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ABSTRACT

The heavy metals that are of most interest in contemporary pollution studies are largely partitioned onto or in solid phases in the marine environment, and the bottom sediments constitute the major reservoir. This laboratory study looks at part of one potential pathway whereby sediment-derived cadmium — a toxic element of no known biological utility — may be taken up by the secondary benthos, and then transferred directly to man.

Two concentrations of inorganic cadmium (0.1 and 1.0 μg ℓ^{-1}), spiked with ^{109}Cd tracer, were added to mono-specific marine bacteria cultures during the log growth phase. Uptake of the cadmium by or onto the bacteria cells was in the range of 87 to ∿100% complete (mean of 96% for six experiments). These bacteria were subsequently presented as the sole additional food source to batches of deposit-feeding clams (*Macoma balthica*) maintained in 11°C aquaria for periods ranging from 14-54 days. In order to lengthen the contact times, the substrates were renewed periodically to introduce additional supplies of the cadmium-labelled bacteria. The experimental design required that the clams ingest the bacteria as their sole or major food source. Test specimens were starved prior to each experiment, and feeding behavior appeared to be normal, but the feeding rate could not otherwise be controlled. At the conclusion of each experimental run, the ratio of cadmium associated with the (non-acid leached) shells to that held by the tissue ranged from 10-50%, suggesting sorption of cadmium (bacteria) onto the shell material. Within the clams, cadmium was found to be concentrated nearly 20x more in the (purged) stomach tissue than in the remaining soft parts. Elevated concentrations of radio cadmium in the aquaria waters (1-23 μg ℓ^{-1}) are attributed to suspension of organic-rich particulate sediment.

In one set of experiments the concentration of cadmium in the bacteria growth medium was set at 0.1 mg ℓ^{-1} and the total quantity of labelled cadmium transferred to the aquarium substrate was computed at 11.3 μg (sediment wet weight concentration range 0.01 - 0.14 μg g^{-1}). After 54 days, 0.1 μg (approximately 1%) of this added cadmium had been taken up (ingested, sorbed) by the clam population. The mean (wet weight) cadmium concentration of the clam tissue at the conclusion of this experiment was close to 1 μg g^{-1}; 33X and 980X greater than that in the coexisting sediment and overlying water, respectively. However, it is estimated that the bacterial concentration of cadmium was around 0.9 μg g^{-1}, so that (assuming this material was the prime food source) no trophic level biomagnification occurred.

The bacterial growth medium concentration of cadmium for a second set of experiments was 1.0 mg ℓ^{-1}. The total quantity of labelled cadmium added to the substrate associated with bacteria was computed to be approximately 160 μg (concentration range 0.15 - 0.70 μg g^{-1} wet sediment) of which 6.0 μg or 3.8% was taken up by or on the clams. At the end of 56 days exposure, half of the remaining clams had died, and the clam tissue cadmium content was determined to be in the range of 12 - 15 μg g^{-1}; > 55X and 530X greater than the concentration of radio cadmium in the sediment and water phases. Again, however, it is estimated that added bacterial cadmium was the same order of concentration as the maximum measured in the clam tissue. The cadmium content of all fecal material was below analytical detection.

These experiments demonstrate that one common deposit-feeding benthic organism may ingest (*via* an identified major food source) and accumulate a non-essential, toxic metal. Food web biomagnification has not been demonstrated here, although tissue concentrations of cadmium increased to one or two orders of magnitude over that present in the substrate and the ambient water (the latter defining the conventionally defined "concentration factor"). Since there was no evidence for elimination of cadmium *via* the feces, a long biological half-life for the tissue-accumulated cadmium is indicated. Tissue concentrations of approximately >12 $\mu g\ g^{-1}$ resulted in 50% mortality of the test clams.

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INTRODUCTION

Metal Uptake by Biota

Biological systems have evolved to use cations in the most efficient and functionally economic fashion. Major factors appear to be the availability and utility of elements which do not form soluble complexes under intra-cellular conditions (that is, pH $\sim$ 7, I $\sim$ 0.3 M; Eh < 0). This means that certain environmentally less abundant elements will be required to perform roles impossible for the major elements. But the number of required trace metals is quite limited. Generally one can think in terms of periodic table group redundancy such that, for example, with the notable exception of Mo, only members of the first transition series are needed. Thus Zn is required to perform certain acid-base functions, whereas Cd — the subject of this report — has no known function and is not required.

It is apparent, then, that biology has developed exceptionally rigorous and complex (many still unknown) chemical reactions to ensure that required elements are extracted from the environment and transported to their functional locations, while chemically very similar elements are excluded. While it is not possible to develop this topic in any detail, a brief outline seems a needed introduction to the work reported here.

Required trace metals may be extracted from the external environment *via* ion exchange reactions on various membrane surfaces, or possibly by means of external chelating agents. After entering the cell, subsequent transport and reaction is usually considered in terms of chelation. Applying basic equilibrium thermodynamic principles, an important characterizing parameter is

$$K'_{ML}\ [M]$$

i.e. the product of the apparent stability constant of a particular metal(M)-ligand and the available concentration of metal [M], which is proportional, other factors being equal, to the amount of metal bound. Figure 1 (from Stumm and Brauner, 1975) illustrates this for a number of metals and ligands. Obviously this simple concept cannot explain a number of vitally important exclusions, and many mechanisms have been proposed to explain specific cases. For example, iron must not effectively compete with Mg in chlorophyll, and kinetic control has been proposed. It has also been proposed that copper, which has very high stability constants with many ligands (see Figure 1) is shunted into special complexes to effectively reduce the [Cu] term.

Zinc and cadmium are of similar size and charge, and thus discrimination may depend upon special ligand binding sites. In this case the ligand concentration must be in excess. Then:

$$Zn + Cd + L_{Zn} + L_{Cd} = ZnL_{Zn} + ZnL_{Cd}$$

where L_{Zn} and L_{Cd} are special binding sites for these metals such that formation of, say, $CdLn_{Zn}$ is excluded because of the subsequent low concentration of [Cd].

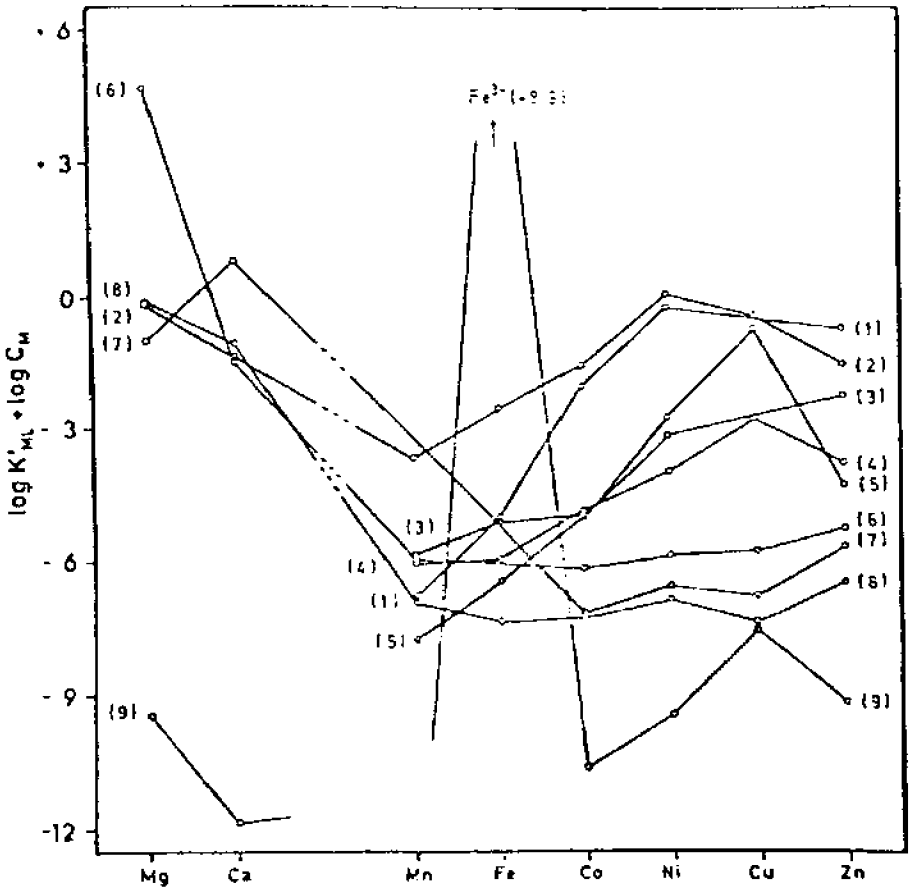

Figure 1. Values of log (K^1_{ML}-C_M) for a series of divalent metal ions and several types of ligands, assuming that these are present in a solution with the inorganic composition of sea water, at pH = S and T = 25°C (W. Stumm and P.A. Brauner in "Chemical Oceanography", ed. J.P. Riley and G.B. Kirrow, Academic Press, London 1975).

Ligands = (1) Cysteine; (2) O-Phenanthroline; (3) Thioglycollic acid; (4) Glycine; (5) Ethylenediammine; (6) Triphosphate; (7) Oxydiacetic acid; (8) Acetic acid; (9) Desferrioxamine B.

The specificity demonstrated by some organisms for certain metals is so extreme that it cannot be explained on the basis of functional group chelation alone. It is believed that certain protein structures possess the necessary geometry to allow the observed separation (Gurd, 1954). Man and certain other mammals (Olafson and Thompson, 1974) are able to synthesi metal-binding proteins — metallothioneins — which sequester unwanted elements, such as Cd. Similar cadmium-binding proteins have also been isolated from *Mytilus* (Noel-Lambot, 1976).

Toxicity of Cadmium

From the above it may be seen that biological systems have evolved to select and reject particular elements largely in terms of their "natural" environmental concentrations. It may be assumed that organisms would evolve naturally to deal with long term changes in environmental conditions if such were to occur, but man-produced pollution can result in conditions outside the "compensation range" of the organisms. This means that althoug non-essential metals, such as Cd, at natural concentration levels may not be able to compete favorably with essential trace metals for binding sites, atypically large, man-induced increases in environmental concentrations of these exotic elements could compensate for their less efficient complexing ability. Hence all metals — even those required in small quantities — may be toxic if administered in sufficiently large doses. And non-essential elements such as cadmium may prove toxic if "external" concentrations are elevated only slightly above ambient. The marine toxicity of cadmium has been extensively reviewed by Simpson (1981).

Chemical Speciation

Since uptake, transport and binding of metals in organisms is generally conceived of in terms of (equilibrium) chelation chemistry, it is clear that the initial, "external" chemical form of the metal is important. Thus, if the "external" form of cadmium is a complex, rather than the "free" cadmium ion, one can envisage a series of intracellular ligand exchange reactions affecting toxicity in which the relative stability constants (and species concentrations) are the controlling factors. Sarsfield and Mancy (1978) suggest such a scheme involving the "aqueous" (external) ligand L_A, the cytoplasmic ligand L_c, and the "toxicity target site" L_T. Then the intracellular equilibrium distribution of the metal is given by:

$$\frac{[CdL_T]}{[Cd][L_t]} = \frac{K_T}{1 + K_c[L_c] + K_a[L_a]}$$

Clearly, if "free" cadmium predominates outside the cell, or if there is excess of a particular ligand, then the species may permeate the cell and additional complexation and ligand exchange reactions are possible. For example, using the terminology of Sarsfield and Mancy:

$$Cd^{2+} + L_T \rightarrow CdL_T \quad \text{(toxicity)}$$

$$L_A + CdL_T \rightarrow CdL_A + L_T \quad \text{(detoxification)}$$

Numerous studies have now been published demonstrating that trace metal toxicity appears to be a function of the concentration of the free (aquo) metal ion and, as a corollary, that toxicity decreases with increase in the proportion of complexed to free metal species. Sarsfield and Mancy (*op. cit.*) have further demonstrated — as might be expected — that the masking effectiveness of an "external" ligand depends upon the stability constant of the metal chelate. Poldoski (1979), considering the uptake of cadmium by *Daphnia magna* in Lake Superior water, also stresses the importance of the stability of the complex in controlling the degree of toxicity.

One of the characteristic features of polluted freshwater is the large amount of dissolved (and colloidal) organic matter usually present, and hence the potentiality for a variety of organo-metallic complexes as the predominating metal speciation in solution. Formation of such complexes may, to a lesser or greater degree depending on relative stabilities, mask the toxicity effects of the metal on the biota. Thus, the results of many aquatic "toxicity experiments", which utilize simple ionic forms of the test metal in the absence of naturally occurring chelation, may be most misleading. Such high-stability complexation is of far less consequence in marine waters. It is conceivable that in the vicinity of sewage outfalls, organic contents could be sufficiently elevated, but relatively high concentrations must be maintained before organo-metallic speciation can quantitatively compete with simple inorganic ligands, in spite of the much larger stability constants of the former class of compound (e.g.: Stumm and Brauner, *op. cit.*; Fig. 3.17). One possible exception might be copper, which forms highly stable complexes with a number of ligands. Sunda and Guillard (1976), Magnuson *et al.* (1979) and other workers, have convincingly demonstrated that, as noted above, copper toxicity is a function of the free cupric ion concentration. Barber and co-workers (e.g. Barber and Ryther, 1969) have further suggested that, in newly upwelled marine water, the free copper ion content may be sufficiently high to inhibit primary productivity unless this metal is partially sequestered by naturally occurring chelators. This concept implies that natural toxicity is the norm under these conditions.

It is possible, however, that bacteria do not conform to this generally observed scheme as noted by Ramamoorthy and Kushner (1975). Mercury-inhibited growth has been demonstrated for example, under conditions where no detectable concentrations of the free metal were present. Ramamoorthy and Kushner (*op. cit.*) suggest that the bacterial cells could successfully compete for binding sites with the ligands present in solution.

Cadmium is one of a small (but important) group of elements which favors stable inorganic chloro speciation in a sea water medium. Sunda, Engel and Thuotte (1978) have shown that cadmium bound in chloro complexes is not toxic to shrimp. These authors have computed the degree of such complexation — the ratio of free (aquo) to chloro cadmium ion — as a function of salinity: at a salinity of 36°/$_{oo}$ the free ion constitutes only 3.5% of the total speciation. The inorganic complex speciation of many (most?) potentially harmful metals in seawater then apparently reduces the toxicity which would result were the metal primarily present as the "free" form. It is, perhaps, surprising that the chloro ligands can "compete" with

intracellular organic groups of potentially higher stability. Possibly these data indicate that cadmium, at least, is initially taken up *via* surface ion exchange, and that the chloro complexes are not as strongly bound as are the more usual hydroxo species. There is some circumstantial evidence for this. The field observations of Preston *et al.* (1972) might be interpreted as possible support for decreased sorption of cadmium in seawater. Mercury, which forms very stable chloro-complexes over a wide pCl range has been shown to strongly desorb in seawater (e.g. Reimers and Krenken, 1974); but Forbes, Posner and Ouirk (1974), in a series of laboratory experiments, showed that Cu, Pb, Se and Cd all adsorb about equally from chloride and (non-complexing) nitrate solutions.

Uptake *via* Ingestion or From Solution

The majority of heavy metal-biota uptake experiments have introduced the test metal in soluble form, either as a stable species (e.g. Eisler, Zaroogian and Hennekey, 1972) or a radio tracer (e.g. Fowler and Benayoun, 1974). This procedure has been adopted mostly because of the far simpler experimental design required. However, it is important to consider how closely these techniques reproduce natural conditions. The problem is at least two-fold, requiring knowledge of the sites of uptake, and also the physico-chemical form of the exotic chemical of interest under environmental conditions. In the case of phytoplankton, and perhaps also bacteria, uptake *via* "adsorption" from solution is usually assumed. This has lead to the concept of "accumulation" or "biomagnification" factors, which are designated as the ratio of metal in the test organism to that in the ambient water. This may not be a useful concept if the uptake pathway is not predominantly or directly from the coexisting solution. Such methodology is certainly questionable when applied to organisms such as certain molluscs which are primarily deposit feeders. However, in all these organisms, a flo of water is maintained over the gills. Experiments using shrimp (e.g. Fowle and Benayoun, *op. cit.*), oysters (e.g. Eisler, Zaroogian and Hennekey, *op. cit.*; Kerfoot and Jacobs, 1976), crab (e.g., O'Hara, 1973), clams (Kerfoot and Jacobs, *op. cit.*), and lobster (e.g., Eisler, Zaroogian and Hennekey, *op cit.*) all indicate significant tissue enhancements when the metal is contact as a soluble species. In many such examples, the uptake site is considered be the gills (e.g., O'Hara, *op. cit.*; Ward, 1982), and the most likely effec a breakdown of respiratory function. Further, when experiments have been designed to test both uptake from solution and from solid phases (food), upt *via* ingestion generally appears to be far lower (e.g. Sick and Baptist, 1979 Kerfoot and Jacobs, *op. cit.*). Jackim *et al.* (1977) have shown that uptake of cadmium, in the absence of a substrate, by the filter-feeding *Mya* is some six times greater than by *Nucula*, a deposit-feeding clam, under the same laboratory conditions.

In man and the higher mammals, ingestion is considered the major entry pathway (i.e., the "food web transfer" of Förstner and Wittman, 1979), and many workers (see review by Kustin and Macleod, 1977) believe ingestion to be of major importance for molluscs also. Boothe and Knauer (1972) note that much of the ingested metal appears to be excreted (and hence that sedimentary feces are an important additional pathway carrying heavy metals

to the sediments); but this would be expected since the organisms are functionally designed to purge themselves of unwanted metals. Possibly there are physiological differences between metals held — at least initially — on gill and other surface membranes and that fraction transported into the body *via* the intestinal tract. This may also have some bearing on the discrepancies reported for biological residence times. For example, cadmium taken up by organisms generally appears to be released very slowly (e.g., Fowler and Benayoun, *op. cit.*); i.e. the biological residence time is long. But other studies (e.g. Jaakkola *et al.*, 1972) have shown quite rapid elimination rates.

One crucial factor is the likely natural and perturbed variation in concentration of cadmium in seawater. Goldberg (1965) calculated that in excess of 100 ppm of the $CdCl^+$ ion pair could theoretically exist in solution in seawater. But, in fact, as with all heavy metals studied to date in any detail, the concentration range of soluble cadmium in open sea water appears to be very low (at a maximum of around 0.1 $\mu g\ \ell^{-1}$). Efficient processes — sorption, biological uptake — will tend to scavenge metals introduced in solution, whether natural or anthropogenic. Heavy metals are present overwhelmingly partitioned onto the sediment phases. It is reasonable to look to this reservoir as a source of metals to the benthic biota, and this seem to be borne out in a general way, inasmuch as deposit feeding species appear to accumulate higher concentrations of inessential elements than do pelagic species (Simpson, 1981). Relative availability depends on the (w/v) concentration of particulate matter present. Thus Houba and Remacle (1982) have calculated that, for river water of bacterial density $3 \times 10^5\ ml^{-1}$, the ratio of soluble to particulate cadmium present would be of the order of 10^3. Filter-feeding organisms might then be exposed to higher concentrations of soluble than particulate metal.

Uptake of Metals From Sediments and the Role of Bacteria

The heavy metal contents of marine sediments may be many orders of magnitude greater than in the overlying water column. We know, too, that metals may not be totally immobilized in the sediments but that some may, depending on the biogeochemical conditions, become remobilized. Heggie and Burrell (1981) have demonstrated this for copper. Deposit-feeding organisms in particular process large quantities of detritus and hence the potentiality for contact of high concentrations of metal with digestive tract membranes is very great. Whether such metals will be assimilated by the organism probably largely depends upon the chemical form of the metal. There is some evidence to show that uptake of metals by deposit feeders may be very small in relation to the total amount present in the sediments, and that organo-complexation is the major reason for this. Thus Luoma and Jenne (1977) demonstrated no detectable uptake from labelled organic detritus, or from organic coated inorganic oxide material *via* ingestion by *Macoma*.

There has been considerable controversy regarding the identification of "available" metals present in the sediments. Working exclusively with oxic zone sediments Luoma and Bryan (1979) have shown that extractable zinc correlates most strongly with the amorphous iron oxide content and is negatively correlated with total organic content. The microbenthos component of these samples was not identified.

Although disputed by some workers (e.g., Hargrave, 1970), the general consensus in recent years is that bacteria constitute a most important source — in some cases perhaps the most important source — of nutrition for benthic deposit feeders (Mann, 1973; Prieur, 1981). Much of the relatively high sedimentary organic material of estuaries is relatively refractory allochthonous material which characteristically has a high C:N ratio, and Tunnicliffe and Risk (1977) and others have drawn attention to a possible "gardening strategy" where deposit feeders may re-ingest feces and pseudo-feces which have been enriched in nitrogen by bacteria. Fenchel and Jørgensen (1977) have noted the propensity for bacteria to adhere to solid surfaces, and Dale (1974) has demonstrated a strong correlation between bacterial biomass and sediment grain-size; i.e. bacteria associate with the size material likely to be selected as food particles by clams. Tunnicliffe and Risk (*op. cit.*) have shown that, for an inlet on the Bay of Fundy, densities of *Macoma* are positively correlated with the density of bacteria in the sediment, and they calculate that, under some circumstances, bacteria may constitute 100% of the food utilized.

It has been frequently noted that heavy metals are taken up by natural bacteria populations (Patrick and Loutit, 1976; Babick and Ptotzky, 1978). Metals may be both incorporated into the bacterial cells or sorbed (Remacle, 1981; the mechanism is immaterial for this set of experiments). McLerran and Holmes (1974) and Remacle (1981) suggest an important role in transporting metals from the water column to the sediments. In this report we are interested in the potential transfer of these exotic elements *via* bacteria to higher organisms.

Use of *Macoma balthica*

Although the situation is not entirely clear, it appears that a single clam species *Macoma balthica* (L.) has ubiquitous circumpolar distribution and is thus ideal for comparative experimental purposes. This small tellin is functionally capable of both filter and deposit feeding *via* an inhalent syphon. De Wilde (1975) suggests that around 60-90% of feeding may be deposit under natural intertidal conditions. Chambers and Milne (1975) have noted that *M. balthica* is an important food species for a large number of predators. These latter authors have measured a maximum density of some 6000 individuals/m^2 in the Ythan Estuary on the east coast of Scotland. They give a production/biomass "ratio" of 2.07 at this locality. *Macoma* is, however, generally a slow growing animal, especially at higher latitudes (Gilbert, 1973): computed P/B ratios in the range 0.25-0.75 are common. Burke and Mann (1974) suggest a general mollusc P/B ratio of around 1.5.

Chambers and Milne (*op. cit.*) record that *Macoma* buries itself in the mud to a degree dependent on size; McGreer (1979) suggests that burrowing behavior can be correlated with sub-lethal heavy metal contamination. The clam feeds from the surface *via* a long incurrent syphon, and this activity results in a characteristic depression or crater in the sediment around 5 cm in diameter. Tunnicliffe and Risk (*op. cit.*) consider that a clam in the 12-14 mm size range will process around 1.5 g of wet sediment per

day. An individual clam need not feed continuously or regularly; this creates some problems with regard to aquaria experimental design.

The primary food source is usually assumed to be benthic algae, but *Macoma* appears to largely select food on the basis of size (so that there may be a tendency to enhance the potential uptake of heavy metals which are generally associated with fine-grained sediment). Rejected material appears as pseudo-feces. Taghorn, Self and Jumars (1978), in their generalized feeding model for deposit feeders, demonstrate that maximum energy efficiency results from ingesting small particles; and they further assume that this fraction will largely consist of bacteria. However, Tunnicliffe and Rick (*op. cit.*) imply that *Macoma* cannot survive by deposit-feeding on bacteria alone. Molluscs in general appear to have a chemosensory system — it can be stimulated by glucose, for example — which influences the filtration-pumping rate. It is not known if toxic heavy metals — or bacteria — can be thus detected to any degree; Prieur (1981) states that the ingestion of bacterial cells by bivalves is unselective. *Macoma* may also function as a second-order consumer of bacteria, *via* microflagellates, as noted by Fenchel (1982).

OBJECTIVES OF STUDY

This work is concerned with the transfer of pollutant heavy metals from the sediments to benthic organisms. The sediments constitute the largest reservoir of such metals in the marine environment. Ultimately we are interested in possible ways that solid phase metals might be made available to organisms; i.e. mechanisms of "remobilization". Solubilization *via* organic chelation is one well considered possibility. Here, we are interested in the potential mediating role of bacteria.

As a background to this study we assumed the basic validity of the following tenets, some of which continue to be controversial.

1. That, under certain biogeochemical conditions, a number of these elements may become remobilized within the sediments to become "available" for transport and biochemical reaction.

It should be noted that it is only in recent years that the flux of major nutrient species out of the sediments has been recognized as a potentially significant contributor to nutrient budgets in the overlying column of estuarine and coastal waters. Mn(II) transport out of, in particular, organic-rich near-shore sediments has also recently been documented by a number of investigators (Owens, Burrell and Weiss, 1980).

For a somewhat longer period it has been appreciated that many trace metals are present in solution in the interstitial waters of anoxic sediments in concentrations which far exceed those predicted assuming simple sulfide equilibria to be controlling. More complex inorganic complexation may partially explain this phenomenon; but organo-metallic complexation is likely to play an important role. And microbial catalysis is believed to be an important component. We know, for example, that bacteria can mediate the conversion of inorganic Hg, and certain other metals, into mobile organic forms.

What is generally poorly understood is whether such transformed metal species are available, or more available, for incorporation into the indigenous benthic biota. This was discussed above.

2. That many deposit feeding organisms (we are concerned only with molluscs in this report) obtain the bulk of their nutrition, not directly from organic detritus (i.e., saprophilic) but from bacteria colonizing this and the surface of other sediment.

The role of sediment bacteria in mediating a number of major, thermodynamically permissable "redox zone" reactions is well known, and their importance in driving many other reactions (e.g., inorganic to methyl mercury) is becoming better known. Many biologists believe bacteria constitute a major portion of the diet of deposit feeding benthos; possibly 100% under favorable circumstances. It is not known whether particular organisms possess the ability to select bacteria *per se*, or if selection by particle size can accomplish the same objective.

3. That direct uptake of pollutant heavy metals by biota in general from solution is a less important pathway than ingestion.

This contention is far more tendentious than the previous. Basically we consider that efficient "buffering" mechanisms exist to prevent significant build-ups of pollutant heavy metals in seawater, and that anthropogenic excesses are removed to the sediments. In very restricted areas it may be argued that organic-metallic complexation may help to augment total soluble contents, but in such cases the sequestered metals are less available for uptake by the biota.

The basic objective of the work reported here, then has been to research the role of bacteria in transporting heavy metals from the sediment reservoir to the indigenous benthos, helping to test the hypothesis that this could represent the most important pathway for toxic metals into the food web. Some previous work (using tubificids and much higher metal concentrations) by Patrick and Loufit (1977) appears to support this concept, but, in general surprisingly few data have been presented by other workers. It appears to be generally believed that heavy metals in sediments, though present in high concentrations, are largely "immobile", a belief probably strengthened by the general inability to define biologically "available" fractions, (Luoma and Jenne, 1977).

This study was originally begun as part of a program to research the potential deleterious effects on natural heavy metal distributions from man-made oil spills. The program sponsors believed that the major problem in this respect would come from the metal contents of the oil itself: in view of the relative volumes and concentrations involved, this appears to us to be incorrect. We considered initially that oil deposited on the sediments might (a) chelate and solubilize metals held in the sediment (b) alter the geochemical (largely redox) environment at the sediment surfaces such that the flux of certain metals out of the sediment was stimulated or increased. At a later stage of these overall investigations it became apparent that oil spills enhanced bacterial activity and this lead to the study described here.

PREVIOUS WORK

Previous work which provided the background to the set of experiments reported here has been reported by Burrell (1978) and Weihs and Burrell (1979). This work utilized stable (non-radioactive tracer) cadmium.

A. Heterogeneous marine bacteria populations were used in the work summarized by Weihs and Burrell (1979). Stable cadmium was added to the broth prior to bacterial innoculation, and the culture was then maintained for 7 days; i.e., well into the stationary growth phase (see later). The concentration of cadmium in the growth medium was initially 0.1 μg $m\ell^{-1}$ (ppm): this concentration did not significantly affect the growth cycle. At the beginning of the experiment, the bacterial concentration of cadmium rose rapidly, but later fell as the biomass increased. The highest concentrations thus occurred during the early stages of the logarithmic growth stage. Concentrations of cadmium in the medium fell below the spectrophotometric detection limit so that, within the analytical limits, the transfer appeared to approximate 100%.

For the second stage of this preliminary experiment, after 7 days, the entire bacterial biomass was spun down, and an attempt was made to make this the primary food source for clams (*Macoma balthica*) held in an aquarium (4-7°C).

An aliquot of spun-down bacterial cell material was analyzed and, from this, it was computed that the entire bacterial food source contained ∿ 25 μg Cd. Thus, the apparent "transfer efficiency" was something less than 10%. Since later experiments (see this report) showed that considerable centrifuge losses occur, the "efficiency" was probably much less than this.

Clams sacrificed beyond this initial one-week period showed decreased contents of cadmium. Although the food source was decreasing, this result suggested *a priori* that either the net biological half-life of cadmium was relatively short (and this appears not to be the case from other workers' data), or the cadmium associated with the clams was not physiologically incorporated.

These preliminary experiments showed an apparent transfer of cadmium from bacteria to clams; but certainly not a biomagnification. This work also pointed up the difficulty of controlling in any meaningful fashion the food source of the clams; a problem hardly better solved in the present set of experiments.

B. Parallel efforts were devoted to the isolation of a mono specific marine bacterial culture and examination of its relevant growth-curve characteristics.

EXPERIMENTAL DESIGN

Basic Considerations

1. For these experiments we utilized a radio isotopic cadmium tracer with a stable carrier. Previous experiments (see above) had shown only a small percent transfer from cadmium-enriched bacteria to clams. It was hoped that use of a radio tracer would permit mass balance computations.

Two sets of experiments were run using two concentrations of total (carrier) cadmium added to the originating bacterial broth: 0.1 and 1.0 $\mu g\ m\ell^{-1}$ (ppm). Previous experiments (see above) had shown that a nutrient broth containing 0.1 $\mu g\ m\ell^{-1}$ of inorganic cadmium did not significantly affect the growth pattern (as compared with controls).

2. It should be noted that, in spite of the primary interest in the potentiality and pathways of sedimentary solid-phase heavy metals to the benthos, this present set of experiments introduces the test metal (cadmium) in soluble form to the bacteria. However, the clams are initially exposed only to solid phase (bacterial) cadmium. The usual — and we believe more artificial — experimental design (e.g., for bioassay, toxicity testing) is to expose the test organism to the toxic chemicals in solution in the ambient water. Of course, since the clams must be maintained in aquaria containing natural seawater which can hold in solution a significant (in terms of these experiments) quantity of soluble cadmium, as the experiment progresses the ambient aqueous reservoir must offer an additional or alternate source of cadmium to the clams. It is apparent from the present experiments (see later) that the test concentrations of cadmium could not be increased without killing the clams, so that there does not appear to be a ready solution to this experimental design problem.

3. This experimental design does not permit the clams being exposed to a constant concentration of cadmium. The bacteria strip out a finite quantity from the nutrient broth, and this quantity is added to the mud in the aquaria. A portion of this discrete spike may be lost to the overlying water; some may be immobilized in the sediment, although since the sediment was not anoxic, this is considered unlikely. More importantly, with time, there is less sedimentary (bacterial) cadmium available for the clams to feed on. To counteract this latter effect the clams were, as described in detail for each specific experimental run below, transferred periodically to new sediment containing fresh bacterial cadmium. This procedure was an attempt to simulate exposure of the clams to an approximately constant source of particulate-phase cadmium.

4. Following isolation of a mono-specific marine culture, noted in the previous work section above, this was innoculated into the growth medium, and the stable and radio-tracer cadmium were added at a specific point in the growth cycle. The bacteria were subsequently spun down and removed at a set point on the growth curve. Details are given below.

5. The clams were maintained in trays of mud set in an 11°C aquarium. The most difficult part of the overall experimental design involved the means of presenting the bacterial food to the clams. Ideally, we would

have wished for the clams to feed consistently and steadily only on the bacteria. And the latter should then be readily accessible to the clams; but it should not be too dilute or diffuse within the mud since, in order to observe an uptake of the tracer, it is necessary that as large a fraction as possible of the total added pellet be ingested within the time frame of the experiment.

The spun-down bacterial pellet was well mixed into a natural mud slurry which was added entire to the individual tray. Each tray (the base of a 1 liter poly bottle) contained approximately 220 g of (wet) mud slurry to which were added 20-22 clams. Thus, each clam was "given" approximately 10 g of mud which could have contained some 5% of the total cadmium spun down from the bacterial growth medium. In actuality, of course, the cadmium "available" for ingestion by the clam depended upon whether the organism fed solely from the surface or not and the feeding range *vis-a-vis* its neighbors. The amount ingested by each organism depended upon its individual — and frequently highly idiosyncratic — feeding habits during the duration of the experiment, and also on such factors as possible uptake of soluble forms of the metal from the water.

6. Although the natural mud used as substrate was frozen prior to each experiment, it was not formally sterilized since it was felt that this would have far increased the problems — difficult at the best of times — of keeping the clams alive and feeding "normally" under aquaria conditions. In this case then, this sediment would have contained detrital carbon as an additional or alternate source of nutrition. It is shown later that the "background" organic carbon content of the substrate is of about the same order of magnitude as that added as bacteria cells.

The Clams

General considerations

Given the program objectives, a deposit feeding mollusc species was required. As noted previously, the clam *Macoma balthica* is ubiquitious in southeast and southcentral Alaska (and apparently has a circumpolar distribution). *Macoma* is primarily a deposit feeder, but can also filter feed where necessary. Food intake is through the incurrent syphon, and the whip-like motion of this appendage may promote some bioturbation and sediment resuspension. It is believed that these clams may feed from beneath the sediment surface. If this is not so, or is relatively unimportant, then, in the absence of extensive bioturbation, a large fraction of the cadmium added to the sediment must have been unavailable.

Food selection is believed to be chemosensory, and, as noted previously, certain size range particles are selected. It would seem likely that these clams might actively select bacteria as a preferred nutrition source, but no direct evidence for this is known to us.

As a general feature, clams can go into non-feeding periods over which we have little experimental control other than to starve prior to each experimental run. Ideally, the organisms are required to ingest (bacteria) at a constant rate through the test runs. Also each random

batch of clams should ingest at about the same overall rate to avoid non-systematic variability between separate batches. In-batch variation between individuals is less important since batch data were integrated.

de Wilde and Berghuis (1978) have recorded that some 25% of the total biomass of *Macoma balthica* may be released into the overlying waters in the form of sexual products: this constitutes an efficient mechanism for dispersing benthic biogenic material into the water column. The clams used in this present set of experiments are not believed to have dispersed material in this way.

Behavior specific to these experiments

The batches of *Macoma* used in these experiments appeared to behave quite "normally". Individuals buried themselves within about an hour of being placed in the mud slurry containers, and syphon action appeared to be regular and normal with the formation of characteristic feeding craters.* Sacrificed samples always contained material in the gut when purged. The special case of organisms exposed to high concentrations of cadmium is noted later.

Sediment processed by clams

In the work reported by Tunnicliffe and Risk (1977), individual clams (*Macoma balthica*) were shown to process 0.6 g dry weight of mud per day; say around 1.5 g wet weight. These individuals were larger (12-14 mm) than the ones used by us (5-10; maximum 12 mm) hence it may be estimated that each of our organisms should have processed around 0.8 g of wet sediment per day; i.e., each would work its entire "share" of the available substrate in around 12-15 days.

Bacteria

The culture

A gram-negative rod (marine pseudomonad) culture was isolated from marine sediment collected in lower Cook Inlet *via* standard soil dilution and plating isolation techniques (at room temperature). This particular isolate was chosen for its suitability as a food source to the test organism, and its ubiquity in the natural habitat.

Note that in experiments preceding this present report, a heterogeneous culture was used (Weihs and Burrell, 1979).

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*Recent work in this laboratory (Winiecki, C. T. and D. C. Burrell), using clams collected from the same locality, in a running seawater aquarium, failed to show evidence for growth over a 6-month period. If this was so for the experiment described here then the apparent uptake of Cd recorded could have been very much underestimated.

The growth medium

Each liter of nutrient broth contained:

trypticase	2 g
soytone	2 g
yeast extract	2 g
agar	15 g
marine mud extract	100 ml

The marine mud extract was prepared by autoclaving 1 kg of wet marine mud in 1 ℓ of seawater for 20 minutes. The medium was adjusted to pH 7.2-7.6, autoclaved, and 1 ℓ aliquots were transferred into sterile flasks and innoculated with the bacteria isolate.

It would be expected that growth medium ligands would sequester heavy metals, thus reducing the fraction of available "free" metal, and competing with bacterial cell binding sites. Ramamoorthy and Kusher (1975) have shown that cadmium is more weakly bound to growth medium constituents than Hg, Pb or Cu; and also that — as noted previously — bacterial uptake of these elements does not seem to be correlated with the free metal fraction.

Growth characteristics

Under control conditions, the culture begins logarithmic growth stage at around 19 hours after innoculation.

In the preliminary set of experiments utilizing a heterogenous population, cadmium was added to the nutrient broth prior to innoculation; i.e., the bacteria were exposed to cadmium from the beginning of the lag phase. Although the 0.1 ppm concentration of cadmium in the medium was shown to have no significant effect on the timing of the growth cycle, a broth with 1 ppm of cadmium seriously inhibited the commencement of the logarithmic growth phase (by around 60–80 hours). It was found, however, that 1 mg Cd added to 1 ℓ of the growth medium during the logarithmic phase did not inhibit or distort the remaining growth cycle. Therefore, for the present set of experiments, the cadmium spike was always introduced at this growth cycle stage. This ensured that all experimental runs reached stationary phase at approximately the same time and simplified the sampling procedures.

The bacterial cells were spun down and processed for addition to the clam substrate always during the stationary growth phase.

The Cadmium Tracer

The isotope ^{109}Cd, a γ emitter with $T_{1/2}$ 453 days was used for this work. This was prepared to give an initial stock solution which, prior to the start of the experiments, contained 6.76 μCi/ml of the tracer. The counter was determined to be 11% efficient so that the stock solution gave $1.0 \cdot 10^6$ dpm/ml. 1 ml of this tracer was added to each liter of culture solution. Two separate concentrations of stable cadmium carrier were used: 0.1 and 1.0 mg/ℓ. At the start of the experiments, cultures

containing 0.1 and 1.0 mg/ℓ of stable cadmium then gave 16,360 and 1636 dpm/μg Cd respectively. These ratios were employed for the first 20 days. To allow for decay, for the second and third 20 day periods the ratios (for a 1.0 mg Cd/ℓ culture) used were 1530 and 1423 dpm/μg Cd respectively.

In all cases 3 ml of solution were counted to maintain a constant geometry. In the case of mud which was freeze-dried and powdered, this material was added to the 3 ml "mark". Absolute concentrations of cadmium computed for the various aquaria phases are based only on distribution of the labelled cadmium added as bacteria. It is believed that equilibration does not occur with the pre-existing reservoir of (inert) cadmium in the sediment. (In the case of water, the original content — around 0.1 μg — is too small to affect computations.) If equilibration with the pre-existing sediment cadmium load occurred during any experimental run, distribution of the radio tracer would have been skewed further in this direction, but, in fact, elevated concentrations were observed for the "water" phase. Such participation of added pools of stable cadmium would not affect the mass balance summation computations given here (since analysis was of cadmium-109 added); but the absolute quantities, based only on stable cadmium added, will be incorrect.

PROCEDURES

General

The work reported here consists of three separate aquaria experiments, in two of which the mollusc substrate was replaced. Each substrate is hereafter referred to as a "slurry". The bacterial growth media contained two levels of cadmium concentration. The periods over which each experiment ran were:

Experiment	Aquarium #1	Aquarium #2	Aquarium #3
Cd conc	0.1 ppm	1.0 ppm	1.0 ppm
Slurry #1	15 days	28 days	14 days
Slurry #2	21 days	26 days	
Slurry #3	18 days		

All weights given subsequently in this report are wet weights. In the case of freeze-dried mud samples, equivalent wet weight values have been computed.

Figure 2 is a flow diagram of the experimental procedures used for each aquaria/slurry experiment. These are described in detail in the following sections.

Bacteria

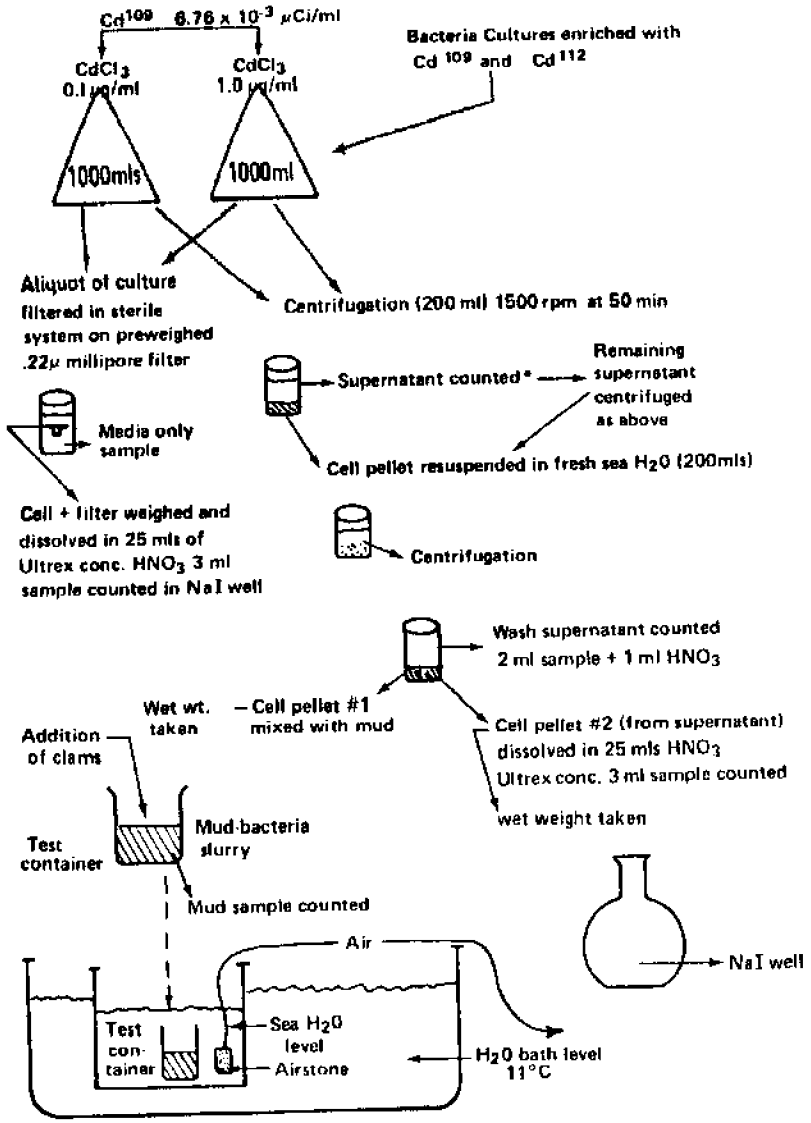
G-rod bacteria were cultured at room temperature as noted. The ^{109}Cd tracer and the Cd enrichment (carrier in solution: either 0.1 or 1.0 ppm) were added during the logarithmic growth stage. The culture was sampled when it reached the stationary growth phase — i.e., the point at which the optical density remains constant — as follows:

(i) 2 ml aliquot of the culture was digested in 1 ml of Ultrex HNO_3 and counted.

(ii) A 15-60 ml aliquot of culture was filtered under sterile conditions through a pre-weighed 22 μm Millepore filter. The cell and filter material was digested in 25 ml Ultrex HNO_3. 3 ml sub-samples of this digested material, and of the filtrate, were counted.

(iii) 200 ml aliquots of the medium were centrifuged to remove the cell material. Details of this step are:

a) Centrifuge tubes weighed dry
b) 200 ml aliquot of cultured added and centrifuged at 1500 rpm for 50 min.
c) Supernatant decanted and counted
d) The remaining pellet resuspended in 200 ml fresh sea-water and spun down as in (b).
e) Wash supernatant decanted and counted
f) Pellet weighed by difference, dissolved in 25 ml Ultrex HNO_3. 3 ml of this solution counted.
g) Procedure (a)-(f) repeated for second or third time. Pellets of bacterial cell material combined and mixed with mud to give particular slurry.



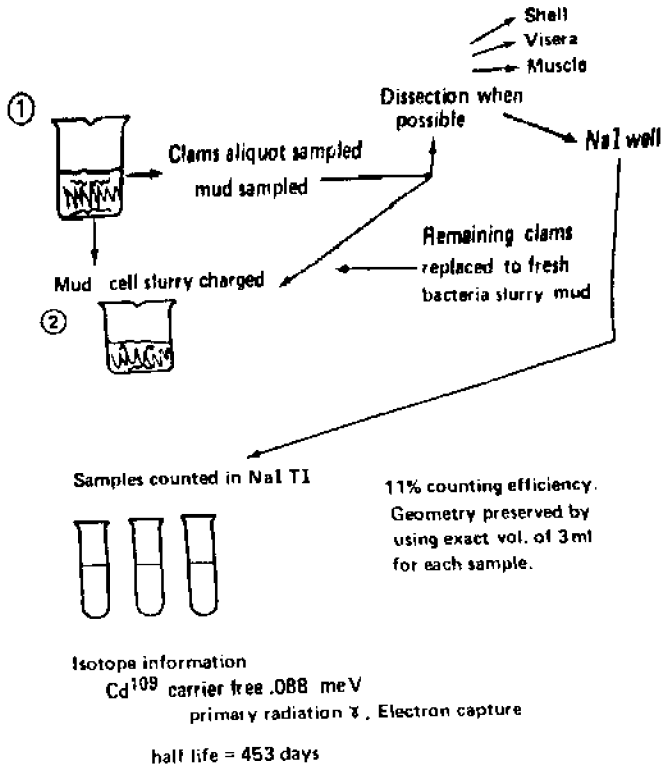


Figure 2. Flow diagram of general experimental procedures employed.

In order to maintain the integrity of the bacterial cells as far as possible, a relatively low centrifugation setting was used: 15,000 g, based on the work of Guthrie *et al.* (1977).

This method for obtaining pellets of labelled bacterial cell material suitable for mixing in with sediment substrate was found to be very inefficient. It proved difficult to spin down the cell material, and variable fractions were lost with the supernatant liquid. This was undoubtedly a major experimental design problem.

Before giving computations of the various transfer (labelled bacterial cell from growth medium to sediment) efficiencies, it should be noted that these losses were <u>not</u> attributable to poor uptake of the cadmium by the bacteria from the medium. As noted above, preliminary work using stable cadmium had shown close to 100% uptake within the analytical limitations. Step (ii) above in which cells were filter-removed from the growth medium showed that the measured uptake efficiency for all aquaria and slurries ranged from 96-102% (counting error).

For a given homogeneous aliquot of medium-plus-cells (in this case the bacterial uptake efficiency is immaterial) the amount of cadmium in each pellet can be computed from the counts on the supernatant and wash (see above). Thus, for the specific case of Aquarium #1, Slurry #2, we have:

Example A: Aquarium #1, Slurry #2

i. The ideal quantity of Cd in 200 ml aliquot of homogeneous cells-plus-medium = 20 µg.

ii. Actual amount measured *via* counting of radio-tracer = 21.4 µg.

iii. Amount determined in supernatant = 18 µg.

iv. Amount determined in wash = 1 µg.

v. So that, in this example, only 2.4 µg maximum can be present in the cell pellet spun down from 200 ml of medium; i.e., the media to sediment transfer efficiency was only 11%.

For this example, it may be computed that the maximum amount of Cd (*via* bacterial cell pellet material) mixed into the mud slurry = 0.04 µg Cd g^{-1} of wet mud.

Example B: Aquarium #1, Slurry #3

Here 86% of the medium cadmium was lost in the decanted supernatant. Since the efficiency of the transfer to the sediment was 14%, the calculated cadmium content of the slurry at the start of this run was 0.05 µg Cd g^{-1} wet mud; the measured content (on a mud aliquot) was 0.04 µg g^{-1}.

Initially, it was hoped to determine the spin-down efficiency by running parallel centrifugation separations in which a second pellet was dissolved and counted. However, it was seen that the centrifugation removal step was not only inefficient, but variable,

so that the results of the parallel run could not be applied. In all subsequent runs the computed cadmium content of each pellet was then determined by measuring the concentration of the medium, and the decanted and wash waste.

Sediment

Approximately 220 g wet weight of mud was used for each experiment and two or more labelled bacterial cell pellets were mixed with this material to make a "slurry". This mix was then allowed to stand at 10°C for some 12 hours. The purpose of this step was to allow "equilibration" of the bacteria without a large amount of growth activity prior to the addition of clams. The slurry was added to the clam feeding chambers (see Figure 2) which was then carefully filled with 350 ml seawater and the whole placed in a aquarium. This step always produced a suspension of fine sediment particles into the overlying water. Water samples collected at this time and counted showed enhanced contents of the cadmium tracer, and at all times measured concentrations of cadmium in the aquaria water are higher than can be explained *via* equilibration with the natural cadmium levels. It is believed that suspended sediment loads remained relatively high throughout the experimental runs, and that this material probably largely consisted of (less dense) bacteria cell material, rich in labelled Cd.

At the end of such individual slurry experiment, the entire mud sample was mixed and freeze dried, homogenized and sub-sampled. These latter were added to the counter to give a volume equivalent to the 3 ml solution samples (to maintain counting cell geometry). The (wet) sediment sample was also weighed at this stage and equivalent dry weights computed.

It was not possible to adequately sample the sediment at the beginning of each run in order to determine the actual quantity of labelled cadmium available. This latter value was computed at the time of pellet separation as noted previously. Only small sub-samples could be spared at this time, and this gave variable results, probably due to sample inhomogeneity.

It has been noted previously that labelled bacterial cell cadmium was added to natural sediment which already contained stable cadmium. We do not believe that the labelled cadmium equilibrated to any significant degree with this pre-existing reservoir. In the first place the labelled cadmium is added in a bound form with living organisms (the bacteria). Some will be released from senescent cells, but presumably the majority remain with the viable bacteria. Also the length of each experimental run is too short to permit significant exchange with the solid-bound cadmium. We compute that the acid extractable solid phase pool is around 20 µg Cd per slurry. If exchange were important, then this reservoir would be dominant, changes in concentration during each experiment run would be insignificant and uptake by the clams probably undetectable. This is not the case. It is, therefore, assumed that the sediment bacterial cadmium functions as an independent labile pool within the inert inorganic reservoir. Mass balance computations given below are for the "excess", labelled cadmium.

The natural sediment used in these experiments was taken from Alaskan fjords and contained around 1% organic carbon which could have constituted

an additional or alternative food source. However, the bacteria pellets added to the sediment amounted to between 1.5 and 3% wet weight organic carbon.

Use of consecutive slurries for the same batch of clams helped provide an approximately constant source of cadmium and food, and also extended the life of the experiment.

It could be argued that fecal material provided an additional source of nutrition and hence a means of recycling cadmium. However, as will be shown, very little (radio) cadmium was found to be excreted.

The Clams

Live *Macoma balthica* samples were collected from within Kachemak Bay, Cook Inlet (Figure 3) and transported to the laboratory. As noted previously, these clams were perhaps smaller than average — 5-10 mm; maximum of 12 mm — and 20-22 individuals were used in each feeding chamber. The clams were starved prior to each experimental run in order to promote feeding. On being placed on the test substrate each clam rapidly buried itself within about 1 hour, and normal syphon activity, with the progressive development of feeding "craters", was observed. It is clear that actual feeding behavior could not be controlled, and all individuals would not have fed at the same rate.* However, the quantity of substrate used, and the length of each run, were designed to promote complete "overturn" of all the available sediment. At the completion of each "slurry" experiment, either half or all of the clams were sacrificed, and the results integrated so that an average uptake rate was obtained.

Sampling of clams

With certain exceptions which are noted in the "Results" section, half of each clam population (i.e., about 10 individuals) was removed at the end of each slurry run. The sampled clams were placed in a fresh seawater container for eight days (a much longer period than usually considered necessary) in order to purge all intestinal tract contents. The purged material, which included fecal pellets, was collected and analyzed as noted below.

Individual clams were washed with distilled water prior to dissection.

Dissection of sacrificed clams

Initial attempts were made to separate out individual organs, but because of the small size, this proved to be a difficult operation. Limite data for fractionation within the clam are given below, but the majority of the results reported here are for total "tissue", and for the shells separately. Tissue material was blotted dry, individually weighed, and dissolved in 3 ml concentrated (Ultrex) nitric acid. The shell material was treated similarly. It should be noted that the shells were rinsed with water only and were not acid leached. It is, therefore, likely

*See note concerning growth characteristics of aquaria clams given previous

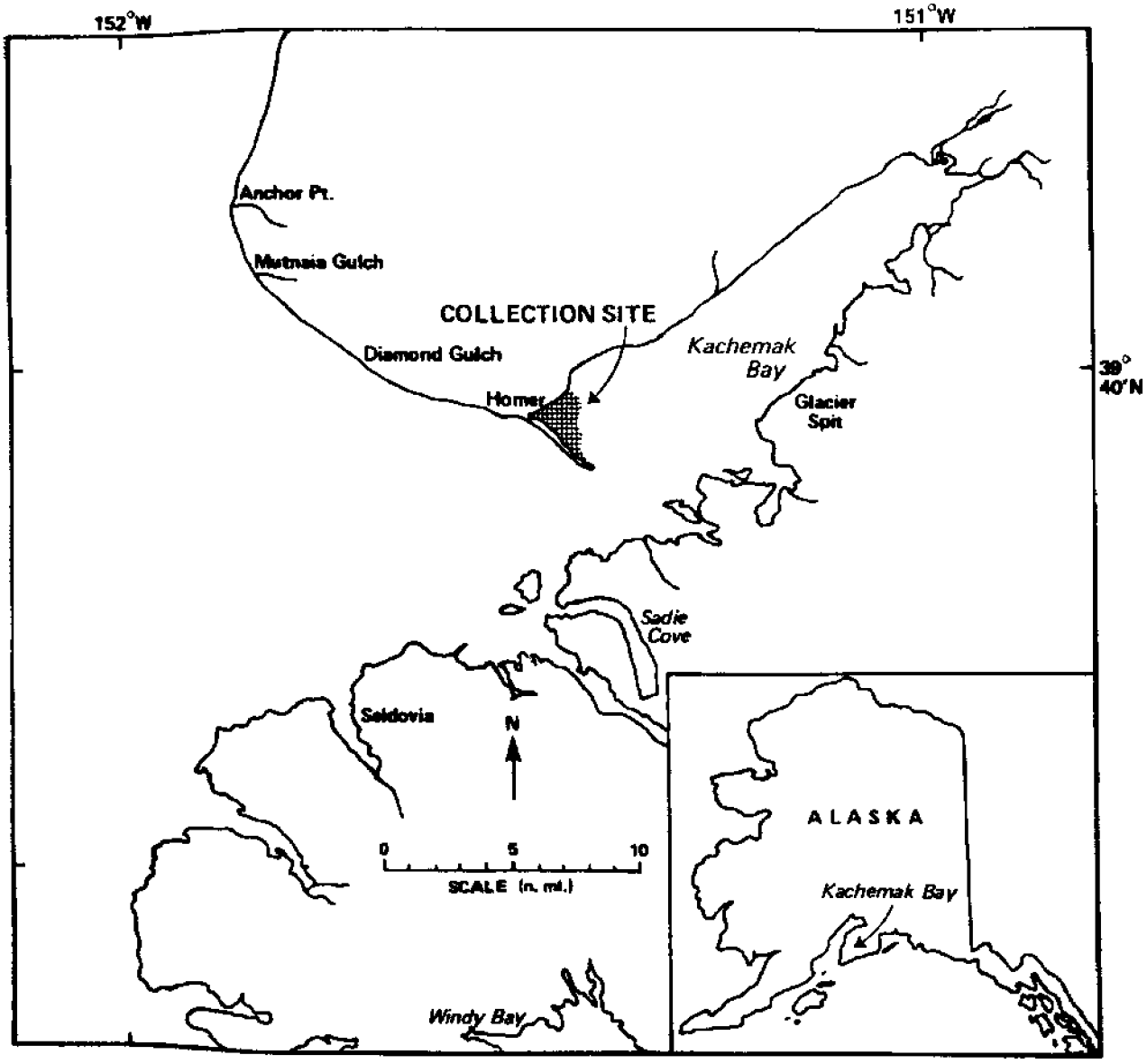


Figure 3. Location of collection site of samples of *Macoma balthica*.

that the relatively high concentrations of cadmium unexpectedly found associated with the shell component were due to surface sorption (and/or epibacteria).

Clam mortalities

The Aquaria #2 and #3 experiments employed starting cadmium concentrations which were x10 higher than Aquaria #1. Aquaria #3 was a short-term experiment. During the second slurry run of Aquaria #2 clams started dying, and at the final sampling point there was almost an exactly 50% mortality. Tissue concentrations given in the "Results" section are for the surviving live specimens: i.e., 12.2 ± 30% μg Cd g^{-1}. But even these individuals appeared to be in poor condition, and tissue concentrations of cadmium for the clams that died — 15.5 μg Cd g^{-1} — were not very much higher. It was not possible to thoroughly purge the dead clams of stomach contents. However, since the cadmium content of this material purged from live samples was very low, this is not considered to be a serious bias.

Fecal pellets

The purge time allowed must have been adequate since visual observation of dissected intestinal tract and stomach showed no sediment. There was also no residue remaining following the acid digestion of the tissue. The material excreted during the seawater purge appeared to consist largely of fecal pellets with some sediment and mucus. It was collected by retention on a 0.22 μm filter. Counts for the cadmium tracer on digested samples of this material were never significantly above background. (It would appear, therefore, that undigested sediment was not a major component.)

Water

Each aquarium contained approximately 4 ℓ of seawater. It was expected that, even during the relatively short experiment times used here this water would tend to equilibrate, as regards soluble cadmium content, with the sediment.

The mean content of Gulf of Alaska open ocean water previously determined by us is 0.03 μg/ℓ. The range determined by Bruland (1980) for the open north Pacific is 0.00016–0.12 μg/ℓ. Taking the higher concentration, the "equilibrated" cadmium content of each aquaria would be expected to be around 0.5 μg. However, as will be seen, measured aquaria "soluble" contents were always higher, by up to an order of magnitude. Measurements taken at the beginning of each experiment, soon after the sediment container was placed in the aquarium, were always very high. It is believed, therefore, that high counts on the aquarium water also included suspended particulate sediment. Labelled bacteria cells, of density less than the inorganic sediment, should form a large fraction of this material, and hence account for the measured high concentrations of cadmium in this "compartment". Although *Macoma* is known to discharge large amounts of organic material into the water, this is thought to be an insignificant factor.

RESULTS

Introduction

Labelling of the bacteria, and the subsequent separation of concentrated "pellets" of cells suitable for addition to the aquaria sediment substrate, have been described in the previous "Methods" section (see Figure 2). In this section the results of the various Aquaria/Slurry experiments are given. The boxed area of Figure 4 indicates the various major sinks for distribution of cadmium in each system. It should be re-emphasized that labelled cadmium was introduced to a complex "ecosystem" which already contained quantities of stable (unlabelled) cadmium, most especially in the natural sediment used. As noted previously, it is considered that the biologically held labelled cadmium did not equilibrate to any major degree with the pre-existing (primarily inorganic) pool during the life of each experiment. In the case of the "soluble" reservoir, it will be seen that the measured content is far larger than would be given by the pre-existing content in solution.

The mass balance distributions given in this section are necessarily based on the total (computed) quantity of labelled cadmium transferred to each individual mud slurry. However, subsequent estimates of the distribution of this material between the various system components does not allow for any equilibration of the spike with pre-existing cadmium. If such does occur, then the measured quantities (based on the amount of stable cadmium carrier added to the original bacteria growth media) will be too low.

Fractionation of Cadmium Within the Clam

A few sacrificed clams were dissected, and portions were analyzed separately in order to determine sites of concentration within the organism. The fractions analyzed were:

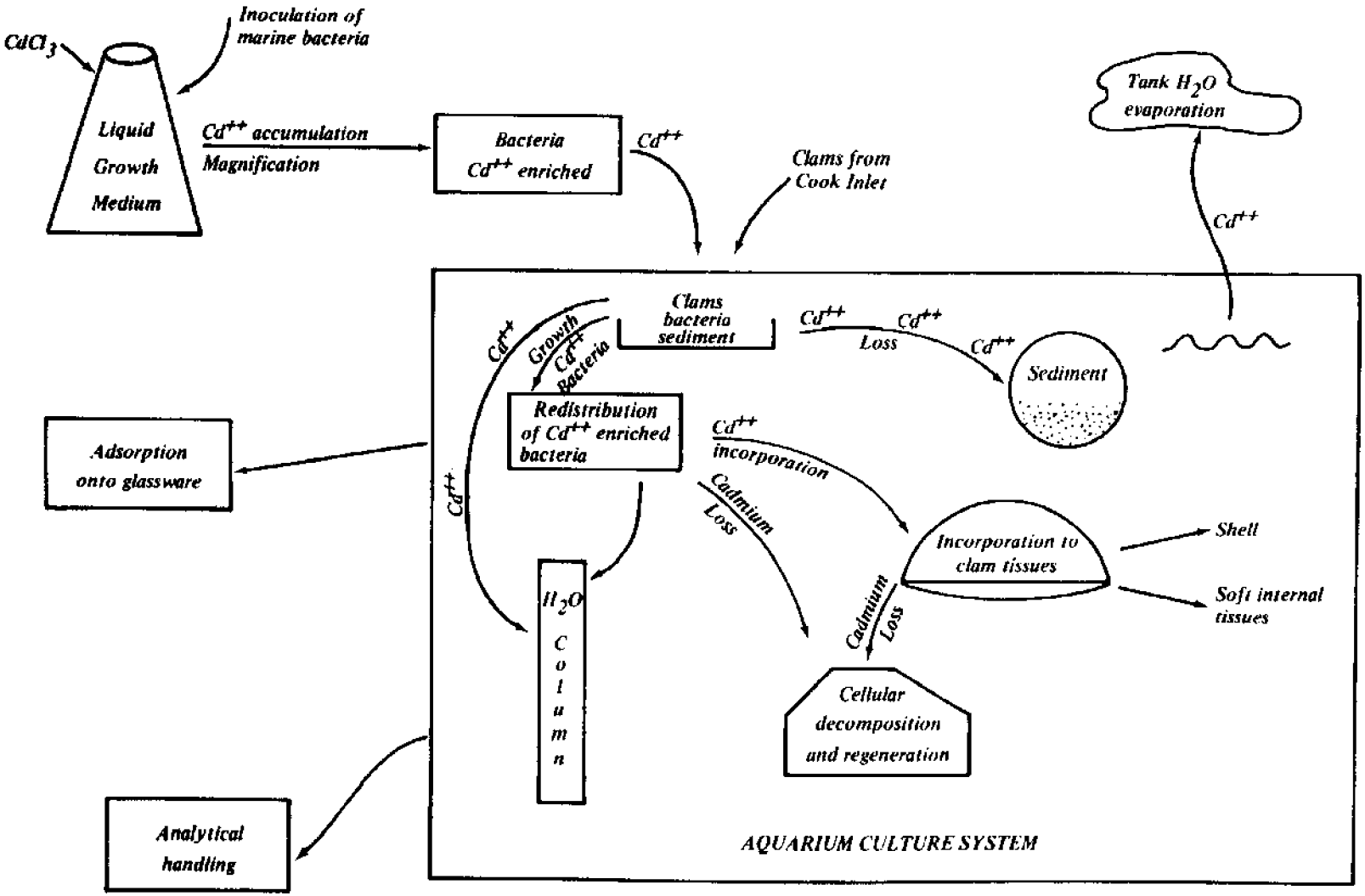
a) stomach
b) remaining internal tissues including foot, mantle, gonads, etc.
c) shell.

The ratio of radio cadmium per unit wet weight held by the stomach to that in the remaining soft tissue was found to be 19 ± 1. (It should be noted that the stomachs had been purged of contents.) The results following in this section are for the total soft tissue and shell fractions only.

Aquarium #1

Slurry #1; Days 0-15:

The concentration of cadmium added to the bacterial growth medium during the logarithmic growth stage was 0.1 mg ℓ^{-1}. Counting of an aliquot of the culture gave 0.11 ± 0.003 µg Cd ml^{-1} (i.e., 44 µg Cd in 400 ml). Filtration separation and analysis of the bacteria gave 0.12 ± 2% µg Cd ml^{-1}. The error cited here is the replicate counting error; the value


$CdCl_3$
Inoculation of marine bacteria
Liquid Growth Medium
Cd^{++} accumulation Magnification
Bacteria Cd^{++} enriched
Cd^{++}
Clams from Cook Inlet
Tank H_2O evaporation
Cd^{++}
Clams bacteria sediment
Cd^{++}
Growth
Cd^{++} Bacteria
Cd^{++} Loss
Cd^{++}
Sediment
Redistribution of Cd^{++} enriched bacteria
Cd^{++} incorporation
Cadmium Loss
Incorporation to clam tissues
Shell
Soft internal tissues
Cd^{++}
H_2O Column
Cadmium Loss
Cellular decomposition and regeneration
Adsorption onto glassware
Analytical handling
AQUARIUM CULTURE SYSTEM

given is 120% of the theoretical amount present. Partition of the cadmium, added in soluble inorganic form into or onto the bacterial cells, appears to be complete (the filtrate count — 19 cpm or around 0.001 µg/ml — is close to background).

Two 200 ml aliquots of the culture were spun down to give cell "pellets" for addition to the clam sediment substrate. (If the separation had been complete 44 µg of labelled cadmium should have been available as noted above.) In fact, from counts on the supernatant and wash, it was calculated that the cell loss during this process was 31% so that the maximum quantity of labelled cadmium added to the sediment (Slurry #1) was 0.14 µg Cd g^{-1} of wet mud.

Clams were transferred to this labelled substrate and the whole contained within an aquarium as described above. After 15 days, 7 clams were removed at random, treated, dissected as described, and analyzed. The computed uptake and fractionation of cadmium by the shell and tissue of these *Macoma* are given in Table I. From these data it may be computed that the total cadmium held by all the clams (both those sacrificed and those remaining at the end of this 15 day period) was .039 µg and .016 µg Cd held by the tissue and shell material respectively. Fecal pellet material purged from the sampled clams weighed 0.27 g and showed non-detectable quantities of cadmium.

At the start of this experiment, prior to placing the sediment container into the aquarium, analysis of an aliquot of the water held in the sediment container showed 0.3 µg in this phase; at the end of the 15-day run, the aquarium water contained 12 µg. This value is too high to be attributable to (inorganic) soluble cadmium species. It is believed, therefore, that particulate sediment, rich in bacteria cells, was included. A count of the sediment taken at this time showed 8.8 µg Cd present. Unfortunately, the sediment sample taken at the beginning of this run was lost. At the conclusion of the run, therefore, the apparent mass balance, as fractions of the labelled cadmium theoretically added, is as shown in Figure 5. It will be seen that this distribution leaves approximately one-third of the labelled cadmium believed to have been added to the sediment unaccounted for. This "loss" may be due to one, or a combination of: (a) additional undetected loss of bacterial cells prior to addition to the mud; (b) the existence of other sinks (e.g. the aquarium walls) not accounted for; (c) error (e.g. sample inhomogeneity) in the determination of cadmium in the sediment; (d) magnified error in the water analysis. Figure 6 shows the fractional distribution, without absolute values, of cadmium within the system actually measured at the end of the first 15-day period.

At day 15 the (wet weight) concentration partition of cadmium between the sediments and the whole clams (tissue and shell) was approximately 0.04 µg g^{-1} for both phases; i.e. no biomagnification. The concentration of cadmium in the tissue was 4 times greater than that associated with the shell. Nevertheless, the shell concentration (mean of 0.02 µg g^{-1}) is surprisingly high. It may possibly be attributed to surface sorption.

TABLE I

Distribution of labelled cadmium ($\mu g\ g^{-1}$) in shell and tissue of *Macoma balthica* - Aquarium #1; Slurry #1

15 day exposure period

	Tissue		Shell	
Specimen	Weight (g)	Cadmium Conc* ($\mu g\ g^{-1}$)	Weight (g)	Cadmium Con ($\mu g\ g^{-1}$)
1	.073	.14 ± 3%	.05	.02
2	.032	.08 ± 7%	.05	.02
3	.023	.10 ± 5%	.05	.01
4	.028	.03 ± 14%	Not above background	
5	.019	.06 ± 4%		
6	.019	.06 ± 4%		
7	.019	.06 ± 4%	.05	.03
	$\bar{X}$	0.08	$\bar{X}$	0.02

* precision refers to counting error

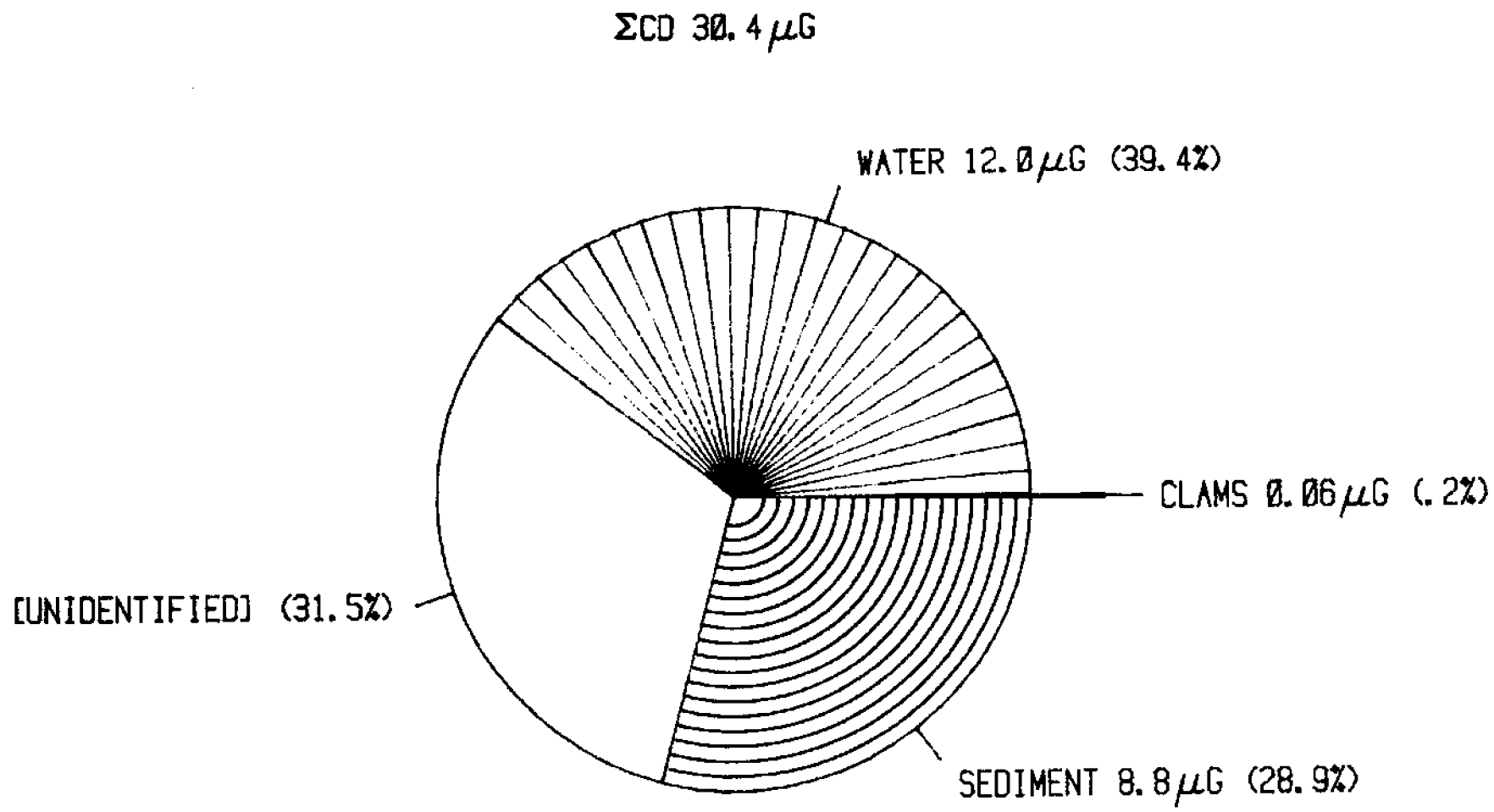
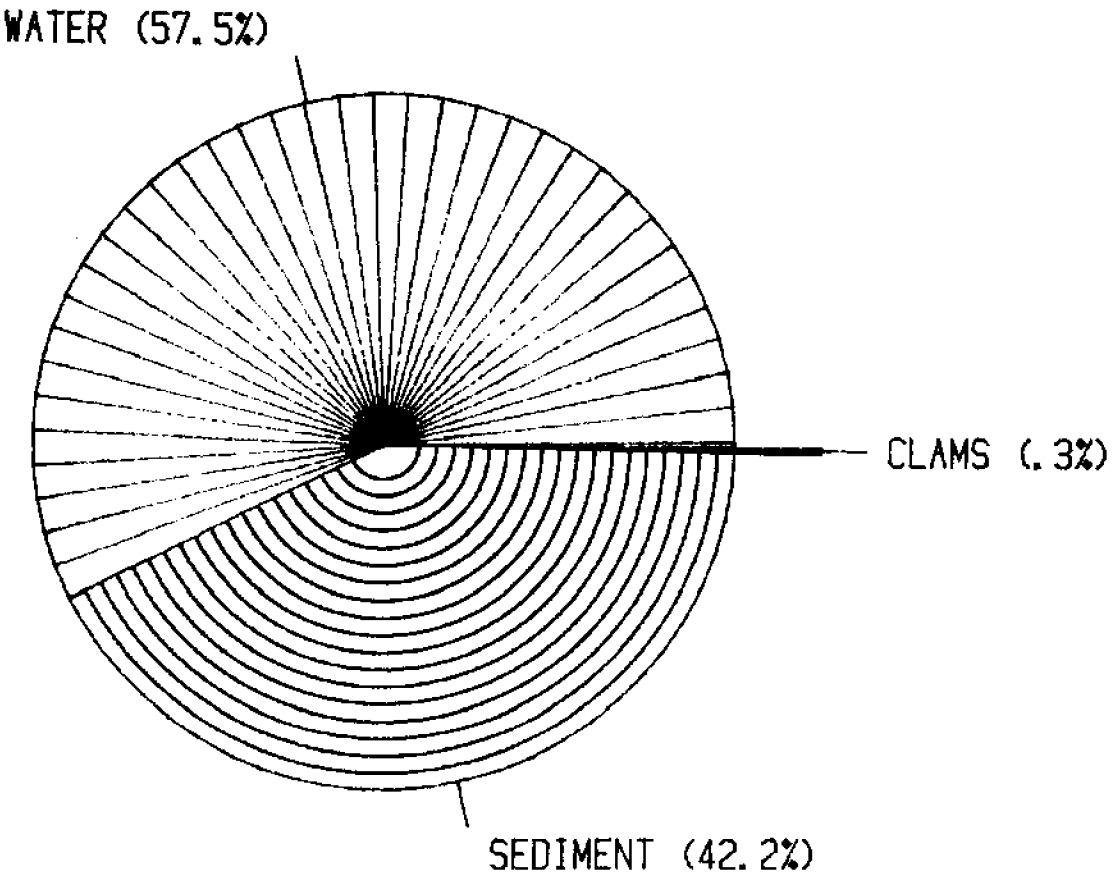


Figure 5. Computed mass balance of labelled cadmium in aquarium system at the conclusion of the first part (15 days) of Aquarium #1 experiment (see text). Distribution in the major reservoirs is shown as % fractions of the quantity of labelled cadmium (μg) theoretically added to the system at the start of the experiment.


WATER (57.5%)
CLAMS (.3%)
SEDIMENT (42.2%)

Slurry #2; Days 16-37:

At the end of the first 15 days of this (Aquarium #1) experiment, a new, labelled sediment slurry was prepared. The bacteria culture was spiked with 0.1 µg ml^{-1} of labelled inorganic cadmium. Counting an aliquot of the total culture prepared for this slurry gave 0.107 µg Cd ml^{-1}. Filtration (0.22 µm) separation of a further aliquot, and counting analysis of the fractionated cells and filtrate (medium), showed that the partition of cadmium onto the bacteria cells was approximately 87% complete.

Four separate 200 ml aliquots of bacteria culture were then spun down to prepare pellets of bacteria cells for addition to a new substrate. The analysis of the supernatant and wash from one of these operations gave:

supernatant - 18.0 ± 0.2 µg Cd
wash - 1.0 ± 0.2 µg Cd

i.e., the computed cell loss here was 89%, and only 2.4 µg labelled Cd was present in the pellet. One other "dummy" run was carried out in which the loss was approximately the same. Measurement of the amount of cadmium lost during the spin-down operation of the other three pellets added to the sediment was not made; it must be assumed that comparable losses occurred. It is estimated, therefore, that only 9.6 µg of labelled cadmium was added to Slurry #2; i.e., 0.04 µg Cd g^{-1} wet sediment. This is the concentration measured in Slurry #1 at the end of the first 15-day period, so that the principal objective in preparing a second slurry-substrate at this juncture — namely, to increase again the concentration of cadmium available to the clams — was thwarted.

The clams remaining at the end of the previous run were resettled on the new substrate in the same manner as described previously. This procedure involved also replacement of the aquarium water. At the start of this second segment of the Aquarium #1 experiment, the distribution of labelled cadmium was then as shown in Figure 7. The total quantity present consisted of labelled cadmium associated with bacteria cell pellets (9.6 µg computed as above), plus cadmium present within the clams which is assumed to be proportionally the same as was found in the sacrificed batch (because of the small amount *vis-a-vis* that is present in the mud, no great error is involved here). Prior to addition of the full volume (4 ℓ) of aquarium water, the 150 ml added to the sediment container was sub-sampled and counted, showing a total of 0.3 µg Cd in this "phase" (presumed to be both water and suspended sediment).

In order to prolong the total exposure time of the remaining clams to sediment-bound cadmium, no clams were sampled at the end of this slurry period, which was terminated after a 21-day period. It was estimated that the clams should have "worked" the mud in this period. Counting an aliquot of sediment at this time identified a total of 2.2 µg Cd remaining in this phase. The computed mass balance is shown in Figure 8. The quantity present in the clams is estimated, based on that present after 53 days; this does not materially affect the distribution. But, again, approximately one-third of the labelled Cd believed to have been added at

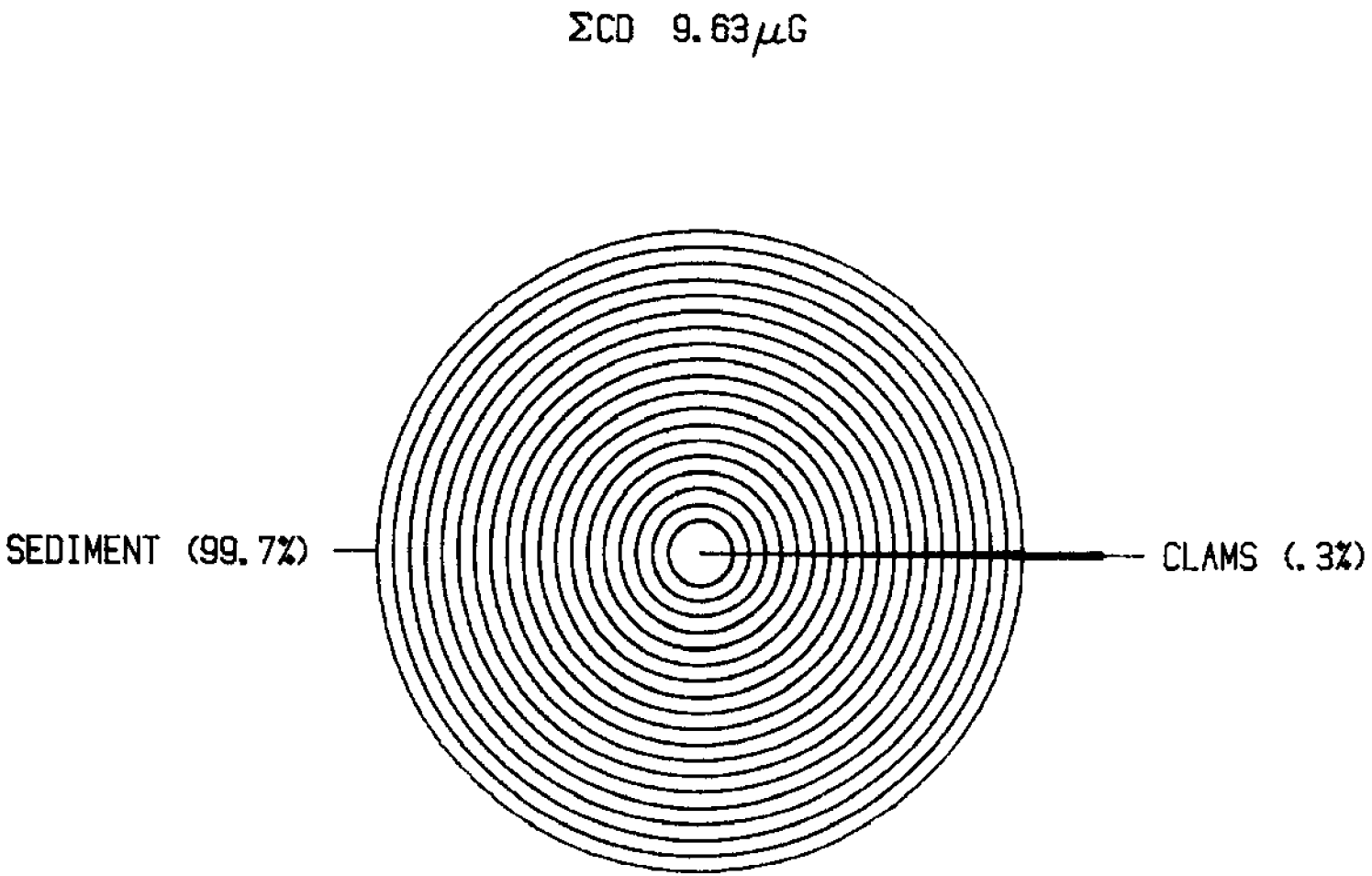


Figure 7. Mass balance distribution of labelled cadmium at the start of the second run of Aquarium #1 experiment (day 16).

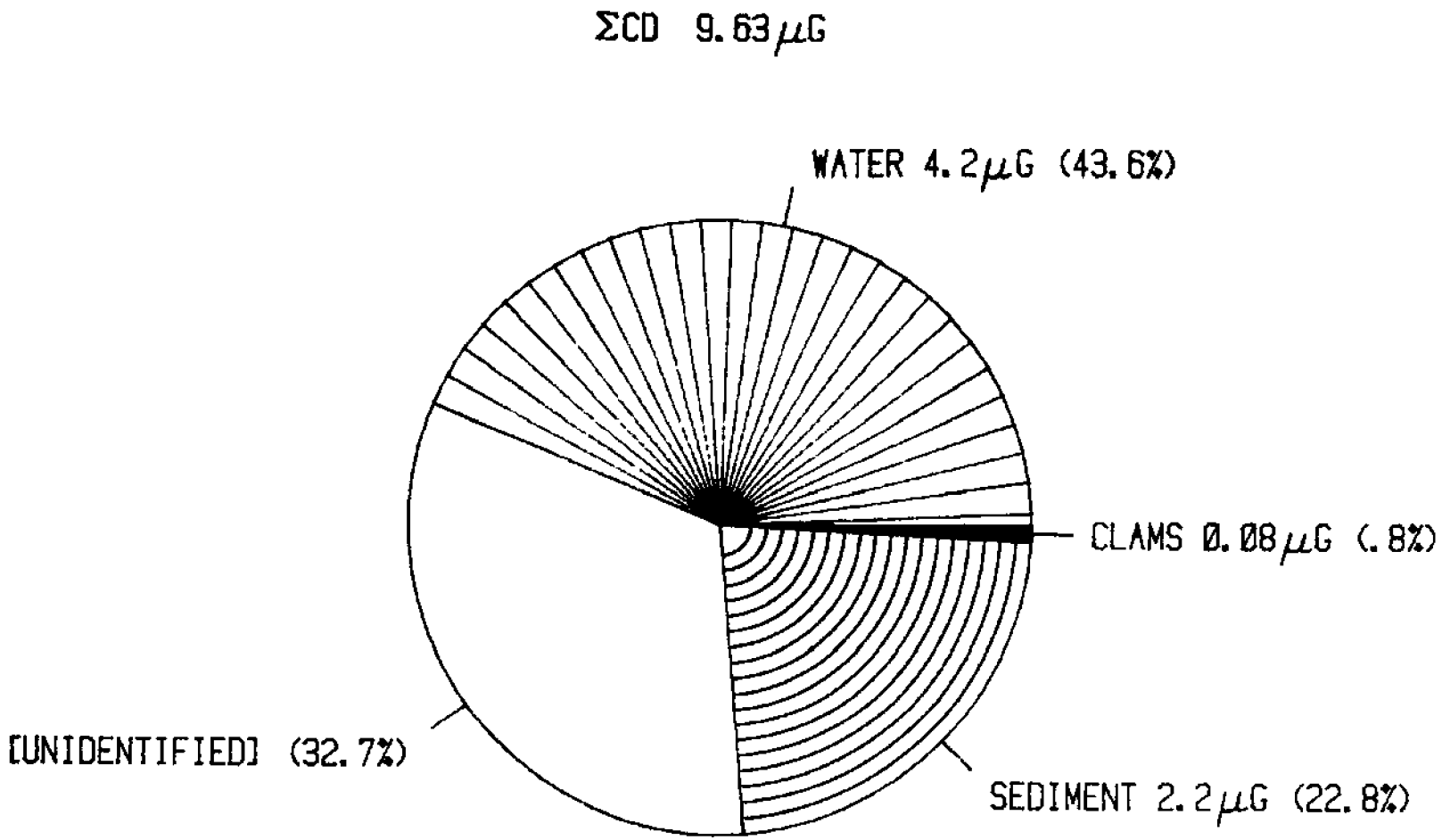


Figure 8. Computed mass balance distribution of labelled cadmium at the conclusion of the second run (Slurry #2) of Aquarium #1 experiment.

the beginning of the slurry run cannot be accounted for. Part of this discrepancy may be because certain of the pellets contained even less labelled cadmium than was determined for the test pellet; but in view of the identified inefficiency of this operation, only a small "loss" can be accounted for in this way.

Slurry #3; Days 38-53:

The clam population was transferred to a third, newly prepared slurry (Slurry #3) and maintained on this substrate for a further 17 days. At the end of this period, this batch of clams had been exposed to cadmium-labelled sediment for a total of 54 days. As described, the cadmium concentration of the sediment (we refer here to cadmium bound to bacteria) did not remain constant over this period but followed a saw-tooth pattern. The nature and objective of this experiment did not permit exposure to a constantly maintained source, but by replacing the substrate twice, the (computed) sediment concentration of labelled cadmium remained between 0.01 and 0.14 μg/g.

Analysis of an aliquot of the (total) culture prepared for this slurry gave a concentration of 0.103 μg ml^{-1} (0.1 μg ml^{-1} of inorganic carrier theoretically added). Filtration separation of a further aliquot showed that > 92% of the added cadmium was partitioned in or onto the bacteria.

Four "pellets" of bacteria cells were prepared, in the same fashion as described previously, for addition to the sediment. Analysis of the supernatant and wash from one of these pellets gave:

supernatant – 16 ± 0.8 μg Cd
wash – 1.2 ± 0.2 μg Cd

The computed loss of labelled cells here was, therefore, 78%, and the total quantity of labelled cadmium added to the sediment substrate 11.2 μg (0.05 μg Cd g^{-1} wet sediment). An aliquot of sediment removed at the start of this run and analyzed gave a measured content of 0.04 μg Cd. At the commencement of this third run, the computed distribution of labelled cadmium was as shown in Figure 9.

The clams from Slurry #2 were resettled on this new substrate and were maintained under the same conditions as previously given for a further 17 days.

Subsamples of water taken immediately after contact with the slurry substrate showed high concentrations of cadmium, which remained essentially constant through the period of this run. This observation supports our belief that suspended sediment — and predominantly less dense bacteria cells — are responsible for this fractionation.

At the end of 17 days exposure to the third substrate, the remaining 9 clams were sampled, dissected, and analyzed as described previously. Fecal pellet material purged from these individuals at this time showed no radio cadmium above the detection limit (counts above background). Table II

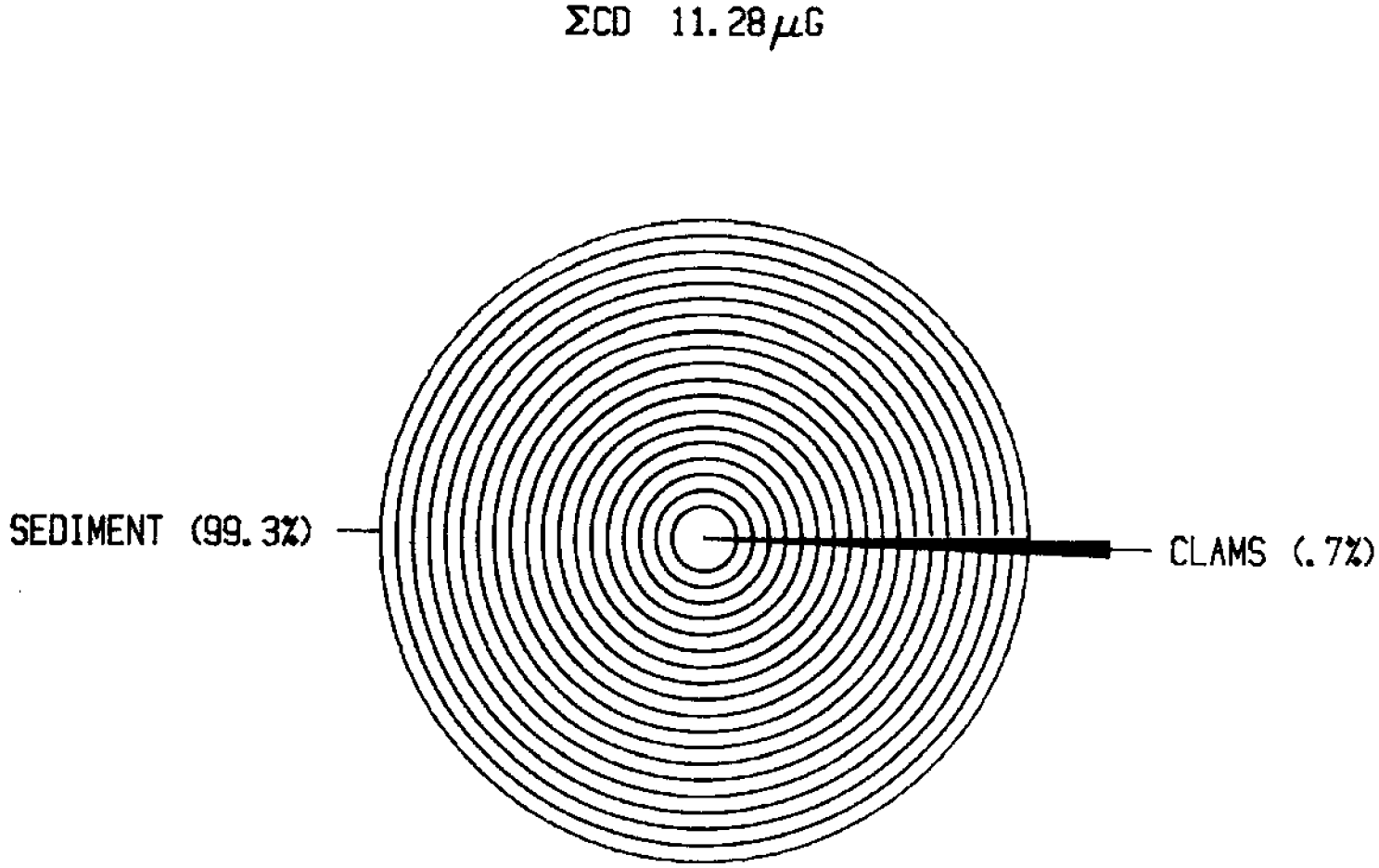


Figure 9. Computed mass balance distribution of labelled cadmium at the start of the third run of Aquarium #1 experiment (day 38).

TABLE II

Distribution of labelled cadmium ($\mu g\ g^{-1}$) in shell and tissue of *Macoma balthica* - Aquarium #1; Slurry #3
53 days total exposure

	Tissue		Shell	
Specimen	Weight (g)	Cadmium Conc* ($\mu g\ g^{-1}$)	Weight (g)	Cadmium Conc* ($\mu g\ g^{-1}$)
1	.008	1.22 ± 2%	.097	.08 ± 2%
2	.010	.84 ± 4%	.092	.09 ± 4%
3	.011	1.06 ± 4%	.079	.07 ± 5%
4	.008	.83 ± 3%	.063	.10 ± 4%
5	.005	1.00 ± 1%	.044	.08 ± 5%
6	.003	1.12 ± 2%	.041	.10 ± 3%
7	.006	.74 ± 3%	.053	.10 ± 3%
8	.005	.97 ± 4%	.047	.09 ± 8%
9	.003	1.03 ± 5%	.024	.11 ± 2%
	$\bar{X}$	0.98	$\bar{X}$	0.09

* Precision shows counting error.

gives the weights of individual shells and tissue material, the concentration of cadmium in each of these fractions, and mean contents of cadmium in the shells and tissue of the 9 pooled *Macoma* at this stage. From these data it may be computed that the total amount of labelled cadmium associated with the clams remaining at the end of the entire Aquarium #1 experiment was 0.11 µg: 0.06 and 0.05 µg with the tissue and shell material respectively. Again, the quantity of cadmium associated with the shell is high. In absolute terms there is about an equal amount associated with either fraction, but the tissue concentration is some order of magnitude higher than for the shell material. The sediment measured at this time showed a residual concentration of 0.03 µg Cd/g wet sediment, and the aquarium water contained a concentration of 0.001 µg Cd/ml. The distribution mass balance from these data is given as Figure 10. This computation accounts for nearly all of the cadmium believed to have been mixed into the sediment. The clam tissue contains only 0.5% of the cadmium added at the start of the final 17-day period of the experiment; but the concentration of tissue cadmium is more than an order of magnitude higher than that measured in the mud, and about 1000 times higher than the concentration in the overlying water.

Aquarium #2

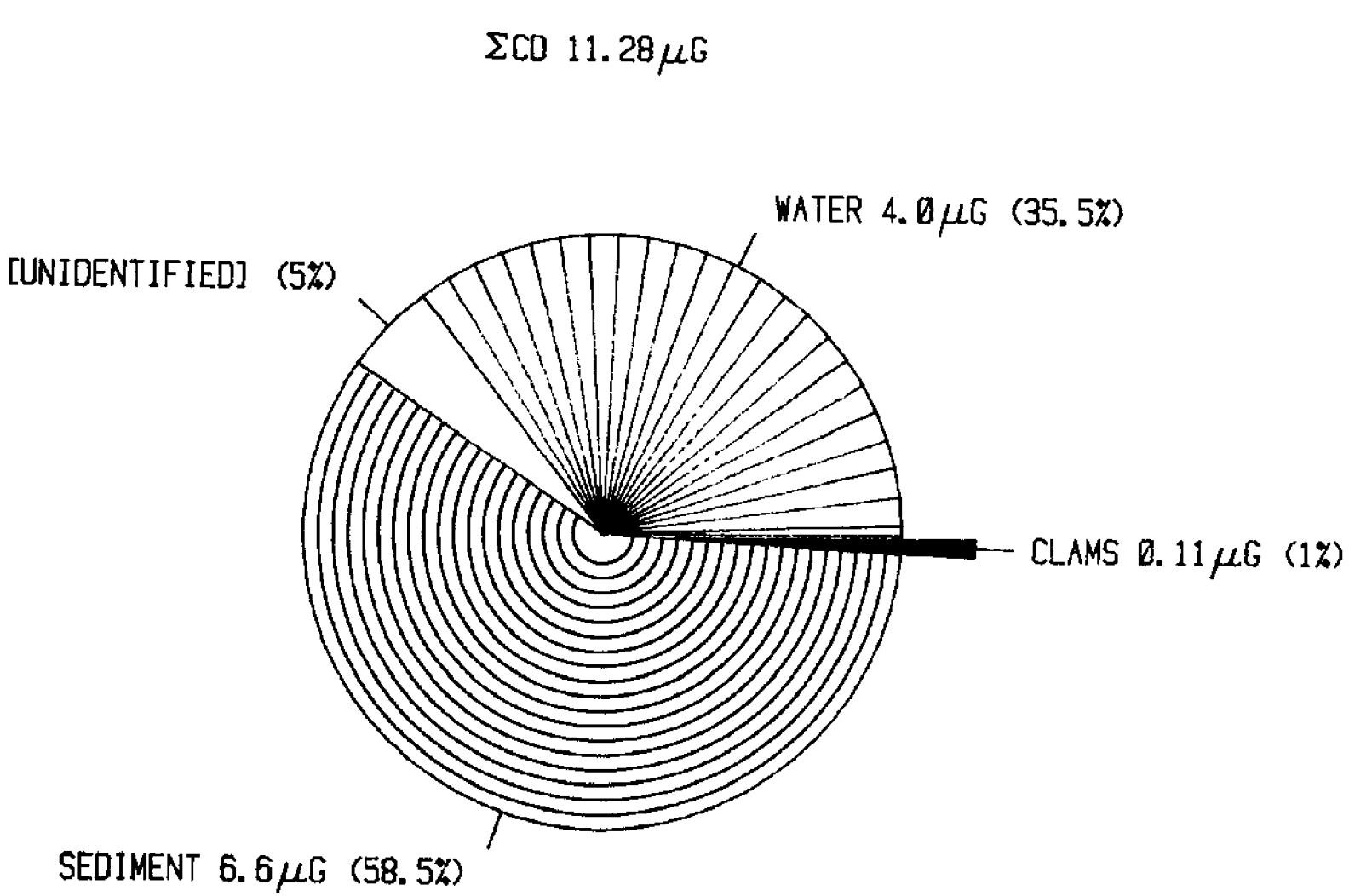
Slurry #1; Days 0-27:

Aquarium #2 experiment was essentially the same as the first experiment described above except that higher concentrations of cadmium were used. To the bacteria media prepared for the slurry-substrates for this aquarium was added 1.0 µg ml^{-1} (1 ppm) of labelled inorganic cadmium; i.e., 1 ℓ of culture contained 1 mg of cadmium which was added, as previously, in solution during the logarithmic growth stage of the monospecific culture.

Counting an aliquot of the culture plus medium prepared for Slurry #1 gave a concentration of labelled cadmium of 1.1 µg ml^{-1} (theoretically 1.0 µg ml^{-1}). Filtration separation of a further aliquot into component medium and cell material showed that partition of the cadmium in or onto the cell material was better than 99% complete. It is apparent that this concentration of available cadmium — an order of magnitude greater than was used in the first aquarium experiment — is not too great for complete removal from solution by the bacteria phase.

200 ml of this culture were spun down to produce a pellet of cell material as described previously. In this case, however, the pellet was not used, but all fractions of the separation treatment were analyzed. The mass distribution of cadmium, based on measurement of Cd-109 tracer, was as follows:

supernatant	160 µg cadmium
wash	8 µg
pellet	50 µg


ΣCD 11.28μG
WATER 4.0μG (35.5%)
[UNIDENTIFIED] (5%)
CLAMS 0.11μG (1%)
SEDIMENT 6.6μG (58.5%)

This distribution (shown diagramatically in Figure 11) sums to 218 µg Cd which is close to the unseparated medium count given above. This method of separating and concentrating bacterial biomass for subsequent mixing with the clam substrate was convenient, but inefficient. In this example (see Figure 11) 77% of the available cell material and contained cadmium was lost.

Two further 200 ml aliquots of culture were centrifuged similarly, and counts were made in both cases of the supernatant and wash. Although we do not have a direct measurement of the pellet cadmium in these cases, based on the dummy run described above, it may be computed with some confidence that the total quantity of cadmium available in the combined pellets was 60.48 µg. This material was mixed in with 220 g of natural marine sediment as in the previous Aquarium experiment to give an initial concentration of 0.275 µg Cd g^{-1} wet substrate.

Twelve clams were added to this prepared substrate, and the aquarium was maintained under standard conditions for a period of 28 days. At this point 4 clams were removed, dissected, and analyzed with the results given in Table III. It may be seen that the concentration of cadmium associated with the shell and the tissue fractions is comparable, which further suggests, in conjunction with Aquarium #1 data, that a sorption phenomenon is involved. (It should be noted that the tissue values are per g wet weight, whereas the shell data is for dry weight.) If it may be assumed that the mean content of cadmium associated with the shell and tissue of the remaining unsacrificed clams is comparable to that of the analyzed clams at this stage, then the total quantity of clam-associated cadmium may be estimated. The values are: 0.19 and 0.28 µg Cd associated with the total clam tissue, and with the total clam shell respectively.

0.27 g of sediment and fecal pellet material was purged from the 4 sacrificed clams; counts on this digested material were not above background. The concentration of cadmium in the sediment and overlying water at the conclusion of this initial 28-day run was determined to be 0.15 µg/g wet sediment, and 0.009 µg/ml respectively. The mass distribution of cadmium computed at this stage was, therefore:

Sediment	33.0 µg
Water	34.2 µg
Clams	0.47 µg
Amount added to system	60.48 µg

The computed "excess" (12%) may be attributed to error either in the sediment or water "phase", or both. It is difficult to subsample the sediment, which tends to be inhomogeneous. And a slight counting error in the exceedingly low concentration water aliquot is multiplied by several orders of magnitude when accounting for the entire volume contained in the aquarium. These computed distribution data are shown schematically in Figure 12.

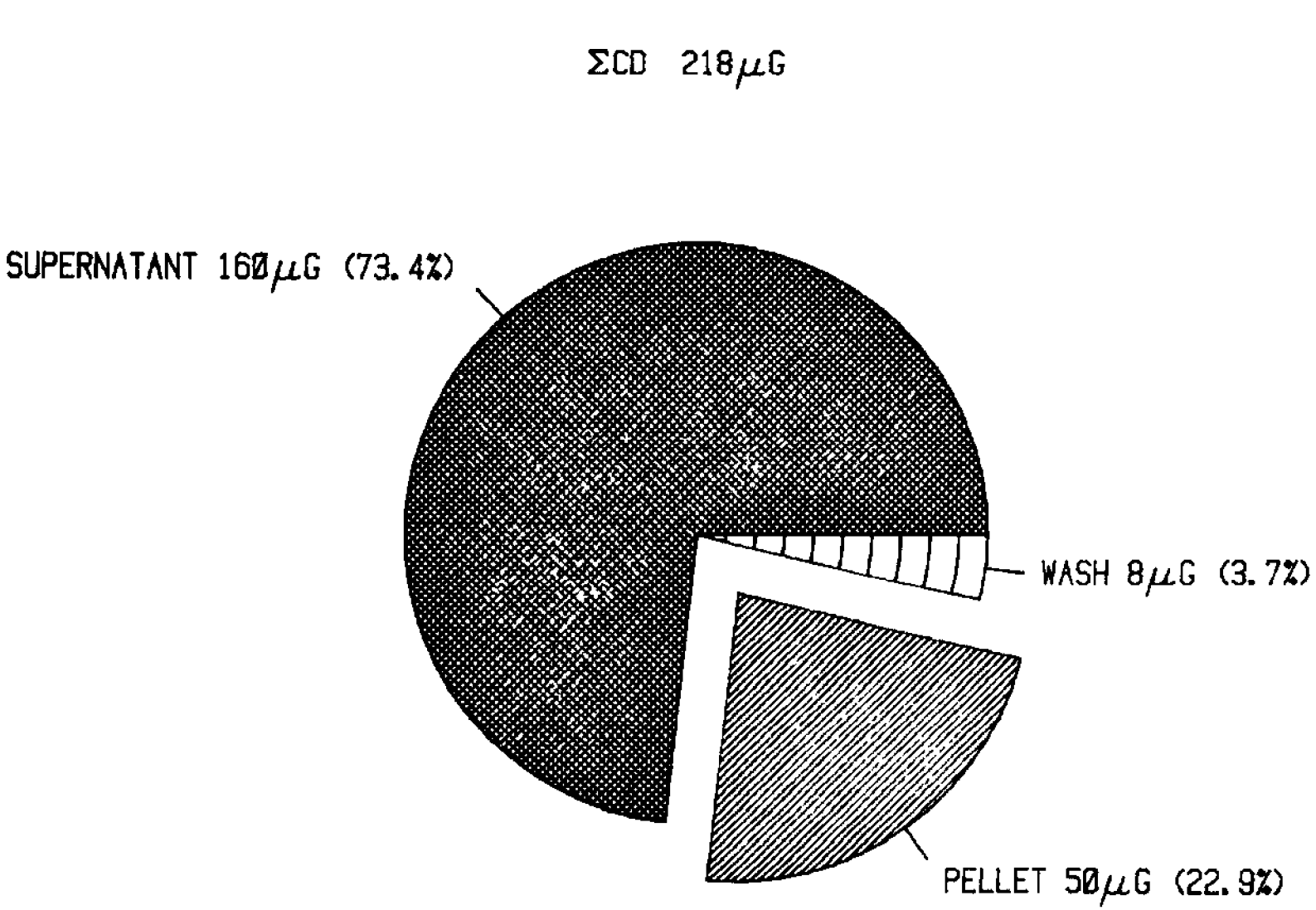

ΣCD 218μG
SUPERNATANT 160μG (73.4%)
WASH 8μG (3.7%)
PELLET 50μG (22.9%)

TABLE III

Distribution of labelled cadmium ($\mu g\ g^{-1}$) in shell and tissue of *Macoma balthica* - Aquarium #2; Slurry #1
28 day exposure period

	Tissue		Shell	
Specimen	Weight (g)	Cadmium Conc ($\mu g\ g^{-1}$)	Weight (g)	Cadmium Conc ($\mu g\ g^{-1}$)
1	.034	.56	.083	.33
2	.035	.68	.098	.34
3	.040	.72	.085	.35
4	.024	.35	.070	.23
	$\bar{X}$	.58 ± .16	$\bar{X}$	.31 ± .06

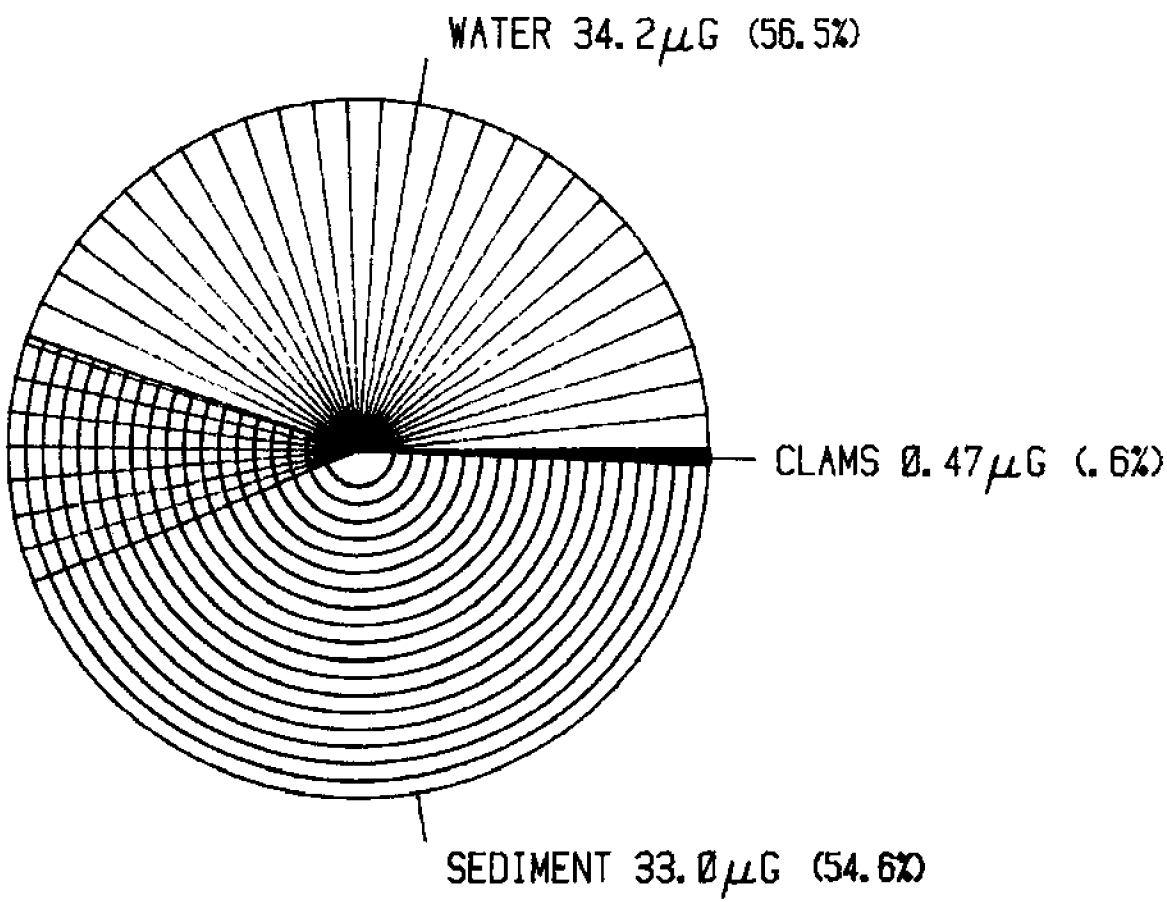


Figure 12. Mass balance of labelled cadmium in aquarium system at the end of the first run (28 days) of Aquarium #2 experiment.

Slurry #2; Days 28-54:

A second labelled substrate was prepared at the conclusion of the initial 28-day exposure period described above. The bacteria culture was prepared as previously, with addition of the Cd-109 spike and inorganic cadmium carrier. Test counting of an aliquot of this new culture gave a cadmium concentration of 1.04 ± .02 μg Cd ml^{-1} (ideal 1.0 μg ml^{-1}). The filtration separation of the cells and medium gave the following values:

Filtered material - 1.08 ± .03 μg ml^{-1}
Filtrate - at detection limit

i.e., the partition efficiency of the cadmium onto the solid (biota) phase was better than 97%.

Four separate 200 ml aliquots of culture medium were spun down to prepare pellets of bacteria cells for mixing into the sediment. Both discard phases (supernatant and wash) were analyzed for each of these separations. The separation efficiency continued to be poor, but relatively uniform. Cadmium separation efficiencies (i.e., efficiency of removal of cell material *via* centrifugation) were: 25, 27, 30, and 30% for each of the four pellets. The combined quantity of cadmium in the pellets then theoretically available for adding to the sediment was 225 μg. However, more was lost during handling prior to addition to the mud. After thorough mixing of the bacteria cells and the sediment, an aliquot of the prepared substrate was counted. This gave a cadmium concentration present of 0.71 μg Cd g^{-1} wet mud, or a total of 157.6 μg. At the start of the Slurry #2 run, then, the quantity of labelled cadmium present in the aquarium consisted of this amount in the sediment, together with that estimated to be present in the clams transferred from Slurry #1; i.e., 0.27 μg.

The eight live clams remaining at the end of the previous slurry run were allowed to remain living in and feeding on this new substrate for a further period of 26 days. However, during this period individual clams died; these were the only mortalities during any of the experiments. By the end of the 26-day exposure (i.e., a total of 54 days for those clams which survived) four clams — 50% of the population — had died. All clams, both those that expired prematurely and those that lived full-term, were removed at the end of the second (26-day) period, dissected, and analyzed. Table IV shows the cadmium content data for the four survivors. The mean concentration of tissue cadmium is 12.2 μg g^{-1}. But one possibly surprising feature is the large variability in concentration between individuals. This applies similarly to the tissue content of those animals which died: these data are shown in Table V. The mean is slightly higher than that for those clams which survived full-term, but in view of the very large individual difference, this has little meaning. It was observed that even the "survivors" appeared to be in poor condition, based on feeding behavior and other factors, so that it was believed that all would have expired close to the cut-off date. It would appear, then, that the overall mean concentration of cadmium in the tissue (13.3 μg g^{-1}) is about at the 50% mortality level. Poor health, with consequent interference in the feeding behavior and ingestion rate might explain the large variation

TABLE IV

Distribution of labelled cadmium ($\mu g\ g^{-1}$) in shell and tissue of clam specimens which survived full term (54 days total) exposure

Aquarium #2; Slurry # 2

	Tissue		Shell	
Specimen	Weight (g)	Cadmium Conc ($\mu g\ g^{-1}$)	Weight (g)	Cadmium Conc ($\mu g\ g^{-1}$)
6	.005	9.70 ± 6%	.044	7.03 ± 4%
7	.004	13.70 ± 4%	.050	7.45 ± 5%
8	.007	7.43 ± 4%	.053	3.55 ± 5%
9	.009	18.12 ± 3%	.088	5.85 ± 4%
	$\bar{X}$	12.24 ± 4.7	$\bar{X}$	5.97 ± 1.7

TABLE V

Distribution of labelled cadmium ($\mu g\ g^{-1}$) in shell and tissue of clam specimens which expired

Aquarium #2; Slurry #2

	Tissue		Shell	
Specimen	Weight (g)	Cadmium Conc ($\mu g\ g^{-1}$)	Weight (g)	Cadmium Conc ($\mu g\ g^{-1}$)
1	0.015	6.32	0.077	6.37
2	-	22.39	0.104	9.81
3	0.019	10.48	0.084	6.61
4	0.014	18.05	0.062	(lost)
	$\bar{X}$	14.4	$\bar{X}$	7.6

in cadmium concentration levels observed between individuals. It should be noted that even at these apparently limiting concentration levels there was no detectable cadmium in the purged stomach contents.

It is computed that the absolute quantity of cadmium held by the clams at the conclusion of this aquarium run – including those that died and were removed early – was greater than 6 µg: 2.14 µg and 3.88 µg associated with the tissue and shell fractions respectively. The apparent substrate concentration of cadmium at the start of this second portion of the Aquarium #2 experiment was some 4X higher than at the beginning; but the tissue concentration has increased by over an order of magnitude.

An aliquot of sediment measured at the termination of this experiment showed a cadmium concentration of 0.22 µg g^{-1}, and the concentration in the overlying water was 0.023 µg ml^{-1}. The overall mass balance of labelled cadmium at this point was, therefore, as shown in Figure 13. The tissue concentration is some 60X higher than that present in the substrate.

Aquarium #3

Aquarium #3 experiment was a short-term experiment (14 days total) using a single prepared and labelled slurry substrate. The bacteria cultured was spiked with a 1.0 µg ml^{-1} concentration of inorganic cadmium. In essentials, therefore, this Aquarium experiment reproduced the conditions of the Aquarium #2, Slurry #1 run. However, a larger quantity of cadmium was introduced to the mud. Details are as follows

During the logarithmic growth phase, 1.0 µg ml^{-1} labelled inorganic cadmium was added to the bacteria culture. An aliquot removed during the stationary phase and separated into cell material and medium *via* a 0.22 µm filter showed that partition of the cadmium onto or in the bacteria cells was better than 98% complete. Two separate 200 ml aliquots were then centrifuged to obtain pellets of cell material. Analysis of the supernatant and wash for each of these showed that, probably coincidently, the removal efficiency was the same (within analytical counting error). This fractionation is shown diagramatically in Figure 14. It is not known why these two centrifugation separations were considerably more efficient than those for the earlier experiments. However, in this case, the total amount of cadmium added to the sediment in the form of pellets of bacteria cells was 295 µg, to give a theoretical well mixed substrate concentration of 1.34 µg Cd g^{-1} wet sediment. The cadmium concentration in the sediment was not, unfortunately, directly measured at this time, and it is believed that there were additional significant handling losses so that the amount actually added to the sediment was less than this. Based on the mass balance distribution at the end of the initial sampling run (see below) it may be computed that the quantity of cadmium actually mixed into the sediment substrate should have been approximately 217 µg.

Twenty clams were added to this substrate; nine were removed at the end of 7 days, and the remainder (11) at the conclusion of the experiment after a total of 14 days. The distribution of cadmium in the initial nine clams is shown in Table VI. The total amount of cadmium "bound" (sequestered,

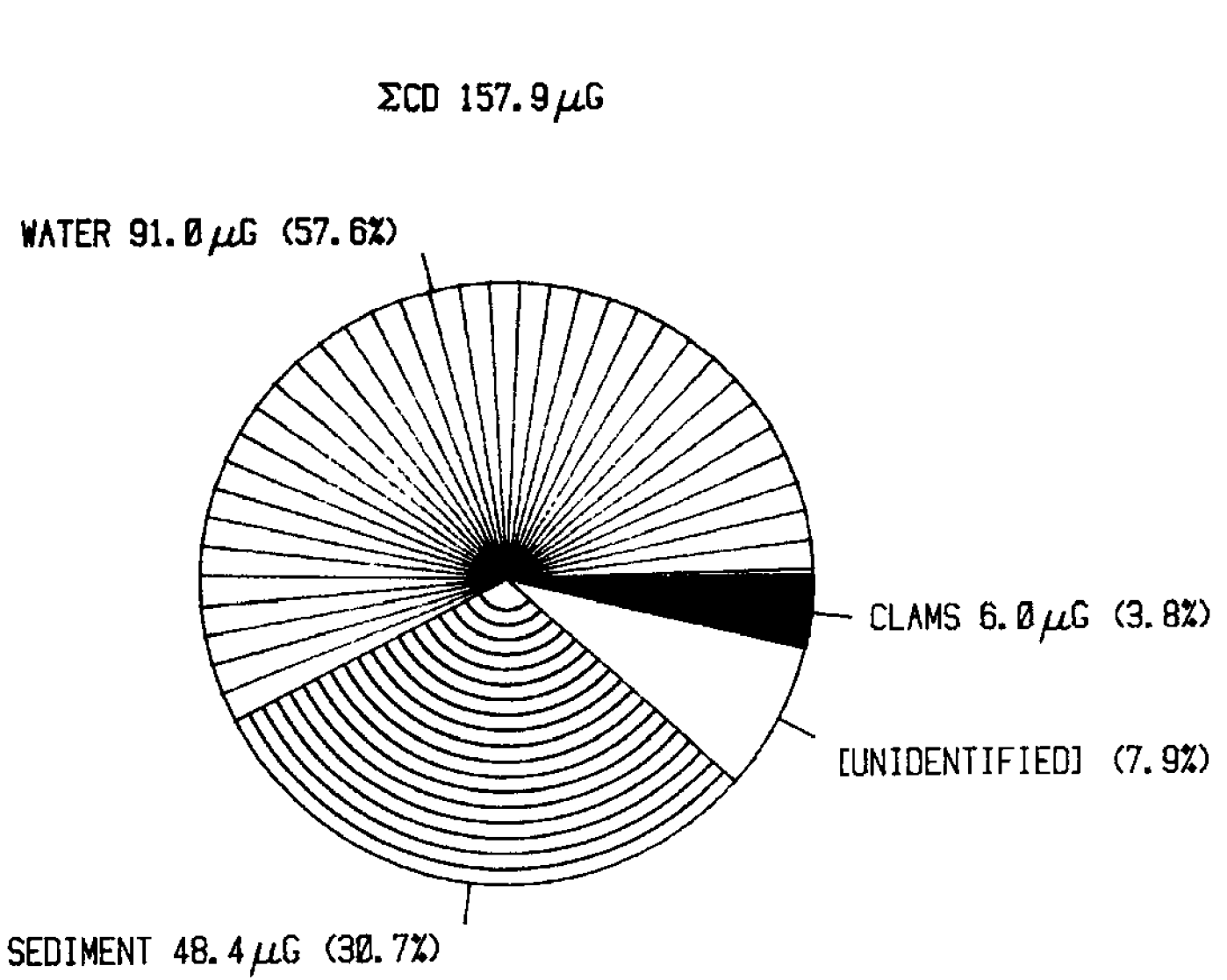


Figure 13. Mass balance distribution of labelled cadmium at the conclusion of Aquarium #2 experiment (54 days).

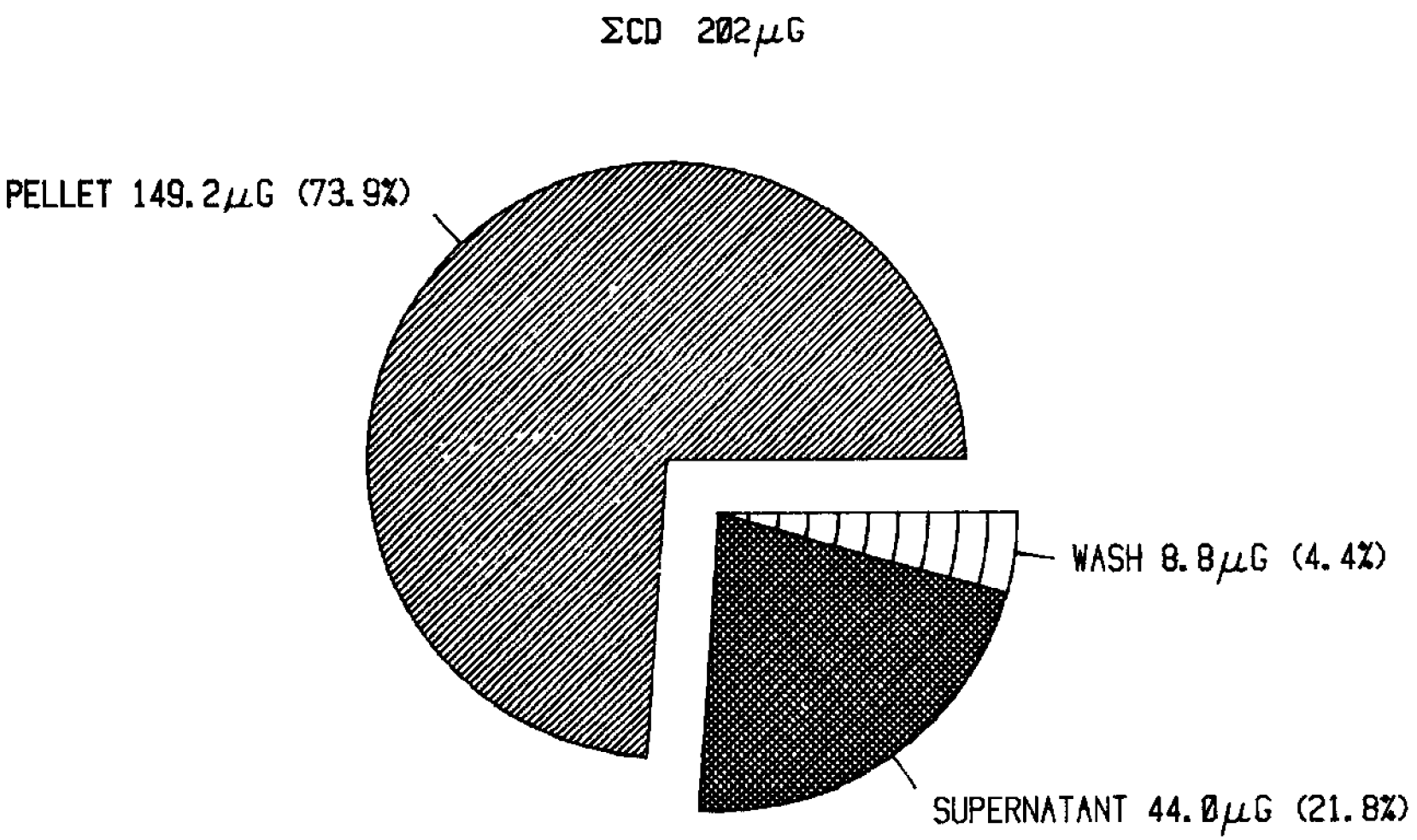


Figure 14. Mass balance distribution of labelled cadmium following bacterial pellet separation. Aquarium #3 experiment.

TABLE VI

Aquarium #3

Distribution of labelled cadmium ($\mu g\ g^{-1}$) in shell and tissue of clam specimens removed after seven days exposure

	Tissue		Shell	
Specimen	Weight (g)	Cadmium Conc ($\mu g\ g^{-1}$)	Weight (g)	Cadmium Conc ($\mu g\ g^{-1}$)
1	0.008	50.9 ± 8%	0.064	1.31 ± 10%
2	0.007	4.02 ± 5%	0.067	0.96 ± 10%
3	0.009	1.95 ± 5%	0.047	1.39 ± 10%
4	0.013	3.73 ± 8%	0.051	0.62 ± 9%
5	0.009	3.51 ± 8%	0.062	0.56 ± 7%
6	0.009	3.12 ± 7%	0.044	0.82 ± 8%
7	0.007	3.41 ± 5%	0.029	0.95 ± 5%
8	0.011	4.60 ± 9%	0.026	0.95 ± 5%
9			0.026	0.89 ± 6%
	$\bar{X}$	3.27	$\bar{X}$	0.895

sorbed, etc.) by the clams was thus 0.61 µg: 0.24 and 0.37 µg associated with the tissue and shell respectively. Aliquots of sediment and water analyzed at this time gave cadmium concentration values of 0.84 µg g^{-1} wet sediment and 0.008 µg ml^{-1} respectively. Based on these data, the mass distribution of labelled cadmium after seven days is shown in Figure 15. The total used in this representation is that calculated to be present in the two centrifuged pellets as shown by the fractionation of Figure 14. The quantity held by the clams includes that estimated to be present in the clams which were not sacrificed at this time. As noted above, further losses were believed to have occurred prior to mixing of the bacteria cells with the sediment. Based on the distribution shown in Figure 15, the quantity of labelled cadmium actually added to the sediment at the start of the experiment should have been around 217 µg; and, as will be shown below, this total agrees well with the mass distribution determined at the conclusion of the experiment. Figure 16 shows the % distribution of the cadmium total identified at this time.

After a further seven days (total of 14 days) the remaining clams were removed from the substrate, purged, dissected, and analyzed for cadmium. These data are given in Table VII, which shows the total amount of cadmium held by the clams to be 0.92 µg: 0.306 and 0.614 µg associated with the tissue and shell material, respectively. Analysis of aliquots of the sediment, and of the overlying water (including suspended sediment) gave concentrations of 0.80 µg/g and 0.010 µg/ml respectively. The mass distribution of labelled cadmium, remaining in the system during the final run, at the termination of the experiment, was as shown in Figure 17.

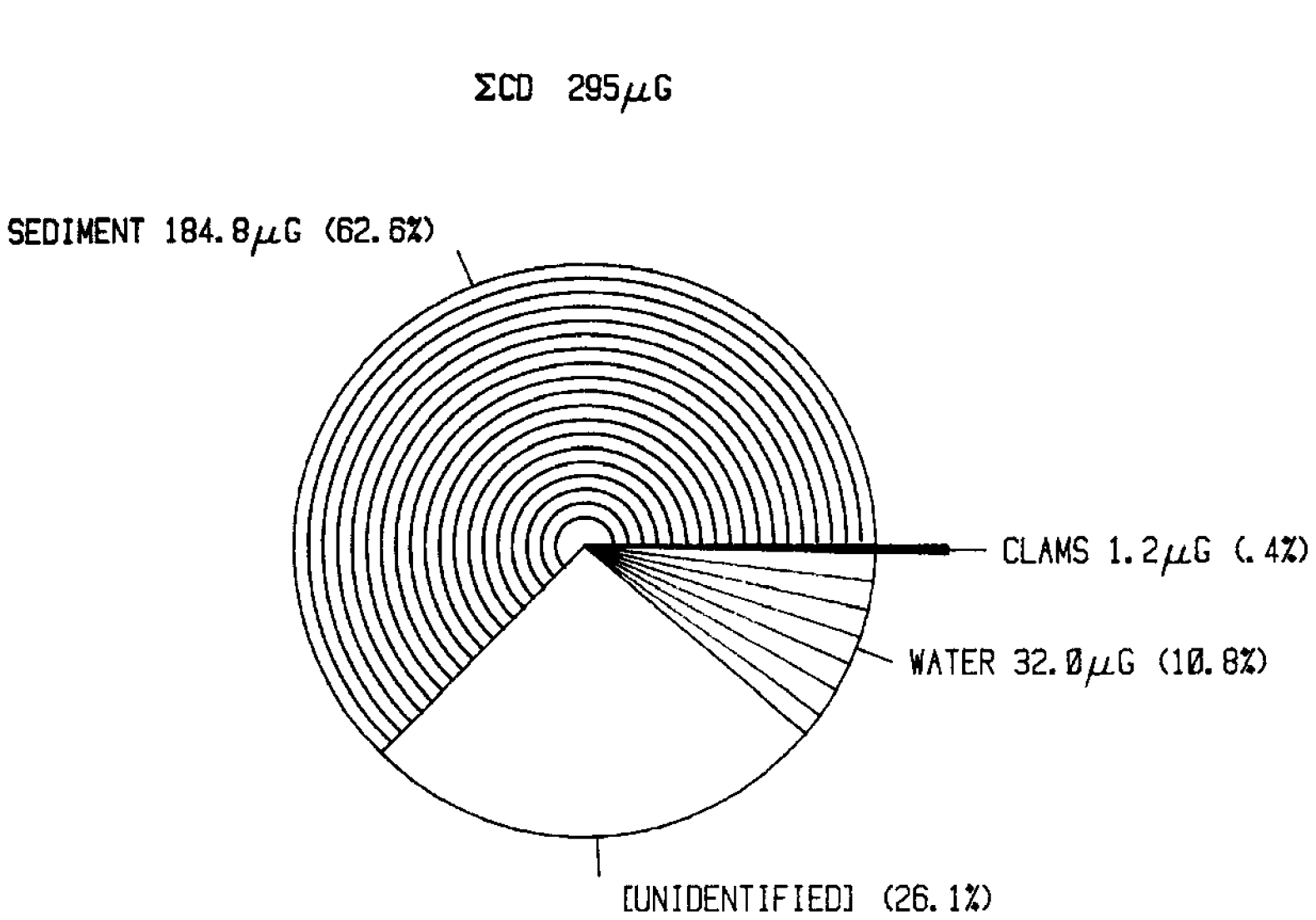


Figure 15. Computed mass balance of labelled cadmium after 7 days of Aquarium #3 experiment (see text). Distribution in the major reservoirs is shown as % fractions of the quantity of labelled cadmium theoretically added on day 1.

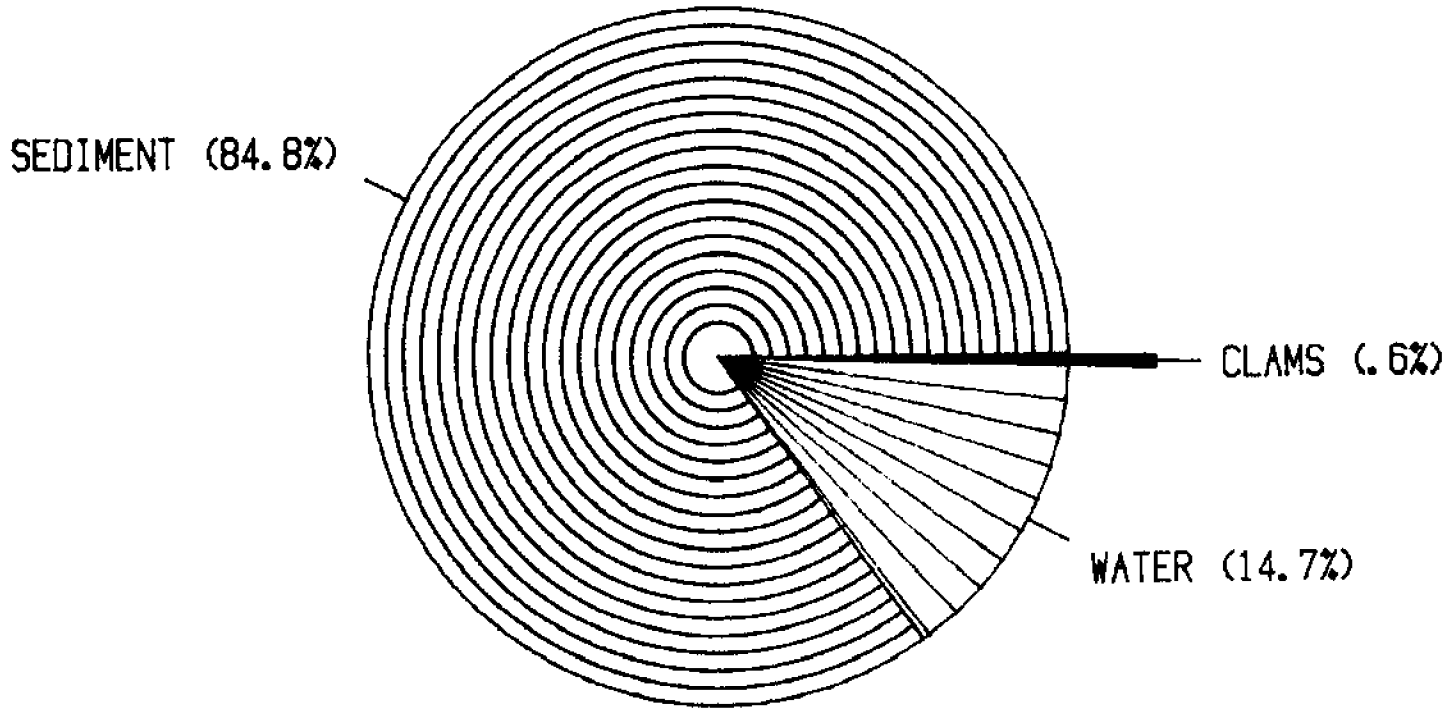


Figure 16. Mass distribution of identified labelled cadmium within the Aquarium #3 system after 7 days (values given in Fig. 15).

TABLE VII

Aquarium #3

Distribution of labelled cadmium ($\mu g\ g^{-1}$) in shell and tissue of clams removed after 14 days exposure

	Tissue		Shell	
Specimen	Weight (g)	Cadmium Conc ($\mu g\ g^{-1}$)	Weight (g)	Cadmium Conc ($\mu g\ g^{-1}$)
10	0.007	5.62 ± 4%	0.080	1.72 ± 4%
11	0.007	5.02 ± 4%	0.060	1.38 ± 4%
12	0.006	5.62 ± 6%	0.054	1.71 ± 3%
13	0.006	7.03 ± 3%	0.050	1.67 ± 5%
14	0.006	6.56 ± 4%	0.048	1.54 ± 3%
15	0.005	11.24 ± 4%	0.050	1.25 ± 4%
16	0.005	7.31 ± 4%	0.037	1.25 ± 4%
17	0.003	3.04 ± 2%	0.026	0.49 ± 3%
18	0.004	5.09 ± 1%	0.030	0.96 ± 4%
19	0.006	4.69 ± 3%	0.020	0.77 ± 8%
20			0.021	1.44 ± 3%
	$\bar{X}$	5.56	$\bar{X}$	1.29

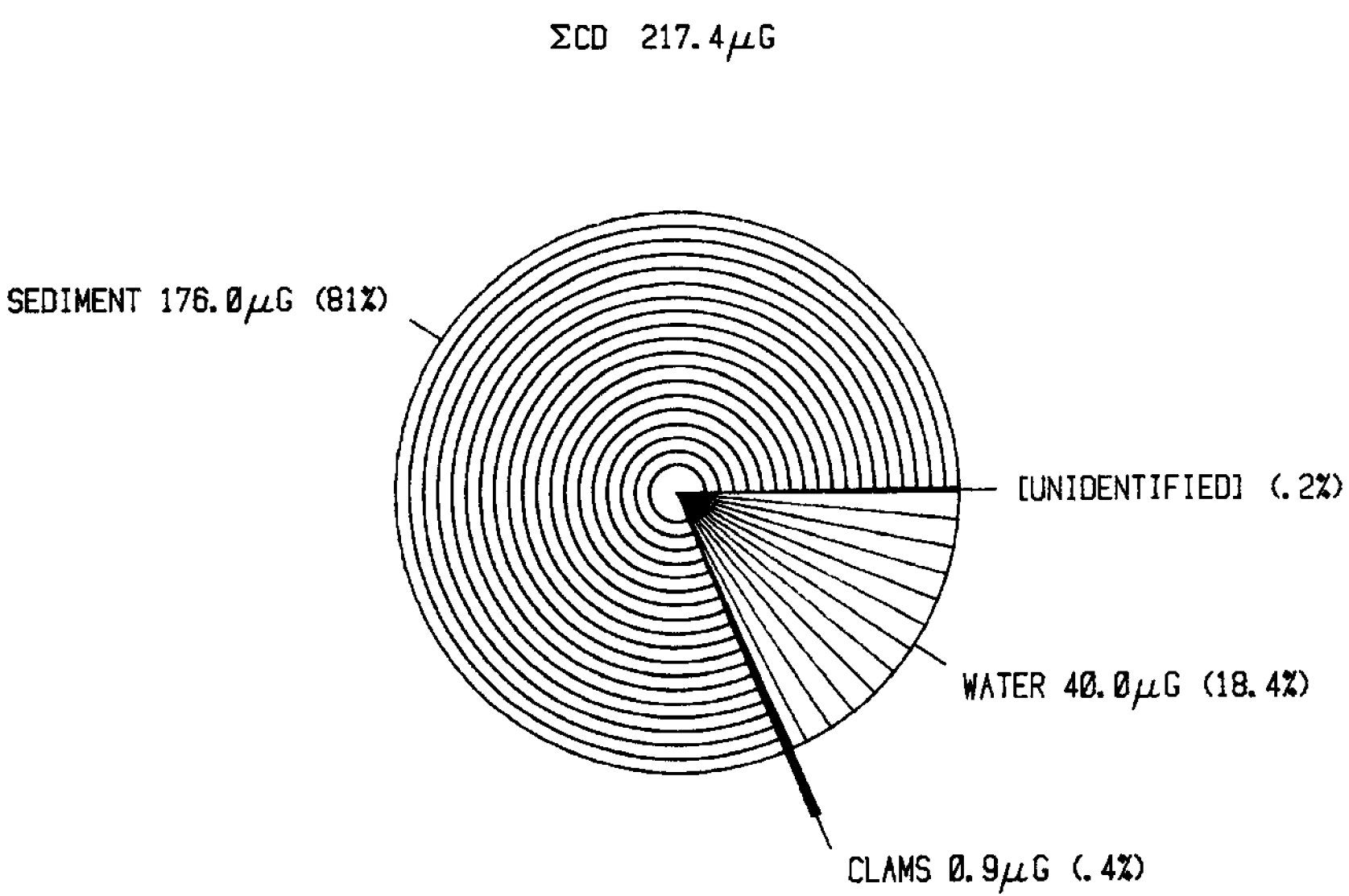


Figure 17. Mass balance distribution of labelled cadmium at the conclusion of Aquarium #3 experiment (14 days).

DISCUSSION

1. Inorganic cadmium added in solution to a mono-specific marine bacteria culture is almost entirely taken up by the bacteria; the uptake measured in the various experiments described here ranges from 87-99%. This result appears to contradict the findings of Houba and Remacle (1982). There was no discernible difference in uptake behavior for either of the two concentrations used; 0.1 and 1.0 $\mu g\ ml^{-1}$. Previous work had shown that if the higher (1 ppm) cadmium concentration was added during the lag phase, onset of the logarithmic growth phase was delayed. In all experiments described here, the cadmium was added during the log phase, and the bacteria cells were spun down after onset of the stationary growth phase.

The mode of attachment of the inorganic cadmium to the bacteria is not known. It is probable that much or most of it is surface sorbed rather than structurally bound. However, this does not affect the experimental design used here. On the assumption, as reported by many other workers, that clams may preferentially feed on bacteria, it was required only that the cadmium be concentrated in this phase. Whatever the mode of attachment, it was unaffected by filtration as described. The results of the centrifugation separations are ambiguous. Large losses have been documented. It is believed that this was due primarily to a very incomplete spin-down of cell material, but remobilization of cell-bound cadmium is an additional possibility.

2. An attempt was made to "present" as much particulate cadmium as possible to the feeding clams. The objective of the experiment was not to introduce the cadmium to the clams in solution. The labelled bacteria cells were mixed into the entire substrate so that maximum uptake opportunity required that the clams — if they fed mainly from the sediment surface — should "work" the substrate. No additional food was added during the course of each experiment. Filter feeding of resuspended organic particulate could have occurred; but such material would have been primarily labelled bacteria. It is possible, even probable, that the clams fed at less than optimum rates. Using the same species collected for similar Alaskan estuarine environments, Winiecki and Burrell (1982) failed to demonstrate (statistically) growth over a 6-month period under flow-through seawater conditions, although all specimens survived full-term. McLusky (1981) has noted that *Macoma balthica* is generally a very slow growing animal, and the aquaria for the present set of experiments were maintained at 11°C to simulate "Alaskan" conditions.

3. Relatively large concentrations of radio cadmium were found in the water of the aquaria after each run. These computed concentrations were larger than could be readily explained by invoking equilibration of the tracer with the pre-existing ("equilibrium") content of cadmium in the natural seawater used. It is considered, therefore, that aliquots of water removed for analysis contained suspended particulate material which, because of its low density relative to alumino-silicate, is likely to have been largely bacterial cell material.

As may be seen from the mass balance computations, a large fraction of the introduced labelled cadmium ended up in this reservoir. This would

have reduced the amount of cadmium in the sediment which was available for uptake *via* ingestion of detritus, except that, if the "aqueous" cadmium pool were largely suspended particulate, this material would have constituted a readily available food source *via* a filter-feeding mode. *Macoma balthica* is capable of either bottom or filter feeding depending on food availability, and other environmental factors. In either case, subsequent Cd uptake would be from a particulate phase.

It should be noted that, because of the large volume of water involved, the mass of cadmium present in this reservoir is relatively large, but the available concentration on a weight/volume basis is around an order of magnitude less than was available in the sediment.

4. The mass balance computations given previously show that most of the introduced labelled cadmium is partitioned onto particulate phases; assuming, as discussed above, that most of the cadmium measured in the "water" is, in fact, associated with suspended particulate material. We do not believe that any major reservoir, including sorption on the walls of the aquaria, has been unidentified. The apparent "losses" given for the Aquarium #1 runs (i.e., difference between theoretical addition, and identified distributions after each time period) are generally much reduced or essentially absent (the first slurry run of Aquarium #2 shows a "gain") in the Aquarium #2 and #3 experiments. There are various explanations for these discrepancies. In some cases additional losses were known to occur during the handling and transfer of the bacterial "pellets" to the sediment substrate. However, the major factor is undoubtedly due to the measurement precision of the small quantities of cadmium present, especially, in the "water" phase. Any error here was magnified by three orders of magnitude on computing the mass balance. It is notable that the distribution balance was improved when higher initial concentrations of cadmium were used (i.e., Aquaria #2 and #3).

5. The uptake concentrations of cadmium by the clam tissue, and the corresponding coexisting substrate concentrations (both on a wet weight basis) are shown in Figures 18 and 19 for Aquarium #1, and Aquarium #2 and #3, respectively. It may be seen that for the Aquarium #1 experiment, the substrate concentration of (labelled) cadmium ranged from .01 to (computed) 0.14 μg Cd g^{-1}, i.e., around an order of magnitude. The tissue concentration of cadmium after a total of 54 days exposure was approximately 1 μg g^{-1}, which was some 30 times greater than the measured substrate concentration at that time. This is the <u>mean</u> substrate concentration. The bacterial concentration would have been probably much higher but we cannot assign a value to this because of the change (and consequent cadmium dilution) of the bacteria biomass during the course of the experiment. It may be estimated that the bacteria cell concentration of cadmium at the <u>start</u> of the second and third slurry run was approximately 0.9 μg g^{-1} and higher than this at the start of the Slurry #1 run, so that no trophic level biomagnification of the cadmium is indicated. However, the "concentration factors", as conventionally defined (tissue conc./ambient water conc.), <u>are</u> very large. Thus, for the concentration distributions (μg g^{-1}) present at the termination of each aquarium experiment:

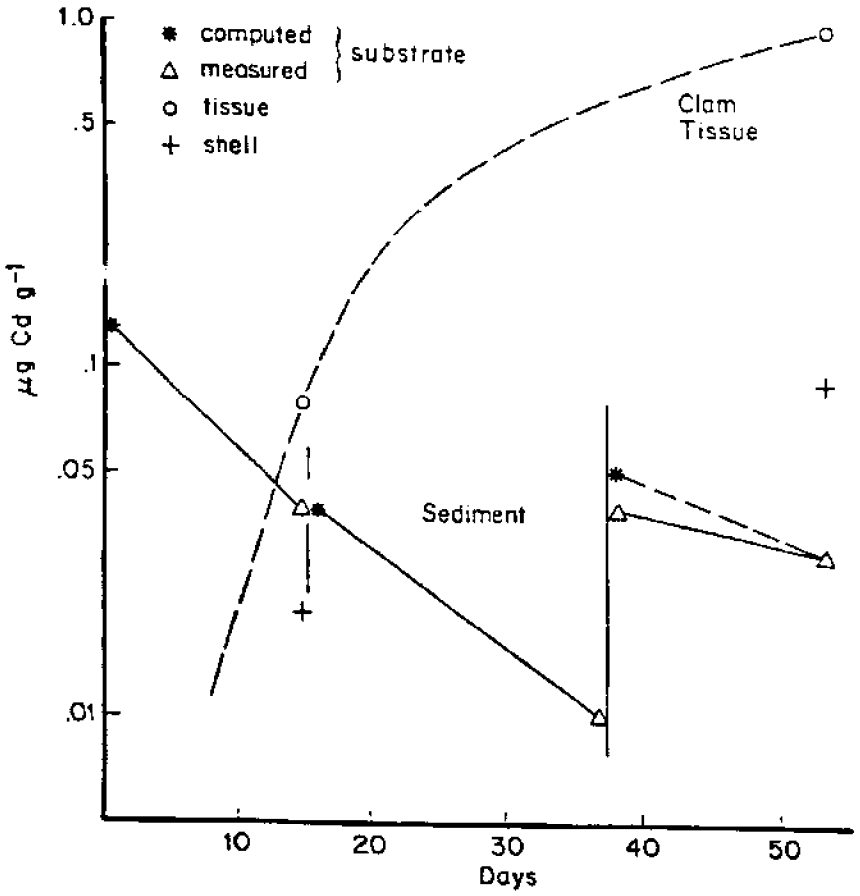


Figure 18. Concentration distribution of labelled cadmium through Aquaria #1 experiment.

* - sediment (wet weight) concentrations computed on basis of bacteria cell material added

Δ & ● - sediment and clam tissue (wet weight) concentrations measured

\+ - concentration of cadmium measured in clam shells (dry weight)

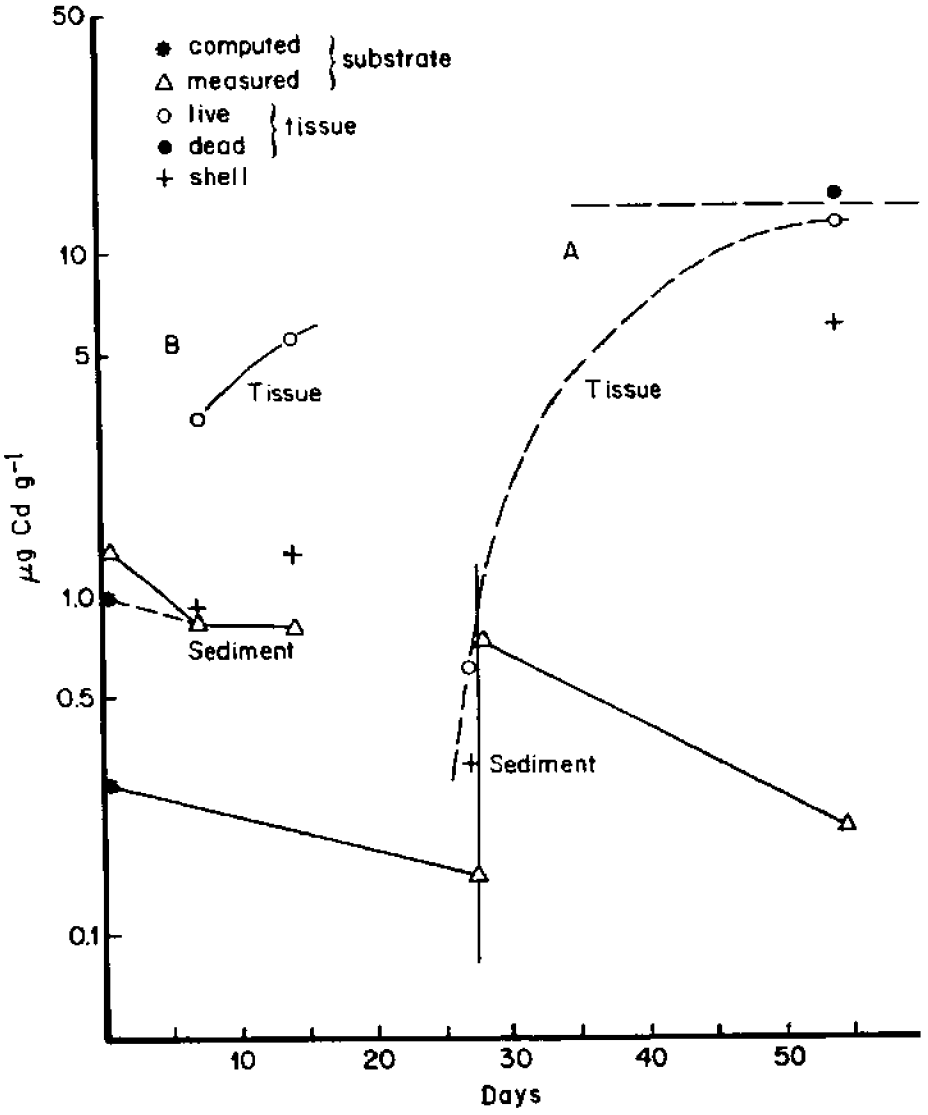


Figure 19. Concentration distribution of labelled cadmium through (A) Aquaria #2 and (B) #3 experiments.

● - mean concentration (wet weight) of cadmium in tissue of clams that died prior to conclusion of 14-day experiment (Aquarium #2)

Δ & o - sediment and clam tissue (wet weight) concentrations measured

\+ - concentration of cadmium measured in clam shells (dry weight)

Aquarium	I	II	III
Tissue	0.98	12.24	5.56
Sediment	0.03	0.22	0.8
Water*	0.001	0.023	0.01
"Concentration factor"	980	530	560

* unfiltered water phase.

For Aquaria #2 (Figure 19) which received a higher (approximately 10X greater) dose of cadmium *via* the bacteria, the tissue concentration of the clams (both live and dead) after 53 days ranged from 12-15 $\mu g\ g^{-1}$ or around 60-70 times higher than in the sediment. Again, however, this is close to or slightly less than the concentration in the bacteria cells. This later experiment is particularly significant in this respect since it appears (with some 50% mortality) that the viable uptake limit of cadmium into the clam tissue had been reached. The Aquarium #3 run was terminated well before this "limit" was reached, and the mean concentration of the metal in the clam tissue after the 14-day exposure was far below that held by the bacteria. Such comparisons are only meaningful, of course, if the clams preferentially selected the bacteria cells as the major or significant food source.

6. Within the clams, cadmium was preferentially concentrated (approximately x 20) in the (purged) stomach tissue. Because all tissue and sediment concentration data given in this report are wet weight, the fraction of shell-associated cadmium appears to be misleadingly high. Assuming tissue dry weight at 10% wet weight, comparable shell:tissue fractionations at the termination of each run (estimated concentrations in $\mu g\ Cd\ g^{-1}$ dry weight) were:

Aquarium	I	II	III
Tissue conc.	9.8	122.6	55.6
Shell conc.	0.09	6.0	1.3
% of total Cd associated with shell	0.9	4.9	2.3

It would appear that the proportion of labelled cadmium held on the shell surface increases with (1) increasing ambient concentrations and (ii) increasing exposure time, supporting a "simple" surface sorption mechanism.

7. From the data shown in Figures 18 and 19, it would appear that uptake of the labelled cadmium was initially slow and then accelerated during the latter half of each experiment (considering here Aquaria #1 and #2, not the short term Aquarium #3). There seems no obvious explanation for this apparent trend, unless the feeding rate of the clams increased as the experiment (and "acclimatization" of the clams) continued.

The change in concentration (C) of a heavy metal in an organism with time is given by

$$\frac{dC}{\partial t} = k_u\, w(t) - k_\ell\, C(t)$$

where k_u and k_ℓ are the uptake and loss rate constants and w is the concentration of the metal in water. Then:

$$C(t) = C_{eq}\,[1 - e^{-k_\ell t}]$$

where C_{eq} is the steady-state concentration in the organisms,

$$k_\ell = 0.693/\tau$$

where τ = the biological half-life.

There have been various estimates of the biological half-life of cadmium in shellfish. Fowler and Benayoun (1974) give $\tau_{1/2}$ > 10 months for a *Mytilus* species under natural conditions, and some 4X greater for laboratory reared mussels. In general it would appear that cadmium retention times in molluscs is relatively long (i.e. small k_ℓ value and slow elimination rates).

Since only two tissue concentrations were measured per experiment, it is impossible to conclude anything definite about the shape of the uptake rate curve. It was not practically possible to collect all the material egested by the clams. However, fecal material purged from each clam prior to dissection showed no measurable radio-cadmium and it would appear that little of the cadmium taken up by the clam tissue was recycled back to the sediment (the absence of fecal cadmium from clams at the end of the Aquarium #2 run, where the clams had either died or appeared to be in very poor condition, is of particular interest).

These observations support a long biological half-life for cadmium in *Macoma*, and it seems safe to conclude that the theoretical equilibrium concentration (C_{eq}) was not approached. Linear uptake curves are therefore shown in Figures 18 and 19.

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