

Diet variably affects the trophic transfer of trace elements in the oyster *Crassostrea gigas*

Short title: Is the oyster *Crassostrea gigas* what it eats?

Simon Pouil¹, Marc Metian^{1*}, Christine Dupuy², Jean-Louis Teyssié¹, Michel Warnau¹, Paco Bustamante^{2,3}

¹ International Atomic Energy Agency, Environment Laboratories, 4a, Quai Antoine 1er, MC-98000, Principality of Monaco

² Littoral Environnement et Sociétés (LIENSs), UMR 7266, CNRS-La Rochelle Université, 2 rue Olympe de Gouges, F-17000 La Rochelle, France

³ Institut Universitaire de France (IUF), 75 rue Descartes, 75009 Paris, France

* Corresponding author: Marc Metian
Radioecology Laboratory
IAEA Environment Laboratories
4a Quai Antoine 1er
MC-98000 Principality of Monaco
Telephone: +377 97 97 72 17
E-mail: m.metian@iaea.org

Highlights

- Trophic transfer of Ag, ^{241}Am , Cd, Co, Mn and Zn was assessed in oysters fed on ciliates and diatoms.
- Assimilation of Ag and ^{241}Am in *C. gigas* differs in response to the different diets tested.
- Highest assimilation of Ag and ^{241}Am in *C. gigas* was observed when it is fed with ciliates.

Abstract

Although it has been shown that trophic transfer of trace elements in oysters can be influenced by the diet, most of the studies investigating the ability of oysters to bioaccumulate trace elements from their diet are based on experiments using phytoplankton alone. Wild oysters feed also on large bacteria, ciliates or detritic organic matter. The present study aimed at examining the influence of quality of food on the assimilation efficiency (AE) of trace elements in the Pacific cupped oyster *Crassostrea gigas*. Oysters were exposed via their food to the radiotracers of essential (^{57}Co , ^{54}Mn and ^{65}Zn) and non-essential ($^{110\text{m}}\text{Ag}$, ^{109}Cd and ^{241}Am) trace elements under different diets (protozoan ciliates *Uronema marinum* and diatoms *Thalassiosira pseudonana*). Significant differences were found only for Ag and ^{241}Am , with lower AEs measured in oysters fed with ciliates than in individuals fed with diatoms (Ag: $54 \pm 3\%$ vs. $67 \pm 4\%$ for Ag and ^{241}Am : $62 \pm 4\%$ vs. $76 \pm 4\%$). Interestingly, no significant difference was found among estimated depuration rates (k_{el}) for all trace elements ingested with the two diets tested. These findings indicate that the differences observed are driven by the digestion process, presumably due to difference of bioavailability of trace elements dependent on the quality of the food ingested.

Keywords: bioaccumulation; bivalve; dietary pathway; metals; radionuclides; radiotracers

1. Introduction

The ability of oysters to bioaccumulate trace elements has been studied intensively in laboratory experiments during the last decades. Several studies have indicated that food is generally the main accumulation pathway of trace elements in oysters (e.g., Hédouin et al., 2010b; Metian et al., 2016). Laboratory studies generally showed also that food quality and quantity affect trophic transfer of trace elements in bivalves (e.g., Hédouin et al., 2010a; Metian et al., 2008; Wang and Fisher, 1996). This is likely caused by the adjustment of feeding processes such as filtration rate in response to variations in the feeding environment (Navarro and Iglesias, 1993; Widdows and Donkin, 1992) and the bioavailability of trace elements in the ingested food (e.g., Ng et al., 2005; Wang and Fisher, 1996). An overlook of the literature (see Table 1) indicates that assimilation efficiency (AE) of trace elements was shown to be highly dependent on the food type in seven oyster species. Most studies quantified trace element AE from phytoplankton exclusively (Blackmore and Wang, 2004; Hédouin et al., 2010b, 2010a; Reinfelder et al., 1997) but some also included sediment (Ke and Wang, 2001; Pan and Wang, 2011), whereas other food items were rarely studied (see Table 1).

Wild oysters are filter-feeders ingesting a variety of food items, including phytoplankton, heterotrophic protists, large bacteria, fungi and detritus (Dupuy et al., 1999; Heral, 1990). For instance, Pacific cupped oyster *Crassostrea gigas* retains efficiently food particles between 4 and 72 μm of size (Barillé et al., 1993; Dupuy et al., 1999); heterotrophic protists (such as ciliates) can be a major food source for *C. gigas* and thus act as a trophic link between picoplankton and oysters (Dupuy et al., 1999). Nevertheless, to the best of our knowledge, trophic transfer of trace elements from ciliates to oysters has been poorly investigated. In a recent work, Metian et al. (2020) showed that oysters fed with ciliates *Uronema marinum* assimilated more efficiently methyl mercury and less efficiently inorganic mercury than oysters fed with diatoms *Thalassiosira pseudonana*.

In the present study, we investigated the influence of the diet quality on the AE of six essential (Co, Mn and Zn) and non-essential (Ag, ^{241}Am and Cd) trace elements in the Pacific cupped oysters *C. gigas*, using pulse-chase feeding method and radiolabeled diatom *T. pseudonana* (2.5-15 μm diameter) and ciliate protozoan *U. marinum*, 20 μm length). Both protists are components of the oyster natural diet (Le Gall et al., 1997; Dupuy et al., 1999).

2. Materials and Methods

2.1. Origin and acclimation of organisms

Pacific cupped oysters *C. gigas* were purchased from a shellfish farm in La Rochelle, France. They were transported to IAEA-EL premises in the Principality of Monaco, and were acclimated to laboratory conditions (constantly aerated, open-circuit aquarium; salinity: 36 ± 1 ; temperature: $19 \pm 1^\circ\text{C}$; pH: 8; light/dark cycle: 12h/12h) for 4 weeks. During acclimation, bivalves were fed with Prymnesiophyceae *Isochrysis galbana* (10^4 cells mL^{-1}).

2.2. Radiotracers and counting

Depuration kinetics of trace elements in oysters were determined using high-specific activity radiotracers (see Table S1 for details). Exposed oysters were whole-body 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 multi-channel analyzer and a computer equipped with a spectra analysis software (Interwinner® 4). The radioactivity was determined by comparison with standards of known activity and of appropriate geometry (Cresswell et al., 2017), and corrected for counting efficiency and physical radioactive decay. The counting time was adjusted to obtain a propagated counting error less than 5% (Warnau et al., 1996, 1997).

2.3. Dietary exposure

Trophic transfer of trace elements to oysters was studied using protozoan and phytoplankton as diets. Ciliate (*Uronema marinum*) and phytoplankton (*Thalassiosira pseudonana*, clone 3H) cells were maintained, respectively, in FAG medium (e.g. L  pinay et al., 2018) and in modified F/2 medium (without EDTA; e.g. Guillard, 1975; Guillard and Ryther, 1962). Cultures were handled axenically throughout the experimentation and were spiked with 1 kBq L⁻¹ for ²⁴¹Am, 2 kBq L⁻¹ for ^{110m}Ag, ⁵⁷Co, ⁵⁴Mn and ⁶⁵Zn and 3 kBq L⁻¹ for ¹⁰⁹Cd during their exponential growing phase. The diatom culture was centrifuged (2500 g for 25 min) whereas the protozoan culture was filtered (25-  m mesh size; Osmonic   filters) and the resulting filtrate centrifuged (1000 g for 15 min). Twenty oysters (68.6    3.4 g wet wt, 8-10 cm shell length) were randomly distributed in two 13-L aquaria 2 weeks prior to the experiment (n = 10 per treatment). An experimental treatment was assigned to each aquarium. Oysters were single-fed (pulse-chase feeding method; Wang and Fisher, 1999a) for 2 h in closed circuit with radiolabeled food by resuspension of the centrifuged pellets (density of 10⁵ cells mL⁻¹ for both food items) to avoid pseudofeces production by the bivalves (Beninger et al., 1999). After the feeding period (2 h), all oysters were whole-body   -counted, flow was restored in the aquaria (~40 L h⁻¹) and organisms were put back in their respective, now open circuit, aquaria. The same counting procedure was regularly repeated for all individuals over a 50-d period in order to determine the whole-body depuration kinetics of the radiotracers ingested with food. Oyster shells were placed as control in each aquarium to check for any possible radiotracer recycling from food during the feeding period. These control shells were radioanalyzed at regular intervals of time. Throughout the 50-d depuration period, oysters were fed daily for 1 h with non-radiolabeled Prymnesiophyceae *I. galbana* (10⁴ cells mL⁻¹). Feeding was carried out in the same aquaria placed in closed system for 1 h, then the water flow was open back. Volumes were adjusted

depending on the algae culture concentrations to keep feeding densities constant throughout the experiment.

2.4. Data analysis

Depuration kinetics were fitted using nonlinear regression routines and iterative adjustment. The depuration kinetics of the radiotracers were best fitted using either a single-component or a double-component exponential model (Warnau et al., 1996). The decision was based on F test and ANOVA tables for two fitted model objects. Kinetic parameters were determined using the R freeware 3.5.2 (R Development Core Team, 2018). They are considered significantly different when 95% of their confidence intervals do not overlap (Payton et al., 2003).

3. Results and Discussion

During the last three decades, dietary pathway has been increasingly recognized as the main source of trace element bioaccumulation in bivalves (e.g., Hédouin et al., 2010b; Metian et al., 2009; Reinfelder et al., 1998; Wang and Fisher, 1999b; Wang et al., 1996). The assimilation efficiency (AE) is among the critical parameters to assess the dietary uptake of trace elements in aquatic organisms (Pouil et al., 2018; Wang and Fisher, 1999a) and numerous studies have been devoted to determine experimentally trace element AEs in different oyster species, including *Crassostrea* sp., *Isognomon* sp., *Malleus* sp. and *Saccostrea* sp. (e.g., Blackmore and Wang, 2004; Hédouin et al., 2010a; Ke and Wang, 2001; Pan and Wang, 2011). However, as shown in Table 1, most of these studies used phytoplankton species as food to assess trophic transfer of trace elements, which does not reflect the diversity of the natural diet of these bivalves (Barillé et al., 1993; Dupuy et al., 1999; Heral, 1990).

We explored the possible influence of the diatom *T. pseudonana* and the ciliate *U. marinum* on the trophic transfer of 6 trace elements in the present study. Depuration kinetics of Ag, Cd, Co, Mn and Zn ingested by *C. gigas* fed with ciliates and diatoms were best described by a double-

exponential model (R^2 : 0.44-0.67 for ciliates and 0.39-0.63 for diatoms, see Table 2 and Figure 1). ^{241}Am is the only element for which both depuration kinetics were best fitted using a single-exponential model (R^2 : 0.31 for ciliates and 0.32 for diatoms). Overall AEs in oysters fed with ciliates ranked as follows: $\text{Cd} < \text{Ag} \leq \text{Mn} < \text{Co} \leq \text{Am} < \text{Zn}$ whereas, when fed with diatoms, they ranked as: $\text{Cd} < \text{Co} < \text{Zn} < \text{Ag} \leq \text{Mn} < \text{Am}$ (Table 2). The AEs estimated were in accordance with previous observations reported for oysters fed with phytoplankton species (e.g., Hédouin et al., 2010a; Ke and Wang, 2001; Reinfelder et al., 1997; Table 1).

Significant differences were found for Ag and ^{241}Am , with lower AEs observed when oysters were fed with ciliates than with diatoms (Ag: $53.77 \pm 3.29\%$ vs. $67.02 \pm 4.30\%$ and ^{241}Am : $62.33 \pm 4.04\%$ vs. $75.63 \pm 3.80\%$; Figure 2). Similar observations were recently reported for Hg(II) by Metian et al. (2020). For the four other tested trace elements, no significance difference was found in AEs between oysters fed on ciliates and the one fed on diatoms (Figure 2).

In the first instance, we could assume that the lower Ag and ^{241}Am AEs in oysters fed with ciliates than in those fed with diatoms could be attributed to a lower bioavailability of these elements in the ciliates compared to diatoms. Reinfelder and Fisher (1991) reported that Ag and ^{241}Am were the less abundant elements in the cytoplasm of *T. pseudonana* (i.e. fraction the most bioavailable) among 10 different trace elements tested.

Our study is among the first ones to use ciliates as food to assess the trophic transfer of trace elements in bivalves. Among the few previous studies on trophic transfer from ciliates, Twining and Fisher (2004) found that AEs of Ag, Cd, Fe, and Zn in copepods fed with ciliates were higher than when fed with dinoflagellates or diatoms. The authors correlated this observation with the higher percentage of trace elements located in the cytoplasm of ciliates, which is considered as being more bioavailable. Such contrasting findings between the study of Twining and Fisher (2004) and ours may be explained by difference in culture conditions of the prey,

which are known to affect the bioavailability of trace elements in phytoplankton species (Lee and Fisher, 2016), or by difference in digestive strategies between predators (copepods vs oysters) that can have a substantial influence on metal assimilation (Wang and Fisher, 1999a). Further investigations are needed to assess whether the difference in AE of Ag and ^{241}Am observed between copepods and oysters is due to difference in subcellular partitioning (see Wallace and Luoma, 2003) in ciliate *U. marinum* and diatom *T. pseudonana* or to specific digestive metabolism in the Pacific cupped oyster *C. gigas* and in the copepods (*Acartia tonsa*, *A. hudsonica*, and *Temora longicornis*). Our study confirmed that trace element AE in oysters can be affected by the food ingested and demonstrated that protozoan ciliates can act as vectors in the trophic transfer of trace elements in aquatic food chains.

Our experimental results are complementary to previous studies as they expand the available knowledge regarding trophic transfer of trace elements in oysters feeding on protozoan ciliates. Because of the major importance of the dietary contribution to trace element bioaccumulation in oysters, it is recommended to pay great attention to the influence of diet on AE and to the dietary composition of the natural food in the field. This would help refining both bioaccumulation model predictions and interpretation of data from field surveys and biomonitoring programs.

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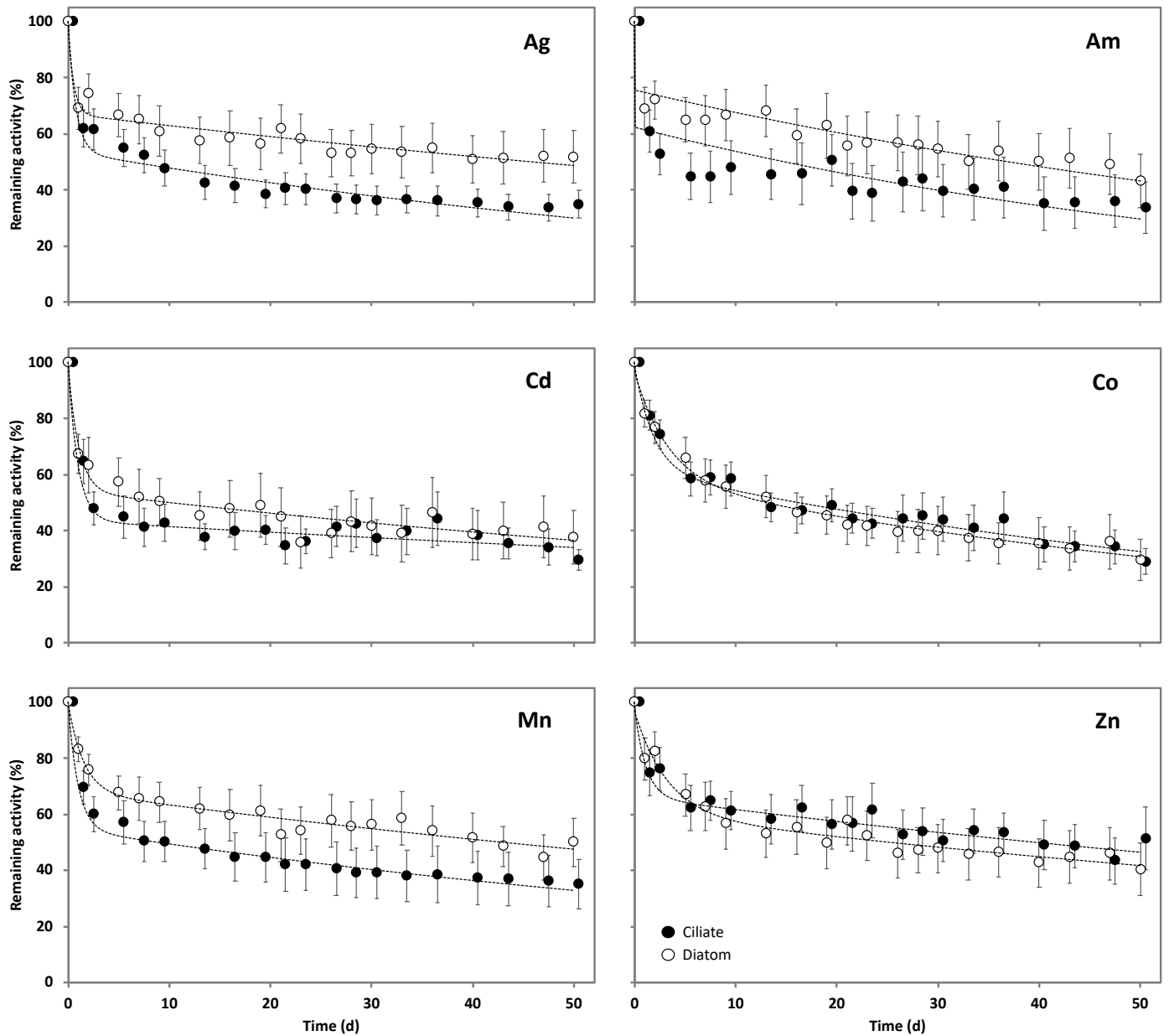


Figure 1. Whole-body depuration kinetics of dietary Ag, Am, Cd, Co, Mn, and Zn in Pacific cupped oysters *Crassostrea gigas* (n=10, % remaining activities, mean \pm SE) fed ciliate *Uronema marinum* and phytoplankton *Thalassiosira pseudonana*. Parameters and statistics of the depuration kinetics are given in Table 2.

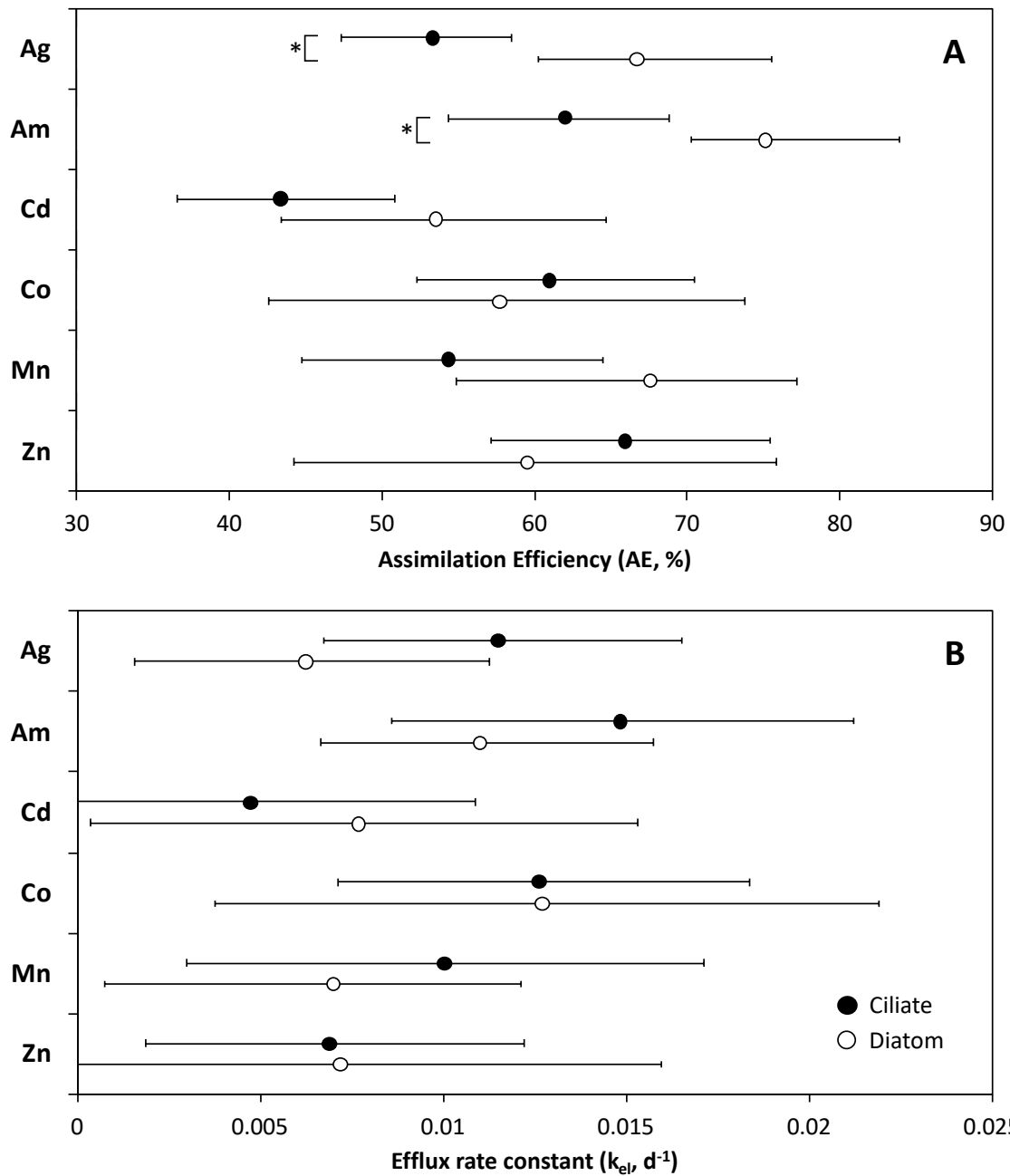


Figure 2. 95% confidence intervals of the main kinetic parameters of dietary Ag, Am, Cd, Co, Mn, and Zn: (A) Assimilation Efficiency (AE) and (B) Efflux rate constant (k_{el}) estimated by fitting exponential models in Pacific cupped oysters fed radiolabeled prey (ciliate *Uronema marinum* and diatom *Thalassiosira pseudonana*). Symbol (*) denotes significant differences.

Table 1. Short review of trace elements' Assimilation Efficiencies (AEs) determined experimentally in different oyster species.

Oyster species	Element(s)	Range of AE (%)	Food tested	Reference(s)
<i>Crassostrea gigas</i>	Hg(II) MeHg	28-90 57-98	diatom <i>Thalassiosira pseudonana</i> ciliate <i>Uronema marinum</i>	Metian et al. (2019)
<i>Crassostrea rivularis</i>	Cd Se Zn	29-83 18-77 33-89	green algae <i>Chlorella autotrophica</i> dinoflagellate <i>Prorocentrum minimum</i> diatom <i>Thalassiosira pseudonana</i> prymnesiophyceae <i>Tetraselmis levis</i> sediment	Ke and Wang (2001)
<i>Crassostrea virginica</i>	Ag Am Cd Co Se Zn	36-52 0-23 60-78 24-44 64-76 65-81	prymnesiophyceae <i>Isochrysis galbana</i>	Reinfelder et al. (1997)
<i>Isognomon isognomum</i>	Ag Cd Co Mn Zn	52-61 55-62 16-62 17-96 48-80	coccolithophoridae <i>Emiliana huxleyi</i> dinoflagellate <i>Heterocapsa triquetra</i> prymnesiophyceae <i>Isochrysis galbana</i>	Hédouin et al. (2010a) Hédouin et al. (2010b)
<i>Malleus regula</i>	Ag Cd Co Zn	31-36 47-55 38-46 56-59	prymnesiophyceae <i>Isochrysis galbana</i>	Hédouin et al. (2010a)
<i>Saccostrea cucullata</i>	Cd Hg(II) MeHg Zn	29-32 29-33 88-94 50-56	diatom <i>Thalassiosira pseudonana</i> diatom <i>Thalassiosira weissflogii</i> sediment	Blackmore and Wang (2004) Pan and Wang (2011)
<i>Saccostrea glomerata</i>	Cd Se Zn	23-78 29-77 28-70	green alga <i>Chlorella autotrophica</i> dinoflagellate <i>Prorocentrum minimum</i> diatom <i>Thalassiosira pseudonana</i> prymnesiophyceae <i>Tetraselmis levis</i> sediment	Ke and Wang (2001)

Table 2. Parameters (mean \pm SE, n = 10) of the whole-body depuration kinetics of dietary Ag, Am, Cd, Co, Mn, and Zn in Pacific cupped oysters (*Crassostrea gigas*) fed on ciliate (*Uronema marinum*) and diatom (*Thalassiosira pseudonana*). Depuration parameters: A_{0s} and A_{0l}(=AE): activity (%) lost according to the short-and the long-lived exponential component, respectively; T_{b½}: biological half-life (d) [T_{b½}= ln2/k_e]; O and T: one-component and two-component exponential model, respectively. R²: determination coefficient.

Element	Food	Model	A _{0s} \pm SE	k _{es} \pm SE	T _{b½s} \pm SE	A _{0l} \pm SE	k _{el} \pm SE	T _{b½l} \pm SE	R ²
Ag	Ciliate	T	45.80 \pm 6.08***	1.34 \pm 0.47**	0.52 \pm 0.18**	53.77 \pm 3.29***	0.012 \pm 0.002***	59.64 \pm 3.64***	0.66
	Diatom	T	32.81 \pm 8.72***	1.90 \pm 1.75 ^{NS}	0.36 \pm 0.33 ^{NS}	67.02 \pm 4.30***	0.006 \pm 0.002**	108.10 \pm 6.94**	0.39
Am	Ciliate	O	-	-	-	62.33 \pm 4.04***	0.015 \pm 0.003***	46.49 \pm 9.98***	0.31
	Diatom	O	-	-	-	75.63 \pm 3.80***	0.011 \pm 0.002***	62.14 \pm 12.87***	0.32
Cd	Ciliate	T	56.51 \pm 6.97***	1.04 \pm 0.31***	0.66 \pm 0.20***	43.73 \pm 3.62***	0.005 \pm 0.003 ^{NS}	141.34 \pm 11.69 ^{NS}	0.59
	Diatom	T	45.25 \pm 9.96***	0.97 \pm 0.50*	0.72 \pm 0.37*	54.05 \pm 5.41***	0.008 \pm 0.004***	88.33 \pm 8.84***	0.44
Co	Ciliate	T	38.07 \pm 6.68***	0.53 \pm 0.22*	1.30 \pm 0.54*	61.37 \pm 4.60***	0.013 \pm 0.003***	54.33 \pm 4.08***	0.67
	Diatom	T	38.78 \pm 8.76***	0.30 \pm 0.16*	2.29 \pm 1.21*	58.18 \pm 7.93***	0.013 \pm 0.005**	53.98 \pm 7.36**	0.63
Mn	Ciliate	T	45.06 \pm 8.65***	1.02 \pm 0.46*	0.68 \pm 0.31*	54.75 \pm 4.77***	0.010 \pm 0.003**	68.24 \pm 5.95**	0.52
	Diatom	T	31.75 \pm 8.12***	0.66 \pm 0.39 ^{NS}	1.05 \pm 0.62 ^{NS}	67.98 \pm 4.80***	0.007 \pm 0.003**	97.05 \pm 6.85**	0.47
Zn	Ciliate	O	32.88 \pm 8.57***	0.91 \pm 0.55 ^{NS}	0.76 \pm 0.46 ^{NS}	66.29 \pm 4.65***	0.007 \pm 0.003**	98.51 \pm 6.91**	0.44
	Diatom	O	37.10 \pm 9.48***	0.31 \pm 0.20 ^{NS}	2.22 \pm 1.39 ^{NS}	60.00 \pm 8.03***	0.007 \pm 0.004 ^{NS}	94.01 \pm 12.58 ^{NS}	0.49

Probability of the model adjustment: ^{NS} p > 0.05, * p < 0.05, ** p < 0.01, *** p < 0.001.