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Impact of Cadmium and Zinc Prior Exposure on ^{110m}Silver, $^{58+60}$Cobalt and ^{137}Cesium Uptake by Two Freshwater Bivalves During a Brief Field Experiment

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In the framework of freshwater radioecology, numerous studies have been carried out on the contamination of aquatic organisms by artificial radionuclides taken separately, whereas the bioaccumulation of radionuclides may be influenced by non-radioactive metals present at significant concentrations in many aquatic ecosystems. To our knowledge, the effect of the physiological disruption induced by these trace metals on radionuclide bioaccumulation by freshwater organisms has never been investigated. This multipollution context must be taken into consideration to improve the biomonitoring of the radioactive liquid wastes released by nuclear power plants. The methodology for efficient monitoring includes the use of bioindicators, which alleviates a number of problems associated with the analysis of trace pollutants in water or sediments and gives a direct indication of their bioavailability to the biocenosis. The possible organisms for use as trace pollutant indicators include bivalve species, e.g. the Asiatic clam *Corbicula fluminea* and the zebra mussel *Dreissena polymorpha*. The two species are complementary, since their ecological specificities are different. *C. fluminea* is a benthic species whereas *D. polymorpha* is an epilithic one.

The aim of this study was to examine the effects of a prior exposure, to waterborne cadmium and zinc, on the uptake of artificial radionuclides by *C. fluminea* and *D. polymorpha*. The metallic contamination site was located downstream from an old zinc ore treatment facility. This prior metallic contamination was followed by radionuclide *in situ* exposure of the bivalves at the nuclear power plant of Golfech (France), in an artificial channel.

MATERIALS AND METHODS

Adult *D. polymorpha* were collected on the banks of the Garonne river (France) whereas *C. fluminea* were collected in the Sanguinet-Biscarosse freshwater lake (Landes, France). Homogeneous groups were constituted (mean antero-posterior length of the shell : 2.26±0.20 cm and 2.70±0.30 cm, respectively for *C. fluminea* and *D. polymorpha*, corresponding to a mean wet weight of 3.97±1.05 and 2.49±0.78 g).

The mollusks were transferred to the prior contamination site (Lot river, France), consisting of three stations making up a natural Cd and Zn concentration gradient.

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The reference station was located on the main river above the confluence with a highly polluted stream, on which the most contaminated station was selected. The moderately polluted station was located downstream of this confluence on the same river as the reference site. At those three stations, about 600 individuals of the two bivalve species were placed in the same cages in order to ensure identical exposure conditions. These consisted of 50 × 30 × 30 cm plastic containers, with highly perforated walls. The zebra mussels were placed at the top of the cages on a cement layer to ensure their fixation, whereas the Asiatic clams were laid on the bottom of the container and partly buried in the sediment. After 3 days of stabilization at the reference site, the cages were firmly fixed on the river bed at each station. This first phase of exposure to metal contamination lasted 2 weeks.

At the end of this phase, the cages containing the bivalves were transported to the nuclear power station of Golfech, on the Garonne river (France), in insulated containers, to be exposed to radionuclides. In this plant, an experimental channel was built in order to receive the pre-diluted radioactive effluents discharged by the nuclear facility, mixed with the water originating from the cooling system. The cages were placed in this channel for 5 days to ensure that the organisms were fully acclimatized to the different physical and chemical conditions. After that period, the diluted effluents were discharged into the channel during 58 hr. It must be underlined that this field exposure to artificial radionuclides could not be extended any longer, in view of the authorized limit concentrations in the field of radioprotection.
At the end of the prior contamination phase, ten specimens of *C. fluminea* and *D. polymorpha* were collected at each station and dissected to remove the soft body. Tissues from 2 animals were pooled, yielding five replicate samples per station. Each sample was mineralised in a glass tube with a screw stopper (HNO_3 65%, 3 hours, 105°C, Blockdigest). Digested samples were diluted with deionised water up to a volume of 20 mL. Cd concentrations were determined by graphite furnace atomic absorption spectrometry (Perkin Elmer 4110 ZL). Zn concentrations were measured by flame atomic absorption spectrometry (Varian AA 200). The detection limits using these techniques were 0.1 µg Cd /L and 10 µg Zn /L.

During the radioactive contamination phase, radionuclide concentrations were measured in whole specimens of *C. fluminea* and *D. polymorpha* at six sampling times (0, 30, 58, 78, 120 and 192 hours after the discharge of diluted radioactive effluents) and in the soft body at 72-hour exposure. Bivalves were rinsed, blotted dry with absorbent paper, weighed and dried at 105°C for 48 hours. Samples were homogenized using a grinder (Vorwerk Thermomix 3300). To achieve greater accuracy of the γ-spectrometry measurement, 60 to 240 animals were pooled. In the case of radionuclide determinations in the soft body, tissues of 120 individuals were pooled. Radionuclide measurements were performed using a high-purity germanium detector connected to a multichannel analyser and geometrical differences between samples were taken into account by referring to similar

radioactive standards. Under these conditions and for a counting time ranging from 24 to 48 hours, the detection limit ranged from 0.05 to 0.1 Bq and the 95 % confidence interval on the counting was about 15 %. The results of the measurements were related to the first day of the experiment by correction for the physical decay of the radionuclide.

To quantify radionuclide concentrations in the water of the experimental channel, a first radioactivity analysis was performed by γ-spectrometry on the pre-diluted effluents before their mixture with the water from the cooling system in order to achieve greater accuracy. Then, over a period of 80 hours after the release of low-level radioactive effluents, tritium concentration was measured. This element is assumed not to interact with the particulate phase and consequently, it can be used as a tracer to estimate the dilution factor of the radioactive effluent in the channel. Based on the initial composition of the effluent, this dilution factor was extrapolated to the other identified radionuclides.
Suspended matter was collected every day during the release of low-level radioactive effluents, by filtration through 0.45 µm Millipore cartridges (CW S 10). Cartridges were dried (105 °C, 24 hours) and incinerated (480 °C, 25 hours), dry and ash weights being measured at each step. The ash radioactivity was analysed by γ- spectrometry.

Statistical analyses were carried out using Systat for Windows (Ver 5, Systat, Illinois, USA). A Tukey's HSD test was performed to assess significant differences among the three metal contamination conditions.

RESULTS AND DISCUSSION

The main chemical characteristics of sampling stations are summarized in Table 1. The three stations made up a natural gradient, particularly marked in the case of Cd^{2+} and SO_4^{2-}. Zn^{2+} and Mg^{2+} also showed appreciable concentration ranges, whereas the remaining ions were somewhat less variable (Ca^{2+}, HCO_3^-). At the reference station, Cd and Zn concentrations were close to geochemical background levels. At the moderately contaminated station, they still remained below the authorized limit (Anonymous, 1995), whereas at the highly contaminated station, they were characteristic of a polluted area. Measurement of Hg, Cu or Ag did not reveal any presence of these metals.

At the end of the 14-day period of prior exposure, metal concentrations in the soft body of *C. fluminea* and *D. polymorpha* also varied along the contamination gradient (Figure 1), though to a lesser extent than in the water. The tissue concentrations measured at the moderately and the highly contaminated stations were significantly higher than those measured at the control station ($p<0.001$). Ranges of concentrations were wider for Cd than for Zn, as the ratio between highest and lowest values were about 5 for Zn and 17 for Cd. Tissue Cd

concentration was significantly greater at the highly than at the moderately polluted station, which was not found for Zn. These results may indicate the existence of an homeostatic control over Zn tissue concentrations in *C. fluminea* and *D. polymorpha* even on the short term, which reflects its role as an essential trace element.

Table 1. Main water characteristics at the prior contamination stations

		Reference	Moderately polluted	Highly polluted
pH		7.7	7.7	7.8
Ca^{2+}	(mg L^{-1})	22	26	65
Mg^{2+}	(mg L^{-1})	3.9	7.1	24
Na^{+}	(mg L^{-1})	5	5.1	10.4
K^{+}	(mg L^{-1})	2	2.8	6.5
HCO_3^{-}	(mg L^{-1})	67	70	98
Cl^{-}	(mg L^{-1})	5.6	5.6	5.8
SO_4^{2-}	(mg L^{-1})	2	30	172
NO_3^{-}	(mg L^{-1})	6.7	7	11.4
PO_4^{3-}	(mg L^{-1})	0.08	0.11	0.26
Zn^{2+}	(mg L^{-1})	<0.05	0.157	1.055
Cd^{2+}	(µg L^{-1})	0.3	2.52	26.6

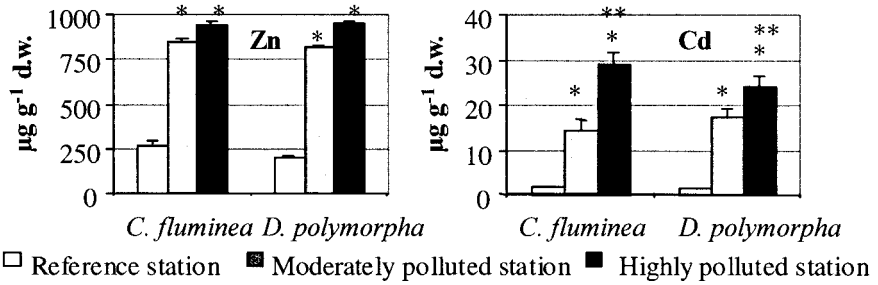


Figure 1. Zn and Cd concentrations in the soft body of bivalves after the 14-day prior exposure period. Error bars represent the standard deviation. * : value significantly different from control ($p<0.001$) ; ** : value significantly different from the moderately polluted station ($p<0.001$)

Apart from ^{3}H, the main identified radionuclides in the pre-diluted effluents were ^{110m}Ag, ^{58}Co, ^{60}Co, ^{137}Cs and ^{54}Mn (Table 2). Radioactive cobalt isotopes accounted for about 65 % of the total non-tritium radioactivity, ^{110m}Ag for 28 %, while ^{137}Cs and ^{54}Mn accounted only for 4 and 3 %. Over the 80 hour period after the effluent release, the greatest values were observed from the 32^{nd} to the 54^{th} hour. During that phase, radionuclide concentrations ranged from 0.034 Bq L^{-1} (^{60}Co) to 0.0024 Bq L^{-1} (^{54}Mn). The radioactivity analysis of the suspended matter revealed the presence of low concentrations of ^{57}Co and ^{134}Cs that were not detected in the channel water due to the great dilution of radionuclides in water

samples (Table 3). Radioactive cobalt isotopes represented 57 % of the total particulate radioactivity, while ^{110m}Ag, radioactive caesium isotopes and ^{54}Mn contributed to 29, 14 and 1% of the radioactivity respectively. The highest radionuclide concentrations in the suspended matter were measured for ^{110m}Ag (356 Bq kg^{-1} d.w.) and the lowest for ^{57}Co (4 Bq kg^{-1} d.w.).

Table 2. Radionuclide water concentrations (Bq L^{-1}) in the experimental channel, after mixing the pre-diluted effluents with the water from the cooling system

Time (hr)	^{3}H	^{58}Co	^{60}Co	^{110m}Ag	^{137}Cs	^{54}Mn
8	170	0.005	0.008	0.006	0.001	0.001
30	560	0.018	0.025	0.019	0.002	0.002
32	740	0.023	0.034	0.025	0.003	0.002
54	700	0.022	0.032	0.024	0.0031	0.002
58	530	0.017	0.024	0.018	0.002	0.002
78	190	0.006	0.009	0.006	0.001	0.0006

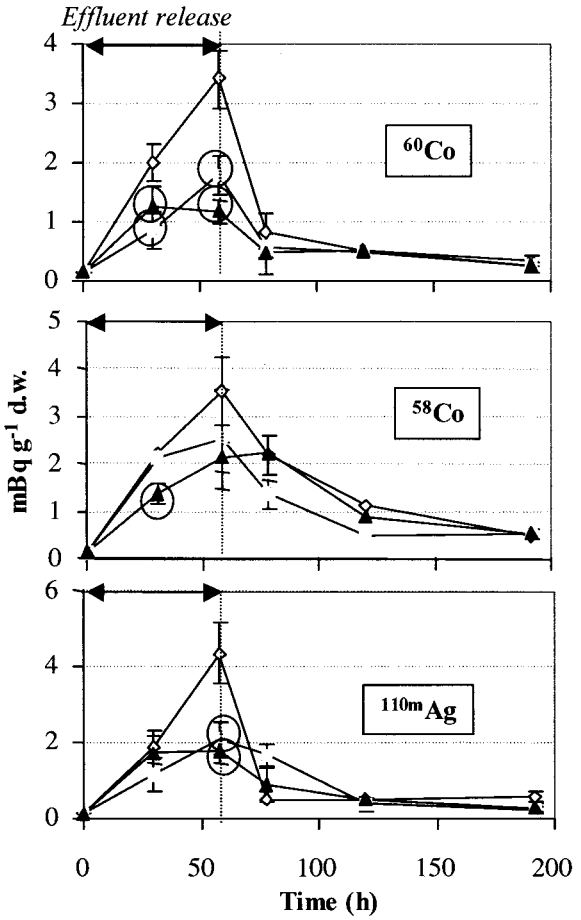
Table 3. Radionuclide concentrations in the suspended solids (Bq kg^{-1} dry weight) in the experimental channel, after mixing the pre-diluted effluents with the water from the cooling system. "-" : below the detection limits

Time (hr)	^{57}Co	^{58}Co	^{60}Co	^{110m}Ag	^{134}Cs	^{137}Cs	^{54}Mn
8	-	15	32	24	4.9	23	-
30	4.6	317	317	330	9.8	47	12
54	4.0	336	257	356	5.0	24	11
78	-	39	68	44	6.2	29	-

Radionuclide uptake by the bivalves *in toto* was totally different between species. While no radionuclide at all was detectable in *D. polymorpha*, ^{58}Co, ^{60}Co and ^{110m}Ag were easily measured in *C. fluminea*. This difference may be attributed to the high suspended matter load, which probably affected the filtration rate of the zebra mussel. Due to an incipient flood occurring in the river supplying the cooling system, the corresponding suspended matter load reached about 90 mg L^{-1} at the end of the discharge. At very high concentrations, this species maintains its filtration rate at a minimum only to meet the oxygen demand, whereas Asiatic clams are known to be much less sensitive to physical and chemical variations (Elder and Collins, 1991 ; Fraysse *et al.*, 2000). Therefore, even though several authors have demonstrated that *D. polymorpha* can bioaccumulate metals to high concentrations, it was not suited to the high suspended matter load observed during the *in situ* implantation.

In the case of *C. fluminea*, only ^{58}Co, ^{60}Co and ^{110m}Ag were detectable in the whole organism. Radionuclide accumulation increased regularly until the end of the effluent release, then the concentrations levelled off and approached the background levels (Figure 2). At the end of effluent release, by 58-hr, the concentrations ranged between c.a. 1 and 4 mBq g^{-1} (d.w.). For the three radionuclides, specimens exposed to metals generally accumulated smaller

amounts than the specimens from the reference group. By 58-hr, metal-exposed bivalves were 1.7, 2.5 and 2.9 fold less contaminated by ^{58}Co, ^{110m}Ag and ^{60}Co respectively, than the control. In the case of ^{58}Co, this difference was statistically significant ($p<0.05$) for the most metal polluted group at 30-hr exposure. A significant difference was also found for the two metal polluted groups at 30-hr and 58-hr exposure for ^{60}Co and at 58-hr exposure for ^{110m}Ag.



◇Reference station Moderately polluted station ▲Highly polluted station

Figure 2. ^{58}Co, ^{60}Co and ^{110m}Ag concentrations in *C. fluminea* as a function of time. Error bars represent the 95 % confidence interval on the γ-spectrometry measurements. ^{134}Cs, ^{137}Cs and ^{54}Mn concentrations remained below the detection limits. Values significantly different from the reference group ($p<0.05$) are indicated by circled symbols.

To examine the possible relationship between metal content and radionuclide uptake by *C. fluminea*, the radionuclide concentrations in just the soft body have to be considered. These measurements, undertaken at 72-hr exposure, enable to eliminate the variability associated to the shell contamination. In contrast with the radionuclide measurements carried out at the whole organism level, ^{137}Cs was detectable in the soft body (Figure 3). For a given condition of metal contamination, higher concentrations of ^{58}Co, ^{110m}Ag, ^{60}Co and ^{137}Cs (in decreasing order) were found. Radionuclide concentrations observed in the soft body were much higher than in the whole organism and ranged from 4.3 to 80 mBq g^{-1} (d.w.), for ^{137}Cs and ^{58}Co respectively. It must be underlined that ^{58}Co, whose released concentration was lower than that of ^{60}Co, was detected at higher concentration in the clam soft body. This isotopic discrimination might be linked to different chemical forms of radiocobalt released in the effluent. As regards to the interactions between the metals (Cd/Zn) and the radionuclides, no statistically significant difference was evidenced at the soft body level, after 72 hours, between control and metal polluted groups for ^{137}Cs. On the contrary, for ^{58}Co, ^{60}Co and ^{110m}Ag, specimens exposed at the moderately metal polluted station were less contaminated by radionuclides than those from the control group. No difference was found for specimens from the highly metal polluted station, which suggests that different mechanisms might be induced depending on Cd and/or Zn concentration.

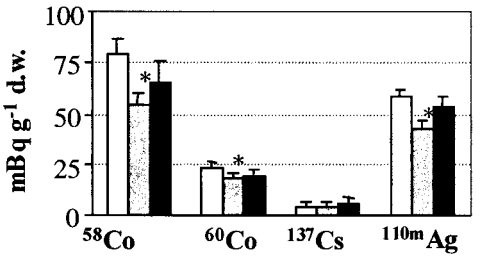


Figure 3. ^{58}Co, ^{60}Co, ^{137}Cs and ^{110m}Ag concentrations measured in *C. fluminea* soft body at the 72nd hour after the beginning of radioactive effluent release. Error bars represent the 95 % confidence interval on the γ-spectrometry measurements. ^{134}Cs and ^{54}Mn concentrations remained below the detection limits. * : value significantly different from the reference group ($p<0.05$).

Similar studies were conducted during laboratory experiments with *C. fluminea*. The same conclusions were drawn, i.e. cobalt and silver decreased with increasing Cd and Zn concentrations. Such effects have been studied for Cd and Zn interactions, as they are often discharged in combination from zinc-producing plants into freshwater systems. The processes involved in these interactions may be of various kinds. It is known that Cd and Zn can induce the implementation of mechanisms of sequestration and excretion of metals, including metallothioneins,

glutathione, calcified concretions and mucus secretion (Canesi *et al.*, 1998 ; Carmichael *et al.*, 1980 ; Naimo *et al.*, 1992 ; Pynnönen *et al.*, 1987). For *Anodonta cygnea*, Hemelraad *et al.* (1987) 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 hypothesized 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 defense mechanisms.

The results of this study present important information on the influence of Cd and Zn on radionuclide accumulation in field conditions. However, there is a need for further experimentations to validate these first findings. In particular, the influence of Zn on ^{57}Co is now being investigated. Kinetic studies are being performed to evaluate the uptake and excretion rates of the radionuclide, and the subcellular localization is being examined by protein fractionation on gel permeation chromatography, autoradiography and X-ray microanalysis.

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