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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**

5 Harriet Kuranchie-Mensah ^{1,2}, Jean-Louis Teysseyé ¹, François Oberhänsli ¹,
6 Yutthana Tumnoi ³, Simon Pouil ¹, Michel Warnau ¹, Marc Metian ¹

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

9 ² *Nuclear Chemistry and Environmental Research Centre, National Nuclear*
10 *Research Institute, Ghana Atomic Energy Commission, P. O. Box LG 80 Legon-*
11 *Accra, Ghana*

12 ³ *Bureau of Technical Support for Safety Regulation, Office of Atoms for*
13 *Peace, Chatuchak, Bangkok 10900, Thailand*

14
15 Telephone: +377 97 97 72 17

16 E-mail: m.metian@iaea.org

17

18 * *Corresponding author: Dr Marc Metian, Radioecology Laboratory, IAEA*
19 *Environment Laboratories, 4a Quai Antoine 1er, MC-98000 Principality of*
20 *Monaco*

21

22 Abstract: Bioaccumulation kinetics of 5 dissolved metals were determined in the mangrove oyster
23 *Crassostrea gasar*, using corresponding radiotracers (⁵⁴Mn, ⁵⁷Co, ⁶⁵Zn, ¹⁰⁹Cd and ^{110m}Ag).
24 Additionally, their bioaccessibility to human consumers was estimated. Results indicated that over
25 a 14-d exposure ⁵⁴Mn and ⁵⁷Co were linearly concentrated in oysters whereas ¹⁰⁹Cd, ⁶⁵Zn and
26 ^{110m}Ag were starting to saturate (steady-state not reached). Whole-body concentration factors at
27 14d (CF_{14d} *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⁻¹; 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
73 10⁴ to 10⁵ cell mL⁻¹.

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- μm filtered seawater (same conditions as above). The seawater was
77 spiked with 0.45 kBq $^{54}\text{Mn L}^{-1}$ (as MnCl_2 , in 0.1M HCl, $T_{b1/2} = 312.2\text{d}$), 0.15 kBq
78 $^{57}\text{Co L}^{-1}$ (as CoCl_2 in 0.1 M HCl, $T_{b1/2} = 271.8\text{ d}$), 0.23 kBq $^{65}\text{Zn L}^{-1}$ (as ZnCl_2 in
79 0.5 M HCl, $T_{b1/2} = 243.9\text{d}$), 0.95 kBq $^{109}\text{Cd L}^{-1}$ (as CdCl in 0.1 M HCl, $T_{b1/2} =$
80 426.6d) and 0.51 kBq $^{110\text{m}}\text{Ag L}^{-1}$ (as AgNO_3 in 1 M 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 γ -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 γ -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 h^{-1} ; other conditions as previously described) and were
96 allowed to depurate for a period of 58 d. Oysters were fed daily and γ -counted
97 hereto at different times to determine the depuration kinetics of the radiotracers.
98 Radioanalyses were carried out using a high-resolution γ -spectrometer system
99 composed of 5 Germanium – N or P type – detectors (EGNC 33-195-R,
100 Canberra[®] and Eurysis[®]) connected to a multichannel analyzer and a computer
101 equipped with a spectra analysis software (Interwinner[®] 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 NaHCO_3 (chemicals and enzymes were purchased from Sigma[®]).
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[®] 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}Mn and ^{57}Co was best fitted using a linear model ($R^2 \geq$
156 0.84), whereas bioconcentration of ^{65}Zn , ^{109}Cd and ^{110m}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 ± 47 (^{110m}Ag ; $p < 0.001$), 897 ± 224 (^{65}Zn ; $p < 0.001$) and 401 ± 231
160 (^{109}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}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 ± 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 Ag_2S (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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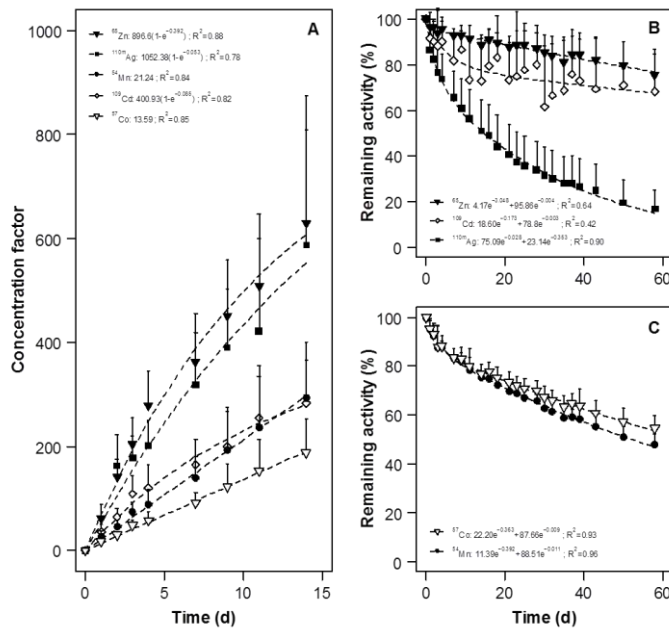
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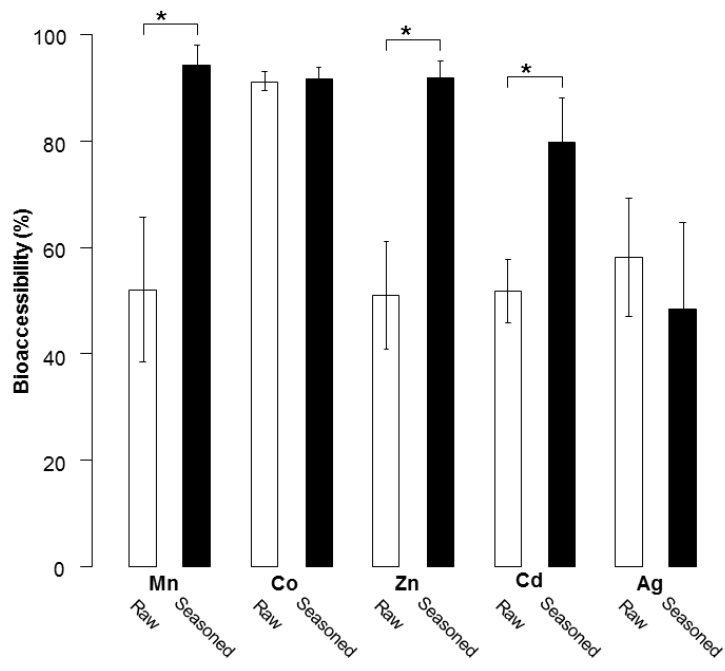
286 **Caption to figures**

287 Figure 1. Uptake and depuration kinetics of dissolved ^{65}Zn , ^{109}Cd , $^{110\text{m}}\text{Ag}$ [A, B], ^{54}Mn and ^{57}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}Mn , ^{57}Co , ^{65}Zn , ^{109}Cd and $^{110\text{m}}\text{Ag}$ (mean \pm SD, n=4) in raw and
 292 lemon-seasoned mangrove oyster *Crassostrea gasar*.



293



294