Accumulation and Retention of Metals in Mussels from Food and Water: A Comparison under Field and Laboratory Conditions

Nicholas S. Fisher, Jean-Louis Teyssié, Scott W. Fowler, and Wen-Xiong Wang

Marine Sciences Research Center, State University of New York, Stony Brook, New York 11794-5000, and International Atomic Energy Agency, Marine Environment Laboratory, MC-98012 Monaco

ENVIRONMENTAL® SCIENCE & TECHNOLOGY

Reprinted from
Volume 30, Number 11, Pages 3232–3242

Accumulation and Retention of Metals in Mussels from Food and Water: A Comparison under Field and Laboratory Conditions

Nicholas S. Fisher, Jean-Louis Teyssié, Scott W. Fowler, and Wen-Xiong Wang

Marine Sciences Research Center, State University of New York, Stony Brook, New York 11794-5000, and International Atomic Energy Agency, Marine Environment Laboratory, MC-98012 Monaco

ENVIRONMENTAL® SCIENCE & TECHNOLOGY

Reprinted from
Volume 30, Number 11, Pages 3232–3242

Environ. Sci. Technol. 1996, 30, 3232–3242

Accumulation and Retention of Metals in Mussels from Food and Water: A Comparison under Field and Laboratory Conditions

NICHOLAS S. FISHER,*,† JEAN-LOUIS TEYSSIÉ,‡ SCOTT W. FOWLER,‡ AND WEN-XIONG WANG†

Marine Sciences Research Center, State University of New York, Stony Brook, New York 11794-5000, and International Atomic Energy Agency, Marine Environment Laboratory, MC-98012 Monaco

Mussels are used as bioindicator organisms to assess bioavailable contaminant concentrations in coastal waters. This study used radiotracer methodologies to examine the bioaccumulation and efflux rates of six metals (Ag, Am, Cd, inorganic Co, organic Co [as cobalamine], Pb, and Zn) in the mussel *Mytilus galloprovincialis*, in which the dissolved phase and ingested phytoplankton food were compared quantitatively as sources. Mussels maintained in the laboratory in running seawater and caged in the field for up to 4 months generally displayed similar metal absorption efficiencies (AEs) and efflux rates from their soft parts. AEs from food were typically lower than radioisotope retention efficiencies from the dissolved phase and ranged from 4% for Am to about 60% for Pb; values were comparable to those reported for the common blue mussel, *Mytilus edulis*. Efflux rate constants from mussel soft parts (1–5% daily) were comparable to *M. edulis* values from short-term depurations; biological half-lives of all metals in soft parts, shells, and whole mussels typically ranged from 10 to 60 days, although exceptions were noted. Metals obtained from food were primarily bound to soft part tissues; metals from the dissolved phase were mostly associated with the mussel shells. Higher AEs and lower efflux rates of cobalamine than of inorganic Co suggest that cobalamine is a more bioavailable form of cobalt for mussels. Mussel fecal pellets were enriched with metals following ingestion, with retention half-times of the metals in the pellets ranging from 18 days for Ag to 107 days for Am. Fecal pellet deposition beneath mussel beds may play an important role in the biogeochemical cycling of these metals in coastal ecosystems. The efflux rate constants and AEs can be used in kinetic models to estimate the relative importance of food and water as source terms for metals in mussels and to predict metal concentrations in mussels under different field conditions. A sensitivity analysis was performed to show that AEs are critical parameters influencing metal concentrations in mussels (particularly for Am, Ag, and Zn), and the results from this study indicate that laboratory-derived estimates of AEs are applicable to field conditions.

Introduction

Interest in the bioaccumulation of trace metals in marine bivalve molluscs stems mainly from their use as bioindicators of coastal contamination (*1–3*) and from concern over public health arising from their consumption by humans as seafood. Various studies have demonstrated that marine mussels can accumulate ambient trace metals from the dissolved phase and from ingested phytoplanton food (*4–12*). Influx rates from the dissolved phase and from food are required in quantitative models of metal accumulation in marine animals (*11, 13, 14*). Influx rates for food intake require quantitative estimates of both ingestion rate and absorption efficiency of the ingested metal, but with rare exceptions absorption efficiencies for metals have only recently been determined in adult bivalves (5, *10–12, 14*). Bioaccumulation models also require efflux rates of the metals out of the bivalves. Most of the earlier studies have measured the retention of metals in mussels over relatively short periods (typically in experiments lasting days to weeks), and there is some question as to whether depuration rate constants determined in longer term depuration periods would be significantly different. There are few published studies that compare the parameters quantified in laboratory experiments with parameters obtained from mussels held in the field over relatively long periods of time. Furthermore, there has been no systematic comparison of dissolved and food source terms for metal accumulation and retention in mussels.

We have therefore conducted an experiment to provide data enabling direct comparisons between the dissolved phase and food as sources of bioavailable metal for mussels and between laboratory- and field-derived depuration rates. The experiment followed depuration rates over relatively long periods to provide depuration rate constants that would be applicable to natural mussel populations. All parameters were determined simultaneously for six different metals: cobalt, zinc, silver, cadmium, lead, and americium. For cobalt, we further compared inorganic cobalt with the organic form cobalamine, which have been reported to have different biological uptake and assimilation patterns (*15*). These metals were chosen because they provide a contrast between oxygen-seeking or class A (Am), sulfur-seeking or class B (Ag), and borderline metals (Co, Zn, Cd, Pb) (*16*) as well as between biologically essential (Zn, Co) and non-essential (Ag, Cd, Pb, Am) metals; they may therefore be representative of other metals that display similar chemical or biological behavior. These metals are

* Corresponding author e-mail address: NFISHER@CCMAIL.SUNYSB.EDU.

† State University of New York.

‡ Marine Environment Laboratory.

S0013-936X(96)00009-0 CCC: $12.00

TABLE 1

Radioisotope Content of Algal Food Cells Fed to Mussels[a]

radioisotope	μBq cell^{-1}	% accumulated on cells
^{57}Co	0.84	22.0 ± 0.2
^{60}Co	3.80	40.6 ± 1.6
^{65}Zn	197.29	74.9 ± 1.0
^{109}Cd	54.07	46.9 + 1.4
^{110m}Ag	32.89	78.3 ± 4.3
^{210}Pb	8.06	36.7 ± 1.7
^{241}Am	20.09	87.4 ± 0.8

[a] Also shown are average (±1 SD) fractions of radioisotope accumulated by algal cells after the 120-h uptake period.

also of concern in some coastal waters, either as contaminants deriving from industrial pollution (Pb, Cd, Ag, Zn) or from the nuclear fuel cycle (Co, Am) and are regularly measured in large national monitoring programs (*17*).

Materials and Methods

Radioisotope Accumulation in Mussels from Food. To prepare radiolabeled phytoplankton cells, the centric diatom *Thalassiosira pseudonana* (clone 3H) was grown in four 1-L batches of sterile-filtered (0.2 μm) Mediterranean surface water enriched with *f*/2 (*18*) nutrients but with no Cu, Zn, or EDTA and with *f*/20 levels of other trace metals. Cultures were swirled every 12 h and maintained at 15 °C under cyclic illumination (200 μEinstein m^{-2} s^{-1}, 14 h light: 10 h dark) provided by cool-white fluorescent lamps. Two batches of radiolabeled diatoms were prepared, one exposed only to ^{210}Pb (which could not be detected together with the other radioisotopes) and the other exposed to a mixture of dissolved radioisotopes (^{57}Co-labeled cobalamine, [$t_{1/2}$ = 272 d], ^{60}Co [$t_{1/2}$ = 5.27 yr], ^{65}Zn [$t_{1/2}$ = 244 d], ^{109}Cd [$t_{1/2}$ = 1.27 yr], ^{110m}Ag from [$t_{1/2}$ = 250 d], and ^{241}Am [$t_{1/2}$ = 433 yr]). These radioisotope solutions, all obtained from Amersham, were in dilute HCl ($^{60}Co^{2+}$, $^{65}Zn^{2+}$, $^{109}Cd^{2+}$), HNO_3 ($^{110m}Ag^{+}$, $^{210}Pb^{2+}$, $^{241}Am^{3+}$), or 0.9% benzyl alcohol solution ([^{57}Co]cobalamine). Microliter quantities of each isotope were added to replicate algal cultures, and the pH of each culture was adjusted with dilute Suprapur NaOH so that the pH of the cultures was 8.0–8.1. The initial cell density was 6 × 10^5 mL^{-1}, and the cells grew to 2–3 × 10^6 mL^{-1} after 120 h. The concentrations of radioisotopes added (kBq L^{-1}, or total metal concentration, considering the specific activity of the isotope stock solutions) were as follows: ^{57}Co, 11.5 (12 pM); ^{60}Co, 28.1 (21 pM); ^{65}Zn, 790 (41 pM); ^{109}Cd, 346 (33 pM); ^{110m}Ag, 126 (49 nM); ^{210}Pb, 44 (74 pM); ^{241}Am, 69 (2.3 nM). All cultures were maintained and handled using trace metal clean techniques to avoid contamination with additional metals.

The radioactivity per algal cell after 120 h exposure, determined using established methods (*19*), is given in Table 1. A 1.6-L sample of multi-labeled cells and an additional 1.6 L of ^{210}Pb-labeled cells were resuspended quantitatively out of their radioactive media via 1-μm Nuclepore filters (*19*) into two 200-mL batches of unlabeled sterile-filtered Mediterranean water to produce one batch containing 1.37 × 10^7 multi-labeled cells mL^{-1} and one batch containing 1.36 × 10^7 ^{210}Pb-labeled cells mL^{-1}. Twenty milliliter of each batch was then added to 10 L of filtered seawater held in an aquarium containing 85 individual *Mytilus galloprovincialis*; uniformly sized mussels (approximately 6 cm shell length and 35 g dry wt) were used. The mussels had been collected 48 h prior to the start of the experiment and held without food in filtered seawater. The initial algal cell density in each feeding suspension was 2.74 × 10^4 mL^{-1}, equivalent to 614 μg dry wt L^{-1}. Every 30 min, an additional 20 mL of radiolabeled diatoms was added to each of the two aquaria (one containing multi-labeled cells and one containing ^{210}Pb-labeled cells) until all the radiolabeled cells were consumed by the mussels, as confirmed by microscopy (5 h). An average ingestion rate of 130 μg dry wt h^{-1} was calculated for each individual mussel. Throughout the course of the radioactive feeding, checks were made to determine the partitioning of the radioisotopes between dissolved and particulate phases (*19*). Immediately following the radioactive feeding, the radioactivity of each of four mussels from each batch was counted, after which the animals were frozen for later weighing and dissection.

Radioisotope Accumulation in Mussels from the Dissolved Phase. Two other batches of identical mussels, 85 individuals per batch, were exposed to the same radioisotopes dissolved in 10 L of 0.2-μm filtered Mediterranean surface water for 16 h. Mussels were exposed for a longer time (16 h vs 5 h) to the dissolved radioisotopes because recycling of the radioisotopes between food, animals, and the dissolved phase is less problematic than when the mussels are exposed to radioactive food (*11*, *14*). The pH of the water was adjusted so that after addition of the microliter quantities of acidic solutions the pH was 8.0. The radioactivity was adjusted to the following concentrations: [^{57}Co]cobalamine, 8.6 kBq L^{-1} (9.0 pM); ^{60}Co, 4.5 kBq L^{-1} (3.4 pM); ^{65}Zn, 27.1 kBq L^{-1} (1.4 pM); ^{109}Cd, 40.7 kBq L^{-1} (3.9 pM); ^{110m}Ag, 20.5 kBq L^{-1} (8 nM); ^{210}Pb, 7.1 kBq L^{-1} (11.9 pM); ^{241}Am, 7.4 kBq L^{-1} (242 pM). The fractionation of the radioisotopes was checked in aliquots of the aquarium water (*19*). There was undetectable particulate radioisotope throughout the 16-h exposure period. Immediately following this uptake period, four mussels from each batch were removed for counting and weighing, as described above for the food uptake.

Depuration. Immediately following the radioisotope exposure periods, the radioactivity of four replicate mussels from each radiolabeled group was determined, after which they were dissected. The retention of accumulated radioisotopes in the remaining mussels was then measured under laboratory and field conditions. Half the animals (n = 40) that had been exposed to radioisotopes via food only or water only were placed in aquaria in flowing unlabeled Mediterranean surface water (flow rate = 500 mL min^{-1}) in the laboratory. This group of 40 mussels was fed daily with a total ration of 2 mg (dry wt) of *T. pseudonana* cells, representing the maintenance requirement for mussels of this size (*12*). The other half of these mussels was placed into nylon mesh cages (0.5 cm mesh) and moored at a site approximately 500 m off the open Monaco coast at a depth of 3 m. Unfortunately, one of the cages (holding mussels labeled from food) on the mooring was lost in a storm in the third month of depuration.

Periodically, two replicate mussels were removed from their depuration vessels, counted, dissected, and then the various tissues were counted for their radioactivity. Radioactivity in whole mussels, pallial fluid (pipetted into radioactive counting tubes), and dissected tissues were compared with the radioactivity in samples at the onset of the depuration period. The release of the radioisotopes from the collected fecal pellets produced by mussels feeding

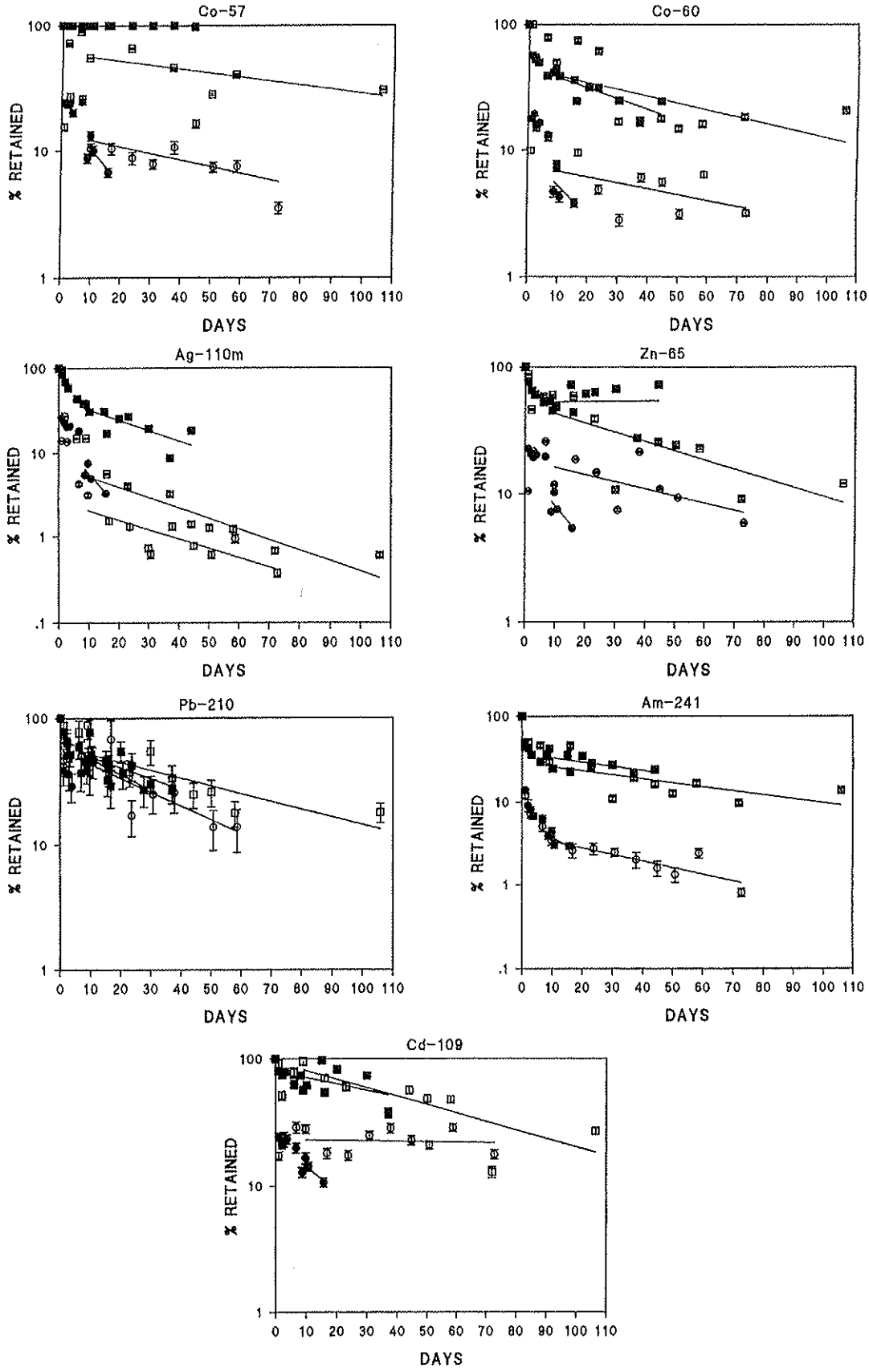

FIGURE 1. Retention of radioisotopes in mussels following uptake from the dissolved phase (squares) or from ingested food (circles). Mussels held in the laboratory throughout the depuration period are denoted by solid symbols; mussels held in the field are denoted by open symbols. Lines drawn are least-squares regressions for the slowly exchanging pool for each treatment, calculated from 8 days after the onset of depuration. Calculated *y* intercepts, representing absorption efficiencies from food or retention efficiencies from the dissolved phase, are given in Table 2; rate constants for loss are given in Table 3. ^{57}Co was in the form of cobalamine; all other radioisotopes were added in inorganic form. All regressions were significant ($P < 0.05$) except ^{65}Zn obtained from the dissolved phase, laboratory depuration; ^{109}Cd obtained from food, field depuration; ^{210}Pb obtained from food, laboratory depuration.

on multi-labeled phytoplankton was also determined using methods described elsewhere (*20*); the retention of ^{210}Pb in fecal pellets was not determined.

Radioactive Counting. The radioactivity of all samples was determined using a high-purity Ge–Li detector linked to a Cosynus multi-channel analyzer and a computer with spectra analysis software (Intertechnique, Grenoble). ^{210}Pb was detected alone on a hyperpure low-energy Ge detector. The detectors were calibrated using appropriate standards for each of the counting geometries used. Dissected tissues were dissolved in 20 mL of 4 N HCl. The counting efficiencies determined took into account the low energy γ-autoabsorption by the mussel shells of the low-energy radioisotopes. γ-emissions were detected as follows: ^{57}Co, 122 keV; ^{60}Co, 1333 keV; ^{65}Zn, 1115 keV; ^{109}Cd, 88 keV; ^{110m}Ag, 658 keV; ^{210}Pb, 46 keV; ^{241}Am, 60 keV. Counting times were adjusted (typically to around 5 min) so that propagated counting errors were < 5%, although some samples of dissected tissues had low radioactivity and required 12-h counts with resultant counting errors up to 15%.

Results

Mussels accumulated all radioisotopes from both the dissolved phase and ingested phytoplankton food. The retention of the seven radioisotopes in the mussels obtained from all sources is shown in Figure 1. Because some of the animals died in the laboratory, mussel depuration after food uptake was followed for only 15 d. Generally, radioisotope release followed a two-compartment exponential model, in which there was a very rapid release followed by a slower release. The rapid release is primarily in the form of fecal pellets if uptake had been from food and desorption primarily from shell if uptake had been from the dissolved phase. The regression lines shown in Figure 1 are least-squares fits for the slowly exchanging pool of radioisotopes in whole mussels. Regressions for the slowly exchanging pool were calculated for data beginning at 8 days after the onset of depuration (rapidly exchanging pools for all treatments were evacuated by this time as indicated in Figure 1) to ensure that all treatments were in the slow, second phase (representing physiological turnover) of element release. The *y* intercepts for these regression lines represent absorption efficiencies (AEs) for the radioisotopes from food and retention efficiencies from the dissolved phase.

Retention efficiencies for radioisotopes obtained from the dissolved phase and AEs from food did not differ appreciably between laboratory and field mussels, with the exception of ^{110m}Ag and ^{57}Co (Table 2). Overall, retention efficiencies were higher for radioisotopes obtained from the dissolved phase than AEs from ingested food, probably reflecting the longer retention times in shell and the greater proportion of total radioisotope in shell following uptake from the dissolved phase. There was more variability for some radioisotopes than others, particularly in mussels exposed to dissolved radioisotopes, leading to bigger ranges in the 95% confidence intervals for the *y* intercepts for these regression lines (i.e., retention efficiencies). For example, among the radioisotopes examined, there was greatest variability for ^{210}Pb, and there was greater variability for dissolved ^{65}Zn than for ingested ^{65}Zn (Table 2).

The calculated depuration rate constants and biological half-lives ($Tb_{1/2}$) of the radioisotopes in whole mussels, shell, and diverse tissues are given in Table 3 and Figure 2. All

TABLE 2

Absorption Efficiencies of Radioisotopes in Mussels from Food and Retention Efficiencies of Radioisotopes from Dissolved Phase under Different Experimental Treatments[a]

	retention efficiency from dissolved phase (%)		absorption efficiency from ingested food (%)	
radioisotope	laboratory	field	laboratory	field
^{57}Co	100 (100, 101)	60 (47, 76)	20 (15, 25)	14 (10, 20)
^{60}Co	46 (39, 55)	44 (26, 77)	9 (7, 13)	8 (5, 11)
^{65}Zn	54 (39, 74)	52 (31, 86)	15 (12, 19)	19 (13, 28)
^{109}Cd	81 (60, 108)	95 (62, 144)	22 (19, 26)	32 (20, 52)
^{110m}Ag	43 (32, 59)	7 (4, 14)	15 (12, 19)	3 (2, 4)
^{210}Pb	61 (53, 70)	60 (43, 84)	64 (48, 85)	56 (38, 84)
^{241}Am	37 (30, 45)	29 (20, 43)	6 (5, 7)	4 (3, 5)

[a] Values shown are *y* intercepts of regression lines for slowly exchanging pools (from Figure 1). Values in parentheses are lower and upper limits of *y* intercepts (95% confidence) calculated from regression analyses.

calculations were based on radioactivities normalized to the dry weights of the samples. (Periodic weighing of mussels just prior to dissection indicated that average mussels weights of the experimental population remained relatively constant [±20%] throughout the course of the experiment.) Overall, many radioisotopes displayed greater $Tb_{1/2}$ values in whole mussels in the field than in the laboratory. This was principally attributable to longer retention in the shells of the animals caged in the field. The $Tb_{1/2}$ values of all radioisotopes except ^{109}Cd were nearly identical in the field and in the laboratory in the soft parts of animals that had accumulated their radioisotope from ingested food. The rate constant for loss of ^{109}Cd was about 2-fold higher in soft parts of mussels held in the lab than those in the field. In the shells, the $Tb_{1/2}$ values for most radioisotopes (^{60}Co, ^{65}Zn, ^{210}Pb, ^{241}Am) were greater in mussels held in the field than those in the laboratory (Figure 2). This longer retention in field animals was probably due to an epiphytic growth observed on the shells of the caged animals, not observed in the laboratory animals, which could have impeded the rate of desorption from the shells.

The tissue distribution of the radioisotopes in the mussels at the beginning and end of the depuration periods is given in Table 4. Generally, radioisotopes obtained from the dissolved phase showed a greater fraction associated with shell than radioisotopes obtained from ingested food, whereas radioisotopes ingested in food were far more enriched in soft parts than radioisotopes obtained from the dissolved phase. Immediately following uptake of radioisotope from ingested food, the fraction of total mussel radioisotope associated with shell was <10% for all isotopes. In the soft parts, radioisotopes from food were especially enriched in the digestive gland, and radioisotopes from the dissolved phase were more evenly distributed in the different tissues and were more prominent in byssal threads. There were no systematic differences in the distribution of radioisotopes from either source in mantle, gills, or adductor muscle. Radioisotopes from the dissolved phase were more

TABLE 3

Depuration Rate Constants (k) (in Units of Day^{-1}) and Biological Half-Lives ($Tb_{1/2}$) of Radioisotopes in Slowly Exchanging Pools in Whole Mussels, Total Soft Parts, Shell, Digestive Gland, Adductor Muscle, Gills, and Mantle[a]

	^{57}Co			^{60}Co			^{65}Zn			^{109}Cd			^{110m}Ag			^{210}Pb			^{241}Am		
tissue	%	k	$Tb_{1/2}$	%	k	$Tb_{1/2}$	%	k	$Tb_{1/2}$	%	k	$Tb_{1/2}$	%	k	$Tb_{1/2}$	%	k	$Tb_{1/2}$	%	k	$Tb_{1/2}$
Dissolved, Laboratory																					
whole	100	−0.0004	1733	46	−0.0198	35	54	−0.0005	1291	81	−0.0116	60	43	−0.0281	25	61	−0.0196	35	37	−0.0114	61
soft		−0.0091	76		−0.0543	13		−1.06	1		−0.0232	30		−0.0511	14		−0.0329	21		−0.0415	17
shell		−0.0081	86		−0.0333	21		−0.032	22		−0.0234	30		−0.0277	31		−0.021	33		−0.0263	26
dig gland		0			−0.0234	43		−0.0028	248		0			−0.0141	49		−0.008	87		−0.0146	47
adductor		0			−0.0218	32		0			−0.0007	990		−0.0376	18		−0.0085	82		−0.0163	43
gills		−0.0272	25		−0.032	22		−0.0019	365		−0.0065	107		−0.0393	18		−0.0123	56		−0.0388	18
mantle		0			−0.0289	24		−0.0167	41		−0.0061	114		−0.0236	29		−0.0347	20		−0.0189	37
Dissolved, Field																					
whole	60	−0.0074	94	44	−0.0126	55	52	−0.0169	41	95	−0.0152	46	7	−0.0287	24	60	−0.0142	49	29	−0.0108	64
soft		−0.0139	50		−0.0276	27		−0.0205	34		−0.0129	54		−0.0432	16		−0.0146	47		−0.0226	31
shell		−0.0144	48		−0.0108	64		−0.0185	37		−0.0108	64		−0.028	25		−0.0093	75		−0.0135	51
dig gland		−0.0149	47		−0.0343	20		−0.0251	28		−0.015	46		−0.0545	13		−0.0165	42		−0.0167	41
adductor		−0.0162	43		−0.0247	28		−0.0181	38		−0.0145	48		−0.05	14		−0.0129	54		−0.0102	68
gills		−0.0223	31		−0.0261	27		−0.0238	29		−0.0188	37		−0.0641	11		−0.0149	47		−0.0243	29
mantle		−0.0135	51		−0.0326	21		−0.0255	27		−0.0127	55		−0.0496	14		−0.0174	40		−0.0135	51
Food, Laboratory																					
whole	20	−0.0647	11	9	−0.0566	12	15	−0.0655	11	22	−0.0444	16	15	−0.1639	4	64	−0.0281	25	6	−0.0472	15
soft		−0.0366	19		−0.0416	17		−0.027	26		−0.0256	27		−0.0658	11		−0.0289	24		−0.1089	6
shell		−0.0458	15		−0.045	15		−0.0601	12′		−0.0535	13		−0.0578	12		−0.0317	22		−0.0277	25
dig gland		−0.074	9		−0.081	9		−0.0904	8		−0.0433	16		−0.1109	6		−0.0002	3,465		−0.1664	4
adductor		−0.0162	26		−0.0282	25		0			−0.0236	29		−0.0695	10		−0.0123	56		−0.0897	8
gills		−0.0223	17		−0.0673	10		−0.0099	70		−0.0249	28		−0.0617	11		−0.0064	108		−0.13	5
mantle		−0.0135	11		−0.053	13		−0.0317	22		−0.028	25		−0.0587	12		−0.6	12		−0.1271	6
Food, Field																					
whole	14	−0.0122	57	8	−0.0107	65	19	−0.0133	52	32	−0.0008	822	3	−0.0254	27	56	−0.0251	28	4	−0.0184	38
soft		−0.0409	17		−0.0365	19		−0.0256	27		−0.0133	52		−0.0559	12		−0.024	29		−0.0561	12
shell		−0.0223	31		−0.0158	44		−0.0245	28		−0.0141	49		−0.0428	16		−0.0197	35		−0.026	27
dig gland		−0.0656	11		−0.061	11		−0.0492	14		−0.0315	22		−0.0729	10		−0.0399	17		−0.0781	9
adductor		−0.043	16		−0.0329	21		−0.0179	39		−0.0121	57		−0.0605	11		−0.0207	33		−0.0381	18
gills		−0.0434	16		−0.0262	26		−0.012	58		−0.0199	35		−0.0653	11		−0.0184	38		−0.0663	10
mantle		−0.0253	27		−0.0319	22		−0.0196	35		−0.0124	56		−0.0583	12		−0.027	26		−0.0227	31

[a] Also shown are the fraction (%) of total radioisotope in whole mussels associated with the slowly exchanging pools. The ^{57}Co was in the form of cobalamine; all other radioisotopes were added in inorganic form.

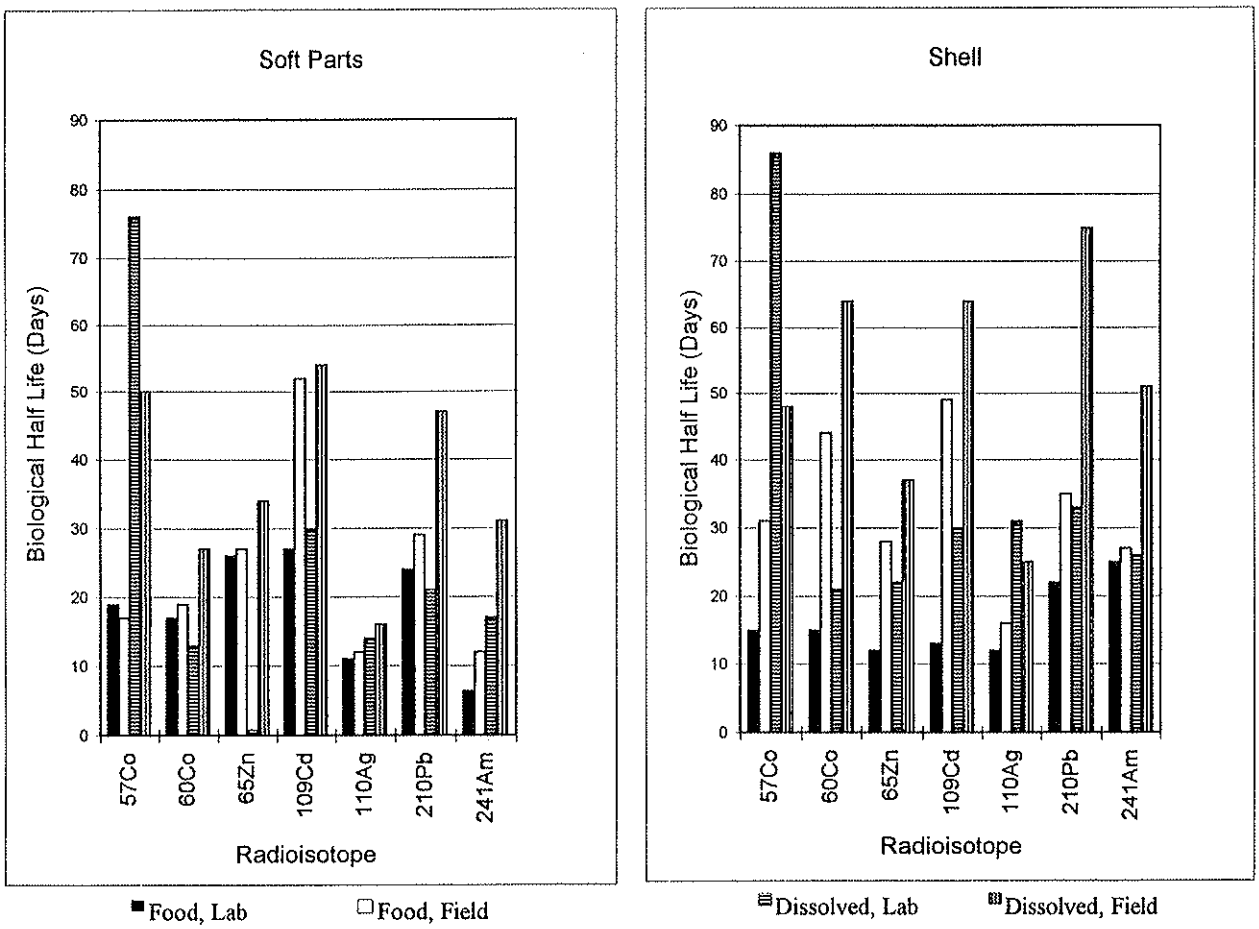

FIGURE 2. Biological half-lives of radioisotopes in mussel soft parts and shells. ^{57}Co was in the form of cobalamine; all other radioisotopes were added in inorganic form.

enriched in gills at the onset of depuration than at the end, whereas radioisotopes obtained from food were most enriched initially in the digestive gland relative to final distributions (especially for ^{65}Zn, ^{110m}Ag, and ^{210}Pb). The biggest differences in radioisotope tissue distributions between mussels held in the lab or in the field were in the byssal threads and shell (greater enrichment in the field mussels, especially for radioisotopes obtained from the dissolved phase) and in the digestive tract (greater enrichment in laboratory mussels).

The retention of radioisotopes in the mussel fecal pellets is shown in Figure 3 and Table 5. As with radioisotope release from mussels, the release of radioisotopes from the pellets followed a two-compartment exponential model, with the exception of ^{241}Am, which followed a single exponential model. ^{241}Am had the longest retention half-time (107 days), followed by inorganic ^{60}Co; ^{110m}Ag had the shortest half-time (18 days). ^{109}Cd and ^{110m}Ag had the greatest fractions in the rapidly exchanging pool and the smallest fractions—15% and 18%, respectively—in the slowly exchanging pool (Table 5).

Discussion

The results suggest that measurements of efflux rate constants for trace metals in marine mussels derived from laboratory experiments are consistent with values that would be obtained from field studies. That is, results from laboratory experiments may be extrapolated to field situations, particularly for metal retention in mussel soft parts. The discrepancy between laboratory and field mussels observed in this study for whole mussels and for mussel shells may have been due to the epiphytic growth observed in the mussels held in the field. Other studies that used shorter depuration periods did not assess rate constants for metal loss specifically for soft parts, so direct comparisons with the rate constants from this study are not possible.

Depuration of all radiotracers was characterized by multi-compartmental loss, consistent with many previous studies on mussels (*4–6, 21–23*). In this study, retention was calculated from different pools of four replicate individuals sampled at different time intervals (using least-squares regression analysis), which may explain the variability in the depuration patterns shown in Figure 1. Following uptake from radiolabled diatom cells, metals were lost much faster in whole mussels maintained in the laboratory than in those maintained in the field (as explained above), but the efflux rates calculated from the soft tissues were comparable between field- and laboratory-depurated mussels. It is noteworthy that the efflux rate constant in soft tissues (measured by tissue dissection at different times) may be more relevant for modeling bioaccumulation in mussels.

The efflux rate constants measured in this study were directly comparable to those measured for the mussel *Mytilus edulis*, in which short depuration periods (≤20 days) were used (1–3% day^{-1} for Ag, Am, Cd, Co, Se, and Zn: *11*). In that study, efflux rate constants were calculated only for whole mussels. Because <5% of the Cd, Se, and Zn were found on the shells following ingestion of radiolabeled

TABLE 4

Distribution of Radioisotopes in Whole Mussels and Mussel Soft Parts at Beginning and End of Depuration Periods[a]

	beginning of depuration								end of depuration							
	% of whole mussel			% of soft parts					% of whole mussel			% of soft parts				
	pallial fluid	soft parts	shell	dig tract	gills	mantle	adductor	byssus	pallial fluid	soft parts	shell	dig tract	gills	mantle	adductor	byssus
								Dissolved, Laboratory								
^{57}Co	29	64	7	3	58	9	28	3	20	77	3	30	20	21	27	2
^{60}Co	19	51	31	8	20	13	23	36	10	59	31	11	11	8	21	61
^{65}Zn	25	38	36	8	23	18	26	24	19	70	11	13	22	9	44	13
^{109}Cd	26	56	18	10	22	23	30	15	35	60	5	20	17	14	42	7
^{110m}Ag	35	55	10	7	41	18	30	5	16	65	19	12	25	29	27	7
^{210}Pb	34	46	19	13	22	31	21	14	13	61	26	20	21	25	25	9
^{241}Am	17	34	48	4	23	8	17	49	14	42	44	15	15	17	28	26
								Dissolved, Field								
^{57}Co	29	64	7	3	58	9	28	3	12	84	4	6	28	36	27	3
^{60}Co	19	51	31	8	20	13	23	36	6	61	33	2	7	4	9	78
^{65}Zn	25	38	36	8	23	18	26	24	0	64	36	6	16	12	35	32
^{109}Cd	26	56	18	10	22	23	30	15	28	63	9	8	15	35	31	10
^{110m}Ag	35	55	10	7	41	18	30	5	7	53	40	2	8	19	15	57
^{210}Pb	34	46	19	13	22	31	21	14	7	58	35	24	22	31	26	22
^{241}Am	17	34	48	4	23	8	17	49	7	29	64	6	14	18	24	38
								Food, Laboratory								
^{57}Co	34	64	2	52	15	17	15	1	2	96	2	55	15	9	19	3
^{60}Co	32	62	2	39	18	20	19	4	3	92	5	29	22	16	27	5
^{65}Zn	27	66	7	48	16	16	16	4	2	96	2	13	28	17	38	4
^{109}Cd	35	58	7	32	17	20	24	8	1	96	3	35	19	18	24	5
^{110m}Ag	40	55	5	49	18	17	14	1	2	93	5	18	35	19	16	12
^{210}Pb	35	58	7	58	8	18	13	3	1	96	3	30	41	20	39	4
^{241}Am	35	61	4	61	18	10	7	4	5	69	26	52	9	6	16	16
								Food, Field								
^{57}Co	34	64	2	52	15	17	15	1	17	56	27	36	12	37	13	2
^{60}Co	32	62	2	39	18	20	19	4	0	69	31	12	33	32	19	5
^{65}Zn	27	66	7	48	16	16	16	4	0	90	10	15	17	32	31	5
^{109}Cd	35	58	7	32	17	20	24	8	16	74	10	22	14	28	30	6
^{110m}Ag	40	55	5	49	18	17	14	1	0	65	35	11	22	18	47	2
^{210}Pb	35	58	7	58	8	18	13	3	0	60	40	17	16	16	36	13
^{241}Am	35	61	4	61	18	10	7	4	9	35	56	52	9	6	16	16

[a] Values shown are percentages of radioactivity for each radioisotope in whole mussels (pallial fluid, total soft parts, and shell) or in total soft parts that are in each tissue (digestive tract, gills, mantle, adductor muscle, and byssus). The ^{57}Co was in the form of cobalamine; all other radioisotopes were added in inorganic form.

diatom cells, the efflux rates calculated for whole mussels are comparable to efflux rates from the soft tissues (*11*). However, these efflux rates were higher than previously reported efflux determinations (e.g., 0.1−1% day^{-1}: *4*, *21*−*25*). Different experimental conditions (e.g., labeling techniques, duration of radiolabeling, depuration conditions for the mussels) may be responsible for the differences observed in efflux rates among different studies. For example, it has been suggested that the food availability at different seasons can largely explain the seasonal changes in trace element efflux rate (*23*). Several studies have also reported that efflux rates are inversely related to the time of exposure (*21*, *26*−*29*), whereas Wang et al. (*11*) demonstrated that the duration of exposure (12 h vs 6 days) to dissolved radiotracers does not significantly influence the efflux rate in mussels.

Efflux rate constants for ^{57}Co and ^{241}Am in soft parts were 2−3 times greater following uptake from the food pathway than from the dissolved phase. Reasons for this discrepancy are not apparent. In *M. edulis*, defecation is mainly responsible for the removal of unassimilable Am and Co, suggesting that this material does not cross the gut epithelium of mussels (*11*). For the other metals examined in this study, efflux rates were comparable when they were accumulated either from the particulate or from the dissolved phase. Wang et al. (*11*) also generally found no significant difference in metal depuration rates in mussels following uptake from the particulate and dissolved phases. However, Ag was lost significantly faster when it was obtained from the food pathway than from the dissolved pathway, perhaps because the ligands that bind Ag vary with the uptake route (*11*). Ag was the only metal that was released principally in feces following ingestion and in excreted form following uptake from the dissolved phase (*11*).

Calculated AEs for mussels held in the laboratory were generally comparable for this study and another laboratory study, which used seven different algal species as food for the mussel *Mytilus edulis* and shorter (4-day) depuration periods (Table 6). The biggest discrepancy was for inorganic Co--9.2% in this study and 25.9% in the earlier work. Reasons for this difference are probably due to different methods for calculating (AE); in the present study we used the *y* intercept of the regression line describing the slowly exchanging compartment, whereas in the earlier work AE was calculated by mass balance assuming that mussels complete their absorption after 3 d. In the case of Co, 3 days may not be sufficient for completion of digestion and therefore the earlier study may have overestimated the AE of Co.

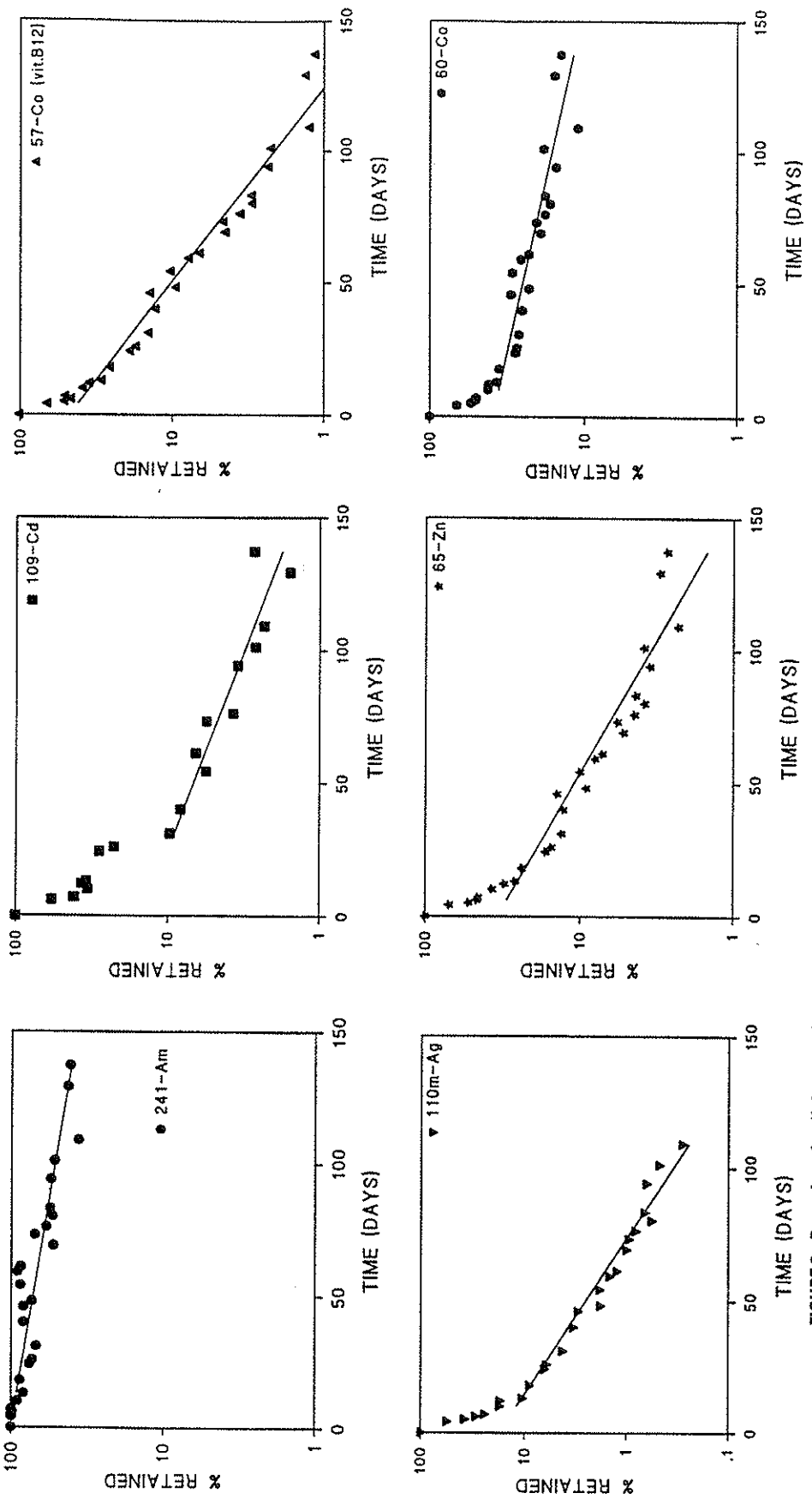

FIGURE 3. Retention of radioisotopes in mussel fecal pellets. Regression lines shown represent least-squares fits for the slowly exchanging pools. All regressions were significant ($P < 0.05$).

TABLE 5

Retention of Radioisotopes in Mussel Fecal Pellets[a]

radioisotope	% in pool	K	$Tr_{1/2}$
^{57}Co	48 ± 1	−0.0312	22 ± 1
^{60}Co	39 ± 1	−0.0087	79 ± 8
^{65}Zn	35 ± 1	−0.0229	30 ± 2
^{109}Cd	15 ± 1	−0.0153	45 ± 6
^{110m}Ag	18 ± 1	−0.0393	18 ± 1
^{241}Am	100 ± 1	−0.0065	107 ± 16

[a] Values shown are fractions (%) in the slowly exchanging pool, rate constants for loss (day^{-1}), and retention half-times ($Tr_{1/2}$) (days).

TABLE 6

Comparison of Absorption Efficiencies of Five Metals between This Study (Mussels Depurated in Field after Feeding on Radioactive Diatoms) and Those from Study of Wang and Fisher (*12*), in Which the Mussel *Mytilus edulis* Fed on Seven Different Algal Species[a]

	absorption efficiency (%)		
metal	this study, field	this study, lab	lab study (*12*)
Co	7.5 (5−11)	9.2 (7−13)	25.9 (20−38)
Zn	18.8 (13−28)	15.0 (12−19)	36.9 (16−48)
Cd	32.4 (20−52)	22.3 (19−26)	21.5 (11−34)
Ag	2.6 (2−4)	14.8 (12−19)	15.6 (4−34)
Am	4.1 (3−5)	6.1 (5−7)	3.3 (1−6)

[a] The Wang and Fisher study only examined laboratory depuration rates (no field component was included) and did not examine Pb or organic Co, so the Co shown in the table is for inorganic Co only. Values are means and ranges for each element.

In general, measured AEs of ingested metals in this study were comparable for mussels maintained in the laboratory and those in the field. For Ag, however, the AE was 5 times greater in mussels held in the laboratory than those in the field (15% vs 3%). This difference may be due to differences in the physiological conditions of the mussels in the field and in the laboratory; Ag absorption in *M. edulis*, for example, is inversely related to temperature (5 vs 15 °C), whereas the absorption of other metals is independent of temperature (Wang and Fisher, unpublished). Because Ag binds so strongly to sulfur ligands in protein (*16*), differences in protein metabolism under different environmental conditions may significantly influence its absorption in mussels.

The findings that trace metals accumulated from the dissolved phase were predominantly bound to shell and those ingested predominantly associated with the soft parts are consistent with results from previous studies (*5*−*7*). Thus, trace metal analysis of dissected mussel tissue of field-collected mussels could provide evidence for the predominant source of each metal at that site.

Model calculations using coefficients derived from controlled laboratory experiments indicate that, for all realistic environmental conditions, Cd is predominantly accumulated from the dissolved phase; Am, Se, and Co are mainly accumulated from ingested food; and Zn and Ag are accumulated significantly from both routes (*11*).

The AE of ^{57}Co (cobalamine) from food and retention efficiency from water was 1.5−2 times that of ^{60}Co (inorganic). This is consistent with the observation that organic Co was found to be more bioavailable to diatoms than inorganic Co (*15*). Furthermore, organic Co was lost at a slower rate than the inorganic Co after accumulation by the mussels from the dissolved phase.

Both absorption efficiency and efflux rate constants are critical in the determination of metal concentration in mussels. Assuming that the influx rate is directly proportional to the total metal concentration in the dissolved and particulate phases, the metal concentration in mussels over time can be described by the following mathematical equation (*11*, *13*, *30*, *31*):

$$dC/dt = (k_u C_w) + (AE \times IR \times C_f) - (k_e + g)C \quad (1)$$

where C is the metal concentration in mussel soft tissues ($\mu g\ g^{-1}$), t is the time of exposure (days), k_u is the uptake rate constant from the dissolved phase ($L\ g^{-1}\ day^{-1}$), C_w is the dissolved metal concentration ($\mu g\ L^{-1}$), AE is the metal absorption efficiency from ingested particles, IR is the ingestion rate of mussels ($mg\ g^{-1}\ day^{-1}$), C_f is the metal concentration in ingested particles ($\mu g\ mg^{-1}$), k_e is the efflux rate constant (day^{-1}), and g is the growth rate constant (day^{-1}).

Under steady-state conditions, the metal concentration in the mussel (C_{ss}) can be calculated as

$$C_{ss} = \frac{(k_u C_w) + (AE \times IR \times C_f)}{k_e + g} \quad (2)$$

If efflux rate constants are dependent of the routes of uptake, eq 2 can be rewritten as

$$C_{ss} = \frac{(k_u C_w)}{k_{ew} + g} + \frac{(AE \times IR \times C_f)}{k_{ef} + g} \quad (3)$$

where k_{ew} is the efflux rate constant after uptake from the dissolved phase and k_{ef} is the efflux rate constant after particulate uptake.

The change in AE is then modeled to determine its importance in affecting metal concentration in soft tissues. Because no data are available for metal uptake rate constants from the dissolved phase (k_u) and for ingestion activity in *M. galloprovincialis*, these data are inferred from studies with *M. edulis* (*11*), a closely related species (*32*). The k_u is 1.794 for Ag, 0.398 for Am, 0.365 for Cd, 0.124 for Co, and 1.044 for Zn (*11*). Only one seston concentration was considered ($5\ mg\ L^{-1}$), and the mussel was assumed to maintain a maximum ingestion rate of $270\ mg\ g^{-1}\ day^{-1}$ (calculated from refs 33 and 34). Both metal concentrations in the particulate (C_f) and dissolved (C_w) phases can be calculated from the total concentration in the water column (C_t), if the partition coefficient (K_d) in ingested particles and total suspended solids (TSS) load are known:

$$C_w = [1/(1 + K_d \times TSS)]C_t \quad (4)$$

$$C_f = [K_d/(1 + K_d \times TSS)]C_t \quad (5)$$

The K_d values employed in this calculation, based on field studies of metal concentrations in suspended particles, are 1.5×10^5 for Ag (*35*), 7.5×10^5 for Am (*36*), 5×10^3 for Cd (*37*, *38*), 5×10^3 for Co (*36*), and 2×10^4 for Zn (*37*). It is recognized that K_d values can vary spatially and tem-

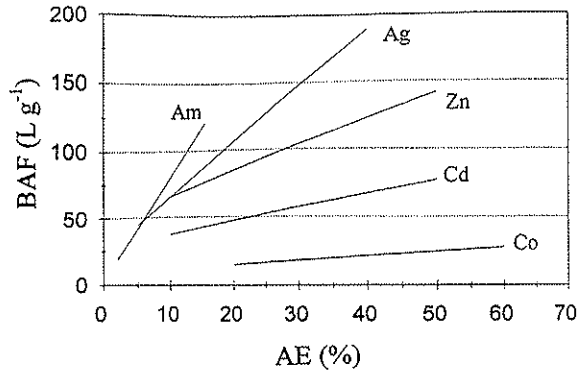

FIGURE 4. Influence of absorption efficiency (AE) on predicted metal bioaccumulation factors in mussels (L g^{-1}). AE ranges considered for each metal are based on the range of AE values measured for mussels for each metal (*11, 12*). No range of Pb AE values has been determined in other studies.

porally, depending on particle loads and composition and on ambient seawater conditions (e.g., salinity, DOC) (*39, 40*). However, the geometric mean K_d values for metals on marine phytoplankton (equal to their concentration factors on dry wt bases), the major food source for mussels, do not vary appreciably among species and are comparable to the values cited here (*36*). The growth rate constant, g, was ignored in the modeling because the efflux rate constant is generally >1 order of magnitude greater than g (0.0019 day^{-1}; *9, 13*). By applying eqs 4 and 5 to eq 3, the metal bioaccumulation factor (BAF), which is the ratio of metal concentration in the mussel at steady state to the ambient total metal concentration, can be calculated as

$$\mathrm{BAF} = \frac{C_{ss}}{C_t} = \left[\frac{k_u}{(k_{ew} + g)} + \frac{(\mathrm{AE} \times \mathrm{IR} \times K_d)}{(k_{ef} + g)}\right]\left[\frac{1}{(1 + \mathrm{TSS} \times K_d)}\right] \quad (6)$$

The BAF is then calculated as a function of AE using the measured k_{ew} and k_{ef} obtained from this study (Figure 4). For Co, only the inorganic form was considered in the model calculation since measurements of k_u are based on inorganic Co species. The model calculation shows that AE is critical in determining metal bioaccumulation in mussels, especially for Am and Ag, for which metal concentrations increase 1.8−2 times over a 2-fold increase in AE (that is, the slope is approximately 1 for these metals). In comparison, the Co concentration in mussels is less dependent on AE, primarily due to a 3-fold higher efflux rate following uptake from ingested particulate matter than from the dissolved phase (which offsets the importance of food ingestion in the overall bioaccumulation of Co). The slopes relating predicted metal concentration in mussels to AE are also lower for Zn and Cd (Figure 4). The contribution of metal uptake from ingested food to the overall bioaccumulation of metals in mussels also depends greatly on the AE. Overall, the model shows that AE cannot be ignored in any bioaccumulation study.

In addition to AE, efflux rates are also important in influencing metal bioaccumulation in mussels and affecting the relative importance of dissolved and food uptake pathways. In bioaccumulation models, it is generally assumed that efflux rate constants are independent of the environmental conditions or are less variable than other parameters described in the model, yet this assumption has not been tested rigorously. Our measurements suggest that efflux rates for some metals (e.g., Am, Co) may be different following uptake from various vectors. If the efflux rate is very different, this should be calibrated in the bioaccumulation model to strengthen the predictive capability of the model.

There are very few studies on the release of trace elements from mussel or other bivalve fecal pellets (*6, 41*), which are the major biodeposition components in many estuarine and coastal environments (such as in mussel beds; *42, 43*). Our measurements demonstrate that Am is very efficiently retained in decomposing fecal material (as it is in zooplankton fecal pellets; *20, 44*) and may therefore be sequestered in the sediments by fecal deposition. If this is the case, there may be very little dissolved Am that can be remobilized into the overlying water. In contrast, Ag, Cd, Co, and Zn are all rapidly released from deposited feces, possibly leading to release into the overlying waters. Thus, mussels may play an important role in the geochemical cycling of trace elements in the coastal and estuarine waters, either by concentrating them from suspended particles in the water column or by regenerating them via their decomposing fecal material.

Acknowledgments

We thank Greg De Villers and Olivier Cotret for technical assistance and three anonymous reviewers for helpful comments. This research was supported in part by grants from the U.S. EPA (R81947201), the New York Sea Grant Institute (NA90AADSG078 and NA46RG0090), the Office of Naval Research (N000149311287), and the National Association of Photographic Manufacturers to N.S.F. This is MSRC Contribution No. 1017. The Marine Environment Laboratory operates under an agreement between the International Atomic Energy Agency and the Government of the Principality of Monaco.

Literature Cited

(1) Goldberg, E. D.; Bowen, V. T.; Farrington, J. W.; Harvey, G.; Martin, J. H.; Parker, P. L.; Risebrough, R. W.; Robertson, W.; Schneider, E.; Gamble, E. *Environ. Conserv.* **1978**, *5*, 101−125.
(2) Phillips, D. J. H. *Quantitative Aquatic Biological Indicators*; Applied Science Publishers: London, 1980.
(3) Goldberg, E. D.; Koide, M.; Hodge, V.; Flegal, A. R.; Martin, J. *Estuarine Coastal Shelf Sci.* **1983**, *16*, 69−93.
(4) Fowler, S. W.; Benayoun, G. In *Proceedings of the Symposium on Comparative Studies of Food and Environmental Contamination*; IAEA: Vienna, 1974; pp 159−178.
(5) Miramand, P.; Guary, J.-C.; Fowler, S. W. *Mar. Biol.* **1980**, *56*, 282−293.
(6) Bjerregaard, P.; Topçuoğlu, S.; Fisher, N. S.; Fowler, S. W. *Mar. Ecol. Prog. Ser.* **1985**, *21*, 99−111.
(7) Fisher, N. S.; Teyssié, J-L. *Mar. Ecol. Prog. Ser.* **1986**, *28*, 197−207.
(8) Langston, W. J.; Spence, S. K. In *Metal Speciation and Bioavailability In Aquatic Systems*; Tessier, A., Turner, D. R., Eds.; John Wiley: Chichester, 1995; pp 407−478.
(9) Thomann, R. V.; Mahony, J. D.; Mueller, R. *Environ. Toxicol. Chem.* **1995**, *14*, 1989−1998.
(10) Wang, W.-X.; Fisher, N. S.; Luoma, S. N. *Mar. Ecol. Prog. Ser.* **1995**, *129*, 165−176.
(11) Wang, W.-X.; Fisher, N. S.; Luoma, S. N. *Mar. Ecol. Prog. Ser.* **1996**, *140*, 91−113.
(12) Wang, W.-X.; Fisher, N. S. *Limnol. Oceanogr.* **1996**, *41*, 197−207.
(13) Thomann, R. V. *Can. J. Fish. Aquat. Sci.* **1981**, *38*, 280−296.
(14) Luoma, S. N.; Johns, C.; Fisher, N. S.; Steinberg, N. A; Oremland, R. S.; Reinfelder, J. R. *Environ. Sci. Technol.* **1992**, *26*, 485−491.
(15) Nolan, C. V.; Fowler, S. W.; Teyssié, J.-L. *Mar. Ecol. Prog. Ser.* **1992**, *88*, 105−116.
(16) Nieboer, E.; Richardson, D. H. *Environ. Pollut. (Ser. B)* **1980**, *1*, 3−26.

(17) O'Connor, T. P. *Recent Trends In Coastal Environmental Quality: Results From The First Five Years Of NOAA Mussel Watch Project*; U.S. Department of Commerce, NOAA: Washington, DC, 1992.
(18) Guillard, R. R. L.; Ryther, J. H. *Can. J. Microbiol.* **1962**, *8*, 229–239.
(19) Fisher, N. S.; Bjerregaard, P.; Fowler, S. W. *Limnol. Oceanogr.* **1983**, *28*, 432–447.
(20) Fisher, N. S.; Nolan, C. V.; Fowler, S. W. *Deep-Sea Res.* **1991**, *38*, 1261–1275.
(21) Van Weers, A. W. In *Proceedings of the Symposium on Radioactive Contamination of the Marine Environment*; IAEA: Vienna, 1973; pp 385–401.
(22) Dahlgaard, H. In *Proceedings of the Symposium on Impacts of Radionuclide Releases into the Marine Environment*; IAEA: Vienna, 1981; pp 361–370.
(23) Dahlgaard, H. *Mar. Ecol. Prog. Ser.* **1986**, *33*, 157–165.
(24) Young, D. R.; Folsom, T. R. *Biol. Bull.* **1967**, *133*, 438–447.
(25) Guary, J. C.; Fowler, S. W. *Estuarine Coastal Shelf Sci.* **1981**, *12*, 193–203.
(26) Heyraud, M.; Fowler, S. W. *Thalassia Jugosl.* **1973**, *9*, 127–137.
(27) Fowler, S. W.; La Rosa, J.; Heyraud, M.; Renfro, W. C. *Mar. Biol.* **1975**, *30*, 297–304.
(28) Amiard, J.-C. *J. Exp. Mar. Biol. Ecol.* **1978**, *34*, 215–225.
(29) Zaroogian, G. E.; Johnson, M. *Arch. Environ. Contam. Toxicol.* **1984**, *13*, 411–418.
(30) Landrum, P. F.; Lee, H.; Lydy, M. J. *Environ. Toxicol. Chem.* **1992**, *11*, 1709–1725.
(31) Newman, M. C. *Quantitative Methods in Aquatic Ecotoxicology*; Lewis: Boca Raton, FL, 1995.
(32) Seed, R. In *Marine Mussels: Their Ecology and Physiology*; Bayne, B. L., Ed.; Cambridge University Press: Cambridge, 1976; pp 13–65.
(33) Bayne, B. L.; Hawkins, A. J. S.; Navarro, E. *J. Exp. Mar. Biol. Ecol.* **1987**, *111*, 1–22
(34) Bayne, B. L.; Iglesias, J. I. P.; Hawkins, A. J. S.; Navarro, E.; Heral, M.; Deslous-Paoli, J. M. *J. Mar. Biol. Assoc. U.K.* **1993**, *73*, 813–829.
(35) Smith, G. J.; Flegal, A. R. *Estuaries* **1993**, *16*, 547–558.
(36) Fisher, N. S.; Reinfelder, J. R. In *Metal Speciation and Bioavailability In Aquatic Systems*; Tessier, A., Turner, D. R., Eds.; John Wiley: Chichester, 1995; pp 363–406.
(37) Kuwabara, J. S.; Chang, C. C. Y.; Cloern. J. E.; Fries, T. L.; Davis, J. A.; Luoma, S. N. *Estuarine Coastal Shelf Sci.* **1989**, *28*, 307–325.
(38) Wood, T. M.; Baptista, A. M.; Kuwabara, J. S.; Flegal, A. R. *Limnol. Oceanogr.* **1995**, *40*, 345–358.
(39) Benoit, G.; Oktay-Marshall, S. D.; Cantu, A.; Hood, E. M.; Coleman, C. H.; Corapcioglu, M. O.; Santschi, P. H. *Mar. Chem.* **1994**, *45*, 307–336.
(40) Sung, W. *Environ. Sci. Technol.* **1995**, *29*, 1303–1312.
(41) Brown, S. L. *Mar. Ecol. Prog. Ser.* **1986**, *28*, 219–231.
(42) Asmus, H.; Asmus, R. M. In *Bivalve Filter Feeders in Estuarine and Coastal Ecosystem Process*; Dame, R. F., Ed.; NATO ASI Series, Vol. G33; Springer-Verlag: Berlin, 1993; pp 57–84.
(43) Dame, R. F. In *Bivalve Filter Feeders in Estuarine and Coastal Ecosystem Process*; Dame, R. F., Ed.; NATO ASI Series, Vol. G33; Springer-Verlag: Berlin, 1993; pp 245–269.
(44) Fisher, N. S.; Bjerregaard, P.; Fowler, S. W. *Mar. Biol.* **1983**, *75*, 261–268.

Received for review January 5, 1996. Revised manuscript received May 28, 1996. Accepted June 10, 1996.[⊗]

ES960009U

[⊗] Abstract published in *Advance ACS Abstracts*, September 1, 1996.