Influence of food on the assimilation of selected metals in tropical bivalves from the New Caledonia lagoon: Qualitative and quantitative aspects

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Abstract

The present study aimed at examining the influence of food quality and quantity on the assimilation efficiency (AE) of metals in two abundant bivalves in the New Caledonia lagoon, the oyster Isognomon isognomon and the clam Gafrarium tumidum. Bivalves were exposed via their food to the radiotracers of three metals of concern in New Caledonia (54Mn, 57Co and 65Zn) under different feeding conditions (phytoplankton species, cell density, and cell-associated metal concentration). When bivalves were fed Heterocapsa triquetra, Emiliania huxleyi and Isochrysis galbana, AE of Mn, Co and Zn was strongly influenced by the phytoplankton species and by the metal considered. In contrast, when fed one given phytoplankton species previously exposed to different concentrations of Co, phytoplankton-associated Co load had no influence on the AE and on the retention time of the metal in both bivalves. Metals ingested with I. galbana displayed generally the highest AE in both bivalve species, except for Mn in clams for which the highest AE was observed for H. triquetra. Influence of food quantity was investigated by exposing bivalves to different cell densities of I. galbana (5 × 10^3, 10^4 or 5 × 10^4 cell ml^-1). As for food quality, food quantity was found to influence AE of Mn, Co and Zn, the highest AE being observed when bivalves were fed the lowest cell density. Overall, results indicate that the two bivalve species are able to adjust their feeding strategies according to the food conditions prevailing in their environment.

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1. Introduction

Changes in coastal ecosystem functioning due to anthropogenic metal inputs is a worldwide issue of concern especially as metals are not biodegradable and enter biogeochemical cycles (Tessier and Turner, 1995). In the coral reef lagoon of New Caledonia, metal contamination is a critical problem in relation with its extreme biodiversity (Labrosse et al., 2000). Indeed, the lagoon is subject to an increasing environmental pressure imposed by urban development and intensive mining activities. In addition, the use of hydrometallurgic process employing heated and pressured sulphuric acid (lixiviation) has been recently developed in New Caledonia and is expected to be implemented at industrial scale early 2010 (Goro-Nickel, 2001, 2003). Such a process will provide new potential to exploit laterite soils that display lower nickel (Ni) contents than garnierite ores currently used in pyrometallurgical plants, such as at the Société Le Nickel, and will allow recovering the cobalt (Co) as a by-product (Mihaylov et al., 2000; Dalvi et al., 2004). However, the Ni and Co extraction based on lixiviation is an unselective process that may result in additional discharges of by-product metals such as chromium (Cr), iron (Fe), manganese (Mn) or zinc (Zn) (Goro-Nickel, 2001; Baroudi et al., 2003).

Although long lasting contamination exists in New Caledonia (Lagonier, 1991; Ambatian et al., 1997) with high levels of metals reported in coastal marine sediments (e.g., Fernandez et al., 2006), few data on contamination levels in marine organisms and possible local marine ecosystem impairments are available so far in the open literature (e.g., Monniot et al., 1994; Dalto et al., 2006; Hédouin et al., 2008a,b; Metian and Warnau, 2008; Chouvelon et al., 2009). Therefore, programmes for monitoring possible impact of the land-based mining activities in the New Caledonia lagoon are needed. Such programmes should largely rely on the use of biomonitor species, as already developed and implemented in temperate areas (e.g., US and EU Mussel Watches; see e.g., Goldberg et al., 1983; Warnau and Acuña, 2007; Thébault et al., 2008). Indeed,
the main advantage of the biomonitoring approach compared to direct measurement in water or sediment is to provide a direct and time-integrated assessment of the metal fraction that is actually available to the organisms (bioavailable fraction) (e.g., Phillips, 1991; Coteur et al., 2003; Danis et al., 2004; Metian et al., 2008b).

In this context, both experimental and field studies have recently identified the oyster *Isognomon isognomon* and the clam *Gafarium tumidum* as promising candidates biomonitoring metal contamination in New Caledonia lagoon waters (Metian et al., 2005; Hédouin et al., 2006, 2007, 2008a; Chouvelon et al., 2009).

It is now well established that food is often a dominant pathway for metal bioaccumulation in marine invertebrates and that food composition and/or quantity can strongly influence metal assimilation efficiency (e.g., Borchardt, 1983; Rissig et al., 1987; Wang and Fisher, 1999a). Furthermore, feeding processes such as filtration rates are flexible in marine filter-feeding organisms and may be adapted according to the changes in environmental conditions such as food quantity and/or composition (e.g., Widdows and Donkin, 1992; Navarro and Iglesias, 1993).

For example, Cd assimilation in the mussel *Mytilus edulis* is inversely related to food quantity (Borchardt, 1983). In the scallop *Pecten maximus*, food is the main bioaccumulation pathway for Ag (~98%) when diet is composed of Bacillariophyceae phytoplankton whereas dietary contribution drops below 40% when the scallop is fed Prymnesiophyceae phytoplankton (Metian et al., 2008a). Furthermore, heterorhabdic bivalves (those which gills are composed of two different filament types) are also able to select the particles that they are ingesting (Ward et al., 1998), which results in a preferential ingestion of nutritionally-rich particles that may also affect metal influx from food (e.g., Bayne, 1993; Wang and Fisher, 1997).

The objective of this study was thus to investigate the possible influence of food quality (i.e., phytoplankton species) and quantity on the assimilation efficiency of three metals of concern in New Caledonia lagoon waters (Co, Mn and Zn) in the oyster *I. isognomon* and the clam *G. tumidum*. The variations in the feeding conditions that were considered are: (1) the phytoplankton species used as food, (2) the phytoplankton density and (3) the metal concentration associated with phytoplankton. Radiotracer techniques were used to enhance the detection sensitivity of metals and to allow for measuring metal flux at environmentally realistic contaminant concentrations (Warnau and Bustamante, 2007).

### 2. Materials and methods

#### 2.1. Collection and acclimation

The organisms (*n* = 100 per species) were collected by SCUBA diving in Maa Bay (oysters *I. isognomon*) or by hand-picking in Dumbeya Bay (clams *G. tumidum*) in October 2003. Both locations are located 15–20 km north of Nouméa City, New Caledonia. Body size is known to affect bioaccumulation of metals in marine organisms (e.g., Boyden, 1974; Warnau et al., 1995); hence, according to previous preliminary studies (Metian, 2003; Hédouin et al., 2006, 2008a), only individuals with a shell longer than 70 mm (*I. isognomon*) or a shell wider than 35 mm (*G. tumidum*) were used in the experiments. After collection, clams and oysters clams were shipped to IAEA-MEL premises in Monaco, where they were acclimated for 2 months to laboratory conditions (open circuit aquaria; water renewal: 30% h⁻¹; salinity: 36 p.s.u.; temperature: *T* = 25 ± 0.5 °C; pH 8.0 ± 0.1; light/dark cycle: 12 h/12 h) simulating the conditions prevailing in the New Caledonia lagoon. During acclimation, bivalves were fed phytoplankton using the Prymnesiophyceae *Isochrysis galbana* (10⁴ cells ml⁻¹). Recorded mortality was lower than 5% over the acclimation period.

#### 2.2. Radiotracers and counting

Investigated elements (Co, Mn and Zn) were introduced into the experimental microcosms as radiotracers of high specific activity, purchased from Amersham, UK (⁶⁵Co in 0.1 M HCl, *T*₅₀ = 271.8 d) and Isotope Product Lab., USA (⁶⁵Mn in 0.1 M HCl, *T*₅₀ = 312.2 d; ⁶⁵Zn in 0.5 M HCl; *T*₅₀ = 243.9 d). Radioactivity was measured using a high-resolution γ-spectrometer system composed of three Germanium N- or P-type detectors (EGNC 33-195-R, Eurusys®) connected to a multi-channel analyzer 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 of appropriate geometry. Measurements were corrected for counting efficiency, background and physical radioactive decay. Counting times were adapted to obtain counting rates with propagated errors less than 5% (Rodriguez y Baena et al., 2006a).

#### 2.3. Experimental procedures

##### 2.3.1. Testing the influence of Co concentration in food

*I. galbana* cells from an axenic stock culture were re-suspended into four erlenmeyer flasks (light/dark cycle: 12 h/12 h at 25 °C). Each flask contained 500 ml sterile-filtered seawater enriched with f/2 nutrients without EDTA and Si (Guillard, 1975). Flasks were spiked with four increasing Co concentrations (0, 5, 50, and 500 ng l⁻¹) and phytoplankton was allowed to grow under these conditions for 6 d. Added Co concentrations were realized using increasing amount of Co(NO₃)₂ (synthesis quality, Merck) and a fixed activity of the corresponding radiotracer ⁵⁷Co (2.5 kBq l⁻¹, corresponding to 0.13 ng Co l⁻¹). The range of concentrations selected covers those encountered in the New Caledonia lagoon waters (Fernandez et al., 2002; Coro-Nickel, 2004). After 6 d of incubation, cell density increased from 10⁴ to 1.5 × 10⁶ cell ml⁻¹. The cells were gently filtered (1 μm-mesh size, Nuclepore® Poly carbonate filters) and re-suspended in clean seawater. The radioactivity of the radiolabelled *I. galbana* in each flask was γ-counted before and after the filtration. The radioactivity of algal cells used in feeding experiments was not significantly different among the different flasks, with an average calculated activity of 0.49 ± 0.14 μBq cell⁻¹.

For each added Co concentration, four groups of nine oysters (shell length from 71 to 94 mm) and four groups of nine clams (shell width from 35 to 40 mm) were placed in four aquaria containing 16 l of 0.45-μm filtered natural seawater (close circuit aquaria constantly aerated; other parameters as previously described). Oysters were acclimated for one week to these conditions and seawater was renewed daily. Bivalves from each aquarium were then allowed to feed for 2 h on one out of the four batches of previously radiolabelled *I. galbana* (10⁴ cell ml⁻¹) (pulse-chase feeding method; see e.g., Warnau et al., 1996b).

Empty shells were placed as control in each aquarium to check for any direct uptake of radiotracers from seawater due to possible recycling from phytoplankton cells during the 2-h feeding period (Metian et al., 2007). These control shells were radioanalysed at regular intervals of time.

At the end of the feeding period, all organisms were γ-counted and open circuit conditions were restored (water renewal rate: 30% h⁻¹; salinity: 36 p.s.u.; *T* = 25 ± 0.5 °C; pH 8.0 ± 0.1; light/dark cycle: 12 h/12 h). From that time on, all individuals were γ-counted at different time intervals over a 25-d period in order to determine the whole-body depuration kinetics of the radiotracers ingested with food. Throughout the depuration period, bivalves were fed daily for 1 h non-radiolabelled phytoplankton (*I. galbana*, 10⁴ cell ml⁻¹).
2.3.2. Testing the influence of phytoplankton species

Two batches of nine oysters (shell length from 71 to 92 mm) and three groups of nine clams (shell width from 35 to 44 mm) were placed in three aquaria containing 16 l of 0.45-μm filtered natural seawater (close circuit aquaria constantly aerated; other parameters as previously described). Clams and oysters were acclimated to these conditions for 1 week (daily seawater renewal) and then fed either radiolabelled Emiliania huxleyi or Heterocapsa triqueta (10^4 cell ml^-1) for 2 h (pulse-chase feeding) in order to assess the possible influence of the phytoplankton species on metal assimilation efficiency and retention capacity in the bivalves. Both phytoplankton species occur naturally in several bays of the New Caledonia lagoon where the clams and oysters are living (Jacquet et al., 2006).

For radiolabelling phytoplankton species, experimental approaches conducted on L. galbana were applied to the Prymnesio- phyceae E. huxleyi and to the Dinophyceae H. triqueta. Cells from axenic stock cultures were re-suspended in two different erlenmeyer flasks (10^4 cell ml^-1), containing 4.5 l sterile-filtered seawater enriched with f/2 for E. huxleyi and enriched with f/2 nutrients without EDTA and Si for H. triqueta (Guillard, 1975). The two cultures were spiked with 5 kBq l^-1 of 54Mn, 57Co and 65Zn, corresponding to 3.6 ng Mn l^-1, 25 ng Co l^-1 and 60 ng Zn l^-1. The cultures were then incubated for 6 d at 25°C (light/dark cycle: 12 h/12 h). After incubation, the cell densities were 7 × 10^5 cell ml^-1 for E. huxleyi and 1.6 × 10^5 cell ml^-1 for H. triqueta. The cells were then gently filtered, re-suspended in clean seawater and γ-counted as described above (Section 2.3.1). The radioactivity of algal cells used in the feeding experiments was 0.26 ± 0.18 Bq cell^-1 for E. huxleyi and 0.96 ± 0.11 Bq cell^-1 for H. triqueta. For 54Mn, 2.1 ± 0.8 and 20.8 ± 12.1 Bq cell^-1 for 57Co and 3.2 ± 1.3 and 3.3 ± 0.1 Bq cell^-1 for 65Zn, respectively.

Empty bivalve shells were used as controls for possible metal recycling and whole-body depuration kinetics of radiotracer ingested with the food were determined in both bivalve species as described in Section 2.3.1.

2.3.3. Testing the influence of cellular density

Three groups of nine oysters (shell length from 71 to 92 mm) and three groups of nine clams (shell width from 36 to 45 mm) were placed in three aquaria containing 16 l of 0.45-μm filtered natural seawater (close circuit aquaria constantly aerated; other parameters as previously described), and acclimated for 1 week (daily seawater renewal) during which time their food was prepared.

To do this, cells of L. galbana from an axenic stock culture were re-suspended in an erlenmeyer flask containing 4.5 l sterile-filtered seawater enriched with f/2 nutrients without EDTA and Si. The culture was then spiked with 5 kBq l^-1 of 54Mn, 57Co and 65Zn and incubated for 6 d at 25°C (light/dark cycle: 12 h/12 h). After incubation, the cell density had increased from 10^3 to 1.4 × 10^4 cell ml^-1. Three sub-samples of 58, 115 and 580 ml of the culture were then gently filtered and re-suspended in clean seawater. These three batches were prepared to obtain final cell density of 5 × 10^3, 10^4 and 5 × 10^5 cell ml^-1 in the 16-l exposure aquaria. The radioactivity of the radiolabelled L. galbana was measured before and after the cellular filtration. The radioactivity of algal cells ranged from 1.11 to 1.80 μBq cell^-1 for 54Mn, 0.83 to 1.37 μBq cell^-1 for 57Co, 2.69 to 4.38 μBq cell^-1 for 65Zn.

Each group of clams and oysters was then fed for 2 h one of the radiolabelled L. galbana batches (5 × 10^3, 10^4 or 5 × 10^5 cell ml^-1). Whole-body depuration kinetics of the radiotracers ingested with the food were then followed as described in Section 2.3.1 and controls (empty shells) were placed in the aquaria for assessing possible radiotracer recycling.

2.4. Data analysis

Depuration of the radiotracers was expressed as the percentage of remaining radioactivity (radioactivity at time t divided by initial radioactivity measured in the organisms just after the feeding period × 100) (Warnau et al., 1996b; Rodriguez y Baena et al., 2006b).

Depuration kinetics for all experiments were fitted using kinetic models and statistical methods as described by Warnau et al. (1996a,b) and Lacoue-Labarthe et al. (2008). Depuration kinetics were always best fitted by a double-component exponential equation (decision based on F test and ANOVA tables for two fitted model objects):

$$ A_t = A_0 e^{-k_s t} + A_0 e^{-k_l t} $$

where $k_s$ is the depuration rate constant (d^-1), $A_s$ and $A_l$ are the remaining activities (%) at time t (d) and 0, respectively, and 's' and 'l' are the subscripts for the short-lived and long-lived components. The short-lived component represents the loss of the radiotracer fraction that remains associated with the faeces and is rapidly eliminated with them, whereas the long-lived component describes the loss of the radiotracer fraction that is actually absorbed by the organism and slowly eliminated (Whicker and Schultz, 1982; Warnau et al., 1996b). The long-lived component allows assessing the assimilation efficiency (AE) of the radiotracer ingested with food (AE = $A_l$). Also, for each exponential component (s and l), a biological half-life can be calculated ($T_{1/2s}$ and $T_{1/2l}$) from the corresponding depuration rate constant ($k_s$ and $k_l$) according to the relation $T_{1/2} = \ln 2/k$.

Constants of the models and their statistics were estimated by iterative adjustments of the model and a Bayesian matrix computation using the nonlinear curve-fitting routines in the Statistica® 5.2.1 software. Differences among the estimated kinetic parameters for the different feeding conditions were tested using comparison tests of the means and possible trends linking metal concentrations to cell densities were assessed using simple linear regression techniques (Zar, 1996). The level of significance for statistical analyses was always set at $\alpha = 0.05$.

3. Results

Depuration kinetics of the radiotracers were followed in the organisms which ingested enough food to display sufficient radioactivity to be accurately counted. Most oysters met this requirement; however some clams displaying very low activities were discarded. No activity was detected on control shells, indicating that no detectable recycling of phytoplankton-associated tracers occurred in the experimental microcosms.

3.1. Effect of Co concentration in phytoplankton

Fitting of the whole-body depuration kinetics of 57Co in oysters fed Co-loaded L. galbana by a double-exponential model was quite satisfactory ($R^2$: 0.86–0.90) for all the food-associated Co concentrations tested (Table 1, Fig. 1). The major fraction (80–85%) of the total radioactivity in oysters was rapidly lost ($T_{1/2s} < 1$ d) whereas the long-lived component accounted for only 15–20% of the 57Co ingested with food that was eliminated with a biological half-life ($T_{1/2l}$) ranging from 13 to 25 d.

Similarly, the fit of the whole-body depuration of 57Co in clams was quite good ($R^2$: 0.27–0.64) for all the Co concentrations tested (Table 1, Fig. 1). However, the estimated AE of 57Co ingested with food was much higher than in oysters (i.e., 76–84%) and this fraction was retained with a $T_{1/2}$ ranging from 36 to 39 d.

In both bivalve species, no significant difference (p always >0.05) was found among the estimated kinetic parameters ($A_0$, $A_s$, $A_l$, $T_{1/2s}$ and $T_{1/2l}$) in the presence of two additional batches of phytoplankton species.
Table 1
Assimilation efficiency (AE, %), depuration rate constant (k_d, d^{-1}) and biological half-life (T_{b,d} d) of 57Co in the oyster Isochrysis isognomon and the clam Gafrarium tumidum fed radiolabelled Isochrysis galbana (10^4 cell ml^{-1}) previously exposed to four increasing Co concentrations (n = 9 oysters per concentration tested, n = 6 clams for 0 and 5 ng l^{-1} and n = 8 clams for 50 and 500 ng l^{-1}). ASE: asymptotic standard error; R^2: determination coefficient.

<table>
<thead>
<tr>
<th>Species</th>
<th>Co concentration added (ng l^{-1})</th>
<th>AE ± ASE</th>
<th>k_d ± ASE</th>
<th>T_{b,d} ± ASE</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. isognomon</td>
<td>0</td>
<td>15.8 ± 7.0^a</td>
<td>0.032 ± 0.036^a</td>
<td>22 ± 24^2</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>16.0 ± 5.4^a</td>
<td>0.054 ± 0.028^a</td>
<td>13 ± 7^2</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>16.6 ± 6.2^a</td>
<td>0.050 ± 0.036^a</td>
<td>14 ± 10^2</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>14.7 ± 6.0^a</td>
<td>0.027 ± 0.033^a</td>
<td>25 ± 30^2</td>
<td>0.90</td>
</tr>
<tr>
<td>G. tumidum</td>
<td>0</td>
<td>77.2 ± 3.9^d1</td>
<td>0.018 ± 0.006^b</td>
<td>37 ± 11^b</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>77.4 ± 3.9^d1</td>
<td>0.019 ± 0.006^c</td>
<td>36 ± 10^c</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>75.7 ± 3.9^d1</td>
<td>0.018 ± 0.006^b</td>
<td>39 ± 12^b</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>84.1 ± 5.9^d1</td>
<td>0.019 ± 0.007^a</td>
<td>36 ± 13^a</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Significance of the estimated parameters:

- ^a p < 0.05.
- ^b p < 0.01.
- ^c p < 0.001.
- ^d p < 0.0001.
- * Not significant (p > 0.05).

3.2. Effect of phytoplankton species

In oysters, depuration kinetics of 54Mn, 57Co and 65Zn ingested with I. galbana (ISO), E. huxleyi (EMI) or H. triquetra (HET) (10^4 cell ml^{-1}) were best described by a double-exponential model (R^2: 0.23–0.63 for ISO, 0.11–0.83 for EMI and 0.57–0.92 for HET) (Table 2, Fig. 2). No significant difference was found among estimated T_{b,57Co} for all radiotracers and all phytoplankton species tested. In addition, no significant difference was found between AEs for Co and Mn in oysters fed EMI and HET, and for Zn in oysters fed ISO (10^4 cells ml^{-1}).

In clams, fitting of the whole-body depuration of the radiotracers ingested with I. galbana, E. huxleyi or H. triquetra (10^4 cell ml^{-1}) were generally somewhat better than in the oyster (R^2: 0.47–0.98 for ISO, 0.47–0.93 for EMI and 0.61–0.89 for HET) (Table 2, Fig. 2). T_{b,54Mn} of 54Mn was significantly longer when it was assimilated from HET than from EMI or ISO (p = 0.03 and 0.004, respectively). Significant differences were also observed among AEs calculated for Co Mn and Zn ingested with the three phytoplankton strains (Mn: ISO < EMI = HET, p < 0.03; Co: ISO = EMI > HET, p < 0.0004; and Zn: ISO > EMI > HET, p = 0.04).

3.3. Effect of cellular density

When oysters were fed 10^4 and 5 x 10^4 cells ml^{-1} of radiolabelled I. galbana, whole-body depuration kinetics of 54Mn, 57Co and 65Zn were fitted with R^2 ranging from 0.23 to 0.63 and 0.38 to 0.60, respectively (Table 2, Fig. 3). No significant difference in T_{b,57Co} and AE between cell densities was found for Co. In contrast, significant differences in AE were found for Mn and Zn, with higher AE calculated at the low cell density (p = 0.001 and 0.0003, respectively).

For clams, examination depuration kinetics of the radiotracers (R^2: 0.33–0.65 at 5 x 10^4 cell ml^{-1} and 0.47–0.98 at 10^4 cell ml^{-1}) indicated that T_{b,57Co} was not significantly different between the two food densities for all three radiotracers (Table 2, Fig. 3). However, when fed the low cell density, clams incorporated Co, Mn and Zn with significantly higher AE (p = 0.003, 0.047 and 0.0003, respectively).

4. Discussion

During the last two decades, dietary pathway has been increasingly recognized as a major source of contaminant accumulation in marine invertebrates (e.g., Wang et al., 1996; Reinfelder et al., 1998; Wang and Fisher, 1999b). The assimilation efficiency (AE) and retention time (T_{b,57Co}) are the critical parameters in assessing and modelling the dietary uptake of contaminants and numerous studies have been devoted to assess these parameters in different...
### Table 2
Assimilation efficiency (AE, %), depuration rate constant ($k_d$ d$^{-1}$) and biological half-life ($T_{1/2}$, d) of $^{54}$Mn, $^{57}$Co and $^{65}$Zn in the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum* fed radiolabeled *Emiliania huxleyi* ($10^4$ cell ml$^{-1}$), *Heterocapsa triquetra* ($10^4$ cell ml$^{-1}$) and *Isochrysis galbana* ($5 \times 10^3$ cell ml$^{-1}$ and $10^4$ cell ml$^{-1}$ for *I. isognomon*; $5 \times 10^3$ cell ml$^{-1}$ and $10^4$ cell ml$^{-1}$ for *G. tumidum*) ($n$ = 8 oysters; $n$ = 7 clams per phytoplankton species tested). ASE: asymptotic standard error; $R^2$: determination coefficient.

<table>
<thead>
<tr>
<th>Species</th>
<th>Phytoplankton strain</th>
<th>Cell density (cells ml$^{-1}$)</th>
<th>Isotope</th>
<th>AE ± ASE</th>
<th>$k_d$ ± ASE</th>
<th>$T_{1/2}$ ± ASE</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. isognomon</td>
<td><em>E. huxleyi</em></td>
<td>$10^4$</td>
<td>$^{54}$Mn</td>
<td>34 ± 6.5</td>
<td>0.028 ± 0.015</td>
<td>24 ± 13</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$^{57}$Co</td>
<td>22 ± 5.6</td>
<td>0.039 ± 0.021</td>
<td>18 ± 10</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^4$</td>
<td>$^{65}$Zn</td>
<td>70 ± 6.5</td>
<td>0.0002 ± 0.007</td>
<td>2783</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td><em>H. triquetra</em></td>
<td>$10^4$</td>
<td>$^{54}$Mn</td>
<td>20 ± 2.6</td>
<td>0.025 ± 0.012</td>
<td>28 ± 13</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^4$</td>
<td>$^{57}$Co</td>
<td>21 ± 4.1</td>
<td>0.050 ± 0.020</td>
<td>14 ± 6</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$10^4$</td>
<td>$^{65}$Zn</td>
<td>51 ± 3.2</td>
<td>0.006 ± 0.005</td>
<td>123 ± 10</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td><em>I. galbana</em></td>
<td>$10^4$</td>
<td>$^{54}$Mn</td>
<td>90 ± 5.6</td>
<td>0.010 ± 0.005</td>
<td>70 ± 32</td>
<td>0.23</td>
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<td>$10^4$</td>
<td>$^{57}$Co</td>
<td>55 ± 7.1</td>
<td>0.026 ± 0.010</td>
<td>26 ± 10</td>
<td>0.63</td>
</tr>
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<td>$10^4$</td>
<td>$^{65}$Zn</td>
<td>76 ± 4.1</td>
<td>0.015 ± 0.004</td>
<td>45 ± 13</td>
<td>0.36</td>
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<tr>
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<td>$5 \times 10^4$</td>
<td>$^{54}$Mn</td>
<td>41 ± 7.8</td>
<td>0.015 ± 0.014</td>
<td>47 ± 48</td>
<td>0.38</td>
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<td>$5 \times 10^4$</td>
<td>$^{57}$Co</td>
<td>37 ± 21</td>
<td>0.027 ± 0.043</td>
<td>26 ± 42</td>
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<tr>
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<td>$5 \times 10^4$</td>
<td>$^{65}$Zn</td>
<td>52 ± 3.5</td>
<td>0.010 ± 0.005</td>
<td>70 ± 34</td>
<td>0.60</td>
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<tr>
<td>G. tumidum</td>
<td><em>E. huxleyi</em></td>
<td>$10^4$</td>
<td>$^{54}$Mn</td>
<td>39 ± 4.3</td>
<td>0.051 ± 0.011</td>
<td>14 ± 3</td>
<td>0.92</td>
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<td>$10^4$</td>
<td>$^{57}$Co</td>
<td>80 ± 3.5</td>
<td>0.010 ± 0.004</td>
<td>70 ± 26</td>
<td>0.47</td>
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<tr>
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<td>$10^4$</td>
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<td>42 ± 2.3</td>
<td>0.014 ± 0.004</td>
<td>48 ± 14</td>
<td>0.93</td>
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<tr>
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<td><em>H. triquetra</em></td>
<td>$10^4$</td>
<td>$^{54}$Mn</td>
<td>56 ± 23</td>
<td>0.021 ± 0.026</td>
<td>34 ± 10</td>
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<td>$10^4$</td>
<td>$^{57}$Co</td>
<td>41 ± 3.1</td>
<td>0.014 ± 0.006</td>
<td>49 ± 9</td>
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<td>$^{65}$Zn</td>
<td>33 ± 7.3</td>
<td>0.005 ± 0.016</td>
<td>143 ± 47</td>
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<td><em>I. galbana</em></td>
<td>$10^4$</td>
<td>$^{54}$Mn</td>
<td>22 ± 3.7</td>
<td>0.044 ± 0.015</td>
<td>16 ± 5</td>
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<tr>
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<td>$10^4$</td>
<td>$^{57}$Co</td>
<td>73 ± 2.7</td>
<td>0.010 ± 0.003</td>
<td>68 ± 21</td>
<td>0.33</td>
</tr>
<tr>
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<td>$10^4$</td>
<td>$^{65}$Zn</td>
<td>51 ± 3.8</td>
<td>0.013 ± 0.006</td>
<td>55 ± 25</td>
<td>0.52</td>
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<td>$5 \times 10^4$</td>
<td>$^{54}$Mn</td>
<td>87 ± 6.4</td>
<td>0.002 ± 0.005</td>
<td>416 ± 135</td>
<td>0.47</td>
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<td>$5 \times 10^4$</td>
<td>$^{57}$Co</td>
<td>87 ± 6.4</td>
<td>0.002 ± 0.005</td>
<td>416 ± 135</td>
<td>0.47</td>
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<tr>
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<td>$5 \times 10^4$</td>
<td>$^{65}$Zn</td>
<td>90 ± 3.0</td>
<td>0.011 ± 0.003</td>
<td>61 ± 16</td>
<td>0.68</td>
</tr>
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</table>

Significance of the estimated parameters:
- $^a p < 0.05$.
- $^b p < 0.01$.
- $^c p < 0.001$.
- $^d p < 0.0001$.
- $^*$ Not significant ($p > 0.05$).

**Fig. 2.** Influence of phytoplankton species (*Isochrysis galbana*, *Emiliania huxleyi* and *Heterocapsa triquetra*; $10^4$ cells ml$^{-1}$) used as food on whole-body depuration kinetics of $^{54}$Mn, $^{57}$Co and $^{65}$Zn in the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum* ($n$ = 9 for *I. galbana* and 8 for *E. huxleyi* and *H. triquetra*; $n$ = 8 for *I. isognomon*) and the clam *Gafrarium tumidum* ($n$ = 9 for *I. galbana* and 7 for *E. huxleyi* and *H. triquetra*). A (%): remaining activity (%) ± SD.
whereas AE of Se in the mussel Perna viridis ingested food appears to depend on the element as well as on rum...
feeders can adjust their filtration rate to ambient phytoplankton density and thereby are able to maintain a stable ingestion rate even at high food concentrations (Jin et al., 1996; Dong et al., 2000; Zhuang and Wang, 2004). Although no conclusion on the impact of this adaptive feeding behaviour could be directly drawn from our results, it is clear that food availability notably influenced the AE of the metals examined in *I. isognomon* and *G. tumidum*. It is nowadays well documented that the dietary pathway is an important contributor to the global bioaccumulation of metals in marine organisms (e.g., Wang and Fisher, 1997; Metian et al., 2008a). Since the present study has shown that the feeding behaviour of *I. isognomon* and *G. tumidum* is influenced by the feeding conditions (quality and/or quantity of food), it is strongly recommended that future studies take into account these parameters so as to refine the prediction of biodynamic models (e.g., Thomann et al., 1995; Metian et al., 2008a; Pan and Wang, 2008). The consideration of such data is also needed to explain bioaccumulation data obtained in the framework of biomonitoring programmes. For example, Bendell-Young and Arifin (2004) demonstrated the influence of mussel feeding behaviour on their predicted tissue concentrations in Cd, especially under conditions of highly variable quantity and quality of suspended particles.

In conclusion, our experimental results suggest that food quality (phytoplankton composition) and quantity (cell density) may play a significant role in the assimilation of metals ingested with food in *I. isognomon* and *G. tumidum*. Because of the major importance of the dietary contribution to global metal bioaccumulation in marine organisms, it is thus recommended to pay great attention to factors influencing AE. This would help refining both bioaccumulation model predictions and interpretation of data from field surveys and biomonitoring programmes.

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References


