

A mechanistic approach to evaluate the influence of Cd and Zn on ^{57}Co , $^{110\text{m}}\text{Ag}$ and ^{134}Cs accumulation and depuration by a freshwater mussel (*Dreissena polymorpha*)

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Abstract. The effects of Cd and Zn on $^{110\text{m}}\text{Ag}$, ^{57}Co and ^{134}Cs uptake and depuration in the freshwater mussel *Dreissena polymorpha* were investigated under laboratory controlled conditions. Differences at the whole body level were identified between groups exposed to radionuclides alone or to radionuclides and metals under different conditions (Cd or Zn alone or a mixture of Cd and Zn). The accumulation of ^{57}Co was reduced in the presence of Zn, at the whole organism level and in the soft body. On the opposite, ^{134}Cs increased in the presence of Cd or Zn. Finally, $^{110\text{m}}\text{Ag}$ uptake decreased in the presence of both metals in the whole organism but increased in the soft tissues. Two hypotheses related to the major mechanisms governing the influence of metals on radionuclides were tested. First, the uptake was hypothesised to be directly related to the filtration rate, which has been shown to decrease with increasing zinc concentrations. On the other hand, whole organism zinc regulation mechanisms may include the radioactive pollutant as well. None of these two hypotheses could be validated. Consequently, further explanations were provided to link metal exposure to the radionuclide uptake by the bivalves.

1 INTRODUCTION

The radioecological assessment concerning radioisotopes present in low-level radioactive liquid effluents released by nuclear power plants during normal operating conditions has been an object of recent intensive research. Most of this research has been focused on the accumulation levels and biological half-lives of individual radionuclides by various biological models. The rationale was that performing experiments in standard controlled conditions enhances reproducibility and allows a better understanding of the complexity of the involved biological and chemical processes. Such procedures are a vital step for the assessment of radionuclide impact on aquatic organisms. Nonetheless, in the liquid effluents, radionuclides are present as mixtures; for example, 8 gamma emitting radionuclides (except tritium) have been identified in the case of Pressurised Water Reactors. Moreover, the radionuclides are not only released as mixtures but, in the receiving waters, many other organic or metallic micropollutants may be present at significant concentrations.

Following measurements performed on biota near Handford reactor or after nuclear testings in the Pacific, showing high ^{65}Zn concentrations in fish, a few number of studies were conducted to evaluate the influence of Zn and Cd on ^{65}Zn uptake by fish. Results were contradictory. Merlini *et al.* [1] found an inhibition of ^{65}Zn uptake by perch with 40 $\mu\text{g/L}$ of cadmium, while Pally and Foulquier [2] showed that the concentration factor of ^{65}Zn in *Anguilla anguilla* was doubled when Cd concentrations were increased from 18 to 50 $\mu\text{g/L}$. For the same species, they observed that ^{65}Zn transfer decreased by a factor of 2 to 3 in the presence of 7.6 mg/L of stable Zn [3]. However, a detailed interpretation of these results is difficult since the stable Cd and Zn concentrations used were not representative of environmental conditions and were well above the guideline standards for surface water quality intended for the production of drinking water (e.g. 1 $\mu\text{g Cd/L}$ and 500 $\mu\text{g Zn/L}$ in France). Recently, only the work of Sugg *et al.* [4, 5] and Jagoe *et al.* [6] have focused on *in situ* measurements of stable metals (Hg, Pb) and radioactive pollutants (^{137}Cs) in fish in the cooling pond of the Chernobyl power plant without, however, seeking to elucidate any possible interactions.

The study presented in this paper aims at evaluating the possible influence of cadmium and zinc chronic waterborne exposures on the kinetics of accumulation and elimination by the zebra mussel of radioactive isotopes of Co, Cs and Ag, all of major radioecological importance. This work is based on the hypothesis that beyond a certain ecotoxicological dose (medium concentration and exposure duration),

exposure to various pollutants induces physiological disruptions that subsequently generate alterations in the uptake characteristics of radionuclides by the organism. The zebra mussel (*Dreissena polymorpha*) was chosen as a biological model because it has been extensively used in biomonitoring programs and ecotoxicological studies. Moreover, given the high population densities often encountered (ranging from hundreds to thousands of individuals/m²), the zebra mussel can represent an important route of radionuclide trophic transfer to fish with pharyngeal teeth capable of crushing mollusc shells, as bream or roach [7]. It must be underlined that the accuracy of the radionuclide transfer kinetic rates is of major importance for that species because of its current use as a bioindicator of radioactive contamination within biomonitoring programs.

2 MATERIAL AND METHODS

2.1 Experimental procedures

Dreissena polymorpha were obtained from Lake Geneva (Haute-Savoie, France). The average wet weight and length of the mussels were 1.44 ± 0.31 g and 2.25 ± 0.17 cm respectively. For 15 days, specimens were acclimated progressively to the experimental conditions and in particular to the chemical characteristics of the water used for the experiments. During this phase, they were fed *ad libitum* with unicellular algae (*Scenedesmus obliquus*) whereas during the experiments they were starved.

60 specimens were placed on a grid in 8 litres of water in plastic tanks. The water used was commercial spring water (Ogeu, France) with a stable physical and chemical composition ($49 \text{ mg L}^{-1} \text{ Ca}^{2+}$, $31 \text{ mg L}^{-1} \text{ Na}^+$, $11 \text{ mg L}^{-1} \text{ Mg}^{2+}$, $1 \text{ mg L}^{-1} \text{ K}^+$, $183 \text{ mg L}^{-1} \text{ HCO}_3^-$, $16 \text{ mg L}^{-1} \text{ SO}_4^{2-}$, $14 \text{ mg L}^{-1} \text{ NH}_4^+$, $44 \text{ mg L}^{-1} \text{ Cl}^-$; pH 7.94) and in which the zinc background concentration was about $10 \text{ } \mu\text{g L}^{-1}$ and the cadmium concentration less than $0.02 \text{ } \mu\text{g L}^{-1}$. During the experiment, the water temperature was maintained at 16 ± 0.3 °C and the mean oxygenation was at 75 ± 4 % of saturation. The daily photoperiod was fixed at 12hr.

The study was designed to simulate a situation that might occur *in situ*, i.e. the discharge by a nuclear facility of radioactive liquid effluent into a freshwater ecosystem chronically contaminated by metallic pollutants. Three stable metal contamination conditions were chosen: $4 \text{ } \mu\text{g L}^{-1}$ of Cd and no Zn (Cd group); $250 \text{ } \mu\text{g L}^{-1}$ of Zn and no Cd (Zn group); $4 \text{ } \mu\text{g L}^{-1}$ of Cd and $250 \text{ } \mu\text{g L}^{-1}$ of Zn (Cd/Zn group). For these three conditions and the control group (no addition of Cd and Zn) two replicates were prepared. The cadmium (Standal, Analys) and zinc (Titrisol, Merck) stock solutions were in the form of bichloride. The radionuclide activities for the radioactive contamination of the water were $5 \times 10^3 \text{ Bq L}^{-1}$ for the ^{113m}Ag and $30 \times 10^3 \text{ Bq L}^{-1}$ for both ⁵⁷Co and ¹³⁴Cs. Taking into account the concentration of the carrier (stable element accompanying the radioactive isotope), the total element concentrations were $0.25 \text{ } \mu\text{g L}^{-1}$ of Ag, 1.3 ng L^{-1} of Co and $1.7 \text{ } \mu\text{g L}^{-1}$ of Cs. The radionuclides, obtained from the Amersham International Radiochemical Center (UK), were added in the form of CsCl₂, CoCl₂ and AgNO₃.

The experiment was carried out over a 31-day period and divided in three consecutive phases during which the water was continually contaminated by the two metals (Zn and/or Cd). The first phase (metallic pollutant(s) alone), lasting 17 days, was designed to constitute different groups of organisms characterized by possible physiological disturbances resulting from the presence of Cd and/or Zn. The second phase (7 days) was devoted to study mollusc uptake of added radionuclides. The third phase (7 days), which represented a return to the initial situation (stable metals alone), was designed to monitor the radionuclide depuration by the mussels. During the experiment, in order to simulate chronic contamination and to maintain its main physical and chemical characteristics constant, the whole medium (water and added pollutants) was renewed daily.

2.2 Sampling and chemical analyses

Three times a day, pH, conductivity, dissolved O₂ and temperature were measured in every experimental unit. Water samples were taken before and after the renewal of the medium and immediately acidified with 2% HNO₃. Cadmium was determined with a graphite furnace AAS with Zeeman correction (4110 ZL, Perkin Elmer; detection limit of $0.1 \text{ } \mu\text{g L}^{-1}$) and zinc with a flame AAS with deuterium correction (Spectro AA 200, Varian; detection limit of $10 \text{ } \mu\text{g L}^{-1}$).

During the radionuclide uptake and depuration phases, the radioactivity of eight individuals from each tank was measured daily by whole-body counting of the gamma emissions for two minutes. The individuals were removed from the tank, rinsed, blotted dry with tissue paper, weighed and placed in a counting tube. Radioactivity measurements were performed with a single-channel analyser connected to a thallium-activated sodium iodide well probe (Ortec). After counting, each mollusc was returned to the tank. At the end of the 31-day experiment, mortality was only about 5% of the organisms for all treatment groups. At the end of the uptake phase, after whole-body counting, four mussels from each group were sacrificed in order to measure metals and radionuclides in the soft body. The soft bodies of two organisms were pooled in a single counting tube to improve the accuracy of the measurements. Each sample was mineralised in a glass tube with a screw stopper (HNO₃ 65%, 3 hours, 105°C, Blockdigest). Digested samples were then diluted with deionised water up to a volume of 20 mL, before being analysed for Cd, Zn and radionuclides.

2.3 Kinetic modelling approach

By combining kinetic modelling and experimental data it is possible to evaluate the relevance of different hypotheses on the major factors controlling metal influence on radionuclide bioaccumulation. The first step is to compare the uptake and depuration kinetic rates characterising the radionuclide transfer to the organisms, to evaluate if Cd and/or Zn contamination leads to a significant decrease or increase of each kinetic rate. The time-course radionuclide concentrations in the mussel is assumed to follow a simple first order differential equation :

$$\frac{dC_{mussel}}{dt} = u.C_{water} - (d+G).C_{mussel} \quad (1)$$

where C_{mussel} represents the radionuclide concentration in the whole organism (Bq g⁻¹ w.w.), C_{water} , the radionuclide concentration in the water (Bq mL⁻¹), t , the time of exposure (days), u (mL g⁻¹ d⁻¹) and d (d⁻¹) are the uptake and depuration kinetic rates respectively and G , the individual growth rate (d⁻¹). For each metal-exposure condition, the kinetic parameters u and d were estimated by numerical calculation (Runge-Kutta 4th order) using the computer program ModelMaker 3.0 (Cherwell Scientific, UK). On the basis of the statistical differences evidenced between the kinetic rates, further mechanistic hypotheses can be developed, by dissociating the parameters u or d .

3 RESULTS AND DISCUSSION

3.1 Effect of metals on radionuclide accumulation

Among treatments with the metals, ⁵⁷Co and ^{110m}Ag on day 24 declined in the whole organisms with the addition of Cd and/or Zn (Table 1). On the opposite, ¹³⁴Cs concentrations increased in metal exposed groups. With regards to the soft tissues, ⁵⁷Co concentrations decreased with Zn addition. In the case of ^{110m}Ag and ¹³⁴Cs, the trend was reversed, since radionuclide concentrations in the soft tissues increased with the addition of Cd and/or Zn.

3.2 Testing different assumptions for involved mechanisms through modelling

3.2.1 Global kinetic model

For ¹³⁴Cs, no obvious differences were observed between the kinetic parameters obtained for the control and the metal contaminated groups (Table 2), except for the uptake parameter u characterising the Cd exposed group. For ⁵⁷Co, the two groups contaminated by Zn were characterised by a decrease of the uptake kinetic parameter u . For ^{110m}Ag, an increase of the parameter u was noted for the Cd/Zn group and of the parameter d for the three metal contaminated groups.

3.2.2 Influence of the filtration rate

A possible explanation of the change in radionuclide uptake is that the filtration rate is modified in the presence of metals. A reduction in filtering activity might be expected to reduce radionuclide uptake,

particularly in the soft tissues. The opposite trend might be expected if the filtration rate was increased in the presence of Cd and/or Zn.

The filtration rates corresponding to each Zn condition were estimated during other experiments using fixed-volume clearance experiments. For the control group, the measured filtration rate was $2041 \text{ mL g}^{-1} \text{ d}^{-1}$ while for the group exposed to $250 \mu\text{g Zn L}^{-1}$, the value decreased to $1355 \text{ mL g}^{-1} \text{ d}^{-1}$. These values were close to those determined by Kraak *et al.* [8] for the same species. In the case of Cd, no difference in the filtration rates were observed by the same authors within the range of Cd concentrations considered in the present paper. Under these hypotheses, the reduced ^{57}Co uptake could be explained by a reduction of the filtration rate. However, reduced uptake of all the radionuclides at higher Zn concentrations would have been expected. On the contrary, silver and caesium concentrations in the soft body increased with metal addition. Furthermore, a reduced uptake would be expected for both Zn and Cd, which is not the case (Table 1).

Table 1: Concentrations of radionuclides in the whole organisms (shell+soft part) and soft tissues (wet weight), for the four metallic contamination conditions at the end of the radionuclide contamination phase (day 24). Values are means \pm S.D. (8 to 15 individuals for the whole organisms; 4 replicates of two soft tissues pooled). * : value significantly different from that characterising the control group using Student's t-test ($\alpha=0.05$). n.d. : not determined.

Exposure condition	Zn ($\mu\text{g g}^{-1}$)	Cd (ng g^{-1})	^{57}Co (Bq g^{-1})	$^{110\text{m}}\text{Ag}$ (Bq g^{-1})	^{134}Cs (Bq g^{-1})
<u>Whole organism</u>					
Control	n.d.	n.d.	3513 ± 300	2183 ± 383	258 ± 32
Cd	n.d.	n.d.	3477 ± 239	$1875 \pm 295^*$	$305 \pm 44^*$
Zn	n.d.	n.d.	$2521 \pm 269^*$	$1852 \pm 394^*$	$328 \pm 43^*$
Cd/Zn	n.d.	n.d.	$2630.2 \pm 219.9^*$	$1859.9 \pm 312.9^*$	$319.0 \pm 46.3^*$
<u>Soft tissues</u>					
Control	15 ± 3	215 ± 54	460 ± 173	1960 ± 47	326 ± 76
Cd	15 ± 3	$1493 \pm 216^*$	557 ± 109	$4229 \pm 1412^*$	$430 \pm 49^*$
Zn	$45 \pm 4^*$	173 ± 21	280 ± 67	$4317 \pm 735^*$	$481 \pm 61^*$
Cd/Zn	$38 \pm 7^*$	$1192 \pm 254^*$	288 ± 38	$2849 \pm 608^*$	327 ± 93

Table 2: Radionuclide kinetic rates related to the uptake (u) and depuration (d) processes in *Dreissena polymorpha* for the different metal exposure groups. Values are mean \pm A.S.E. * : value significantly different from that of the control group.

Radionuclide	Exposure condition	u ($\text{mL g}^{-1} \text{ d}^{-1}$)	d (d^{-1})	R^2
^{57}Co	Control	22.3 ± 0.48	0.020 ± 0.003	0.995
	Cd	22.7 ± 0.44	0.021 ± 0.003	0.995
	Zn	$15.1 \pm 0.44^*$	0.025 ± 0.005	0.987
	Cd/Zn	$15.4 \pm 0.39^*$	0.024 ± 0.004	0.991
$^{110\text{m}}\text{Ag}$	Control	62.1 ± 4.0	0.032 ± 0.010	0.915
	Cd	66.0 ± 3.7	$0.055 \pm 0.010^*$	0.944
	Zn	72.3 ± 6.5	$0.064 \pm 0.015^*$	0.863
	Cd/Zn	$100.5 \pm 6.9^*$	$0.065 \pm 0.011^*$	0.912
^{134}Cs	Control	2.45 ± 0.20	0.14 ± 0.019	0.844
	Cd	$2.91 \pm 0.17^*$	0.15 ± 0.014	0.935
	Zn	2.64 ± 0.14	0.13 ± 0.012	0.943
	Cd/Zn	2.58 ± 0.16	0.12 ± 0.013	0.932

3.2.3 Assuming a co-regulation of radionuclides with Zn

The second approach used to deepen our understanding of metal-radionuclide interactions, aims to describe a possible competition phenomenon, based on the hypothesis of a co-regulation of radionuclides with Zn. The assumption required is that zinc acts as a biochemical analogue of one or all the radioisotopes studied.

A mass balance equation can be developed to describe the balance of radionuclides in mussels, based on the approach of Koulikov [9]:

$$\frac{dm_{mussel} \cdot C_{mussel}^R}{dt} = q_{in}^R - q_{ex}^R - \lambda_p \cdot m_{mussel} \cdot C_{mussel}^R \tag{2a}$$

$$\frac{dm_{mussel} \cdot C_{mussel}^{Zn}}{dt} = q_{in}^{Zn} - q_{ex}^{Zn} \tag{2b}$$

where $C_{mussel}^{R(Zn)}$ represents the radionuclide (Zn) concentration in the mussel, $q_{in}^{R(Zn)}$ and $q_{ex}^{R(Zn)}$, the radionuclide (Zn) ingestion and excretion rates, λ_p , the physical decay of the radionuclides and m_{mussel} the mussel mass (w.w.). Based on the co-regulation hypothesis, the ingestion and excretion rates can be expressed as:

$$q_{in}^R = q_{in}^{Zn} \cdot \frac{C_{water}^R}{C_{water}^{Zn}} \cdot \frac{\alpha_R}{\alpha_{Zn}} \tag{3 a}$$

$$q_{ex}^R = q_{ex}^{Zn} \cdot \frac{C_{mussel}^R}{C_{mussel}^{Zn}} \cdot \frac{\alpha_R}{\alpha_{Zn}} \tag{3 b}$$

where $\alpha_{R(Zn)}$ is the fraction of radionuclide (Zn) taken up from the dissolved phase through the biological barriers and $C_{water}^{R(Zn)}$, the radionuclide (Zn) concentration in the water.

Inserting Zn regulation ($\frac{dC_{mussel}^{Zn}}{dt} = 0$) and mussel exponential growth ($\frac{dm_{mussel}}{dt} = G \cdot m_{mussel}$), into eq. 2b:

$$q_{in}^{Zn} = q_{ex}^{Zn} + G \cdot m_{mussel} \cdot C_{mussel}^{Zn} \tag{4}$$

Finally, under the hypothesis of Zn regulation by excretion only, which is valid for invertebrates [10], into eq. 4 :

$$q_{in}^{Zn} = k_{w-m}^{Zn} \cdot m_{water} \cdot C_{water}^{Zn} \tag{5a}$$

$$q_{ex}^{Zn} = k_{w-m}^{Zn} \cdot m_{water} \cdot C_{water}^{Zn} - G \cdot m_{mussel} \cdot C_{mussel}^{Zn} \tag{5b}$$

where k_{w-m}^{Zn} is the kinetic constant of Zn exchange between water and mussel.

Inserting eq. 5a and 5b for ingestion and excretion rates into eq. 2a transforms the differential equation into:

$$\frac{dC_{mussel}^R}{dt} = \left(\frac{\alpha_R}{\alpha_{Zn}} \cdot \frac{m_{water}}{m_{mussel}} \cdot k_{w-m}^{Zn} \right) \cdot C_{water}^R - \left(\frac{\alpha_R}{\alpha_{Zn}} \cdot \frac{m_{water}}{m_{mussel}} \cdot k_{w-m}^{Zn} \cdot \frac{C_{water}^{Zn}}{C_{mussel}^{Zn}} \cdot \frac{\alpha_R}{\alpha_{Zn}} \cdot G + \lambda_p + G \right) \cdot C_{mussel}^R \tag{6}$$

In the case of this experiment, data are corrected of the physical decay and no growth was evidenced; consequently, eq. 6 simplifies into:

$$\frac{dC_{mussel}^R}{dt} = \left(\frac{\alpha_R}{\alpha_{Zn}} \cdot \frac{m_{water}}{m_{mussel}} \cdot k_{w-m}^{Zn} \right) \cdot C_{water}^R - \left(\frac{\alpha_R}{\alpha_{Zn}} \cdot \frac{m_{water}}{m_{mussel}} \cdot k_{w-m}^{Zn} \cdot \frac{C_{water}^{Zn}}{C_{mussel}^{Zn}} \right) \cdot C_{mussel}^R \tag{7}$$

Let $U = \frac{\alpha_R}{\alpha_{Zn}} \cdot \frac{m_{water}}{m_{mussel}} \cdot k_{w-m}^{Zn}$ and $D = \frac{\alpha_R}{\alpha_{Zn}} \cdot \frac{m_{water}}{m_{mussel}} \cdot k_{w-m}^{Zn} \cdot \frac{C_{water}^{Zn}}{C_{mussel}^{Zn}} = U \cdot \frac{C_{water}^{Zn}}{C_{mussel}^{Zn}}$, eq. 7 simplifies finally into:

$$\frac{dC_{mussel}^R}{dt} = U \cdot C_{water}^R - D \cdot C_{mussel}^R \tag{8}$$

This simple model implies that the uptake parameter U remains constant between the control group and the Zn exposed groups. On the contrary, the depuration parameter D takes a different value according to the change in C_{water}^{Zn} , this value being increased from the control to the metal conditions groups.

These hypotheses cannot be validated according to the kinetic parameters determined for the different Zn exposure conditions and the three radionuclides (Table 2), which suggests that the radionuclides are not co-regulated with Zn. This is consistent with the experiments conducted by Kraak *et al.* [8] who reported that Zn is not regulated anymore by *Dreissena polymorpha* above 191 $\mu\text{g L}^{-1}$.

Assuming that neither a reduction in the filtering activity nor a co-regulation of radionuclides with Zn were determining factors, some possible explanations can be put forward. A direct competition among pollutants for sites on the shell and on gill epithelia may have reduced ^{57}Co and ^{110m}Ag uptake at the whole organism level. This first hypothesis has been reported for Co and Zn. Price and Morel [11] have shown that Co can substitute for Zn in a marine diatom, while in bivalve, cobalt depress the rate of ^{63}Zn

uptake by oyster soft tissue and shell [12]. To validate this hypothesis and develop a mechanistic model for the accumulation of radionuclides in the presence of metals, ^{57}Co and $^{110\text{m}}\text{Ag}$ uptake should be characterised for wider ranges of Zn concentrations, in order to fit a competitive inhibition model to the data, in a similar way to what was done for Sr^{2+} and Ca^{2+} by Chowdhury *et al.* [13]. However, in the case of Ag, it must be underlined that the reverted trend is observed at the soft tissue level. Consequently, if a competition phenomenon exists, it occurs only at the shell surface. An increase of Ag accumulation in the presence of Zn has also been shown for a fish (*Brachydanio rerio*) [14].

Such interactions have been largely studied for Cd and Zn, which are known to induce the implementation of metal sequestration and excretion mechanisms, including metallothioneins, glutathione, calcified concretions and mucus secretion [15-18]. For *Anodonta cygnea*, Hemelraad *et al.* [19] reported that zinc competes with Cd for metal binding sites at the cellular level and accelerates Cd transport from the gills towards the internal organs. It can be hypothesised that the uptake and/or excretion rates of radionuclides may be modified in bivalves exposed to Cd and Zn, because of the greater occurrence of these various defence mechanisms. Another explanation can be put forward, based on the work of Rainbow and White [20] who proved that Zn regulation in a decapod species is an active phenomenon, increasing uptake rates being matched with increasing excretion rates. Under that assumption, this active process will necessarily cost energy, which cannot be used for other metabolic processes. This may also explain the differences in radionuclide uptake and excretion observed between the control and metal exposed groups.

Current knowledge of the radionuclide behaviour in the context of a metal pollution is insufficient to allow for a broad generalisation of these results. However, this experiment provides the first elements to understand some of the mechanisms involved in the influence of metals on radionuclide uptake, which is an important prerequisite to any detailed accumulation modelling that goes beyond describing the observation by global kinetic parameters.

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