# Bioconcentration of Ag, Cd, Co, Mn and Zn in the mangrove oyster (Crassostrea gasar) and preliminary human health risk assessment: A radiotracer study

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Abstract: Bioaccumulation kinetics of 5 dissolved metals were determined in the mangrove oyster *Crassostrea gasar*, using corresponding radiotracers ($^{54}$Mn, $^{57}$Co, $^{65}$Zn, $^{109}$Cd and $^{110m}$Ag). Additionally, their bioaccessibility to human consumers was estimated. Results indicated that over a 14-d exposure $^{54}$Mn and $^{57}$Co were linearly concentrated in oysters whereas $^{109}$Cd, $^{65}$Zn and $^{110m}$Ag were starting to saturate (steady-state not reached). Whole-body concentration factors at 14d ($CF_{14d}$ *in toto*) ranged from 187±65 to 629±179 with the lowest bioconcentration capacity for Co and the highest for Ag. Depuration kinetics were best described by a double-exponential model with associated biological half-lives ranging from 26 days (Ag) to almost 8 months (Zn and Cd). Bioaccessible fraction of the studied elements was estimated using *in vitro* digestions, which suggested that oysters consumed seasoned with lemon enhanced the accessibility of Cd, Mn and Zn to human consumers, but not Ag and Co.

*Keywords: Metals, Bioaccumulation, Tropical African bivalve, Seafood safety.*

# Introduction

The use of bivalves to assess trace metal contamination in aquatic environment is well described in the literature (e.g. Liu and Deng 2007; Birch et al. 2014). Among bivalves, oysters are often used as bioindicators; they are strong accumulators of both essential and non-essential metals and display strong retention capacities for some elements (Hédouin et al. 2010b). Their place in food webs makes their study of further interest, since their trace metals content is susceptible to be transferred to upper trophic levels, humans included, and they thereby provide valuable information for seafood safety assessment (Wang and Rainbow 2008; Metian et al. 2009a).

Biokinetic studies using non-destructive radiotracer techniques have proven to be a powerful tool to investigate the differences in the behavior of metal accumulation among species (e.g. Wang et al. 1996; Metian et al. 2008a; 2009b; Hédouin et al. 2010a), especially in determining uptake and depuration kinetic parameters. Additionally, the latter approach combined with *in vitro* digestion simulation has shown to provide crucial information for metal risk assessment to humans (e.g. Metian et al. 2009a).

However, so far little attention has been paid to metal bioaccumulation capacities of bivalves from the African Sub-Saharan region although substantial levels of metals have been measured in some species from this region (e.g. Otchere 2003; Obodai et al. 2011). Among these bivalves, the mangrove oyster *Crassostrea gasar* is widely distributed in the region and commonly consumed by coastal populations. Bodin et al. (2013) indicated that this species tended to accumulate metals efficiently compared to other molluscs from the region. However, to the best of our knowledge, no study has been conducted to characterize its bioaccumulation capacities.

The present study aimed at: (1) investigating the metal bioconcentration capacities of the mangrove oyster (through dissolved pathway) and (2) determining the metal dietary bioaccessibility to human consumers from raw and lemon-seasoned oysters following dissolved exposure.

# Materials and Methods

In September 2013, 100 mangrove oysters *Crassostrea gasar*, collected by handpicking on the shores of Abidjan (Côte d'Ivoire), were transported to IAEA-EL premises in Monaco. They were acclimated to laboratory conditions for 4 weeks prior to the experiment (constantly aerated, open-circuit, 300-L tank; flux: 150 L $h^{-1}$; salinity: 20±1 p.s.u.; temperature: 25 ± 0.5 °C; pH: 8.0±0.1; light/dark cycle: 12 h/12 h). During the period of acclimation and throughout the experiment, the oysters were fed daily on a mixed diet of phytoplankton (*Isochrysis galbana* and *Skeletonema costatum*) with algal densities ranging from $10^4$ to $10^5$ cell $mL^{-1}$.

Twenty individuals of similar size (shell length: 62±6 mm and wet weight: 28.9±5.5 g) were tag-identified and placed in a 70-L closed circuit glass aquarium filled with 0.2-µm filtered seawater (same conditions as above). The seawater was spiked with 0.45 kBq $^{54}Mn$ $L^{-1}$ (as $MnCl_2$, in 0.1M HCl, $T_{b1/2}$ = 312.2d), 0.15 kBq $^{57}Co$ $L^{-1}$ (as $CoCl_2$ in 0.1 M HCl, $T_{b1/2}$ = 271.8 d), 0.23 kBq $^{65}Zn$ $L^{-1}$ (as $ZnCl_2$ in 0.5 M HCl, $T_{b1/2}$ = 243.9d), 0.95 kBq $^{109}Cd$ $L^{-1}$ (as CdCl in 0.1 M HCl, $T_{b1/2}$ = 426.6d) and 0.51 kBq $^{110m}Ag$ $L^{-1}$ (as $AgNO_3$ in 1 M $HNO_3$, $T_{b1/2}$ = 249.8d). Oysters were then exposed to the tracers for a period of 14 d. Seawater and spikes were renewed each day for the first five days and then every second day in order to keep radioactivity in seawater as constant as possible. In terms of stable metal equivalent, each spike corresponded to an addition of 10 ng/L of Zn, 130 ng/L of Cd and 2 ng/L of Ag (i.e. concentrations that are lower than the background concentrations of these metals in open sea; Bruland 1983). No change in pH and salinity was detectable after radiotracer additions. Water samples were collected before and after each water renewal, and γ-counted to determine the time-integrated activities in water (Warnau et al. 1996; Rodriguez y Baena et al. 2006) and the organisms were briefly fed for 30 min during the water renewal step (same microalgae species and density than during acclimation phase). At different time intervals, 10 tag-identified individuals were γ-counted alive to determine uptake kinetics of the radiotracers. At the end of the 14-d exposure period, radiolabelled oysters were transferred into a new, constantly aerated, 70-L aquarium (flux: 50 L $h^{-1}$; other conditions as previously described) and were allowed to depurate for a period of 58 d. Oysters were fed daily and γ-counted hereto at different times to determine the depuration kinetics of the radiotracers. Radioanalyses were carried out using a high-resolution γ-spectrometer system composed of 5 Germanium – N or P type – detectors (EGNC 33-195-R, Canberra® and Eurysis®) connected to a multichannel analyzer and a computer equipped with a spectra analysis software (Interwinner® 6).

At the end of the 58-d depuration period, 8 oysters were randomly collected and edible parts (*i.e.* whole soft parts) were removed. Four of them were used as is (defined as "raw") whereas the 4 remaining edible parts were seasoned with lemon juice (2 mL per oyster for an action time of 30 seconds) in order to assess effect of seasoning on bioaccessible fraction of the studied elements. Right after, *in vitro* digestions were performed on each individual raw and seasoned soft-part to assess the bioaccessible fraction of elements for human consumers of oysters, following the method described by Versantvoort et al. (2005) and adapted for radiotracer by Metian et al. (2009a). Briefly, homogenized oyster tissues were exposed step by step to artificial saliva, gastric juice and mixture of duodenal juice, bile and $NaHCO_3$ (chemicals and enzymes were purchased from Sigma®). Following the *in vitro* digestion, the resulting chyme was centrifuged and the radiotracer activities were counted in supernatant, which is considered as containing the bioaccessible fraction (Versantvoort et al. 2005).

Whole-body uptake kinetics of radiotracers were expressed in terms of changes in bioconcentration factor over time (CF, ratio between activity of the radiotracer in the whole organism or in a body compartment –Bq $g^{-1}$ wet weight– and time-integrated activity of radiotracer in seawater –Bq $g^{-1}$–; Warnau et al. 1996, Rodriguez y Baena et al. 2006). Radiotracer uptake kinetics were best described using either a simple linear regression model (Eq. 1), or a saturation exponential model (Eq. 2) if the observed kinetics tended to reach a steady- state equilibrium:

$$CF_t = k_u t \qquad \text{(Eq. 1)}$$

$$CF_t = CF_{ss} \, (1 - e^{-k_e t}) \qquad \text{(Eq. 2)}$$

where $CF_t$ and $CF_{ss}$ are the bioconcentration 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.

Depuration of radiotracers was expressed as the percentage of remaining radioactivity over time (radioactivity at time *t* divided by the initial radioactivity measured in the organism at the beginning of the depuration period × 100; Warnau et al. 1996). The depuration kinetics for all the radiotracers were best described using a double-component exponential model (Eq. 3):

$$A_t = A_{0s} \, e^{-k_{es} t} + A_{0l} \, e^{-k_{el} t} \qquad \text{(Eq. 3)}$$

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. The short- and long-lived components biological half-life ($T_{b½s}$ and $T_{b½l}$) can be calculated ($T_{b½s}$ and $T_{b½l}$) from the corresponding depuration rate constants ($k_{es}$ and $k_{el}$, respectively) according to the relation $T_{b½} = \ln 2 / k_e$ (Warnau et al. 1996).

Whole-body uptake and depuration kinetics parameters were determined through iterative adjustment of the model using the nonlinear curve-fitting routines in the Statistica© software 5.2.1 and statistical methods described by Warnau et al. (1996) and Hédouin et al. (2010a). Criteria used for selecting best fitting models were the coefficient of determination ($R^2$) and results from an ANOVA on residuals (Metian et al. 2015)._

Metal bioaccessibility in raw and lemon-seasoned oysters was compared using non-parametric Mann-Whitney U test. The level of significance for statistical analyses was always set at α = 0.05. All the statistical analyses were performed using R software 3.0.1 (R Development Core Team, 2014).

# Results and Discussion

Figure 1A displays the whole-body uptake kinetics of the studied radiotracers. Metals were readily accumulated by oysters, with contrasting patterns: bioconcentration of $^{54}Mn$ and $^{57}Co$ was best fitted using a linear model ($R^2 \geq 0.84$), whereas bioconcentration of $^{65}Zn$, $^{109}Cd$ and $^{110m}Ag$ was best described by a saturation exponential model ($R^2 \geq 0.78$).

For these latter elements, the estimated bioconcentration factors at steady state ($CF_{ss}$) were 1052±47 ($^{110m}Ag$; $p<0.001$), 897±224 ($^{65}Zn$; $p<0.001$) and 401±231 ($^{109}Cd$; $p>0.05$). Using CFs observed in the whole organisms at the end of the exposure period ($CF_{14d}$ *in toto*), radiotracer bioavailability can be ranked as $^{65}Zn$

(629±179) = $^{110m}$Ag (587±288) > $^{109}$Cd (283±82) = $^{54}$Mn (294±105) > $^{57}$Co (187±65). Concurrently, similar trends were observed for the derived uptake rate constants ($k_u$). The uptake rate constant values were 72.3±7.9 d$^{-1}$ (Zn), 55.9±9.7 d$^{-1}$ (Ag), 34.2±4.7 d$^{-1}$ (Cd), 21.2±0.8 d$^{-1}$ (Zn) and 13.6±0.5 d$^{-1}$ (Co). In previous experimental studies investigating similar elements uptake kinetics in tropical bivalves, Cd, Zn, and Ag are generally the elements most rapidly and highly bioconcentrated (e.g. Metian et al. 2008b; Hédouin et al. 2010a). This can be attributed to their strong affinity for sulphur-containing proteins such as metallothioneins, which facilitate their transport across biological membranes. In contrast Mn and Co are transported by passive diffusion (Wang and Dei 1999).

After 58d of depuration, whole-body depuration kinetics were all best described by a double-component exponential model (Fig. 1B & 1C). $^{54}$Mn, $^{57}$Co, $^{65}$Zn and $^{109}$Cd were efficiently absorbed ($A_{0l}$ > 78%), whereas $^{110m}$Ag was less ($A_{0l}$ = 23±5%). Metal absorption capacities in tropical oysters have been already shown. For example, absorption efficiencies over 74% have been described in the oysters *Isognomon isognomon* and *Malleus regula* for Ag, Cd, Co, Cr and Zn (Hédouin et al. 2010a). The latter result for Ag (much higher that the 23% measured in *C. gasar*) suggests the occurrence of different processes of accumulation and/or storage of Ag among tropical oyster species.

Dissolved Ag integrated in *C. gasar* was rapidly lost compared to other elements ($T_{b½l}$ of 25±3 d for $^{110m}$Ag *vs.* 259±259 d, 63±2 d, 82±4 d, 187±19 d for $^{109}$Cd, $^{54}$Mn, $^{57}$Co and $^{65}$Zn, respectively), although it is usually known to be strongly retained by bivalves (e.g. Metian et al. 2008a; Hédouin et al. 2010a). Some detoxification mechanisms protecting against Ag intoxication are well documented in bivalves such as binding to metallothioneins (Bebianno and Langston, 1993) or storage as $Ag_2S$ (very stable amorphous compound; e.g. Berthet et al., 1992), and thus *C. gasar* could have a less efficient detoxification mechanism than other bivalves against Ag toxicity.

The overall results of the simulated *in vitro* digestion experiments showed that the bioaccessible fraction of the metals in mangrove oysters varied from 51% (Mn in raw oysters) to 94% (Mn in lemon-seasoned oysters; Fig. 2). Our results also indicate that oysters seasoned have significantly higher bioaccessible fraction of Cd, Mn and Zn than raw oysters (respectively 51-52% *vs.* 80-94%, $p<0.05$; Fig. 2). Lemon-seasoning dietary habits have already been showed to influence significantly the bioaccessibility of trace metals in seafood for humans. For instance, Houlbrèque et al. (2011) observed a significantly higher bioaccessible fraction of Cd (68.1 ± 4.4%) in lemon-seasoned mussels *Mytilus chilensis* contaminated via a similar dissolved pathway than in cooked mussels (42.4±5.5%). This higher bioaccessibility related to the lemon-seasoned samples results probably from the accelerated, acidic lyse of the oyster cell membranes and organelles prior the digestion *per se*. Interestingly, lemon-seasoning seems to increase the nutritional benefit of oysters (increase in bioaccessible oligo-elements Mn and Zn) while it also increases their potential toxicity through increased bioaccessibility of Cd.

In conclusion, the present study showed that the mangrove oyster *C. gasar* concentrates efficiently all five studied elements. The rather fast depuration pattern observed for Ag in *C. gasar* differs from the one of the other four elements and from its behavior generally observed in other oysters. Such pattern might be of relevance in coastal contamination assessments. Unusual high Ag levels in *C. gasar* might reflect recent events whereas the other elements might rather help surveying chronic contamination. This study further provides better understanding on the risk related to *C. gasar* consumption. Dietary habits such as seasoning raw oysters with lemon before consumption may provide a nutritional benefit for essential elements such as Mn and Zn, but, on the other hand, may pose increased risk to the consumers of the mangrove oyster especially for non-essential metals such as Cd.

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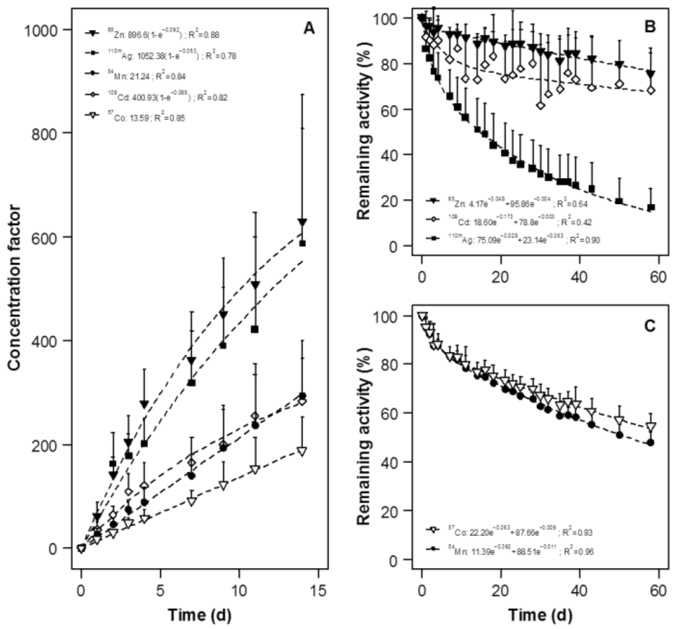
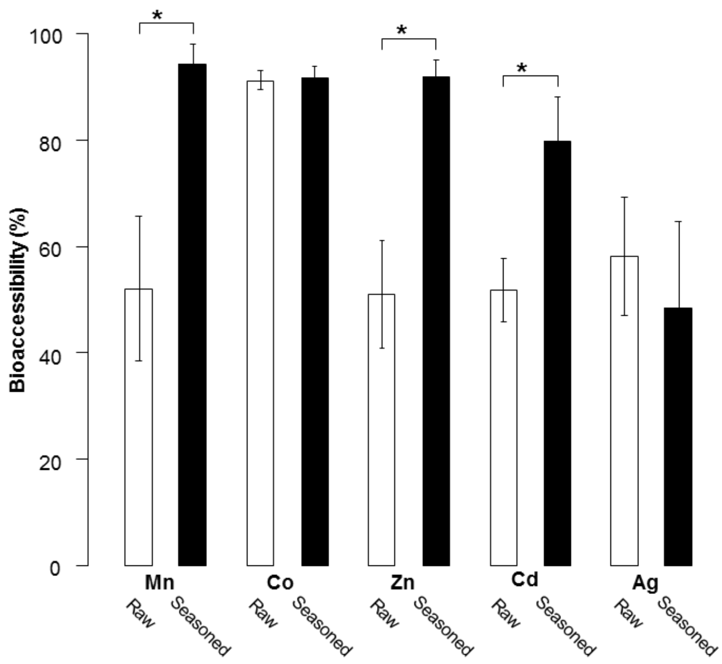
# Caption to figures

Figure 1. Uptake and depuration kinetics of dissolved $^{65}Zn$, $^{109}Cd$, $^{110m}Ag$ [A, B], $^{54}Mn$ and $^{57}Co$, [A, C] in the mangrove oyster *Crassostrea gasar* exposed for 14 d to radiolabelled seawater (Concentration factors, mean ± SD; n = 10), and then maintained in non-contaminated conditions for 58 d (remaining activity, %; mean ± SD; n = 15).

Figure 2. Bioaccessibility (%) of $^{54}Mn$, $^{57}Co$, $^{65}Zn$, $^{109}Cd$ and $^{110m}Ag$ (mean ± SD, n=4) in raw and lemon-seasoned mangrove oyster *Crassostrea gasar*.

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*
*
100
80
60
40
20
0
Bioaccessibility (%)
Mn
Co
Zn
Cd
Ag
Raw
Seasoned
Raw
Seasoned
Raw
Seasoned
Raw
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Raw
Seasoned