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Bioaccumulation, organotropism and fate of cadmium in *Gammarus fossarum* exposed through dietary pathway

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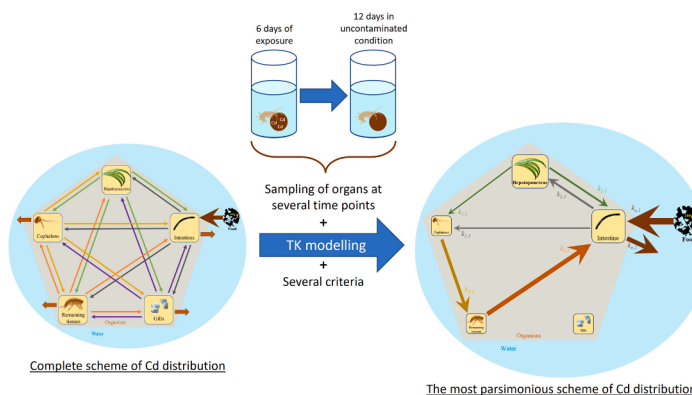
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HIGHLIGHTS

- Importance of intestine and hepatopancreas for Cd bioaccumulation through the trophic pathway.
- Absence of Cd accumulation in the gills throughout the experiment.
- The intestine is the only organ to eliminate Cd to the outside environment.
- Obtain a different pattern of Cd distribution and fate from that obtained previously for the trophic pathway.

GRAPHICAL ABSTRACT



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ABSTRACT

Despite a good knowledge of cadmium accumulation in *Gammarus fossarum*, studies to date have focused on Cd accumulated via the dissolved pathway, leaving aside the trophic pathway. The aim of this study was to assess cadmium organotropism and bioaccumulation processes following a trophic exposure of the species *Gammarus fossarum*. Adult male gammarids were fed with ¹⁰⁹Cd contaminated alder leaves discs for 6 days and then with clean alder leaves for 12 days. During both phases, some gammarids were collected and dissected, and intestines, hepatopancreas, cephalons, gills and remaining tissues were separated to measure their Cd concentrations. Their relative proportions of Cd and their respective BMFs were estimated. The ingestion rate (IR) measured during the exposure phase was divided by 3 between days 2 and 6, indicating that gammarids reduced their feeding activity and therefore the exposure pressure. A multi-compartments TK model was developed, and an iterative inference process was run to select the most parsimonious model that best fits all organ datasets simultaneously. The results showed that: i) intestine and hepatopancreas bioconcentrate Cd the most; ii) no cadmium was quantified

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in gills, meaning that they do not appear to play a role in Cd storage or elimination with a trophic exposure; iii) Cd elimination occurs only through the intestine; and iv) the general pattern of Cd fate in gammarids, obtained here after dietary highlights once again the importance of the intestine and hepatopancreas, as for the dissolved pathway.

1. Introduction

Cadmium (Cd) is a non-essential metal element found in aquatic environments, due to natural (e.g. volcanism, geochemical background) and/or anthropogenic (e.g. industrial and agricultural activity) releases [1]. Its toxicity on aquatic organisms makes Cd a priority substance by the European Water Framework Directive (WFD) justifying its monitoring [2]. In active biomonitoring, gammarids (*Gammarus fossarum*) are widely used to determine environmental contamination, as this freshwater invertebrate is known to bioaccumulate metals from its environment [3,4]. Furthermore, they fulfill the characteristics of sentinel species since they are widely distributed in Europe, easily manipulable, and they occupy a key place in food webs as detritivores recycling organic elements [5–9].

It is now commonly accepted that invertebrates can accumulate metals via both pathways (i.e. dissolved and trophic) but with contrasting respective contribution [10–12]. Indeed, the dominance of the trophic pathway in Cd accumulation in the clams *Corbicula fluminea* and *Macoma balthica* has been demonstrated, while in the amphipod *Hyalalea azteca*, Cd is accumulated equally by both exposure routes [13,14]. In the genus *Gammarus*, the trophic pathway would mainly contribute to Cd accumulation [4] compared to the dissolved one. Interestingly, the route of exposure could control the organotropism of metals, as previously demonstrated, mainly in fish and marine invertebrates [15,16]. In invertebrates, the crustacean *Cancer pagurus* accumulates 94 % of the dietary Cd in its hepatopancreas and the pectinidae *Chlamys varia* and *Pecten maximus* accumulate, respectively, 97 % and 82 % of the dietary Cd in their digestive glands [17]. This tissue distribution contrasts with the metal accumulation in gills and hepatopancreas (or equivalent) following waterborne exposure, which has been classically reported in invertebrates [16–19]. Not surprisingly, this difference results from the fact that the most contaminated organs are those in direct contact with the Cd source (e.g. gills with contaminated water or intestine and hepatopancreas with metal contaminated food). However, most of the studies have been carried out at the end of experimental period (dissolved Cd exposure or following a pulse-chase-feeding of Cd-enriched food) with the assumption of equilibrium and a static view of the tissue distribution. A dynamic approach could have to describe the fate of Cd over periods of exposure and non-exposure revealing the metal uptake, the transfer between tissues, and identifying key physiological processes and role of organs in Cd handling.

Toxico-kinetic (TK) models are useful tools to describe and formalize the key processes of accumulation, distribution, and elimination at both whole organism (one-compartment TK models) or organ/tissue levels (multi-compartments TK models or physiologically based toxico-kinetic (PBTK) models). In addition, multi-compartments (PB)TK models can also help to understand the metal transfer within the organism and the role of organs in the internal contaminant fate over time [15,20–23]. Today, multi-compartments (PB)TK models have been developed mainly for organic compounds in fish [20,22,24,25], relatively poorly used for scenarios involving trophic contamination and not to our knowledge in invertebrates species. Estimation of Cd exchanges among the internal organs of trout *Oncorhynchus mykiss* and the carp *Cyprinus carpio* with PBTK models corrects the underestimation of the metal accumulation in the liver and kidney observed with static approaches, due to the saturation that occurs in these organs [15]. In the fish *Terapon jarbua*, the description of mercury (Hg) transfers between tissues demonstrated that the liver plays an important role in its distribution and that the carcass a sink role for this metal [23]. Moreover, the use of

PBTK models confirmed the role of the gills in the elimination of dietary Hg [23]. For invertebrates, (PB)TK models are scarce: to the best we know, multi-compartments TK models were applied to describe the fate of waterborne Cd in the gammarids *Gammarus fossarum* [26–28].

In this context, the objective of our study is to improve the knowledge of bioaccumulation, organotropism and fate of Cd accumulated through a dietary pathway, in the ecotoxicological model *G. fossarum*. Thus, the relative distribution of dietary Cd in each organ and their BMFs were estimated, and a multi-compartments TK model was fitted to the data to describe dietary Cd organotropism and ADME processes during time.

2. Material and methods

2.1. Origins, collection and maintaining of gammarids and alder leaves

Adult males of *Gammarus fossarum* were collected in an unpolluted former cress pond (Saint-Maurice-de-Rémens, France), before being calibrated to 22.1 ± 1.3 mg wet weight (ww). Then, gammarids were sent to LIENSs laboratory (La Rochelle) where they were kept at a temperature of 12 ± 0.5 °C, under constant bubbling in Evian® water (see characteristics in Table S1), fed *ad libitum* with alder leaves (from *Alnus glutinosa*), and under a dark/light cycle of 8:16 h for a period of 10 days to be acclimated.

On a clean site (Les Ardillats, France), alder leaves (*A. glutinosa*) were collected, and transported to the ecotoxicology laboratory of INRAE Lyon-Villeurbanne to be leached in order to eliminate the tannins they contained (i.e. soaked in well water for several days, with regular renewal of the water). Then, avoiding the large veins, the wet leaves were punched into 20 mm diameter discs. At last, the discs were placed in a teflon bottle filled with well water (see characteristics in Table S1) before being transferred to the LIENSs laboratory premises.

2.2. Contamination of alder leaves

The ^{109}Cd carrier-free solution (CdCl_2 ; 0.1 M HCl) was purchased in Eckert & Ziegler Isotope Products Inc., Valencia, USA. The stock solution of ^{109}Cd was diluted (0.1 M HCl) into an intermediate solution in order to reach an appropriate radioactivity in water for the alder leaves radiolabelling. Thus, 375 leaf discs were contaminated by soaking. All of them were distributed among 25 Cauberes® tubes (36 mm diameter and 0.075 L; with 15 discs per tube), containing 15 mL of Evian® water contaminated with a concentration of $200 \text{ Bq}\cdot\text{mL}^{-1}$ of ^{109}Cd for 7 days. This concentration of radioactive activity corresponds to a concentration of $0.037 \mu\text{g Cd}\cdot\text{L}^{-1}$, which is relevant from an environmental point of view, since it falls within the low range of Cd concentrations measured in French river water [12]. Then the discs were rinsed with clean Evian® water and placed in new clean Cauberes® tubes (36 mm diameter and 0.075 L; with 15 discs per tube) and 15 mL of clean Evian® water for 7 days, with a renewal every two days, in order to remove the weakly adsorbed and potentially remobilizable portion of radioisotopes during gammarid exposure.

2.3. Contamination of gammarids by dietary pathway

Two days before the beginning of the experiment, gammarids were starved and transferred in beakers where they were separated individually in hand-made baskets to avoid cannibalism [29]. Each beaker contained 8 gammarids (for a total of $n = 208$) and was filled with

uncontaminated Evian® water (i.e. without radiotracer). The experimental protocol included two phases. First, during the 6-day accumulation phase, each gammarid was exposed *ad libitum* to discs of alder (*Alnus glutinosa*) leaf previously contaminated with ^{109}Cd ($0.585 \mu\text{g}$ of $\text{Cd}\cdot\text{mg}^{-1}$ of alder leaf). Then, the gammarids were transferred to clean (i.e. without radiotracer) baskets and beakers, filled with clean water and were fed with uncontaminated leaves, for a 12-day depuration phase. At different sampling time (days 2, 4, 6, 8, 11, 14 and 18), twenty gammarids were randomly sampled, rinsed in clean water, gently dried before being weighted ($\pm 0.1 \text{ mg}$) and dissected according to the method described in Gestin et al. [28,29]. Cephalon, hepatopancreas, intestine, gills and remaining tissues (mostly muscles and cuticle) were collected, to obtain 4 replicates of 5 pools of each organ per sampling day. Before being gamma-counted (i.e. to determine the ^{109}Cd radioactivity in the sample), each pool of organ was stored, at ambient temperature, in $500 \mu\text{L}$ of HCl (3.4 %). The weight of each organ was estimated from their respective proportion previously defined [29], relatively to the gammarid total weight (Table S2). At each sampling time, the contaminated leaves discs were also sampled and photographed. The captures were then processed using ImageJ software to determine the surface area of disc eaten by the gammarids (Table S3). Then the surface was converted in mg of leaves and was used to estimate the Ingestion Rate (IR) between the beginning of the experiment (t_0) and the sampling time, expressed as mg of leaves ingested per mg of gammarid per day (Table S3). The mortality of gammarids was checked every day. The survival rates of gammarids at the end of the experiment was 74 %.

All along the experiment, the water was aerated and maintained at $12 \pm 0.5 \text{ }^\circ\text{C}$, since the beakers were placed in a thermostated bath. The ^{109}Cd radioactivity in water due to recycling (i.e. from ^{109}Cd desorption from leaves or ^{109}Cd elimination by gammarids) was daily monitored. The water was changed as much as necessary to maintain ^{109}Cd radioactivity below the detection limit and a good water quality. At least, the water was changed every two days.

Inhouse calibrated standards with appropriate geometry of contains were used to determine the radioactivity of samples. Using InterWinner 7.0 software (ITECH Instruments®) coupled to NaI detector, the samples were analyzed, to obtain runs ranged from 10 min to 48 h of counting with an uncertainty below 5 %. To obtain the concentrations of Cd in stable equivalent for each organ, the measured radiotracer activity (Bq) was converted into concentration of stable Cd (μg of $\text{Cd}\cdot\text{g}$ of organ $^{-1}$; Tables S2).

2.4. Data analysis

As commonly used in studies on bioaccumulation, the relative Cd burden of organs and the biomagnification factor (BMF) were calculated for each organ (see the [supplementary materials](#) for details).

2.4.1. Estimation of the uptake rate

With a trophic contamination, the rate of accumulation from the food is the result of two processes: the ingestion and the assimilation. Indeed, to be accumulated from food, a contaminant should firstly be ingested (depending on the quantity of food the organism eats) and thus be assimilated (according to the efficiency of the organism to assimilate this type of food).

First, the Ingestion Rate (IR) is the amount of food ingested by an organism over time, here expressed in mg of leaves ingested per mg of gammarid per day ($\mu\text{g}\cdot\text{mg}^{-1}\cdot\text{d}^{-1}$). This parameter was estimated from the data obtained at different time steps of the accumulation phase (days 2, 4 and 6). Because we observed a linear decrease with time, we fitted to the data the following model:

$$IR(t) = IR_0 - b \times t \quad (3)$$

where $IR(t)$ is the ingestion rate at time t (day^{-1}), IR_0 is the ingestion

rate at day 0 (day^{-1}) and b the slope of IR according to the time t (days).

After being ingested, only the fraction of Cd assimilated by the intestine is available to be distributed to the other organs. This fraction is the assimilation efficiency (AE), i.e. the proportion of contaminant (here Cd), that crosses the intestinal epithelial barrier. The estimation of AE can be done from data obtained in a previous pulse-chase-feeding experiment performed [30]: i) under similar physico-chemical and temperature conditions; ii) with the same type of food (alder leaves, *A. glutinosa*); and iii) in adult male gammarids of the species *G. fossarum* from the same population. Because most of the time, the elimination pattern of a contaminant is biphasic, the short-lived and long-lived elimination kinetic parameters and the assimilation efficiencies of Cd ingested by gammarids were estimated with the double component exponential equation (Eq. 4) [3,31,32]:

$$p(t) = (1 - AE) \times e^{-k_{es} \times t} + AE \times e^{-k_{el} \times t} \quad (4)$$

with $p(t)$ is the proportion of remaining activity in gammarid at time t , AE is the assimilation efficiency of Cd by gammarids, k_{es} and k_{el} represent the elimination rates of Cd with k_{es} corresponding to the first phase of elimination, i.e. the part that is quickly depurated ("short-lived"); and k_{el} corresponding to the second phase of elimination, i.e. the assimilated part that is slowly depurated.

2.4.2. Multi-compartments TK models

Biologically, organs of gammarids are all interconnected and can potentially exchange metals, thus fulfilling their physiological functions (e.g. storage, and excretion). The modeling of these flows makes it possible to estimate the importance of the exchanges among organs, but also to know the role played by each organ in ADME processes and in the fate of the contaminants into the organism. The multi-compartments toxicokinetic (TK) models, already used for several species, was recently developed for gammarid but with the dissolved pathway as the only source of contamination [27,28].

Since the bioaccumulation studied here is carried out by contaminated food only, intestine is necessarily the only way of entry of the contaminant into the organism and thus the first organ of distribution to other organs. As a consequence, the equation describing the Cd concentration over time in intestine is different from the other organs during the first phase (Eq. 5): the concentration in intestine increases due to the ingestion and assimilation of Cd from food and the Cd coming back from the other organs; it decreases due to the distribution to the other organs and the excretion in water. For the other organs, the concentration could vary during the exposure phase only due to the exchanges with the other organs and the excretion to water (Eq. 6). During the depuration phase, the equation is the same for all organs, the exchanges being maintained (Eq. 7). The complete model (Fig. S1) is thus composed of a set of 10 ordinary differential equations (Eqs. (S1) to (S10) in SI), generalizable as follows:

where $C_i(t)$ corresponds to the internal concentration of Cd in the organ i ($i = 1.5$, with $i = 1$ for intestine, $i = 2$ for hepatopancreas, $i = 3$ for cephalon, $i = 4$ for remaining tissues and $i = 5$ for gills) ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight) at time t (days), $IR(t)$ is the Ingestion Rate ($\mu\text{g}\cdot\text{mg}^{-1}\cdot\text{day}^{-1}$) at time t (estimated with Eq. 3), AE is the Assimilation Efficiency (AE) (without unit) (estimated with Eq. 4), C_f is the concentration of Cd in alder leaves discs ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight), k_{ji} is the transfer rate from the organ j to the organ i (day^{-1}), k_{ij} is the transfer rate from the organ i to the organ j (day^{-1}), $k_{e,i}$ is the elimination rate from the organ i to the surrounding media (day^{-1}), and t_c is the time t separating the accumulation phase from the elimination phase (days).

For the stochastic part, it was assumed that the Cd concentrations in organs follow a Gaussian distribution:

$$C_{obs,i}(t) \sim \mathcal{N}(C_i(t), \sigma_i) \quad (8)$$

where $C_{obs,i}(t)$ is the observed concentrations in the organ i ($i = 1.5$) at

$$\begin{cases} \frac{dC_1(t)}{dt} = IR(t) \times AE \times C_f + \sum_{j \neq 1} k_{j1} \times C_j(t) - \sum_{j \neq 1} k_{1j} \times C_1(t) - k_{e,1} \times C_1(t) & \text{for } 0 \leq t \leq t_c \quad (5) \\ \frac{dC_i(t)}{dt} = \sum_{j \neq i} k_{ji} \times C_j(t) - \sum_{j \neq i} k_{ij} \times C_i(t) - k_{e,i} \times C_i(t) & \text{for } i = 2 \dots 5 \text{ and } 0 \leq t \leq t_c \quad (6) \\ \frac{dC_i(t)}{dt} = \sum_{j \neq i} k_{ji} \times C_j(t) - \sum_{j \neq i} k_{ij} \times C_i(t) - k_{e,i} \times C_i(t) & \text{for } i = 1 \dots 5 \text{ and } t > t_c \quad (7) \end{cases}$$

time t , \mathcal{N} represents the Normal law with the standard deviation σ_i for the organ i ($i = 1..5$), and a mean $C_i(t)$ stands for the internal concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight) in the organ i predicted by the model at time t (Eqs. 5 to 7).

2.4.3. Parameters estimation by Bayesian inference

All the parameters were estimated by fitting simultaneously: i) the TK multi-compartments model (Eqs. 5 to 7) to all organ concentrations measured during time simultaneously; ii) the model describing the ingestion rate during time (Eq. 3) to ingestion data measured during the exposure phase; and iii) the model describing the proportion of remaining activity (Eq. 4) to the data previously observed in a pulse-chase feeding experiment. Bayesian inference was used with R software and JAGS [33,34] (for more details, see the [supplementary materials](#) and Gestin et al., 2021).

As previously seen, few prior information exist on IR for gammarids [4,35]. Moreover, we know that the assimilation efficiency (AE) is by definition necessarily between 0 and 1. So, for IR(0) and AE parameters, semi-informative priors were used: a Uniform law bounded from 100 to

700 and from 0 to 1, respectively (Table S4). For the other kinetic parameters, there is no information in the literature. A Uniform law is thus used on the decimal log scale (i.e. non-informative prior) (Table S4). Lastly, for the uncertainty parameters (corresponding to the stochastic part) a Gamma law was used (Table S4). The IR, AE, k_{es} and k_{el} parameters were estimated simultaneously with the k_{ij} , k_{ji} and $k_{e,i}$ parameters because all data are used simultaneously and, as mentioned above, the models describing IR(t) and p(t) (Eqs. (3) and (4)) were fitted to their respective datasets at the same time that the multi-compartments model was fitted to bioaccumulation data observed in all organs. The estimate of each parameter is summarized by the median and the 95 % credibility interval of the marginal posterior distribution obtained for each parameter.

3. Results

The measured Cd amounts, the estimated organ weights, and the calculated Cd concentrations in each organ at each sampling day are presented in Table S2.

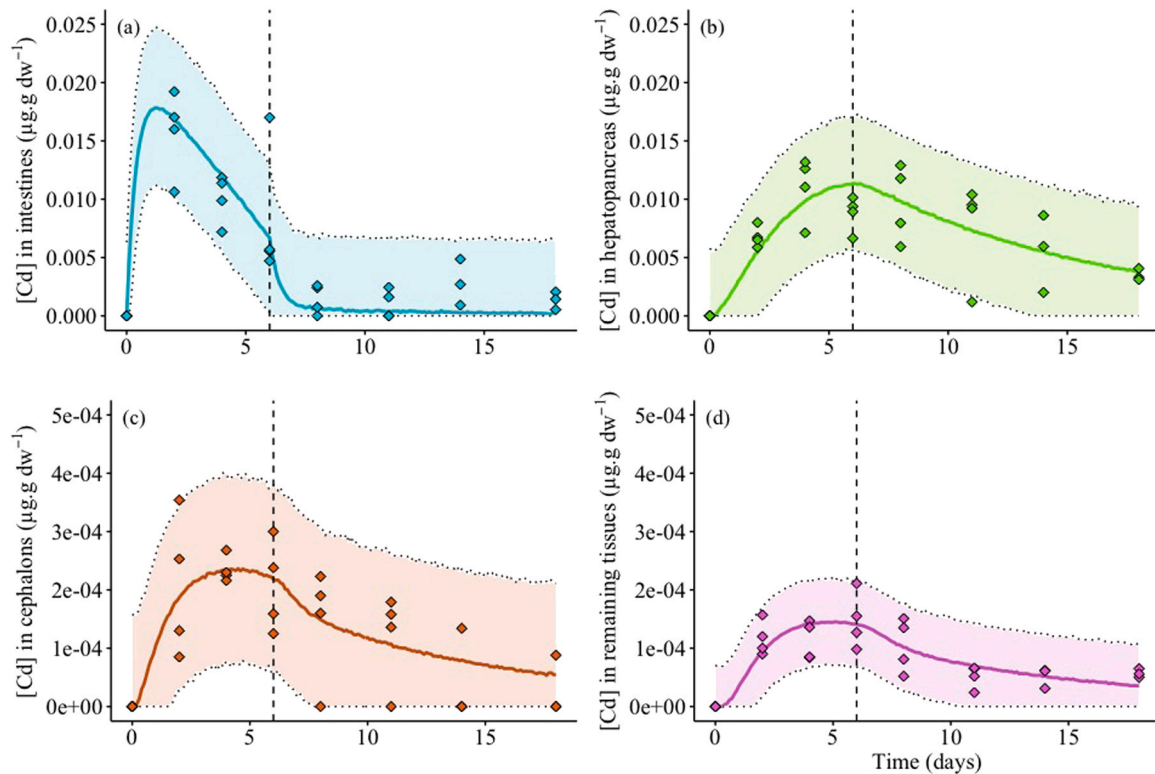


Fig. 1. Measured (diamonds) and predicted, with the final multi-compartments TK model, (Eqs. (S1) to (S10)) (95 % credible interval in dashed lines and median in solid line) concentrations of Cd in a) intestines, b) hepatopancreas, c) cephalons and d) remaining tissues of gammarids exposed to ^{109}Cd contaminated alder leaves during the accumulation phase (6 days) followed by a depuration phase (12 days). The black dotted vertical line separates the two phases. The y-scales of the plots differ between the graph a) b) and c) d).

3.1. The organotropism of Cd by dietary pathway

At the end of the exposure period (at day 6), the BMF of the organs were ranked from the highest to the lowest as follows: hepatopancreas \approx intestine $>$ cephalon \approx remaining tissues $>$ gills, with values of 16, 9.6, 0.35, 0.24 and null, respectively (see Table S2 for the concentrations). Noteworthy, the 26 replicates obtained for gills during the experiment, radioactivity was detected in only 6 replicates, at very low levels, implying thus a non-accumulation of Cd in this tissue.

Along the accumulation and depuration phases (Fig. 1), the concentration of Cd in the hepatopancreas increased until day 4 and then stabilized until day 6 around $0.010 \mu\text{g}\cdot\text{g}^{-1}$ (Fig. 1). Once gammarids were placed in depuration condition, the hepatopancreas concentration gradually decreased from 0.010 ± 0.0033 (day 8) to $0.0035 \pm 0.00051 \mu\text{g}\cdot\text{g}^{-1}$ at the end (Table S2). In intestine, the Cd concentration reached its highest value at day 2, with $0.013 \pm 0.004 \mu\text{g}\cdot\text{g}^{-1}$ (Fig. 1) and then decreased by two-fold between day 2 and day 6 (Fig. 1; Table S2), related to a reducing ingestion rate of gammarids during this period (see thereafter). In depuration condition, this concentration dropped until a plateau at around $0.001 \mu\text{g}\cdot\text{g}^{-1}$. Finally, the low Cd concentrations recorded in remaining tissues and cephalons quickly increased and remained then stable until day 6, showing a plateau around $1.4\text{e-}4$ and $2\text{e-}4 \mu\text{g}\cdot\text{g}^{-1}$, respectively (Fig. 1). Then, the cephalon concentration gradually decreased but 2/3 of sample values passed below the detection limit from the day 14. The concentrations in the remaining tissues reached a plateau at day 11 around $5\text{e-}5 \mu\text{g}\cdot\text{g}^{-1}$ (Fig. 1).

The relative proportion of Cd in the hepatopancreas accounted for more than half of the total metal burden in the whole organism (from 59 to 82 %) whatever the sampling time, except at day 2 (Fig. 2) when intestine and hepatopancreas showed similar proportions (44 % vs. 42 %, respectively; Fig. 2). During the accumulation phase, the Cd proportion in intestine decreases from 44 % at day 2 to 24 % and 22 % at day 4 and 6, respectively, and then dropped to 4 % as soon as the gammarid were placed in the depuration condition. Finally, from day 11, the Cd fraction in intestine increased 3 to 4-fold concomitantly with a decrease of 17 % of Cd proportion in hepatopancreas (Fig. 2). Regarding the other tissues, the “remaining” remained stable (between

10 % and 16 %) as the cephalon (< 3 %) all along the experiment.

3.2. Fate of Cd into *G. fossarum*

The uptake rate of Cd by intestines resulted from IR and AE, estimated from the simultaneous fits of (i) the multi-compartments TK model (Eqs. (5) to (7)) to the measured concentrations in each organ during time, (ii) the models describing IR variation over time (Eq. (3)) and (iii) the proportion of Cd assimilated over time (Eq. (4)) to their respective observed data. The predictions of these models, superimposed to the observations, are presented in Fig. S3 and S4 for the IR and the AE respectively. Gammarids reduced IR throughout the accumulation phase (Table S3), resulting in an IR estimate at t_0 (IR_0) of $244 \pm 9.42 \mu\text{g}\cdot\text{mg}^{-1}\cdot\text{d}^{-1}$ with a slope coefficient (b) of -31 ± 2.2 (Fig. S3). The AE was estimated to 32.4 ± 3.13 %.

The most parsimonious model finally selected from the full model, after successive fits, was schematized in Fig. 3. The inference process converged, and thin posterior distribution was obtained for each parameter (Fig. S2), meaning that data contained enough information to precisely estimate all the parameters. Each parameter was summarized, from the joint posterior distribution, by their respective median and 95 % credibility intervals (Table S4). The median internal concentrations predicted by the model, and their 95 % credibility intervals were superimposed to observed data in Fig. 1, which indicates the goodness of fit.

The final scheme of the most parsimonious model (Fig. 3) shows that Cd was accumulated in the intestines before being transferred to the hepatopancreas and the cephalon. A fraction of the Cd in hepatopancreas was distributed to the cephalon whereas another part returned to intestines. The Cd accumulated by the cephalon was passing through the remaining tissue before being eliminated through the intestine. The intestine was the only organ that eliminate Cd toward the external medium.

4. Discussion

This study improved our knowledge of the bioaccumulation, organotropism and fate of dietary Cd in the gammarid *G. fossarum*, for which

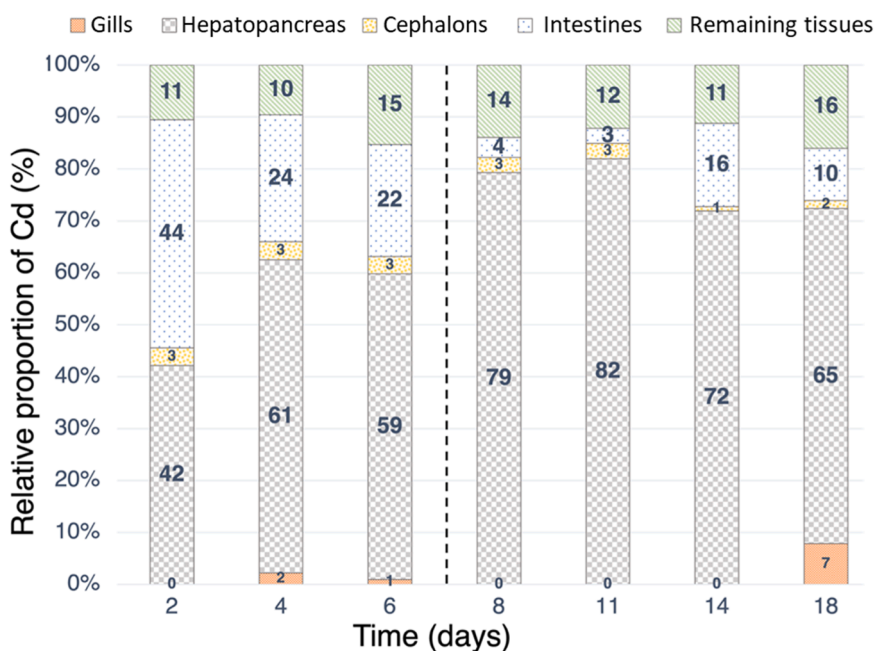


Fig. 2. Mean of the relative proportions (%) of metal burdens per organ (gills, hepatopancreas, cephalons, intestines and remaining tissues) with respect to the whole-body burden at each sampling time (days) during accumulation (days 0–6) and depuration phases (days 7–18). These two phases are separated with the black dotted vertical line.

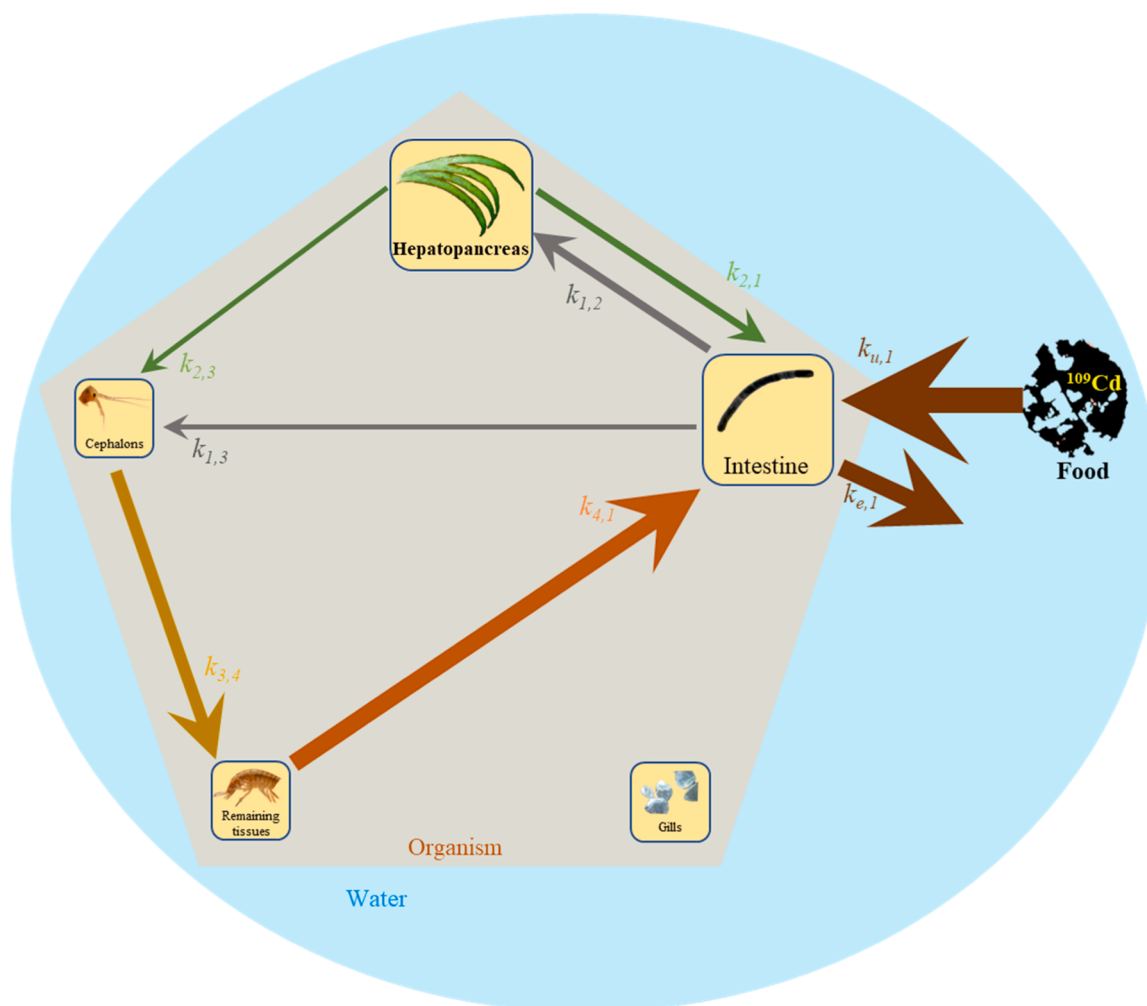


Fig. 3. Final pattern of the multi-compartments model selected at the end of the iterative process. The large blue outer border represents the medium surrounding the organism (i.e. water), the nibbled black circle on the right represents a nibbled disc of alder leaf (food) contaminated by ^{109}Cd , the beige pentagon represents a whole gammarid, and the yellow boxes inside represent the organs of interest associated. The difference in the size of the boxes is an indication of the amount of Cd accumulated in the different organs. The parameters $k_{u,1}$ and $k_{e,1}$ associated to the different arrows correspond, respectively, to the rates of uptake from food to intestines and the rate of elimination of Cd from intestines to water, while the flow from an organ i to an organ j is symbolized by the parameters k_{ij} (with $i = 1$ for intestines, $i = 2$ for hepatopancreas, $i = 3$ for cephalons, $i = 4$ for remaining tissues and $i = 5$ for gills and $i \neq j$).

no organ-level kinetic data were still available. The present work showed that the dietary Cd bioaccumulation mainly involve intestines and hepatopancreas. Both these tissues can be considered as highly accumulative organs, with BMF values well above 1 at day 6 [36–38]. In many invertebrates, the gastro-intestinal tract (encompassing intestine and hepatopancreas) acts as a site of metal sequestration and detoxification [39,40]. This makes hepatopancreas and intestines critical organs in dietary Cd accumulation. In addition, these two organs together account for about 84 % of the total Cd bioaccumulated into the whole organism at all time steps (Fig. 2) and are also those that bioconcentrate the most Cd, about 40 times more than the cephalons and the remaining tissues at the end of the accumulation phase (Fig. 1). In fact, remaining tissues and cephalons displayed a BMF of less than 1 at the end of the exposure period, making them organs that would have little influence in the overall handling of Cd and no (or a very minimal) role in its fate after a trophic contamination.

4.1. The importance of the trophic pathway in the contamination of organisms

In this experiment, the Cd accumulation was driven by the ingestion rate and the assimilation efficiency estimates. We observed that IR

decreased all along the accumulation phase. Inhibition of IR has already been demonstrated in gammarids (*G. fossarum* and *G. pulex*) after *in situ* and lab exposure [35,41–43]. Croteau and Luoma, (2008) showed an IR divided by 10 when the dietborne Cd concentration increased from 0.14 to 12.42 $\mu\text{g}\cdot\text{g}^{-1}$ after an 18 h-exposure in *Lymnaea stagnalis*. Rather than a toxic effect of Cd on intestines, this IR inhibition would be attributed to a behavioral response of avoidance to pollutants [44,45]. Nevertheless, given the low Cd concentration used in this study, it remains unclear whether this IR inhibition was due to a difference in leaf palatability, resulting from Cd detection and therefore avoidance behavior by gammarids, or if it resulted from an increased appetite of individual due to the 2-day starvation period preceding the experiment. Overall, this demonstrates the importance of measuring IR and including these values in TK models to better describe the bioaccumulation kinetics of trophically ingested contaminants.

The AE estimate showed that one third of ingested Cd was assimilated by gammarids. This value was of the same order of magnitude than those measured in other invertebrates such as shrimp (42.4 ± 5.1 %) and copepod (30.4 %) [46,47], and the gammarid *Gammarus pulex* (5–47 %) [4]. This AE was in the lower range of values determined in the daphnid *Daphnia magna* (30–77 %) and the mussel *Dreissena polymorpha* (19–72 %), which show great variability [31,48]. In contrast, very low

AE of 2.7 %, was reported for dietary Cd in fish *Menidia menidia* and *M. beryllina* [49]. Thus, the estimated AE value of 32.4 ± 3.13 % indicates a good bioavailability of Cd in alder leaves allowing a significant accumulation of dietborne Cd by gammarids.

The development of toxicokinetic (TK) models is a relevant tool to describe and understand (in a mechanistically and physiologically way) all the processes governing bioaccumulation into organisms [45,50–52]. The present study shows that Cd reached the highest concentration in the intestine before being transferred mainly to the hepatopancreas and, to a lesser extent, to the cephalon. In the killifish *Fundulus heteroclitus* fed by multiple Cd pulses, 86–94 % of total Cd was shown to be associated primarily to the intestine due to its key role in the digestive physiology [53]. This organotropism was also described in the trout *O. mykiss* where Cd was bound to metallothioneins of intestinal cells, before being released into the lumen before elimination [54]. The high Cd concentration in intestine suggests that this organ both accumulates this metal in its cells and has a permeability allowing metal to diffuse to the other internal organs. In addition, during the depuration phase, the intestines received Cd back from the other organs, which explains why the relative proportion in the intestine increases from 4 to 10 % (Fig. 2). This demonstrates that the intestines is the organ the most linked to the others in term of Cd exchanges with a dietary exposure, which is confirmed by the TK model outputs.

Based on the final scheme obtained for the TK model (Fig. 3) and the associated k_{12} parameter (Table S4), most of the Cd moved from the intestine to the hepatopancreas, consistent with the biological link between the two organs for the nutrient assimilation. Consequently, the hepatopancreas showed the greatest relative proportion and highest concentration of Cd among organs from day 4. Furthermore, as long as the gammarids were exposed *via* the diet, Cd concentrations in the hepatopancreas increased until day 4 before reaching a plateau by day 8 (Fig. 1). Kinetically, the parameter k_{12} , which represents the rate of Cd which entering in the hepatopancreas, was in median 2.3 and 11.5-fold higher than k_{21} and k_{23} , respectively, the two rates leaving the hepatopancreas. Thus, the digestive physiology logically drives this important transfer of Cd to the hepatopancreas and overall leads to the key role of this tissue in metal storage as shown in crustaceans [17,46] cephalopods [55] or fish [54,56,57]. Hepatopancreas is already known to be a central sink organ in metal detoxification especially through its sequestration in mitochondria [46,58,59]. Metals such as Cd can complex with metallothionein-like proteins or with MRGs (i.e. Metal-Rich Granules), hindering metal interaction with biological molecules and toxic effects [58–60].

The TK model showed that the cephalon was also supplied with Cd by both the intestines and the hepatopancreas with low transfer rate values indicating that the Cd sequestration by the hepatopancreas is not total. Cephalons should play a negligible role in ADME processes since they: i) represented less than 3 % of the total body burden; and ii) concentrate 30 to 40 times less Cd than the hepatopancreas and the intestines during the exposure phase. Moreover, after 8 days in the elimination phase, only one third of the cephalon samples contained radioactivity above the detection threshold, suggesting an efficient elimination of Cd by this organ.

During the accumulation phase, the remaining tissues showed an accumulation pattern roughly identical to the one of cephalons (Fig. 1). Interestingly, this was consistent with the model outputs which estimated that cephalons were the organ transferring Cd to the remaining tissues (Fig. 3). Unlike cephalons, it appears that the remaining tissues have a role of Cd storage site, showing a plateau around $5e-5 \mu\text{g}\cdot\text{g}^{-1}$ from day 11 to day 18, at the end of the elimination period (Fig. 1). However, considering the low magnitude of the exposure concentrations and the fact that this organ accounts for less than 16 % of the total amount of Cd in the organism while representing 77.5 % of its weight, this storage role is nevertheless minimal in the context of dietary Cd accumulation. This is in agreement with very low Cd concentrations reported in the carcass (i.e. muscle, bone and skin) of the trout *O. mykiss*

[57].

Finally, the gills did show no significant Cd accumulation all along the experiment. The very small activities detected in few samples (3 pools of gills in each accumulation and elimination phase) might be more attributed to a waterborne gills contamination due to Cd recycling from the gammarid feces than a real metal transfer from intestine or hepatopancreas. Thus, we consider that dietborne Cd did not accumulate in the gills, which represent a relative proportion of Cd < 2 %. The final selection of the global Cd handling scheme from the most parsimonious multi-compartment TK model showing that the gills do not exchange Cd with the other organs, with $k_{5,j}$ and $k_{i,5}$ set to 0. We can conclude that the gills are not a storage and/or elimination organ for Cd accumulated through the trophic pathway.

4.2. The importance of considering the different pathways of contamination

An accurate knowledge of metal bioaccumulation in organisms, taking into account all the different contamination pathways, is important for interpreting the concentrations reported in biomonitor species with respect to their physiological processes [11,12,61,62]. Through trophic pathway, the final scheme of Cd handling here in *G. fossarum* shows a two-ways exchange between intestine and hepatopancreas and a one-way exchange from hepatopancreas to cephalons, from cephalons to remaining tissues and from remaining tissues to intestines. When gammarids are contaminated by the trophic pathway, the intestine is the only organ that eliminates Cd to the external environment, as also demonstrated for the dissolved pathway [28]. Therefore, whether for the dissolved or trophic pathway, the intestine plays a major role in the accumulation, distribution, and elimination of Cd in gammarids.

The other organ that has a consistent role in Cd handling whatever the exposure pathways is the hepatopancreas. It remained the most concentrated organ and plays an undisputed storage role for Cd [29] which could be attributed to its capacity for metal detoxification. However, the relative proportion of Cd in hepatopancreas represents 42 to 82 % vs. 26 to 47 % of the total body burden (excluding gills) following a diet- and waterborne exposure, respectively [29]. This suggests that the potential of Cd sequestration and entrapment by the hepatopancreas is more effective when Cd is associated with food nutrient.

Finally, the major changes between both exposure pathways concern the gills. Indeed, gills play no role in Cd storage or elimination following a trophic exposure, whereas they play an important role in the accumulation and long-term storage following waterborne Cd exposure [29]. This divergence of Cd organotropism according to the pathway of accumulation has also been observed in another aquatic invertebrate, the Norway lobster *Nephrops norvegicus* [63]. Since in gammarids the Cd accumulated by the trophic pathway never reaches the gills, this confirms that the gills are full-fledged organ, which do not appear to be connected to other organs for the handling of Cd [29]. The gill is an organ that accumulates and stores Cd, but to do so it must be directly exposed to it, as in the case of dissolved contamination. This is an interesting feature, as it could make it possible to determine whether the organisms studied as part of biomonitoring programs have accumulated cadmium by dissolved or trophic pathways.

5. Conclusion

In this study, we successfully contaminated gammarids *via* the trophic pathway, continuously for 6 days, and recovered the organs to measure the amount of bioaccumulated Cd. The hepatopancreas and intestines together accounted for about 83 % of the total organism burden and concentrated about 40 times more Cd than cephalons and other tissues. The cephalons and remaining tissues are very lightly burdened, each accounting less than 3 % and 14 % of the total organism

burden, respectively. This shows a very similar pattern to that demonstrated for the dissolved pathway if the gills are disregarded. The final pattern of Cd handling shows bidirectional Cd exchange between the intestines and hepatopancreas, unidirectional exchange from hepatopancreas to cephalons, cephalons to remaining tissues, and remaining tissues to the intestines, and elimination to the surrounding media occurring only through the intestine. Thus: i) the intestines combine the roles of organs for Cd uptake, distribution, and elimination; and ii) the hepatopancreas store the major amount of Cd presumably in a detoxified form. This study, coupled with other recent studies concerning organotropism following waterborne contamination, greatly improve the understanding of Cd fate in gammarids and the role that organs play in the overall Cd process.

Environmental implication

To date, most studies on metal bioaccumulation mechanisms in small freshwater invertebrates have been carried out at the level of the whole organism and only considered the dissolved pathway. This work described the dynamic of bioaccumulation, the organotropism and the fate of dietary cadmium in the sentinel species *Gammarus fossarum*, currently used in biomonitoring studies for environmental risk assessment. The organisms were exposed to cadmium (Cd), a non-essential metal of priority concern for the European Water Framework Directive, using ^{109}Cd radiolabeled alder leaves, to work at environmental relevant concentrations. Studying gammarids' organotropism help to understand their exposure (from trophic or dissolved pathway).

CRedit authorship contribution statement

Ophélie Gestin: Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Olivier Geffard:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Nicolas Delorme:** Methodology. **Laura Garnero:** Methodology. **Thomas Lacoue-Labarthe:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Christelle Lopes:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jhazmat.2024.135965](https://doi.org/10.1016/j.jhazmat.2024.135965).

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