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**Delineation of heavy metal uptake pathways (seawater and food) in the
variegated scallop *Chlamys varia* using radiotracer techniques**

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ABSTRACT: The bioaccumulation and depuration kinetics of selected metals (Ag, Co, Hg, Mn and Zn) were determined in the European variegated scallop *Chlamys varia* following exposures via seawater and food, using highly sensitive radiotracer techniques (^{110m}Ag , ^{57}Co , ^{203}Hg , ^{54}Mn and ^{65}Zn). Body distribution of Ag, Co, Mn and Zn was similar for both waterborne and dietary metals. Ag was mainly present in the digestive gland (> 80%); Co and Mn were generally localized in similar proportions in the digestive gland and kidneys, whereas Zn was mainly found in the kidneys (> 40%). In contrast, Hg was mainly present in gills during seawater exposure whereas it was mainly distributed in the digestive gland after food exposure. The results from all the experiments have been integrated into a bioaccumulation model in order to delineate the relative contribution of each metal uptake route in *C. varia*. Computation indicated that food is the main uptake route of Ag, Co, Mn, and Zn in scallop, whereas waterborne and dietary pathways were shown to contribute similarly in the global bioaccumulation of Hg in *C. varia*. Except for Hg, dietary transfer was investigated using two different phytoplankton species (*Isochrysis galbana* and *Skeletonema costatum*). For Mn and Zn, the dietary contribution was not influenced by the phytoplankton species used. In contrast, food quality played a major role for Ag and Co intake, (e.g., dietary pathway contributed for 97% of global Ag bioaccumulation when *S. costatum* was used as food vs. 58% for Ag ingested with *I. galbana*).

KEYWORDS: Trace elements; Bioaccumulation kinetics; Depuration kinetics; Bioaccumulation model; Bivalves

INTRODUCTION

Marine invertebrates are exposed to essential and non essential metals from both the dissolved and the particulate phases. Many of the available studies on invertebrates have focussed on the bioaccumulation of dissolved metals and/or their toxic effects. However, more recently numerous evidences have suggested or shown that food can contribute significantly to metal uptake (e.g. Luoma 1983, 1989; Wang & Fisher 1999).

Among Bivalves, Pectinidae accumulate high metal levels in their tissues (e.g. Bryan 1973; Bustamante & Miramand 2004, Metian et al. 2008), even in areas far from anthropogenic sources such as the Antarctic Ocean (e.g. Mauri et al. 1990; Viarengo et al. 1993). In this particular Family, the variegated scallop *Chlamys varia* is a common species on the Mediterranean and Atlantic European and North African rocky shores, where it is living fixed on rocky substrata (Quéro & Vayne 1998). Several field studies have shown that this species displays high levels of several trace metals, rare earth elements and radionuclides in its tissues (Bustamante et al. 2002; Bustamante & Miramand 2004, 2005ab). Since *C. varia* is targeted by commercial fishery as well as for leisure activities (see e.g. Bustamante & Miramand 2004, 2005a), its high metal bioaccumulation capacity could potentially play a significant role in contaminant transfer to consumers.

The high capacity of pectinids to bioaccumulate metals stresses the need to better understand metal behaviour and fate in these edible organisms. Field studies have inferred that the dietary pathway could be of importance in the bioaccumulation of some elements in scallops (Palmer & Rand 1977; Uthe & Chou 1987; Bustamante & Miramand 2005a). However, to the best of our knowledge, an actual assessment of the relative contribution of different exposure pathways in pectinids has been carried out in only two recent studies and only for Cd (Metian et al. 2007) and Ag (Metian et al. in press (a)).

The aim of the present study was to characterize the bioaccumulation of selected metals (Ag, Co, Hg, Mn and Zn) in the scallop *Chlamys varia* exposed via the seawater or its food under controlled laboratory conditions. In addition, the influence of food quality (i.e., phytoplankton species used as food, viz. *Isochrysis galbana* or *Skeletonema costatum*) on the assimilation efficiency of metals has been investigated. Whenever possible, a global bioaccumulation model (Landrum et al. 1992) has been used to determine the relative contribution of each uptake pathway on the bioaccumulation of the studied metals in *C. varia*.

MATERIALS AND METHODS

Sampling. In spring 2005, one hundred variegated scallops *Chlamys varia* were collected from the French Atlantic coast (Pertuis Breton, Charente-Maritime) by SCUBA diving. This location was selected because it shelters the main variegated scallop population along the French coast and is intensively exploited by local fishermen. Local exposure conditions to metals are low as previously reported in Bustamante & Miramand (2005b). Scallops were carefully transported to IAEA-Marine Environment Laboratories premises in Monaco where they were acclimated to laboratory conditions (constantly aerated open water circuit aquarium; flux: 50 l h⁻¹; salinity: 36 p.s.u.; temperature: 17 ± 0.5°C; pH: 8.0 ± 0.1; light/dark cycle: 12 h/12 h) for 8 weeks prior to experiment. During this period, the scallops were fed daily an algal diet (5 10⁴ cells ml⁻¹) composed of the Bacillariophyceae *Skeletonema costatum* and the Prymnesiophyceae *Isochrysis galbana*.

Radiotracers and counting. Bioaccumulation and depuration kinetics of ^{110m}Ag, ⁵⁷Co, ²⁰³Hg, ⁵⁴Mn and ⁶⁵Zn in scallops were determined using radiotracers of high specific activity purchased from CERCA, France (^{110m}Ag as AgNO₃, T_{1/2} = 249.8 d), from Amersham (⁵⁷Co as

CoCl₂, T_{1/2} = 271.8 d), or from Isotope Product Lab (²⁰³Hg as HgCl₂, T_{1/2} = 46.59 d; ⁵⁴Mn as MnCl₂, T_{1/2} = 312.2 d; ⁶⁵Zn as ZnCl₂; T_{1/2} = 243.9 d).

The radiotracers were counted using a high-resolution γ -spectrometer system composed of three Germanium -N or P type- detectors (EGNC 33-195-R, Canberra[®] and Eurysis[®]) connected to a multichannel analyser and a computer equipped with a spectra analysis software (Interwinner[®] 6). The radioactivity of the samples was determined by comparison with standards of known activities and appropriate geometries and was corrected for background and physical decay of the radiotracers. The counting time was adjusted to obtain a propagated counting error less than 5% (Rodriguez y Baena et al. 2006a).

Seawater exposure. Twenty-five *C. varia* (average wet weight \pm SD: 30 \pm 7 g) were placed in a 70-l glass aquarium (constantly aerated closed circuit aquarium; same salinity, temperature, pH and light/dark conditions as previously indicated) and exposed for 7 d to ^{110m}Ag (0.4 kBq l⁻¹), ⁵⁷Co (0.5 kBq l⁻¹), ²⁰³Hg (1.4 kBq l⁻¹), ⁵⁴Mn (0.5 kBq l⁻¹), and ⁶⁵Zn (0.75 kBq l⁻¹) dissolved in filtered (0.45 μ m) seawater, according to the methodology described in Warnau et al. (1996, 1999). In terms of stable metal concentration, these additions corresponded to Ag (15 pmol l⁻¹), Co (0.5 pmol l⁻¹), Hg (12 pmol l⁻¹), Mn (0.4 pmol l⁻¹) and Zn (138 pmol l⁻¹), which were 1 to 3 orders of magnitude lower than the natural concentrations of metals in seawater (Bruland 1983). No change in pH was detectable after the tracer addition. Spikes and seawater were renewed twice a day the first two days and then daily in order to keep radiotracer concentrations constant in seawater. Activity of the radiotracers in seawater was checked before and after each spike renewal, yielding time-integrated activities in seawater for the 5 radiotracers of 0.25 \pm 0.21 kBq ^{110m}Ag l⁻¹, 0.31 \pm 0.11 kBq ⁵⁷Co l⁻¹, 1.39 \pm 0.72 kBq ²⁰³Hg l⁻¹, 0.34 \pm 0.23 kBq ⁵⁴Mn l⁻¹ and 0.73 \pm 0.14 kBq ⁶⁵Zn l⁻¹ (Rodriguez y Baena et al. 2006b). Each day, during renewal of seawater and spike, the

scallops were fed briefly (30 min) *S. costatum* and *I. galbana* (5×10^4 cells ml^{-1}) in clean seawater.

Nine tag-identified scallops were collected daily from day 1 to 4, then on day 7 and were whole-body radioanalyzed alive (same individuals each time). At the end of the 7-d exposure period, 5 scallops (not belonging to the tag-identified batch) were sacrificed and dissected. Shell, digestive gland, kidneys, gills, gonad, mantle, intestine, adductor muscle and the remaining soft tissues were separated and radioanalyzed in order to assess the radiotracer body distribution.

The remaining scallops were placed in non contaminating conditions (constantly aerated open circuit; flux: 50 l h^{-1} ; same salinity, temperature, pH and light/dark conditions as previously indicated) for 36 d (91 d for ^{203}Hg) and the nine tag-identified individuals were radioanalyzed regularly in order to follow the depuration kinetics of the radiotracers from the scallops. During the whole depuration period, scallops were fed daily *S. costatum* and *I. galbana* (5×10^4 cells ml^{-1}). Four scallops were collected at the end of the depuration period and dissected into their body compartments as previously described.

Food exposure. The Prymnesiophyceae *Isochrysis galbana* and the Bacillariophyceae *Skeletonema costatum* were separately used to study the influence of food quality (phytoplankton species) on the dietary transfer of $^{110\text{m}}\text{Ag}$, ^{57}Co , ^{54}Mn , and ^{65}Zn in *C. varia*. Indeed the contrasting nature of the two phytoplankton species (*I. galbana* is a naked flagellate whereas *S. costatum* is a Si-walled diatom) allows encompassing the complexity of the phytoplankton diet of scallops (Mikulich and Tsikhon-Lukamina 1981; Shumway et al. 1987). Transfer of ^{203}Hg in *C. varia* was investigated with *I. galbana* only.

One culture of each phytoplankton species was exposed to radiotracers (see Table 1) during their growing phase (7 d for *I. galbana* and 10 d for *S. costatum*) and then separated from the

spiked seawater (1- μm filtration on Osmonic filters) according to the method described in Metian et al. (2007). The radiolabelled phytoplankton species were γ -counted before and after the filtration in order to determine the distribution coefficient (K_{df}) of each radiotracer for each species. Each phytoplankton species was resuspended in a 70-l closed-circuit aquarium to reach a cell density of $5 \cdot 10^4 \text{ cell ml}^{-1}$ (this cell density was selected in order to avoid pseudofaeces production; Metian et al. 2007). Twelve scallops (6 in each of the two 70-l aquaria; average whole-body wet weight \pm SD: $18 \pm 4 \text{ g}$) had been acclimated for one week prior starting the feeding experiments. Scallops were allowed to feed radiolabelled phytoplankton for 2 h (pulse-chase feeding method; see e.g. Warnau et al. 1996; Metian et al. 2007). After the feeding period, all scallops were whole-body γ -counted and then placed in non contaminated, flowing seawater conditions (50 l h^{-1}), with daily feeding on *S. costatum* and *I. galbana* ($5 \cdot 10^4 \text{ cells ml}^{-1}$).

All individuals were then whole-body γ -counted at different time intervals to follow the depuration kinetics of ingested $^{110\text{m}}\text{Ag}$, ^{57}Co , ^{203}Hg , ^{54}Mn and ^{65}Zn . All individuals were collected after 30 d of depuration for *I. galbana* experiment, dissected and radioanalyzed to determine the body distribution of the radiotracers (shell, digestive gland, kidneys, gills, gonad, mantle, intestine, adductor muscle and the remaining soft tissues). For *S. costatum* experiment, the depuration period lasted for 18 d and no dissection was carried out.

Preliminary feeding experiments were also carried out in order to assess the ingestion rate (IR, $\text{g g}^{-1} \text{ d}^{-1}$) of both phytoplankton species by *C. varia* fed $5 \cdot 10^4 \text{ cells ml}^{-1}$ according the method described in Metian et al. (in press (a)).

Data analyses. Bioaccumulation of radiotracers from seawater was expressed in term of concentration factors (CF, ratio between the radioactivity in scallops $\text{-Bq g}^{-1} \text{ wet wt-}$ and time-

integrated activity in the seawater $-Bq\ g^{-1}$) over time. Bioaccumulation kinetics were fitted using a simple exponential kinetic model (eq.1) or a linear model (eq.2).

$$CF_t = CF_{ss} (1 - e^{-k_e t}) \quad (\text{eq.1})$$

$$CF_t = k_u t \quad (\text{eq.2})$$

where CF_t and CF_{ss} ($CF_{ss} = k_u/k_e$) are the concentration factors at time t (d) and at steady state, respectively; k_u and k_e are the uptake and depuration rate constants (d^{-1}), respectively (Whicker & Schultz 1982, Warnau et al. 1996).

Depuration of radiotracers (seawater and food experiments) was expressed in terms of percentage of remaining radioactivity (radioactivity at time t divided by initial radioactivity measured in scallops at the beginning of the decontamination period multiplied per 100). The percentage of remaining activity was plotted against time and depuration kinetics were described by a single- (eq.3) or a double-component exponential model (eq.4):

$$A_t = A_0 e^{-k_e t} \quad (\text{eq.3})$$

$$A_t = A_{0s} e^{-k_{es} t} + A_{0l} e^{-k_{el} t} \quad (\text{eq.4})$$

where A_t and A_0 are the remaining activities (%) at time t (d) and 0, respectively; k_e is the depuration rate constant (d^{-1}); 's' and 'l' are the subscripts for the 'short-lived' and 'long-lived' components, respectively. For each exponential component (s and l), a biological half-life can be calculated ($T_{b/2s}$ and $T_{b/2l}$) from the corresponding depuration rate constant (k_{es} and k_{el} , respectively) according to the relation $T_{b/2} = \ln 2/k_e$. Regarding feeding experiments, the 'long-lived' exponential term describes the proportion of the radiotracer ingested with food that is actually absorbed by the organism and slowly eliminated; the corresponding A_{0l} represents the assimilation efficiency (AE) of the considered radiotracer (Warnau et al. 1996). Model constants and their statistics were estimated by iterative adjustment of the model and Hessian matrix computation, respectively using the nonlinear curve-fitting routines in the Statistica[®] 6 software. Best fitting models were selected according to the highest

determination coefficient and examination of residuals. The level of significance for statistical analyses was always set at $\alpha < 0.05\%$.

Bioaccumulation model. The relative contribution of each exposure pathway was determined using the bioaccumulation model originally proposed by Thomann (1981) and Landrum et al. (1992), and further used, revised and developed by other authors (e.g., Thomann et al. 1995; Wang et al. 1996; Reinfelder et al. 1998; Metian et al. in press (a)). The introduction of the term A_{0l} in this model allowed considering only the fraction of the radiotracer taken up by the organisms from seawater or from food that was actually absorbed (Metian et al. in press (a)). In addition growth rate in the original model was considered as negligible over the experiment duration (e.g., Reinfelder et al. 1998).

The total activity concentration of the radiotracer in the organism, C_t (Bq g^{-1}) was defined as the sum of each concentration resulting from the uptake through the dissolved and food pathways:

$$C_t = C_{w,ss} + C_{f,ss} \quad (\text{eq.5})$$

where $C_{w,ss}$ is the radiotracer concentration in scallops (Bq g^{-1}) at steady state taken up from the dissolved phase (eq.6), and $C_{f,ss}$ is the radiotracer concentration in scallops (Bq g^{-1}) at steady state obtained from food (eq.7):

$$C_{w,ss} = (A_{0l,w} k_{u,w} C_w) / k_{e,w} \quad (\text{eq.6})$$

$$C_{f,ss} = (AE IR C_f) / k_{e,f} \quad (\text{eq.7})$$

where A_{0l} is the proportion (%) of radiotracer taken up from seawater that was lost according to the long-lived compartment, k_u and k_e are the uptake and depuration rate constants (d^{-1}), C_w is the activity concentration of the radiotracer in seawater used in the experiment (Bq g^{-1}), AE is the assimilation efficiency (%) of the radiotracer ingested with the food, IR is the phytoplakton ingestion rate by the scallop ($\text{g g}^{-1} \text{d}^{-1}$), C_f is the activity concentration of the

radiotracer measured in the food (Bq g^{-1}) used in the experiment, and “w” and “f” subscripts are referring to water and food exposure pathways, respectively.

The relative contribution of each exposure pathway is then assessed from the relations:

$$\% \text{ Seawater} = C_{w,ss} / (C_{f,ss} + C_{w,ss}) \quad (\text{eq.8})$$

$$\% \text{ Food} = C_{f,ss} / (C_{f,ss} + C_{w,ss}) \quad (\text{eq.9})$$

RESULTS

Seawater pathway. The bioconcentration of $^{110\text{m}}\text{Ag}$, ^{57}Co , ^{54}Mn and ^{65}Zn in whole-body *C. varia* was best described by a saturation exponential model ($r^2 \geq 0.81$), whereas ^{203}Hg bioconcentration was best fitted by a linear model ($r^2 = 0.82$) (Fig. 1, Table 2). After 7 d of exposure, estimated uptake rate constant (k_u) allow ranking the radiotracers according to the following order of bioavailability: $^{110\text{m}}\text{Ag} > ^{203}\text{Hg} > ^{54}\text{Mn} > ^{65}\text{Zn} > ^{57}\text{Co}$.

Table 3 shows the concentration factors reached at the end of the exposure period by the whole organisms ($\text{CF}_{7\text{d}}$ *in toto*) and their body compartments. Based on the measured $\text{CF}_{7\text{d}}$ *in toto*, radiotracer bioavailability can be ranked quite similarly than when based on k_u : $^{110\text{m}}\text{Ag}$ ($2,586 \pm 1,186$) $> ^{203}\text{Hg}$ (652 ± 179) $> ^{54}\text{Mn}$ (138 ± 36) $= ^{65}\text{Zn}$ (155 ± 31) $> ^{57}\text{Co}$ (92 ± 36).

Among the tissues and organs, the digestive gland, the kidneys, and the gills displayed the highest bioconcentration capacities. $^{110\text{m}}\text{Ag}$ was hyperconcentrated by all scallop tissues ($\text{CF}_{7\text{d}} > 2,000$) and more particularly by the digestive gland ($\text{CF}_{7\text{d}} = 132,346 \pm 38,091$). ^{203}Hg was highly concentrated in gills ($\text{CF}_{7\text{d}} = 13,377 \pm 3,280$) whereas essential metals (^{57}Co , ^{54}Mn and ^{65}Zn) were mainly concentrated in the kidneys ($\text{CF}_{7\text{d}} = 107 \pm 57$ for ^{57}Co , 172 ± 78 for ^{54}Mn and $8,749 \pm 5,259$ for ^{65}Zn).

Regarding the radiotracer body distribution, $^{110\text{m}}\text{Ag}$ and ^{203}Hg were mainly present in the digestive gland ($87 \pm 2\%$) and the gills ($61 \pm 1\%$), respectively, whereas ^{65}Zn was mainly

found in kidneys ($40 \pm 12\%$), and ^{57}Co was more present in the digestive gland (Fig. 2). Finally, ^{54}Mn was distributed quite homogeneously among the different tissues and organs.

After the exposure period, non-contaminating conditions were restored and depuration kinetics of radiotracers were followed for 36 d for all radiotracers, except ^{203}Hg (91 d). The whole-body depuration of $^{110\text{m}}\text{Ag}$ was best fitted by a single-component exponential model, whereas depuration kinetics of ^{57}Co , ^{203}Hg , ^{54}Mn and ^{65}Zn were best described by a double-component exponential model (Fig. 1). All radiotracers were efficiently absorbed ($A_{01} > 50\%$) and retained ($T_{b/21} \geq 10$ weeks) by *C. varia* (Table 2).

After 36 d of depuration, the dissections highlighted the storage role of the digestive gland for $^{110\text{m}}\text{Ag}$, of the gills for ^{203}Hg and of the kidneys for ^{57}Co , ^{54}Mn and ^{65}Zn (Fig. 2). Interestingly, the proportion of $^{110\text{m}}\text{Ag}$ ($86 \pm 2\%$) in the digestive gland and of ^{65}Zn ($59 \pm 20\%$) in the kidneys were similar at the end of the bioaccumulation and depuration periods, whereas the proportion of ^{57}Co , ^{203}Hg and ^{54}Mn in kidneys and those of ^{203}Hg in the digestive gland increased with the time during the depuration phase (Fig. 2).

Dietary pathway. The preliminary assessment of the ingestion rate (IR) of the two phytoplankton species indicated that *C. varia* ingested both *S. costatum* and *I. galbana* with similar ($p = 0.01$) rates, i.e. $\text{IR} = 0.087 \pm 0.028 \text{ g g}^{-1} \text{ d}^{-1}$.

In order to evaluate whether different phytoplankton species influence assimilation and retention of $^{110\text{m}}\text{Ag}$, ^{57}Co , ^{54}Mn , and ^{65}Zn by *C. varia*, depuration kinetics were followed after 2-h pulse-chase feeding experiments using one or another of two radiolabelled phytoplankton species (*S. costatum* and *I. galbana*).

Whole-body depuration kinetics of all radiotracers in *C. varia* fed radiolabelled *S. costatum* or *I. galbana* were best fitted by a double-component exponential equation (Fig. 3). Assimilation efficiency (AE) of radiotracers ingested with *S. costatum* was higher than when ingested with

I. galbana (Table 4), whereas the biological half-lives of radiotracers ingested with one or the other species did not show significant difference, except for ^{65}Zn which was more strongly retained when ingested with *S. costatum*. Comparison of the retention capacities of the scallops exposed through seawater and food indicated that the $T_{b/2l}$ of all dietary metals were shorter than that of waterborne elements.

Dietary transfer of ^{203}Hg in *C. varia* has been investigated with only *I. galbana* as food source. This element was assimilated with an efficiency of 32% and retained with a $T_{b/2l}$ of 10 ± 4 d.

At the end of the depuration period of the *I. galbana* feeding experiment, the distribution of ^{110m}Ag and ^{65}Zn (Fig. 2) was similar to the one determined after seawater exposure. When assimilated via the food, ^{110m}Ag was mainly present in the digestive gland ($87 \pm 5\%$) and ^{65}Zn in the kidneys ($64 \pm 9\%$). For the other radiotracers, the digestive gland contained higher proportion than observed in the seawater experiment. Indeed, the main part of ^{203}Hg ($67 \pm 15\%$) and ^{57}Co ($52 \pm 13\%$) was present in the digestive gland whereas ^{54}Mn was occurring in similar proportion in the digestive gland ($39 \pm 17\%$) and the kidneys ($34 \pm 17\%$).

Bioaccumulation model. The relative contribution of each exposure pathway in the global bioaccumulation (see Material and Methods) were calculated using the parameters determined in the different experiments and given in Tables 2 and 4 or in the text ($\text{IR} = 0.087 \text{ g g}^{-1} \text{ d}^{-1}$). Relative contribution of each exposure pathway for the considered metals is shown in Fig. 4. The dietary pathway appears to play a major role in metal bioaccumulation in *C. varia*.

The comparison of the runs of the model fed the parameters obtained from the two feeding experiments (viz. using *S. costatum* or *I. galbana* as food source) indicated that the influence of food quality on the dietary contribution to global bioaccumulation is metal-dependent. Indeed, when the scallops were fed ^{110m}Ag -labelled *S. costatum*, the food was the main uptake

pathway (contributing for 96% of the global ^{110m}Ag bioaccumulation), whereas when they were fed ^{110m}Ag -labelled *I. galbana*, food and seawater pathways contributed rather similarly to global bioaccumulation of ^{110m}Ag in *C. varia* (53% and 47%, respectively). A more pronounced trend has been observed for ^{57}Co : the main ^{57}Co uptake route was food (100% contribution) when scallops were fed *S. costatum*, whereas a dramatic shift to seawater as the predominant uptake pathway (contributing for 82% to global bioaccumulation) occurred when scallops were fed *I. galbana*. In contrast, ^{54}Mn and ^{65}Zn were always mainly accumulated from the food ($\geq 91\%$) with both phytoplankton species. For ^{203}Hg , the food tested (*I. galbana*) and seawater contributed equally to the global bioaccumulation of this metal in *C. varia*.

DISCUSSION

In the available literature on metal bioaccumulation in Pectinidae, the importance of dietary transfer to the global metal bioaccumulation has been suggested (e.g., Palmer & Rand 1977; Uthe & Chou 1987; Bustamante & Miramand 2005b) but experimentally demonstrated only for Cd and Ag (Metian et al. 2007, in press (a & b)). For example, according to Bustamante & Miramand (2005b) the very high metal concentrations typically recorded in the digestive gland and kidneys of *C. varia* and the relatively low levels in the gills suggest that the food route is a major pathway of metal bioaccumulation in this species. However, interpretation of organotropism data from the field as an indicator of metal uptake pathway should be done with great caution. Indeed, the results presented here showed for example that Ag was efficiently bioaccumulated and mainly stored in the digestive gland whether the scallops were exposed via their food or via seawater. Moreover food was found to not always be the very

dominant contributing pathway (e.g. similar seawater and dietary contributions when scallops were fed *I. galbana*).

To the best of our knowledge, the present study is the first to delineate the contributions of the uptake pathways of the 5 considered metals in *C. varia* thanks to the use of radiotracer techniques and modelling. Results of the bioaccumulation model indicate that the dietary pathway plays an important role in the global metal bioaccumulation in *C. varia*. When scallops were fed *S. costatum*, the food pathway was always the main route of bioaccumulation for all studied metals (food contributing for $\geq 96\%$). When *C. varia* were fed *I. galbana*, either the dietary pathway was the main route of bioaccumulation (for Mn and Zn; food contribution $\geq 91\%$) or both seawater and food contributed similarly (for Ag and Hg, the latter element having not been tested with *S. costatum*). In a previous study on the blue mussel *Mytilus edulis*, Wang et al. (1996) showed that the dietary pathway was the main route of bioaccumulation for Se but not for Ag, Am, Cd, Co and Zn, for which dissolved and dietary pathways contributed equally. The trend observed in *C. varia* for Zn (dietary pathway contributing for $\geq 99\%$) was opposite to that of the one reported in *M. edulis*. Interestingly, the data on the bioaccumulation and depuration kinetics of Mn and Zn in *C. varia* indicate that these elements are not efficiently bioconcentrated from seawater ($k_u \sim 30 \text{ d}^{-1}$) compared to Ag or Hg ($k_u = 466$ and 100 d^{-1} , respectively). Moreover, even though dietary Mn displayed limited assimilation and retention in *C. varia*, the food came out to be the main uptake pathway for this metal.

Several studies have investigated mechanisms influencing dietary metal bioavailability in filter-feeders (e.g., Borchardt 1983; Wang & Fisher 1996; Ng et al. 2005). Factors such as the cytosolic proportion of the metal in phytoplankton cells (Reinfelder et al. 1997) or the nature of the metal/phytoplankton binding (Ng & Wang 2005) have been shown to influence the bioavailability of a given metal ingested with food. Accordingly, our results show that metal

bioaccumulation characteristics of phytoplankton influences the bioavailability of the metals ingested by *C. varia*. In this study, all studied metals displayed higher AE in *C. varia* when ingested with *S. costatum* than when ingested with *I. galbana*. In particular, for Ag and Co, the phytoplankton species determined the relative contribution of the dietary pathway to global metal bioaccumulation. Indeed, according to whether *S. costatum* or *I. galbana* was ingested, the main route of Co bioaccumulation in the scallops switched between food and seawater, respectively. Since the retention capacity of Co did not differ according to the phytoplankton species used, the observed difference would most probably due to the difference in AE ($AE_{I. galbana} = 17\%$ vs $AE_{S. costatum} = 31\%$) and/or to the difference in the K_{df} of the two phytoplankton species ($K_{df I. galbana} = 10^3$ vs. $K_{df S. costatum} = 8 \cdot 10^5$). An overview of our results suggests that the affinity of the metal for the phytoplankton species (viz. the value of K_{df} measured during the food experiments; see Table 1) is a key factor in determining the relative contribution of the dietary pathway. Indeed, the K_{df} of Mn and Zn for both phytoplankton species was always higher than 10^5 , whereas K_{df} of Ag and Co showed lower value for *I. galbana* ($K_{df} = 4 \cdot 10^4$ for Ag and 10^3 for Co) than for *S. costatum* (K_{df} 1 and 3 orders of magnitude higher, respectively).

At the end of the seawater exposure and at the end of the depuration phase following seawater and food exposures, body targeting of the different metals has been investigated in *C. varia*. Results found are consistent with previous studies carried out on different scallop species (e.g., Bryan 1973; Mauri et al. 1990; Bustamante & Miramand 2005a). The storage capacity of an organ is generally thought to be related to its role in the organism metabolism and to detoxification processes occurring in this organ. For example, metal storage in the tissues of Pectinidae is typically attributed to metal binding to cytosolic proteins in the digestive gland and gills, to co-precipitation with sulfur in the digestive connective tissue, and to their precipitation on mineral granules in kidneys (e.g., George et al. 1980; Stone et al. 1986;

Fowler & Gould 1988; Martoja et al. 1989; Mauri et al. 1990). The body distribution of the tested metals was generally relatively similar for seawater and food exposure, except for Co and especially for Hg. Indeed, Hg was mainly distributed in gills when scallops were exposed via seawater, whereas it was mainly accumulated in the digestive gland after dietary exposure. Comparison with other pectinids is difficult due to the scarcity of published data related to Hg. To the best of our knowledge, only Bargagli et al. (1998) reported a higher Hg concentration in the gills compared to the digestive gland of the Antarctic scallop *Adamussium colbecki* (0.86 ± 0.22 vs. $0.35 \pm 0.08 \mu\text{g g}^{-1}$ dry weight). Nevertheless, given that (1) gills and digestive gland of *C. varia* bioaccumulate most efficiently dissolved and dietary Hg, respectively, and (2) both seawater and food pathways contribute quite similarly to the global bioaccumulation of this metal, our results suggest that the gills and the digestive gland of *C. varia* could be used in the field as biomonitor organs for Hg as they would provide information on both the environmental contamination level and on the source of the contamination.

It is known that methylation of Hg and its accumulation at the base of the food chain can determine its transfer to higher organisms (see e.g., Mason et al. 1996). Whereas relatively low AEs have been previously reported for inorganic Hg in several marine organisms (e.g., Fowler et al. 1978; Riisgaard and Hansen 1990; Metian et al. in press (b)) methyl-Hg is more readily bioaccumulated in bivalves than inorganic Hg (e.g., Fowler et al., 1978; Mason et al., 1996). In addition methyl-Hg is also retained more strongly in bivalve tissues than the inorganic form ($T_{b1/2}$ is 2.7 to 4.7 times longer). However, this paper did not focus on methyl-Hg and additional research should be carried out to further assess possible difference in availability and retention of inorganic vs. methyl-Hg in scallops.

The behaviour and fate of Ag in *C. varia* tissues has been well studied (e.g. Martoja et al. 1989; Metayer et al. 1990; Bustamante & Miramand 2005a). The exposure to elevated dietary

and/or dissolved concentrations of Ag decreased the ability *C. varia* to produce byssus, preventing adequate fixation on the substratum (Metayer et al. 1990). In these conditions, the foot was shown to contain both high concentrations and proportions of Ag. In the present study which considered realistic environmental exposure concentrations, Ag was mainly bioconcentrated and distributed in the digestive gland of *C. varia* whatever the exposure mode (food and seawater), which is consistent with reported results from the field (Bustamante & Miramand 2005a). Both experimental and field works support the central role of scallop digestive gland in detoxification and storage of Ag, likely due to its precipitation with sulphur within the cells (Ballan-Dufrançais et al. 1985; Martoja et al. 1989).

CONCLUSION

The present study determined the relative contribution of the two main exposure pathways of the variegated scallop *C. varia* to metals and confirmed the suspected importance of the dietary pathway in metal bioaccumulation in scallops. The dietary contribution was shown to depend upon the considered metal and/or the phytoplankton species used as food. The results presented here will be a useful tool for interpreting field data on metal concentrations in *C. varia* organs and tissues. In addition, as the dietary pathway was generally the most important one, metal concentrations in scallop food need to be considered with attention during field studies. This is more particularly true if metal toxicity is an issue to be assessed, since most toxicity tests currently available/used are focussing on dissolved metal toxicity assessment.

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Table 1. *Isochrysis galbana* and *Skeletonema costatum*. Radiotracer activity (kBq l⁻¹) added in the phytoplankton culture media and distribution coefficient (K_{df}) between phytoplankton and seawater determined at the end of the phytoplankton exposure period.

species	^{110m} Ag		⁵⁷ Co		²⁰³ Hg		⁵⁴ Mn		⁶⁵ Zn	
	Activity (kBq l ⁻¹)	K _{df}	Activity (kBq l ⁻¹)	K _{df}	Activity (kBq l ⁻¹)	K _{df}	Activity (kBq l ⁻¹)	K _{df}	Activity (kBq l ⁻¹)	K _{df}
<i>Isochrysis galbana</i>	4	4.43 10 ⁴	3	1.35 10 ³	4.5	1.74 10 ⁴	3.5	1.61 10 ⁵	3.5	1.12 10 ⁵
<i>Skeletonema costatum</i>	5	6.86 10 ⁵	5	8.47 10 ⁵	-	-	5	4.42 10 ⁶	5	6.62 10 ⁶

Table 2. *Chlamys varia*. Parameters of the bioaccumulation and depuration kinetics of selected radiotracers following exposure for 7d to the radiotracers in seawater (n = 9) and then depuration for 36d (91d for Hg) in non contaminating conditions (n = 9).

CF_{ss}: concentration factor at steady state; k_u, k_e: uptake and depuration rate constant (d⁻¹), respectively; A_{0l}: activity (%) lost according to the long-lived exponential component; T_{b½}: biological half-life (d); ASE: asymptotic standard error; R²: determination coefficient of the kinetics.

Metal	Uptake phase			Depuration phase			
	CF _{ss} ± ASE	k _u ± ASE	R ²	A _{0l} ± ASE	k _e ± ASE	T _{b½} ± ASE	R ²
^{110m} Ag	3,169 ± 978 ^b	466 ± 59 ^d	0.81	97.8 ± 1.7 ^d	0.003 ± 0.001 ^d	211 ± 46 ^d	0.14
⁵⁷ Co	161 ± 120 ^b	16.7 ± 2.1 ^d	0.82	86.0 ± 4.4 ^d	0.009 ± 0.003 ^c	73 ± 20 ^c	0.56
²⁰³ Hg	-	99.3 ± 3.9 ^d	0.82	50.4 ± 7.9 ^d	0.007 ± 0.003 ^a	95 ± 34 ^a	0.93
⁵⁴ Mn	223 ± 70	27.0 ± 2.4 ^d	0.91	85.4 ± 2.2 ^d	0.010 ± 0.002 ^d	69 ± 10 ^d	0.66
⁶⁵ Zn	253 ± 69 ^c	30.9 ± 2.3 ^d	0.93	89.1 ± 1.9 ^d	0.006 ± 0.001 ^d	115 ± 23 ^d	0.45

Probability of the model adjustment: ^a p < 0.05, ^b p < 0.01, ^c p < 0.001, ^d p < 0.0001.

Table 3. *Chlamys varia*. Concentration factors (mean CF \pm SD, n = 5) in the body compartments of the scallop.

Tissues	^{110m}Ag	^{57}Co	^{203}Hg	^{54}Mn	^{65}Zn
Digestive gland	132,346 \pm 38,091	49 \pm 20	9,545 \pm 1,412	44 \pm 24	355 \pm 127
Gills	5,200 \pm 1,446	18 \pm 6	13,377 \pm 3,280	23 \pm 6	282 \pm 88
Kidneys	15,437 \pm 10,849	107 \pm 57	3,923 \pm 3,074	172 \pm 78	8,749 \pm 5,259
Intestine	2,501 \pm 1,796	8 \pm 8	1,381 \pm 357	19 \pm 16	189 \pm 166
Gonad	9,972 \pm 8,601	7 \pm 5	1,822 \pm 1,293	15 \pm 13	266 \pm 341
Foot	9,488 \pm 5,549	4 \pm 3	875 \pm 148	30 \pm 16	239 \pm 63
Mantle	2,092 \pm 1,852	8 \pm 4	1,608 \pm 229	13 \pm 6	145 \pm 24
Adductor	2,008 \pm 793	4 \pm 1	300 \pm 114	8 \pm 2	243 \pm 96
Remaining	8,471 \pm 6,327	18 \pm 12	2,576 \pm 914	34 \pm 19	222 \pm 131
Whole body	2,586 \pm 1,186	92 \pm 36	652 \pm 179	138 \pm 36	155 \pm 31

Table 4. *Chlamys varia*. Parameters of the depuration kinetics of selected radiotracers after a 2-h feeding on radiolabelled *Isochrysis galbana* (n=6) and *Skeletonema costatum* (n=6) followed by 30d and 18d in non contaminating conditions, respectively.

A₀₁: activity (%) lost according to the long-lived exponential component (= assimilation efficiency, AE); k_{el}: depuration rate constant (d⁻¹); T_{b/2l}: biological half-life (d); ASE: asymptotic standard error; R²: determination coefficient of the kinetics.

Experiment	Metal	A ₀₁ ± ASE	k _{el} ± ASE	T _{b/2l} ± ASE	R ²
<i>I. galbana</i>	^{110m} Ag	44.4 ± 3.9 ^d	0.011 ± 0.008 [*]	64 ± 49 [*]	0.49
	⁵⁷ Co	17.7 ± 3.6 ^d	0.061 ± 0.029 ^a	11 ± 5 ^a	0.93
	²⁰³ Hg	31.6 ± 4.1 ^d	0.067 ± 0.024 ^b	10 ± 4 ^b	0.77
	⁵⁴ Mn	8.6 ± 1.3 ^d	0.058 ± 0.023 ^a	12 ± 5 ^a	0.99
	⁶⁵ Zn	70.0 ± 4.1 ^d	0.008 ± 0.005 [*]	83 ± 45 [*]	0.39
<i>S. costatum</i>	^{110m} Ag	79.0 ± 5.5 ^d	0.014 ± 0.007 ^b	48 ± 25 ^b	0.42
	⁵⁷ Co	31.2 ± 5.3 ^d	0.071 ± 0.022 ^c	10 ± 3 ^c	0.92
	⁵⁴ Mn	23.7 ± 2.5 ^d	0.054 ± 0.013 ^c	13 ± 3 ^c	0.98
	⁶⁵ Zn	87.8 ± 5.1 ^d	0.022 ± 0.007 ^b	31 ± 10 ^b	0.41

Probability of the model adjustment: ^{*} p > 0.05; ^a p < 0.05, ^b p < 0.01, ^c p < 0.001, ^d p < 0.0001.

Caption to figures.

Figure 1. *Chlamys varia*. Bioaccumulation kinetics (mean concentration factor \pm SD, n = 9) and depuration kinetics (mean % remaining activity \pm SD, n = 9) of selected radiotracers.

Figure 2. *Chlamys varia*. Body distribution (mean % \pm SD) of selected radiotracers after a 7-d exposure to the radiotracers via seawater (n = 5), at the end of the 36-d (91-d for Hg) depuration period following the seawater exposure (n = 4) and after a 2 h feeding on radiolabelled *Isochrysis galbana* followed by 30d in non contaminating conditions (n = 6).

Figure 3. *Chlamys varia*. Depuration kinetics (mean % remaining activity \pm SD, n = 6) of selected radiotracers after a 2-h feeding on (1) radiolabelled *Isochrysis galbana* (black dots) followed by 30-d in non contaminating conditions and (2) radiolabelled *Skeletonema costatum* (white squares) followed by 18-d in non contaminating conditions.

Figure 4. *Chlamys varia*. Contribution (%) of dissolved (grey bars) and dietary (black bars) exposure pathways to global bioaccumulation of the selected elements. Dietary exposures considered two phytoplankton species (*Skeletonema costatum* -SKE- and *Isochrysis galbana* -ISO-).

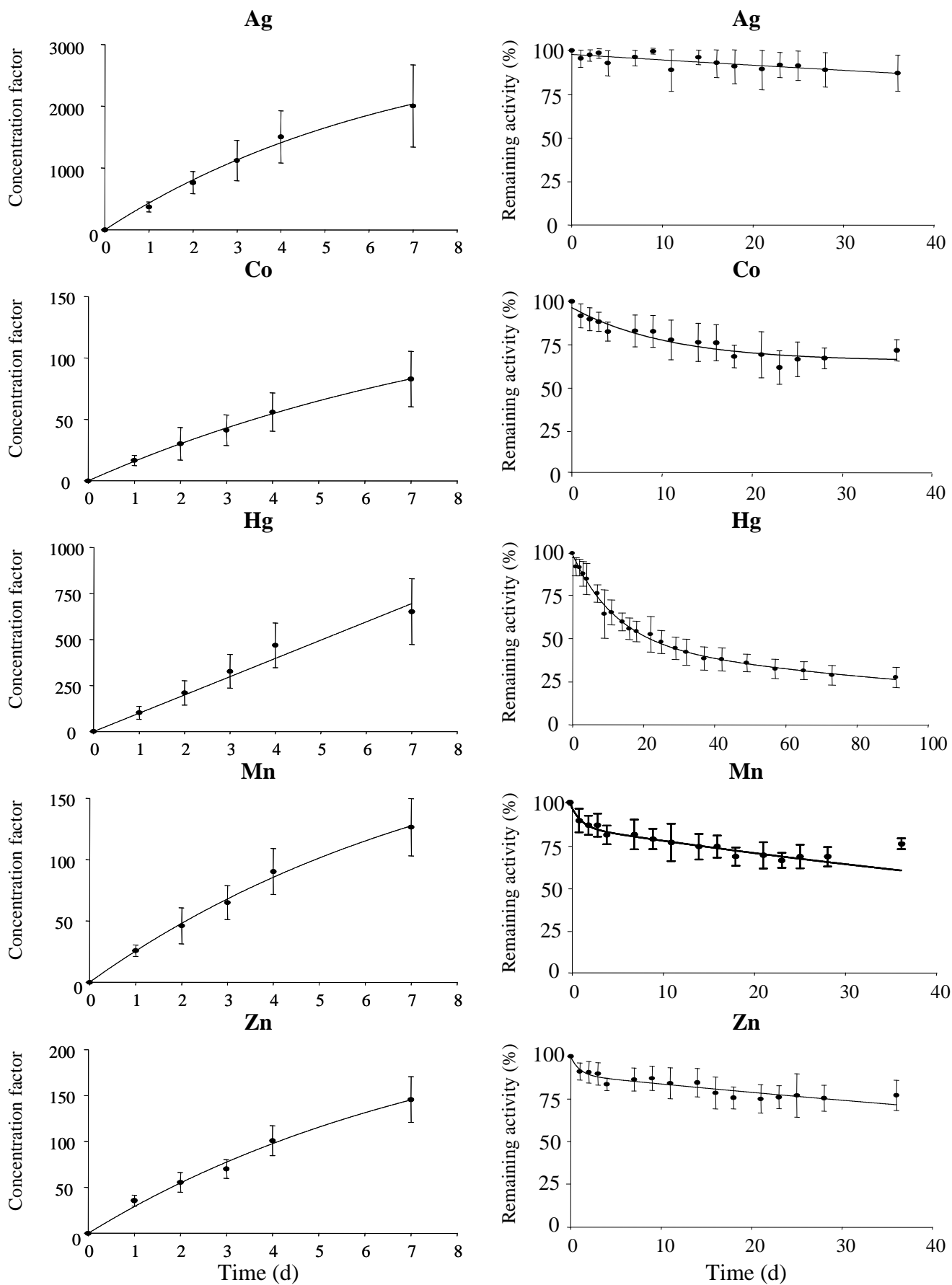


Figure 1.

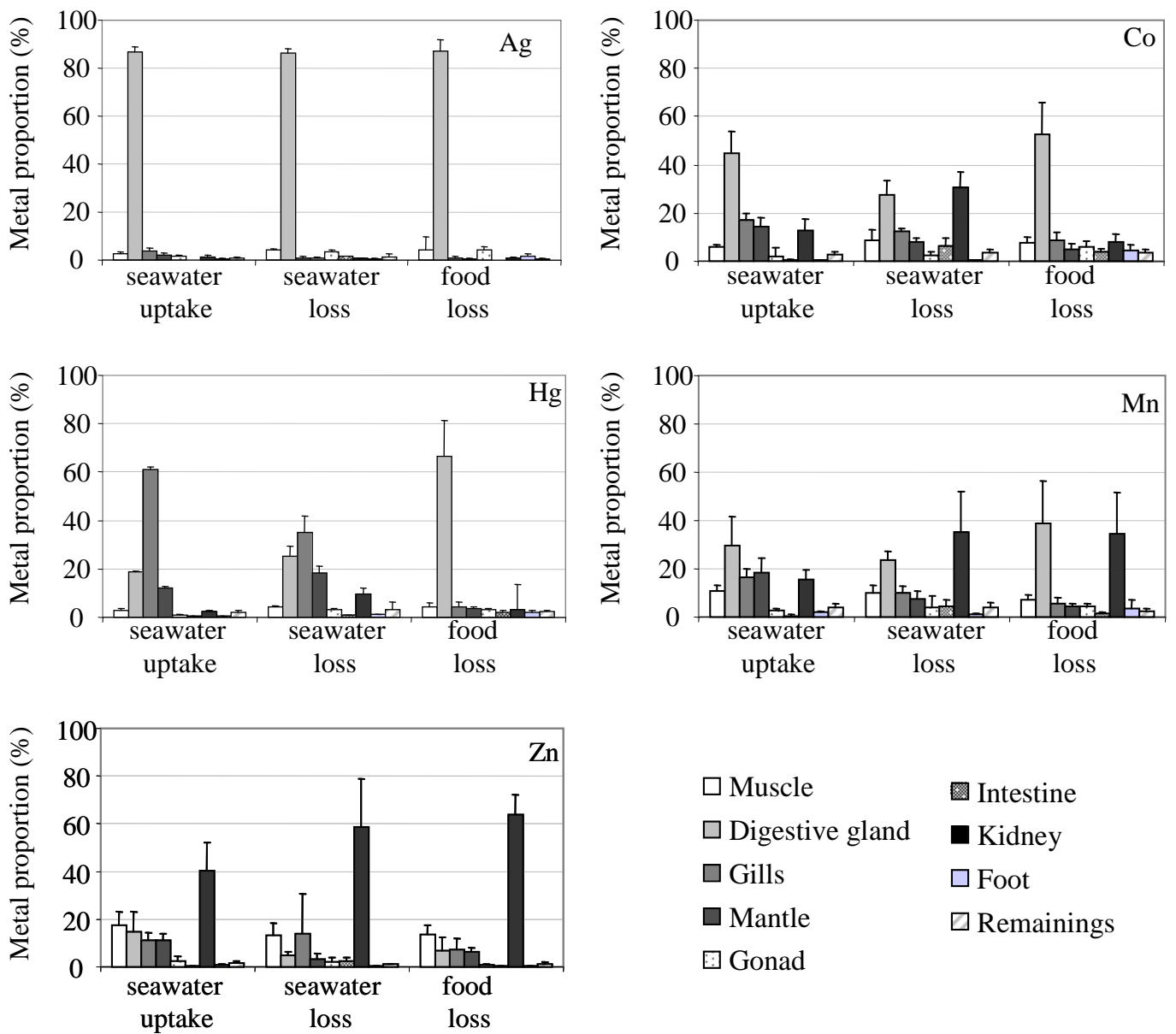


Figure 2.

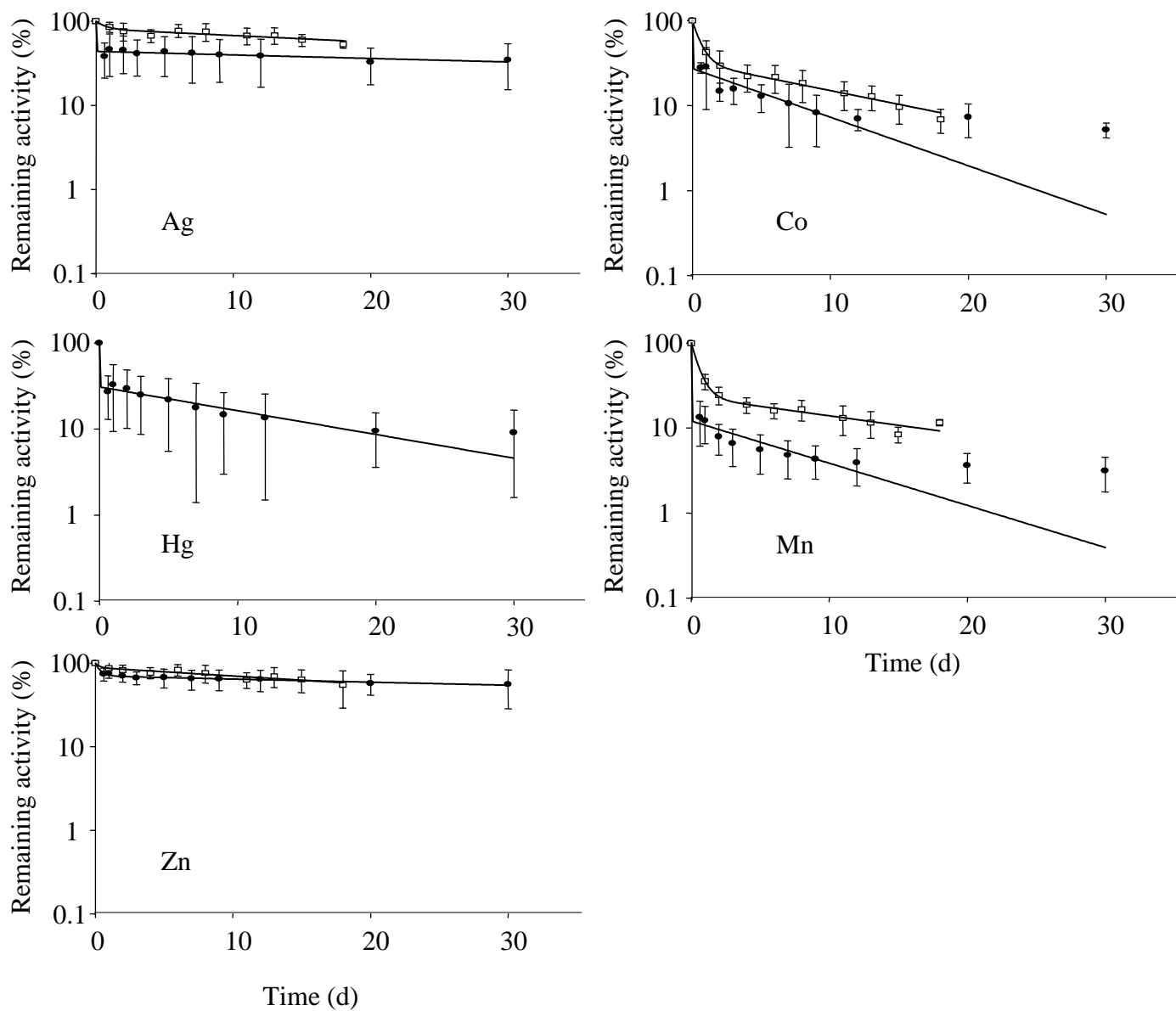


Figure 3.

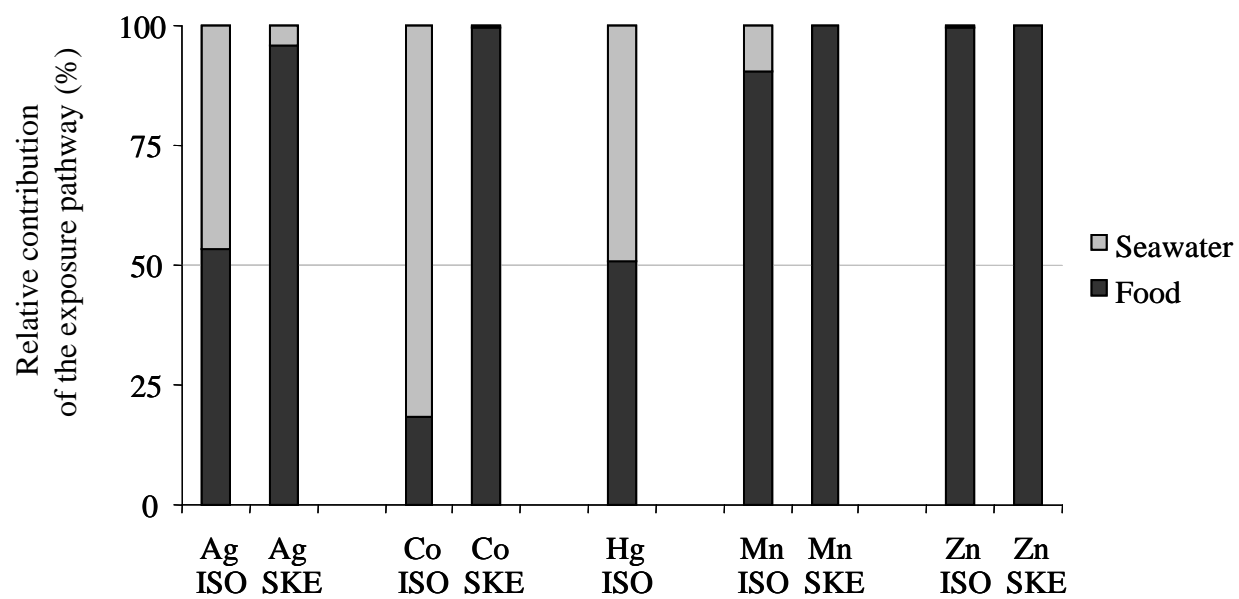


Figure 4.