

1 **Bioconcentration of Ag, Cd, Co, Mn and Zn in**  
2 **the mangrove oyster (*Crassostrea gasar*) and**  
3 **preliminary human health risk assessment: a**  
4 **radiotracer study**

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22 Abstract: Bioaccumulation kinetics of 5 dissolved metals were determined in the mangrove oyster  
23 *Crassostrea gasar*, using corresponding radiotracers (<sup>54</sup>Mn, <sup>57</sup>Co, <sup>65</sup>Zn, <sup>109</sup>Cd and <sup>110m</sup>Ag).  
24 Additionally, their bioaccessibility to human consumers was estimated. Results indicated that over  
25 a 14-d exposure <sup>54</sup>Mn and <sup>57</sup>Co were linearly concentrated in oysters whereas <sup>109</sup>Cd, <sup>65</sup>Zn and  
26 <sup>110m</sup>Ag were starting to saturate (steady-state not reached). Whole-body concentration factors at  
27 14d (CF<sub>14d</sub> *in toto*) ranged from 187±65 to 629±179 with the lowest bioconcentration capacity for  
28 Co and the highest for Ag. Depuration kinetics were best described by a double-exponential model  
29 with associated biological half-lives ranging from 26 days (Ag) to almost 8 months (Zn and Cd).  
30 Bioaccessible fraction of the studied elements was estimated using *in vitro* digestions, which  
31 suggested that oysters consumed seasoned with lemon enhanced the accessibility of Cd, Mn and  
32 Zn to human consumers, but not Ag and Co.

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

## 34 Introduction

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

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

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

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

## 64 Materials and Methods

65 In September 2013, 100 mangrove oysters *Crassostrea gasar*, collected by  
66 handpicking on the shores of Abidjan (Côte d'Ivoire), were transported to IAEA-  
67 EL premises in Monaco. They were acclimated to laboratory conditions for 4  
68 weeks prior to the experiment (constantly aerated, open-circuit, 300-L tank; flux:  
69 150 L h<sup>-1</sup>; salinity: 20±1 p.s.u.; temperature: 25 ± 0.5 °C; pH: 8.0±0.1; light/dark  
70 cycle: 12 h/12 h). During the period of acclimation and throughout the  
71 experiment, the oysters were fed daily on a mixed diet of phytoplankton  
72 (*Isochrysis galbana* and *Skeletonema costatum*) with algal densities ranging from  
73 10<sup>4</sup> to 10<sup>5</sup> cell mL<sup>-1</sup>.

74 Twenty individuals of similar size (shell length: 62±6 mm and wet weight:  
75 28.9±5.5 g) were tag-identified and placed in a 70-L closed circuit glass aquarium

76 filled with 0.2- $\mu\text{m}$  filtered seawater (same conditions as above). The seawater was  
77 spiked with 0.45 kBq  $^{54}\text{Mn L}^{-1}$  (as  $\text{MnCl}_2$ , in 0.1M HCl,  $T_{b1/2} = 312.2\text{d}$ ), 0.15 kBq  
78  $^{57}\text{Co L}^{-1}$  (as  $\text{CoCl}_2$  in 0.1 M HCl,  $T_{b1/2} = 271.8\text{ d}$ ), 0.23 kBq  $^{65}\text{Zn L}^{-1}$  (as  $\text{ZnCl}_2$  in  
79 0.5 M HCl,  $T_{b1/2} = 243.9\text{d}$ ), 0.95 kBq  $^{109}\text{Cd L}^{-1}$  (as  $\text{CdCl}$  in 0.1 M HCl,  $T_{b1/2} =$   
80 426.6d) and 0.51 kBq  $^{110\text{m}}\text{Ag L}^{-1}$  (as  $\text{AgNO}_3$  in 1 M  $\text{HNO}_3$ ,  $T_{b1/2} = 249.8\text{d}$ ).  
81 Oysters were then exposed to the tracers for a period of 14 d. Seawater and spikes  
82 were renewed each day for the first five days and then every second day in order  
83 to keep radioactivity in seawater as constant as possible. In terms of stable metal  
84 equivalent, each spike corresponded to an addition of 10 ng/L of Zn, 130 ng/L of  
85 Cd and 2 ng/L of Ag (i.e. concentrations that are lower than the background  
86 concentrations of these metals in open sea; Bruland 1983). No change in pH and  
87 salinity was detectable after radiotracer additions. Water samples were collected  
88 before and after each water renewal, and  $\gamma$ -counted to determine the time-  
89 integrated activities in water (Warnau et al. 1996; Rodriguez y Baena et al. 2006)  
90 and the organisms were briefly fed for 30 min during the water renewal step  
91 (same microalgae species and density than during acclimation phase). At different  
92 time intervals, 10 tag-identified individuals were  $\gamma$ -counted alive to determine  
93 uptake kinetics of the radiotracers. At the end of the 14-d exposure period,  
94 radiolabelled oysters were transferred into a new, constantly aerated, 70-L  
95 aquarium (flux: 50 L  $\text{h}^{-1}$ ; other conditions as previously described) and were  
96 allowed to depurate for a period of 58 d. Oysters were fed daily and  $\gamma$ -counted  
97 hereto at different times to determine the depuration kinetics of the radiotracers.  
98 Radioanalyses were carried out using a high-resolution  $\gamma$ -spectrometer system  
99 composed of 5 Germanium – N or P type – detectors (EGNC 33-195-R,  
100 Canberra<sup>®</sup> and Eurysis<sup>®</sup>) connected to a multichannel analyzer and a computer  
101 equipped with a spectra analysis software (Interwinner<sup>®</sup> 6).

102 At the end of the 58-d depuration period, 8 oysters were randomly collected and  
103 edible parts (i.e. whole soft parts) were removed. Four of them were used as is  
104 (defined as “raw”) whereas the 4 remaining edible parts were seasoned with  
105 lemon juice (2 mL per oyster for an action time of 30 seconds) in order to assess  
106 effect of seasoning on bioaccessible fraction of the studied elements. Right after,  
107 *in vitro* digestions were performed on each individual raw and seasoned soft-part  
108 to assess the bioaccessible fraction of elements for human consumers of oysters,  
109 following the method described by Versantvoort et al. (2005) and adapted for  
110 radiotracer by Metian et al. (2009a). Briefly, homogenized oyster tissues were  
111 exposed step by step to artificial saliva, gastric juice and mixture of duodenal  
112 juice, bile and  $\text{NaHCO}_3$  (chemicals and enzymes were purchased from Sigma<sup>®</sup>).  
113 Following the *in vitro* digestion, the resulting chyme was centrifuged and the  
114 radiotracer activities were counted in supernatant, which is considered as  
115 containing the bioaccessible fraction (Versantvoort et al. 2005).

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

123

124  $CF_t = k_u t$  (Eq. 1)

125  $CF_t = CF_{ss} (1 - e^{-k_e t})$  (Eq. 2)

126

127 where  $CF_t$  and  $CF_{ss}$  are the bioconcentration factors at time  $t$  (d) and at steady  
128 state, respectively;  $k_u$  and  $k_e$  are the uptake and depuration rate constants ( $d^{-1}$ ),  
129 respectively.

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

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

136 where  $A_t$  and  $A_0$  are the remaining activities (%) at time  $t$  (d) and 0, respectively;  
137  $k_e$  is the depuration rate constant ( $d^{-1}$ ); 's' and 'l' are the subscripts for the 'short-  
138 lived' and 'long-lived' components respectively. The short- and long-lived  
139 components biological half-life ( $T_{b/2s}$  and  $T_{b/2l}$ ) can be calculated ( $T_{b/2s}$  and  $T_{b/2l}$ )  
140 from the corresponding depuration rate constants ( $k_{es}$  and  $k_{el}$ , respectively)  
141 according to the relation  $T_{b/2} = \ln 2 / k_e$  (Warnau et al. 1996).

142 Whole-body uptake and depuration kinetics parameters were determined through  
143 iterative adjustment of the model using the nonlinear curve-fitting routines in the  
144 Statistica<sup>®</sup> software 5.2.1 and statistical methods described by Warnau et al.  
145 (1996) and Hédouin et al. (2010a). Criteria used for selecting best fitting models  
146 were the coefficient of determination ( $R^2$ ) and results from an ANOVA on  
147 residuals (Metian et al. 2015).

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

## 152 Results and Discussion

153 Figure 1A displays the whole-body uptake kinetics of the studied radiotracers.  
154 Metals were readily accumulated by oysters, with contrasting patterns:  
155 bioconcentration of  $^{54}\text{Mn}$  and  $^{57}\text{Co}$  was best fitted using a linear model ( $R^2 \geq$   
156 0.84), whereas bioconcentration of  $^{65}\text{Zn}$ ,  $^{109}\text{Cd}$  and  $^{110m}\text{Ag}$  was best described by a  
157 saturation exponential model ( $R^2 \geq 0.78$ ).

158 For these latter elements, the estimated bioconcentration factors at steady state  
159 ( $CF_{ss}$ ) were  $1052 \pm 47$  ( $^{110m}\text{Ag}$ ;  $p < 0.001$ ),  $897 \pm 224$  ( $^{65}\text{Zn}$ ;  $p < 0.001$ ) and  $401 \pm 231$   
160 ( $^{109}\text{Cd}$ ;  $p > 0.05$ ). Using CFs observed in the whole organisms at the end of the  
161 exposure period ( $CF_{14d}$  *in toto*), radiotracer bioavailability can be ranked as  $^{65}\text{Zn}$

162  $(629 \pm 179) = {}^{110m}\text{Ag} (587 \pm 288) > {}^{109}\text{Cd} (283 \pm 82) = {}^{54}\text{Mn} (294 \pm 105) > {}^{57}\text{Co}$   
163  $(187 \pm 65)$ . Concurrently, similar trends were observed for the derived uptake rate  
164 constants ( $k_u$ ). The uptake rate constant values were  $72.3 \pm 7.9 \text{ d}^{-1}$  (Zn),  $55.9 \pm 9.7 \text{ d}^{-1}$   
165 (Ag),  $34.2 \pm 4.7 \text{ d}^{-1}$  (Cd),  $21.2 \pm 0.8 \text{ d}^{-1}$  (Zn) and  $13.6 \pm 0.5 \text{ d}^{-1}$  (Co). In previous  
166 experimental studies investigating similar elements uptake kinetics in tropical  
167 bivalves, Cd, Zn, and Ag are generally the elements most rapidly and highly  
168 bioconcentrated (e.g. Metian et al. 2008b; Hédouin et al. 2010a). This can be  
169 attributed to their strong affinity for sulphur-containing proteins such as  
170 metallothioneins, which facilitate their transport across biological membranes. In  
171 contrast Mn and Co are transported by passive diffusion (Wang and Dei 1999).

172 After 58d of depuration, whole-body depuration kinetics were all best described  
173 by a double-component exponential model (Fig. 1B & 1C).  ${}^{54}\text{Mn}$ ,  ${}^{57}\text{Co}$ ,  ${}^{65}\text{Zn}$  and  
174  ${}^{109}\text{Cd}$  were efficiently absorbed ( $A_{01} > 78\%$ ), whereas  ${}^{110m}\text{Ag}$  was less ( $A_{01} =$   
175  $23 \pm 5\%$ ). Metal absorption capacities in tropical oysters have been already shown.  
176 For example, absorption efficiencies over 74% have been described in the oysters  
177 *Isognomon isognomon* and *Malleus regula* for Ag, Cd, Co, Cr and Zn (Hédouin et  
178 al. 2010a). The latter result for Ag (much higher than the 23% measured in *C.*  
179 *gasar*) suggests the occurrence of different processes of accumulation and/or  
180 storage of Ag among tropical oyster species.

181 Dissolved Ag integrated in *C. gasar* was rapidly lost compared to other elements  
182 ( $T_{b/2l}$  of  $25 \pm 3 \text{ d}$  for  ${}^{110m}\text{Ag}$  vs.  $259 \pm 259 \text{ d}$ ,  $63 \pm 2 \text{ d}$ ,  $82 \pm 4 \text{ d}$ ,  $187 \pm 19 \text{ d}$  for  ${}^{109}\text{Cd}$ ,  
183  ${}^{54}\text{Mn}$ ,  ${}^{57}\text{Co}$  and  ${}^{65}\text{Zn}$ , respectively), although it is usually known to be strongly  
184 retained by bivalves (e.g. Metian et al. 2008a; Hédouin et al. 2010a). Some  
185 detoxification mechanisms protecting against Ag intoxication are well  
186 documented in bivalves such as binding to metallothioneins (Bebiano and  
187 Langston, 1993) or storage as  $\text{Ag}_2\text{S}$  (very stable amorphous compound; e.g.  
188 Berthet et al., 1992), and thus *C. gasar* could have a less efficient detoxification  
189 mechanism than other bivalves against Ag toxicity.

190 The overall results of the simulated *in vitro* digestion experiments showed that the  
191 bioaccessible fraction of the metals in mangrove oysters varied from 51% (Mn in  
192 raw oysters) to 94% (Mn in lemon-seasoned oysters; Fig. 2). Our results also  
193 indicate that oysters seasoned have significantly higher bioaccessible fraction of  
194 Cd, Mn and Zn than raw oysters (respectively 51-52% vs. 80-94%,  $p < 0.05$ ; Fig.  
195 2). Lemon-seasoning dietary habits have already been showed to influence  
196 significantly the bioaccessibility of trace metals in seafood for humans. For  
197 instance, Houllbrèque et al. (2011) observed a significantly higher bioaccessible  
198 fraction of Cd ( $68.1 \pm 4.4\%$ ) in lemon-seasoned mussels *Mytilus chilensis*  
199 contaminated via a similar dissolved pathway than in cooked mussels  
200 ( $42.4 \pm 5.5\%$ ). This higher bioaccessibility related to the lemon-seasoned samples  
201 results probably from the accelerated, acidic lyse of the oyster cell membranes and  
202 organelles prior the digestion *per se*. Interestingly, lemon-seasoning seems to  
203 increase the nutritional benefit of oysters (increase in bioaccessible oligo-elements  
204 Mn and Zn) while it also increases their potential toxicity through increased  
205 bioaccessibility of Cd.

206 In conclusion, the present study showed that the mangrove oyster *C. gasar*  
207 concentrates efficiently all five studied elements. The rather fast depuration

208 pattern observed for Ag in *C. gasar* differs from the one of the other four elements  
209 and from its behavior generally observed in other oysters. Such pattern might be  
210 of relevance in coastal contamination assessments. Unusual high Ag levels in *C.*  
211 *gasar* might reflect recent events whereas the other elements might rather help  
212 surveying chronic contamination. This study further provides better understanding  
213 on the risk related to *C. gasar* consumption. Dietary habits such as seasoning raw  
214 oysters with lemon before consumption may provide a nutritional benefit for  
215 essential elements such as Mn and Zn, but, on the other hand, may pose increased  
216 risk to the consumers of the mangrove oyster especially for non-essential metals  
217 such as Cd.

218

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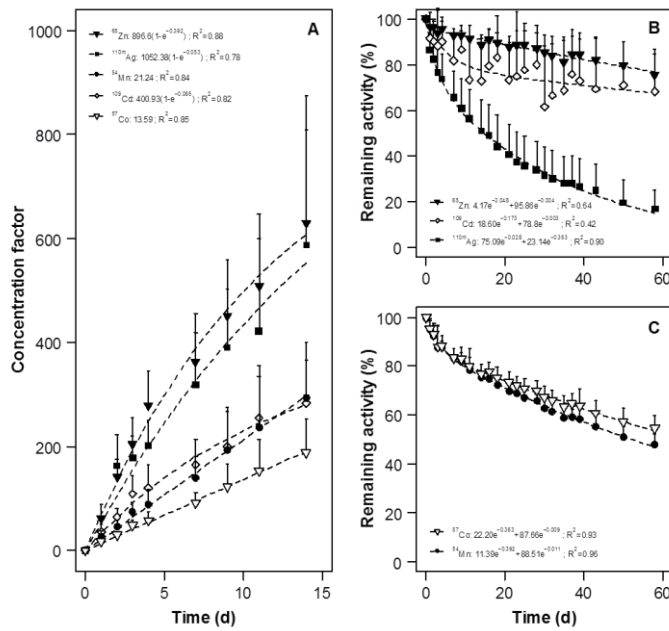
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286 **Caption to figures**

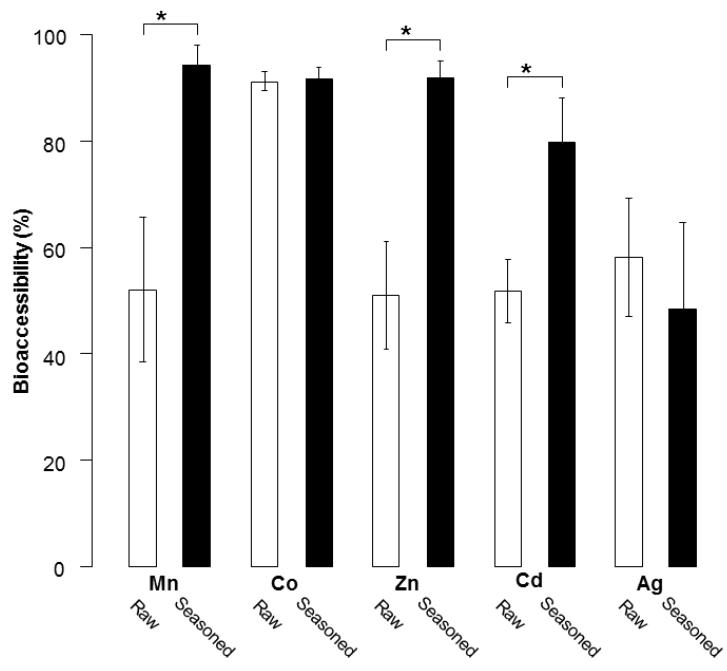
287 Figure 1. Uptake and depuration kinetics of dissolved  $^{65}\text{Zn}$ ,  $^{109}\text{Cd}$ ,  $^{110\text{m}}\text{Ag}$  [A, B],  $^{54}\text{Mn}$  and  $^{57}\text{Co}$ ,  
 288 [A, C] in the mangrove oyster *Crassostrea gasar* exposed for 14 d to radiolabelled seawater  
 289 (Concentration factors,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

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



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294