma ray peak at 88 keV (3.79%), implying the need to have enough activity in a sample to stand out from the background compared to ^{65}Zn (1115 keV) or ^{110m}Ag (658 keV for its main gamma ray). Thus, to obtain precise and accurate measurements, only individuals reaching whole body activity of 150 Bq and above just after the unique feeding (t0) were kept to follow the metal loss kinetics (Fig. 1 and S1). This means that individuals that did not graze enough during the exposure period due to repletion or stress were dismissed. For the gammarids fed with Cd-enriched chironomid (n=11), only 4 gammarids in a first set of 14 individuals showed a body activities above 150 Bq at t0 (Fig S1). In a second 14-gammarids batch, 7 individuals ingested more than 150 Bq of ^{109}Cd activities (Fig S1, Tables S2 and S3). For the leaves-feeding Cd condition (n=13), only 1 on 14 gammarids ingested less than 150 Bq of ^{109}Cd at t0, and so was removed from the experiment. In each condition (n=13) of dietary Zn exposure (i.e. both leaves and larvae as sources), one gammarid died during the first day of experiment. In the leaves-feeding Ag condition (n=14), all the 14 gammarids stayed alive all along the depuration phase.

3.2. The metal elimination kinetics patterns

Kinetics models were fitted to whole-body concentrations over time following a pulse chase feeding with metal contaminated alder leaves. The metal elimination kinetics displayed a biphasic pattern (Figure 2), expressing the presence of two metal pools: a first pool poorly

retained, characterized by a high rate of elimination (k_{es}), and a second pool slowly eliminated (k_{el}).

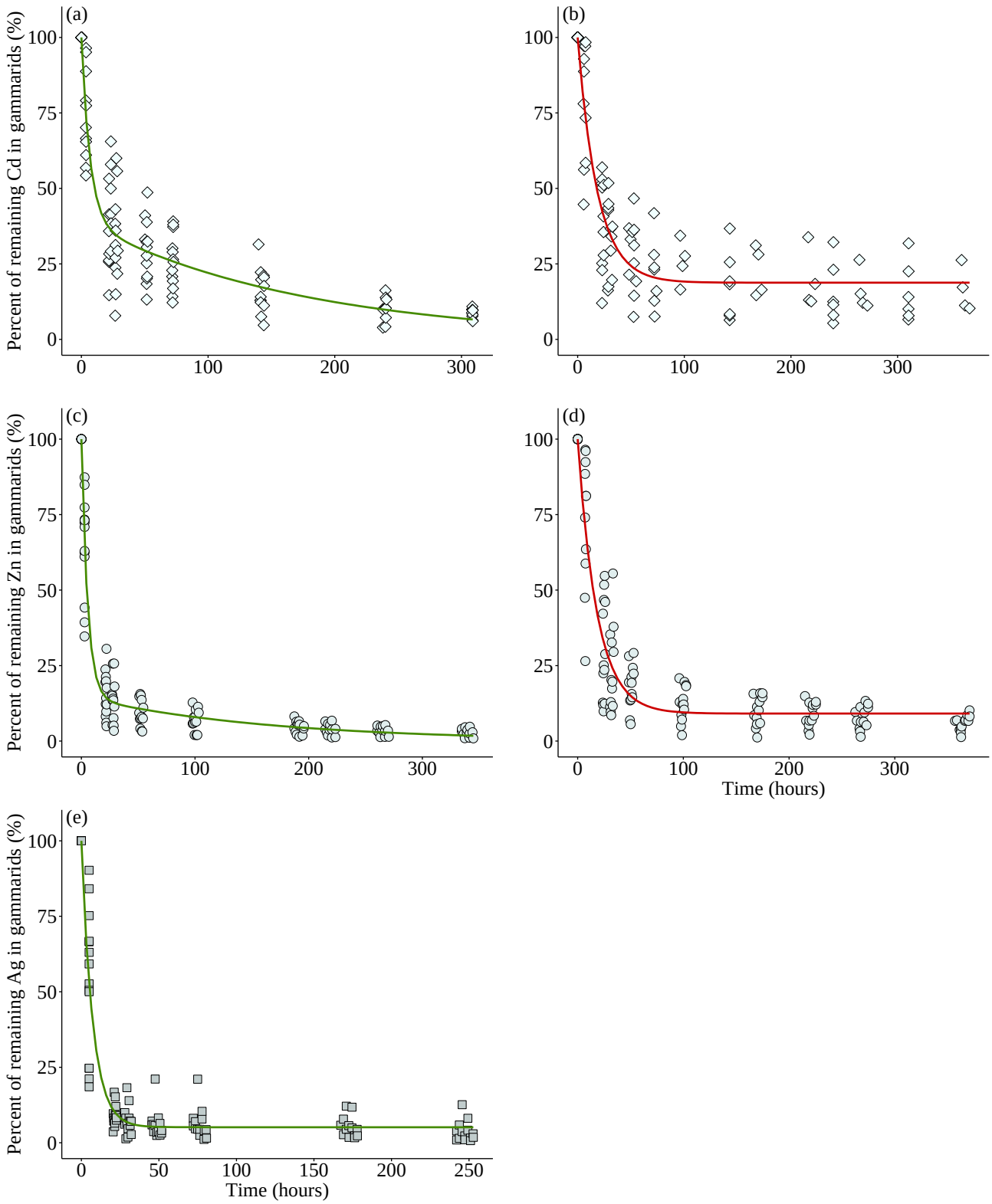

Figure 2. Influence of the metal and the type of matrix used for feeding on the elimination of metals ingested by the gammarid during the depuration phase, expressed as the percentage of activity remaining in the gammarid compared to the end of the pulse-chase phase (t0) as a function of the depuration time (hours).

Whatever the metal, gammarids eliminated the highest proportions of ingested trace elements during the first 48 h, following exposure to contaminated leaves. The elimination rates were similar among metals with kes values of 0.18 ± 0.01 h^{-1}, 0.15 ± 0.03 h^{-1} and 0.14 ± 0.01 h^{-1} for Zn, Cd and Ag respectively (Table 1). Thus, these values imply that, after a 5 hours period of grazing, these pools of metals showed a $Tb_{s1/2}$ of 4-5 hours (Table 1). These biological half-lives, similar among metals, are coherent with the gut transit time reported in the *Gammarus pulex* (16 hours; (Pellet et al., 2014)), suggesting that these fractions were not assimilated and were eliminated through the feces. Stimulated by the ingestion of non-radiolabeled alder leaves since gammarids were place in depuration conditions, this hypothesis is reinforced by this significant release of radiotracers during the first 48 hours of the elimination phase.

Table 1. Parameters estimated by fitting a nls model from pulse-chase-feeding data, with EA (%), k_{el} (d^{-1}) and k_{es} (d^{-1}) with their respective standard deviations, t value, Pr(>ItI) and percentiles (95%). Calculated biological half-life times of metals as a function of food type with their respective standard deviations and percentiles (95%).

Metal	Food type	Parameters	Assimilation efficiencies					Biological half-life		
			Estimate values	t value	Pr(>ItI)	Percentiles 2.5%	Percentiles 97.5%	Calculated values	Percentiles 2.5%	Percentiles 97.5%
Ag	Alder leaves	k_{es}	0.14 ± 0.0098	14	<2E-16	0.12	0.16	5.1 ± 0.37	4.4	5.9
		AE	5.2 ± 1.1	4.8	4.2E-6	3.0	7.3	/	/	/
		k_{el}	0	/	/	/	/	∞	/	/
Cd	Chironomid larvae	k_{es}	0.053 ± 0.0049	11	<2E-16	0.044	0.063	13 ± 1.2	11	16
		AE	19 ± 1.7	11	<2E-16	15	22	/	/	/
		k_{el}	0	/	/	/	/	∞	/	/
	Alder leaves	k_{es}	0.15 ± 0.027	5.5	2.95e-7	0.094	0.20	4.9 ± 1.0	3.6	7.2
		AE	39 ± 3.2	12	<2E-16	33	45	/	/	/
		k_{el}	0.0058 ± 0.0010	5.6	1.8e-7	0.0037	0.0078	124 ± 25	89	185
Zn	Chironomid larvae	k_{es}	0.055 ± 0.0036	15	<2E-16	0.047	0.062	13 ± 0.86	11	15
		AE	9.1 ± 1.2	7.3	2.6E-11	6.7	12	/	/	/
		k_{el}	0	/	/	/	/	∞	/	/
	Alder leaves	k_{es}	0.18 ± 0.014	13	<2E-16	0.16	0.21	3.8 ± 0.29	3.3	4.4
		AE	15 ± 1.7	8.4	8.0E-14	11	18	/	/	/
		k_{el}	0.0061 ± 0.0014	4.4	2.3E-5	0.0034	0.0089	120 ± 35	78	203

The second kinetic phase was characterized by a lower elimination rate, with k_{el} values of 0.0061 ± 0.0014 h^{-1}, 0.0058 ± 0.001 h^{-1} and null for Zn, Cd and Ag, corresponding to k_{el} of

$Tb_{l1/2}$ = 120 ± 35 h, 124 ± 25 h and ∞, respectively (Table 1). This longer elimination rate is considered as resulting from the physiological turnover of the metal fraction assimilated. It is assumed that this pool crosses the intestinal barrier, is distributed to different organs and is taken over by detoxification mechanisms, as already described for Cd and Zn in invertebrates. (Nunez-Nogueira et al., 2006). It is noteworthy that the $Tb_{l1/2}$ of Cd and Zn are equivalent, suggesting that both trace elements metabolism requested similar physiological mechanisms. Contrasting to this, the assimilated pool of Ag was not eliminated by the gammarid which contrasts with shorter Ag retention time than other metals such as Cd or Zn reported in the daphnia (Lam & Wang, 2006), in the cuttlefish and in the turbot fish (Bustamante et al., 2004; Pouil et al., 2015). This phenomenon was already observed in lymnea (k_{el} not different from 0) suggesting that Ag is strongly retained in internal tissues (Croteau et al., 2011). This could be explained by the fact that Ag is an element that reacts very strongly with sulfur compounds (Lam & Wang, 2006). In bivalves, Ag has in fact been shown to be stored in the epithelial cells of the intestine cells, as Ag_2S, (Berthet et al., 1992) which are already known in gammarids to be a site of accumulation and detoxification of other metals such as Cd and Cu (Pellet et al., 2014). In this highly stable Ag_2S form, silver is found in the insoluble fraction (Berthet et al., 1992), and remain infinitely entrapped in tissues under a non-metabolically available form (Fowler et al., 2004).

3.3. The AE values of metals after feeding by contaminated alder leaves

According to the second depuration kinetic components, the assimilation efficiencies of metals in alder leaves fed gammarids were ranked as follow: Cd (39 ± 3.2%) > Zn (15 ± 1.7%) > Ag (9.1 ± 1.2%) (Fig. 2; Table 1). This contrasting AE values among elements were also found in *Daphnia magna*, with AE of 30-70%, 7-66% and 1-2%, for Cd, Zn and Ag, respectively (Yu & Wang, 2002; Lam & Wang, 2006). In line with our results, a higher AE for Cd (5-47%) than for

Zn (1.4%) was also found in the gammarid *Gammarus pulex* (Xu & Pascoe, 1994; Pellet et al., 2014) fed with leaves. To our knowledge, no data exists on Ag AE in amphipods, by similar AE values ranged from 3 to 23% were reported in the marine copepods *Acartia torua*, *Acartia hudsonica* and *Temova longicornis*. Noteworthy, higher AE values for Ag were reported for the cephalopod *Sepia officinalis* (19 ± 3%) (Bustamante et al., 2004) and for the gastropod *Lymnaea stagnalis* (73 ± 5%) (Croteau et al., 2011) whereas it did not exceed 1% in the fish *Scophthalmus maximus* (0.32%) (Reinfelder & Fisher, 1991; Lam & Wang, 2006; Pouil et al., 2015). Thus, all these data show great disparities confirming that there is no general rules for predicting AE among metals and biological species (Wang & Fisher, 1999a; Pouil et al., 2018).

3.4. The effect of type food on metal trophic transfer

The AE values are intrinsically linked to both biotic and abiotic factors that modulate the digestive physiology, metal bioavaibility and bioaccessibility (Pouil et al., 2018). Among these factors, the type of food and its amount, substantially affect the AE values as reported in fish (Pouil et al., 2017) whereas it is less evident in copepods as reviewed by Wang & Fisher (1999b). More specifically, the AE of Cd in *Dreissena polymorpha* was estimated to be between 19 and 72% for 8 types of food (Roditi & Fisher, 1999; Lam & Wang, 2006; Pellet et al., 2014). The AE of Zn has also been reported highly variable in fish, ranging from 2 to 97% (with a median of 20%), as a function of the predator species and the food sources (Pouil et al., 2018). Mechanistically, these AE variations thought to be driven by the subcellular distribution of metals in prey (i.e. the storage form). In theory, metals are considered to be trophically available for transfer to consumers when they are associated with organelles, enzymes and metal-bindings proteins (i.e. Trophically Available Metals), i.e. metals found in the soluble fraction. In turn, metals associated with insoluble fraction (i.e. metal riche granule, or cellular

debris) may not be assimilated (Wallace & Lopez, 1996; Wallace & Luoma, 2003). Nevertheless, in practice, the relationship between TAM and AE are less evident, because of complex metal organ- and subcellular distribution in metazoan preys (see (Pouil et al., 2016)).

In the present study, the AE of Cd and Zn were 2- and 1.5-fold higher when the ingested food was alder leaves (39 ± 3.2% and 15 ± 1.7%, respectively; Fig. 2 and Table 1) compared to larvae of chironomid (19 ± 1.7% and 9.1 ± 1.2%, respectively; Fig. 2 and Table 1). This is in contrast to what is usually reported for omnivorous organisms, for which the trophic transfer of metals from plant sources is less than that from animal prey because the digestive process of vegetable materials is slower and more complex (Xu & Pascoe, 1994; Nunez-Nogueira et al., 2006). For instance, the shrimp *Penaeus indicus* fed with Cd-contaminated algae showed an AE of 42.4 ± 5.1%, a value 1.8 fold higher than this reported in shrimp fed with Cd-contaminated squid (*i.e.* 74.6 ± 8.5%). Contrasting to this, the AE of Zn was not dependent on food type (i.e. AE of *P. indicus* fed with squid = 57.9 ± 7.3%; AE of *P. indicus* fed with algae = 59.1 ± 8.4%) (Nunez-Nogueira et al., 2006).

With regard to our results, it is noteworthy that live chironomid larvae were contaminated with the radiotracers whereas only dead leaves were radiolabeled. More than the plant or animal character, we presume a large passive adsorption of metals on the walls of inert dead leaves compared to a poor absorption within leaf structures. Once ingested, the fraction of metals is supposed to be easily desorbed under a free form ion and thus more bioavailable, increasing the assimilation efficiency for the gammarid. On the contrary, metals actively taken up by live chironomids larvae have been distributed among tissues, incorporated in cells and taken over by the mechanisms of detoxification, and thus bound to various subcellular components. Therefore, the higher AE obtained for leaf-fed gammarids than for chironomid-fed gammarids

would be related to the sorption processes and the TAM fractions in living vs. inert food sources rather than the contrasting efficiencies of plant and animal materials digestion.

As found for AE, both k_{es} and k_{el} of Cd and Zn varied more according to the food matrix than the metal itself. With $Tb_{s1/2}$ of 13.0 ± 1.2 and 13.0 ± 0.3 h, for Cd and Zn, respectively, the short-lived metal pool was 2- to 3-fold longer in chironomid-fed gammarids than in leaf-fed ones (Table 1). In addition, the long-term biological half-life $Tb_{l1/2}$ tends to infinity for both elements when gammarids were fed with chironomid (Table 1). This strong retention of metals is consistent with the previously stated hypothesis that the both food sources have different TAM (Rainbow et al., 2011) which, once assimilated, should also determine the fate of the metal in the gammarid. Thus, the leaf-adsorbed metal assimilated in a free form or in a weak ionic complex is likely to be taken over more rapidly by detoxification and/or elimination processes than the assimilated metal strongly bound to components and concretions of chironomid cells. Nunez-Nogeira et al (2006) also found a higher k_e of Cd in the shrimp *P. indicus* contaminated with squid meat (0.009 ± 0.003 d^{-1}) than when contaminated with algae (0.004 ± 0.001 d^{-1}), confirming that Cd assimilated from a vegetal matrix is more rapidly eliminated than from complex multicellular organism. However, Zn is eliminated more rapidly after ingestion of animal than plant matrix (Nunez-Nogueira et al., 2006), in opposition to what we found in *G. fossarum*. These observations suggest that Zn and Cd undergo different physiological bioaccumulation processes depending on the type of food ingested, hence the importance of better understanding the toxicokinetic of ingested metals and what influences it.

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Conflict of interest disclosure

The authors declare that they comply with the PCI rule of having no financial conflicts of interest in relation to the content of the article.

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