Separation of solute and particulate vectors of heavy metal uptake in controlled suspension-feeding experiments with *Macoma balthica*

Ronald W. Harvey & Samuel N. Luoma
U.S. Geological Survey, Water Resources Division, 345 Middlefield Road, Menlo Park, CA 94025, U.S.A.

Keywords: heavy metal uptake, suspension-feeding, *Macoma balthica*, bacteria, San Francisco Bay, uptake vectors

Abstract

Radioisotope labelling experiments with the estuarine clam, *Macoma balthica*, are described, in which a filter chamber device was used to separate solute metal uptake from uptake of metals associated with suspended bacteria. Solute uptake contributed a majority of the 14-day total body burdens of ^{65}Zn and ^{109}Cd, whereas ^{57}Co uptake largely resulted from ingestion of isotope-laden bacteria. In contrast to those for ^{109}Cd and ^{65}Zn, ^{57}Co tissue distributions at 3 weeks differed significantly ($p < 0.05$) between feeding and non-feeding clams (housed within filter chambers).

Introduction

One of the more perplexing problems in assessing the biological implications of trace metal contamination in aquatic environments is determining the fate of particulate-bound metals. Sediments represent the most concentrated physical pool of heavy metals in aquatic systems. Metal uptake from sediments is considerably slower than uptake from solution if concentrations are equal. In nature, however, higher biological availability of solute metals is countered by much higher concentrations of sediment-bound metals (Luoma & Bryan, 1979; Luoma, 1983). Studies in estuaries indicate concentrations of many metals in deposit-feeders, indeed, are dominated by metal uptake from sediments (Bryan & Uysal, 1978; Luoma & Bryan, 1982).

Interpretation of controlled studies of particulate metal uptake by benthic macrofauna is hampered by the often extreme variability in uptake among individual organisms, and by the tendency of metals to rapidly establish a solute-solid equilibrium when contaminated particles are placed in uncontaminated water. Because animals in such experiments are exposed to both solute and solid vectors of uptake, determination of metal availability from the solid itself is often difficult (Luoma & Jenne, 1976).

The following describes an easy-to-use filter-chamber device that was used to separate solute metal uptake from uptake of metals associated with suspended bacteria, in experiments with the estuarine clam, *Macoma balthica*. We also describe a multi-label, repeated counting approach which facilitates interpretation of metal uptake data in controlled suspension-feeding experiments.

Methods

Bacteria

Pseudomonas atlantica NCMB301 (ATCC# 19262) was grown at 25 °C in Fernback flasks containing sterile nutrient media (filtrable (0.3 μm) seawater, 7.5 gl^{-1} peptone and 10 gl^{-1} dextrose). After 5 days, cells were spun down at 10 000 × g for 15 min in 250 ml polypropylene centrifuge bottles, rinsed and resuspended in filtered seawater. Bacterial suspensions were then incubated for 1 h at

Hydrobiologia 121, 97–102 (1985).

25 °C with 3.7×10^4 Bg each of ^{57}Co, ^{109}Cd and ^{65}Zn (8.44×10^6, 4.57×10^6, and 9.28×10^6 Bg:Mole, respectively). To promote active uptake, 20 mM (final conc.) dextrose was added at the beginning of the incubation. Amounts of isotope taken up by bacteria were determined by comparing quantities of ^{57}Co, ^{109}Cd and ^{65}Zn in suspension to that remaining in solution after centrifugation (10 000 × g for 15 min). Isotope-laden cells were then resuspended with freshly filtered seawater for addition to aquaria.

Filter Chamber

The separation of solute and particulate vectors of metal uptake was accomplished using a filter chamber, which excluded particulates from non-feeding clams housed within, while allowing free passage of dissolved material. This device, designed after the 'membrane filter chamber' described by McFeters & Stuart (1972), was constructed of one 25 mm- and two 6.4 mm-thick machined plexiglass plates, six nylon bolts and nuts, nylon eyehook, threaded PVC plug and two polycarbonate membrane filters (Fig. 1). Several modifications were necessary to facilitate use of the filter chamber in deposit- and filter-feeding experiments with benthic invertebrates. Use of all plastic components, which may be acid washed, lessened heavy metal contamination. Interbolt distances had to be increased from 84 to 98 mm (diagonal) to permit use of standard 90 mm (diam.), 0.4 μm (pore-size) Nuclepore membrane filters. Use of 25 mm- in place of 6.5 mm-thick plexiglass for the middle plate and a redesign of the central circular cavity into an 'archway' configuration facilitated accommodation of a number of control specimens within the same filter chamber.

Once assembled, filtrable (0.3 μm) seawater and *M. balthica* were added through an 18 mm (diam.) threaded sampling port. The chambers were then plugged and lowered into the aquaria by means of a nylon line attached to the nylon eyehook. During the course of the experiment, removal of clams

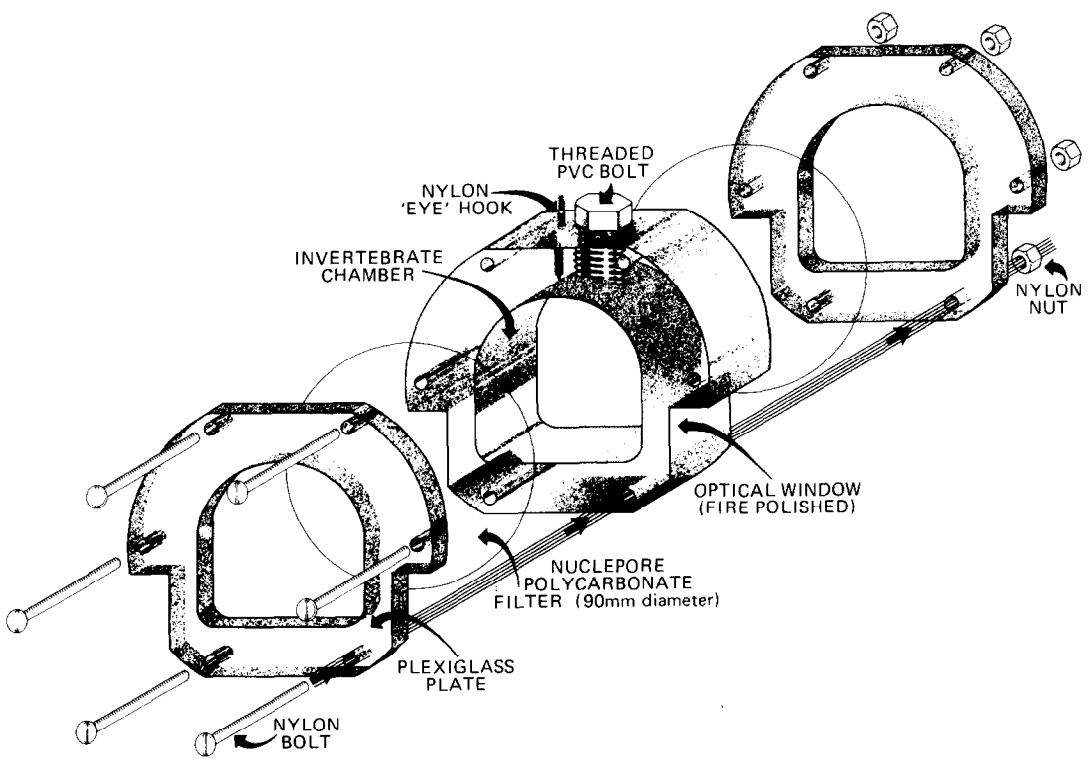

Fig. 1. Exploded diagram of filter chamber device used to preclude particulates from control clams in controlled suspension feeding experiments.

through the chamber sampling port was accomplished with teflon-wrapped forceps while looking sideways through the optical windows (Fig. 1). Liquid samples were withdrawn by pipet.

Experimental set-up and design

Macoma balthica in the 20–21 mm size class was collected from south San Francisco Bay mudflats near Newark, CA. Clams were acclimated to laboratory conditions for 5 days at 12° ± 1 °C prior to the experiment. During the acclimation, salinity was gradually adjusted from ambient levels in the mudflat to 32‰.

Three unstirred aquaria (two experimental and one control) were set up to investigate uptake of Co, Cd and Zn associated with suspended bacteria by *Macoma balthica*. Each aquarium contained 2.5 l of filtrable (0.3 μm) seawater, suspended *P. atlantica* contaminated with the gamma-emitting radioisotopes, ^{57}Co, ^{109}Cd and ^{65}Zn, and a filter chamber housing 5 non-feeding clams, which were used in estimation of radionuclide uptake from the solute vector. In two of the aquaria, clams were placed outside the chamber and allowed to feed on the added bacterial suspensions. Concentrations of Co, Cd, and Zn were $\leqslant 10^{-8}$ M in all aquaria.

One aquarium was set up for a kinetic study. In this system, 5 non-feeding clams from the filter chamber and 7 feeding clams were removed at 1, 2, 4, 7, 11 and 14 days, analyzed for whole body amounts of each isotope and returned to the aquarium. This repeated counting procedure was performed by placing each clam, which had been marked with a diamond pen, in a 25 ml (vol.) wide-mouth scintillation vial containing 10 ml of seawater and counting for 10 min in a gamma detector. Holes in the vial caps facilitated gaseous exchange during counting. To examine tissue distribution of isotopes, five feeding clams were removed from a second aquarium at 1, 2, 5, 9, 14 and 22 d (30 clams total) and dissected into foot, digestive gland, mantle, and gill tissue. Tissue samples were then dried at 90 °C for 24 h, weighed and analyzed for radionuclide content. Five non-feeding clams were also processed on day 22. A third aquarium contained radiolabelled bacteria, but no feeding clams. This 'control' system was used in monitoring declines in bacterial populations and changes in solute: bacteria partitioning of radionuclides in the absence of feeding clams.

Determination of bacterial number and radioactivity

Numbers of suspended *P. atlantica* in each aquarium were calculated from absorbance (520 nm) measurements read on a spectrophotometer. Absorbance was correlated with direct cell counts using the acridine orange epifluorescence procedure described by Hobbie *et al.* (1977). Counts were made to within ±9% at the 90% confidence interval.

Differential counts for ^{109}Cd (88 KeV), ^{57}Co (136 KeV), and ^{65}Zn (1.11 MeV) in the same samples were made on a gamma counter, employing NaI crystal and three energy windows. Counting efficiencies (79.6% for ^{57}Co, 43.7% for ^{109}Cd and 15.0% for Zn) were significantly higher than for comparable GeLi detectors. However, corrections had to be made for overlapping energy ranges for the three isotopes due to lower resolution of our instrument. Counting errors were generally ⩽1%.

Results

Accumulation of total, dissolved, and bacterially-associated ^{57}Co, ^{109}Cd and ^{65}Zn by *M. balthica* in the presence of suspended *P. atlantica* is depicted in Figure 2. Whole body levels of each isotope in suspension-feeding clams, which reflect uptake from both solute and particulate vectors, reached an apparent equilibrium at ~2 d, whereas levels in non-feeding clams, representing uptake of dissolved isotope only, increased throughout the course of the experiment. Calculated levels of ^{57}Co, ^{109}Cd and ^{65}Zn in *M. balthica* derived from assimilation of isotope-contaminated bacteria increased during the first two days of the experiment and decreased thereafter. These results may be explained, in part, by a depletion of much of the bacterial population during the first two days and by the increase in dissolved levels of isotope (Table 1), probably due to net isotope efflux by both bacteria and clams. The apparent equilibrium may be the result of the declining contribution of the food vector and the rising contribution of the solute vector to whole body burdens.

Significant differences in contributions of bacterially-associated isotopes to whole body burdens in *M. balthica* are evident for the three radionuclides. For example, uptake from bacteria alone resulted in 81.6% of the 14-d total uptake for ^{57}Co (Fig. 2A),

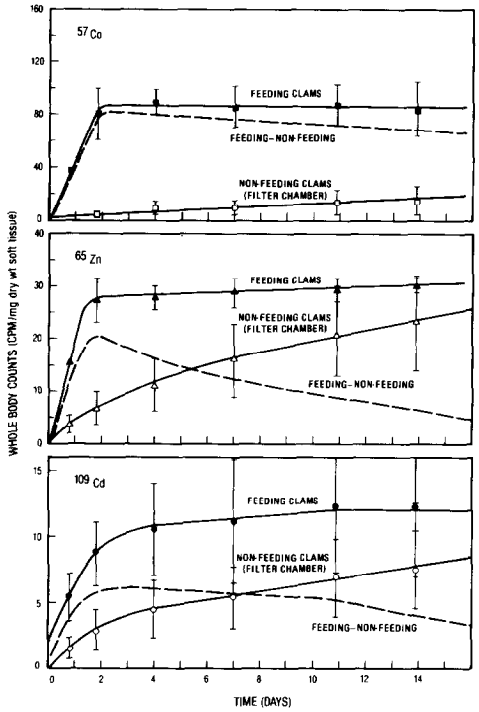

Fig. 2. Whole body uptake of bacterially-associated (*Pseudomonas atlantica*) and dissolved radionuclides by *Macoma balthica*. Uptake of (A) ^{57}Co (B) ^{65}Zn and (C) ^{109}Cd. 'Feeding–non-feeding' refers to calculated uptake of isotope from assimilation of bacteria alone. Error bars represent the standard deviation among replicates.

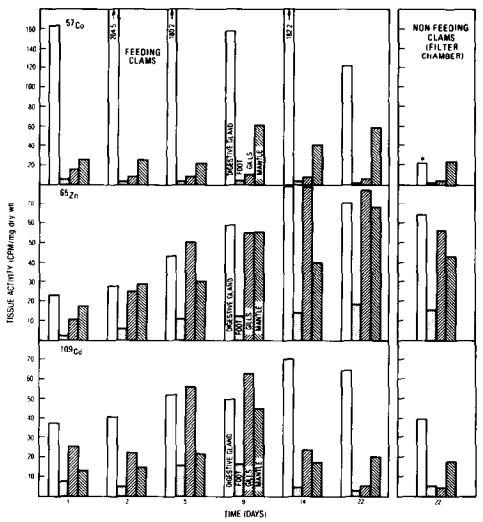

Fig. 3. Levels of ^{57}Co, ^{65}Zn, and ^{109}Cd in digestive gland foot, muscle, gills, and mantle tissue in *M. balthica* feeding on suspended, isotope-contaminated bacteria versus time. Non-feeding clams (right-hand side of figure) were housed within a filter chamber and exposed only to solute metal for 22 d. * Indicates concentration significantly different between feeding and non-feeding animals.

but only 23.4% and 39.0% for ^{65}Zn (Fig. 2B) and ^{109}Cd (Fig. 2C), respectively. At 2 d, before the effects of bacterial depletion were evident, 94.9% of the ^{57}Co uptake was from food, as was 74.9% of the ^{65}Zn uptake and 66.7% of the ^{109}Cd uptake.

Differences in distributions of ^{57}Co, ^{65}Zn and ^{109}Cd in digestive gland, foot, gill, and mantle tissue in *M. balthica* are depicted in Figure 3 as a function of time. Mean tissue levels of ^{57}Co were highest in digestive gland for all time points, ranging up to 204 ± 83.6 C.P.M.: mg dry wt on day 2 and were lowest in foot tissue, which had a maximum ^{57}Co content of only 4.9 ± 0.8 C.P.M.: mg dry wt, which occurred on day 1. Maximum levels of ^{57}Co in gill and mantle tissue were also much lower than for digestive gland, i.e., 15.5 ± 6.1 C.P.M.: mg dry wt (day 1) and 59.4 ± 44.6 C.P.M.: mg dry wt (day 22), respectively. Little variation in tissue ^{57}Co was ob-

Table 1. Distribution of bacteria, ^{57}Co, ^{65}Zn and ^{109}Cd in solution during the uptake-kinetic study.

Elapsed time (d)	Bacteria* (×10⁶: ml)	Isotope activity*					
		Total (C.P.M.: ml)			Bacteria-associated (% of total)		
		Co^{57}	Zn^{65}	Cd^{109}	Co^{57}	Zn^{65}	Cd^{109}
0	30.8 (26.7)	161 (161)	123 (125)	48 (48)	100.0 (100)	100.0 (100)	100.0 (100)
1	12.5 (18.3)	125 (145)	109 (128)	43 (43)	51.6 (52.8)	10.0 (18.5)	15.1 (9.3)
2	2.1 (11.7)	108 (145)	963 (105)	39 (46)	30.4 (35.1)	4.3 (6.0)	3.2 (22.3)
4	0 (10.0)	109 (145)	103 (114)	40 (48)	20.9 (26.1)	3.5 (9.7)	6.7 (23.7)
11	0 (3.3)	99 (136)	101 (108)	38 (46)	12.5 (19.5)	14.7 (16.5)	2.3 (10.7)

* All values in parentheses are for the control system, in which suspended bacteria but no feeding clams were present.

served with time, except for mantle tissue which was two-three fold higher during the second week. Significant differences ($p < 0.05$) in ^{57}Co levels between suspension-feeding and non-feeding clams on day 22 was observed for digestive gland, but not in the foot or gill.

Levels of ^{65}Zn in feeding clams appeared to be fairly similar among tissues with the exception of foot tissue. Maximum ^{65}Zn levels in digestive gland, gill, and mantle tissue were 79.4 ± 25.2 (day 14), 79.9 ± 15.5 (day 17) and 68.1 ± 46.2 C.P.M.: mg dry wt (day 22), respectively, but only 18.3 ± 5.06 C.P.M.: mg dry wt (day 22) for foot tissue. Zinc-65 appeared to accumulate with time in all tissue types.

Distribution of ^{109}Cd among tissues appeared to change over the course of the experiment. Levels of this radionuclide increased in all tissues during the first 9 days, thereafter decreased in foot, gill, and mantle, but continuing to increase in digestive gland. No statistically significant ($p < 0.05$) differences were found in levels of ^{109}Cd and ^{65}Zn between feeding and non-feeding clams on day 22 for the tissues assayed. This, at least partly, reflected the short exposure to labelled bacteria and the dominance of whole body burdens by the solute vector contribution by the end of the experiment.

Discussion

In studies examining the bioavailability of particulate forms of heavy metals to deposit-feeding clams, separation of dissolved from particulate metal uptake has been accomplished by sequestering non-feeding (control) animals in individual dialysis bags, which lay in contact with metal-laden sediment (Luoma & Jenne, 1976, 1977). Since controls enclosed in dialysis bags are only exposed to solute metals, contributions of particle-bound metal to total uptake could then be determined by subtracting tissue metal concentrations in control specimens from those of test animals. In spite of the obvious advantages of being relatively simple and inexpensive, a number of drawbacks render the dialysis bag technique problematic. For example, these bags became quickly fouled when placed in contact with bacteria-laden sediments, necessitating frequent changing. This is a time-consuming operation and can represent significant handling problems in experiments involving use of radiotracers. In addition, the absence of sampling ports in the bags makes continuous monitoring of their aqueous metal content difficult. Alternatively, control animals may be poisoned to prevent their ingestion of radiolabelled particulates. However, this approach is unsuitable for experiments involving radiotracer uptake from food particles, since the poisoned controls do not adequately account for active uptake of dissolved isotope (Montagna, 1983). Consideration of these problems aided in the design of the filter chamber apparatus used in this study.

As demonstrated by McFeters & Stuart (1972), the rate of diffusion of inorganic ions or organic compounds into a filter-chamber device can be comparable to that for dialysis tubing. In our chamber, a pulse of ^{109}Cd, ^{57}Cd and ^{65}Zn added to distilled water (pH 7) in a static system reached equilibrium with the filter-chamber contents within 12 h and was 60% equilibrated within 6 hours. Circulation of water by the activities of enclosed bivalves would likely increase equilibration rates. However, rates of equilibration must still be considered in experimental timeframe and design and may not be high enough to permit use of this device in experiments of short duration, e.g., hours.

Although classified as a deposit-feeder, ^{14}C labelling experiments suggest that *M. balthica* readily filters and digests suspended bacteria (Harvey & Luoma, 1984). The use of filter chambers to exclude suspected bacteria from control clams was essential in interpreting the time-dependent uptake of ^{109}Cd, ^{57}Co, and ^{65}Zn by *M. balthica*. Efflux of the radionuclides from bacteria quickly resulted in significant levels of dissolved (solute) radionuclide which had measurable, but differential effects upon total metal uptake into clam tissue. The magnitude of the solute contribution varied significantly with the metal, however. For ^{57}Co, little uptake from the solute vector was observed, even though most of the Co after the first two days was dissolved in solution (Table 1). The dominance of the food vector in Co uptake also was reflected in significant differences in tissue distribution of Co between feeding and non-feeding clams. These data suggest a high biological availability of bacterially-associated Co.

Recently, Ueda *et al.* (1982) have demonstrated that the chemical form of Co (inorganically versus organically bound) significantly affects its tissue

and molecular distribution within the marine bivalve, *Tridacna crocea*, as well as its biological availability. Concentration factors for Co (tissue Co/seawater Co) were about twice as high for cyanocobalamin (vitamin B_{12}) as for $CoCl_2$. The high biological availability of cyanocobalamin in their experiments may reflect the inability of macroorganisms to synthesize this required vitamin (Lehninger, 1976). In our experiments, rapid incorporation of bacterially-associated ^{57}Co relative to dissolved ^{57}Co into digestive gland of *M. balthica*, in part, may represent cyanoco balamin scavenging from assimilated ^{57}Co-labelled bacteria.

In contrast to solute contribution for ^{57}Co uptake, the solute vector contributed most of the 14-d total body burdens of ^{109}Cd and ^{65}Zn, suggesting the Cd and Zn taken up from food was more labile than Co, and that bacterially-associated Cd and Zn was less important to overall uptake. These results suggest that the solute vector must be accounted for in any investigations of food source contribution to heavy metal accumulation in benthic invertebrates. The importance of solute uptake depends, in part, upon the particular metal.

Acknowledgement

We thank D. Cain and R. Smith of the U.S. Geological Survey for their review of this manuscript.

References

Bryan, G. W. & H. Uysal, 1978. Heavy metals in the burrowing bivalve *Scrobicularia plana* from the Tamar estuary in relation to environmental levels. J. mar. biol. Ass. U.K. 58: 89–108.

Harvey, R. W. & S. N. Luoma, 1984. The role of bacterial exopolymer and suspended bacteria in the nutrition of the deposit-feeding clam, *Macoma balthica*. J. Mar. Res., in press.

Lehninger, A. L., 1976. Biochemistry. Worth Publishers, Inc., N.Y., 1104 pp.

Luoma, S. N., 1983. Bioavailability of trace metals to aquatic organisms: A review. Sci. Total Envir. 28: 1–22.

Luoma, S. N. & G. W. Bryan, 1979. Trace metal bioavailability: Modeling chemical and biological interactions of sediment-bound zinc. In E. A. Jenne (ed.), Chemical Modeling – Speciation, Sorption, Solubility and Kinetics in Aqueous Systems. Am. Chem. Soc. Wash., D.C.: 577–611.

Luoma, S. N. & G. W. Bryan, 1982. A statistical study of environmental factors controlling concentrations of heavy metals in the burrowing bivalve *Scrobicularia plana* and the polychaete *Nereis diversicolor*. Estuarine, Coastal & Shelf Sci. 15: 95–108.

Luoma, S. N. & E. A. Jenne, 1976. Factors affecting the availability of sediment-bound cadmium to the estuarine, deposit-feeding clam, *Macoma balthica*. In C. E. Cushing (ed.), Radioecology and Energy Resources. Proc. 4th Natn. Symp. Radioecol. Dowden, Hutchinson & Ross, Inc.: 2283–2291.

Luoma, S. N. & E. A. Jenne, 1977. The availability of sediment-bound cobalt, silver, and zinc to a deposit-feeding clam. In R. E. Wildung and H. Durcker (eds.), Biological implications of Metals in the Environment. NTIS: 213–231.

McFeters, G. A. & D. G. Stuart, 1972. Field and laboratory studies with membrane-filter chambers. Appl. Microbiol. 24: 805–811.

Montagna, P. A., 1983. Live controls for radioisotope tracer food chain experiments using meiofauna. Mar. Ecol. Prog. Ser. 12: 43–46.

Ueda, T., Y. Suziki, R. Nakamura & M. Nakahara, 1982. Accumulation of Co by bivalve *Tridacna crocea*. Bull. Jap. Soc. Sci. Fish. 48: 993–997.

Received 14 October 1983; in revised form 23 May 1984; accepted 23 May 1984.