This dissertation is dedicated to my family

THE OCCURRENCE AND DISTRIBUTION OF Ca, Sr, Ba, AND Pb IN MARINE ECOSYSTEMS

Thesis by

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ABSTRACT

Processes of enrichment and depletion of Pb, Ba, and Sr relative to Ca in transfers between trophic levels of selected marine ecosystems were studied using ultra-clean laboratory techniques and stable isotope dilution mass spectrometry. Metal relationships between cellular subfractions of marine algae and major Ca reservoirs of consumer animals show that at the primary producer level large bioenrichments in the Pb/Ca ratio occur on algal surfaces as a result of selective chelation of soluble Pb by extracellular algal polysaccharides. This is followed by biopurification of Ca with respect to Pb during active transport of Ca from surfaces to interiors of algal cells and during subsequent transfers to consumer animals at successively higher trophic levels of marine food chains.

Ca, Sr, Ba, and Pb share covariant and coincident distributions in marine organisms, residing chiefly in cell wall mucilages of algae and skeletons of animals. Pb/Ca ratios of major Ca reservoirs in marine biota correlate with variations in the Pb/Ca ratio of source reservoirs which result from different ambient concentrations of industrial Pb in seawater. Pb/Ca ratios of gastropod shell layers correlate directly with Pb/Ca ratios of food but inversely with organism size. The partitioning of Pb between shell layers generally favors calcite relative to aragonite but is influenced by species, size, and ambient environmental Pb concentrations.

A linear model of the pelagic food web based on data of this and another recent study indicates that recycling of organic matter in food chains does not act as a continuous extraction process by which Pb becomes most concentrated at the highest trophic levels.

A model of biological fluxes of Pb through the surface mixed layer of the NE Pacific indicates that sinking of zooplankton fecal pellets containing Pb-rich phytoplankton debris satisfies geochemical constraints requiring a short residence time of Pb in these waters and is an important mode of vertical mass transport of Pb.

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Chapter I
INTRODUCTION AND OVERVIEW

1.1 Purpose and objectives of the investigation

The purpose of this study is to outline and characterize processes of enrichment and depletion of Pb, Ba, and Sr relative to Ca which occur in the nutritive pathways of Ca during its flow from seawater to marine plants and then through successive consumer animals.

The objectives are to assess the extent of biological fractionation of Pb, Ba, and Sr relative to Ca in transfers of these metals between trophic levels in selected marine ecosystems having well-defined food links, to evaluate the response of organisms to variations in external Pb concentration, and to construct a model for the flux of biologically transported Pb through the mixed surface layer of the NE Pacific. Pb is studied because it is a metal whose natural geochemical cycle has been extensively perturbed by the activities of man.

1.2 Interpretive concepts

The conceptual framework of this study is based on physiological studies on metal metabolism which indicate that in biological systems Sr, Ba, and Pb behave as analogues of Ca by paralleling the metabolic pathways of Ca (11,46,61,116,118,133,146,189,204,234). In plants and animals the physiological similarity between abundant, nutritive Ca and the non-essential trace metals Sr, Ba, and Pb is reflected as covariant and coincident distributions of Sr, Ba, and Pb within major tissue reservoirs of Ca (32,59-62,96,159,170,204). Biochemical mechanisms which directly regulate Ca appear to inadvertently and less efficiently process Sr, Ba,

and Pb as a consequence of large differences in relative abundances but small differences in biochemical properties between Ca and trace metal analogues of Ca, such as Sr, Ba, and Pb (11, 146, 164). For this reason, enrichments and depletions of Sr, Ba, and Pb which occur in major Ca reservoirs of different trophic levels in ecosystems are more clearly shown when the abundances of these metals are compared on the basis of corresponding Ca abundances rather than by contrasting bulk concentration measurements. The former approach is therefore used in this study. Findings of other investigations that form the scientific foundation upon which the approach of this study is based are reviewed in the second chapter.

1.3 Techniques of sample collection and analysis

Ultra-clean collection and laboratory procedures designed to exclude artifact Pb and the absolute primary standardization technique of stable isotope dilution mass spectrometry (IDMS) were used to study the occurrences of Ca, Sr, Ba, and Pb in selected benthic and pelagic marine food chains. To minimize artifact contamination during sample collection, preparation, and analysis, field collections of organisms and their seawater nutrient media were made using special techniques described in Chapter III. The samples were processed according to procedures, described in Chapter III and elsewhere (174), which have evolved from the techniques developed by investigators studying the natural occurrence of ^{204}Pb.

1.4 Systems in which metal transfer relationships were studied

Fractionations of Pb, Ba, and Sr relative to Ca occurring as the result of transfers of these metals between major Ca-reservoirs in organisms were studied in the following four systems:

1) seawater → giant unicellular alga (Valonia)

2) seawater → kelp → gastropod (kelp snail, abalone)

3) particulates → pelecypod (scallop, mussel)

4) seawater → phytoplankton → zooplankton → anchovy → tuna

Systems 1, 2, and 3 were studied under different degrees of Pb-pollution. Analytical results are presented and characterized in Chapter IV. Discussion of the significance of these results is contained in Chapter V.

1.4.1 Sample collection localities

Specimens of Valonia ventricosa studied in the first system were collected in an area of the Florida Keys remote from urban centers of Pb-pollution. For comparison, specimens of Valonia macrophysa which had been grown in a Pb-polluted seawater media were obtained from a laboratory culture.

The primary collection sites for the benthic organisms comprising systems 2 and 3 were Cortes Banks and Abalone Cove. Both areas are located within the Southern California Bight. The former is within a zone of minimum pollution on the outer continental shelf while the latter is a highly polluted site on the Palos Verdes peninsula. Concentrations of dissolved Pb in seawater increase from about 15 ng Pb/ℓ at Cortes Banks to about 50 ng Pb/ℓ in more heavily polluted Abalone Cove (172,193). This difference in dissolved Pb concentrations approximates the range of ambient dissolved Pb concentrations in coastal waters of the Southern California Bight.

1.4.2 A food chain model of the pelagic food web

The linear food-chain model of the pelagic food web represented by

system 4 was formulated using data obtained in this study as well as published data (170). The phytoplankton level of this system was modeled using data on Valonia because the present technology of plankton collection does not exclude artifact Pb. Valonia is similar to marine phytoplankton species (56, 179), yet its large 1-2 cm diameter size allows sampling of cellular subfractions with minimal potential for cross-contamination. Zooplankton were modeled using data obtained for the spiny lobster Panulirus because the widespread occurrence of inorganic sediment particles in the gut contents and fecal pellets of zooplankton (65, 103, 104) has caused investigators to suspect the significance of published zooplankton trace metal analyses (65). Metal concentrations measured in spiny lobster tissues were assumed to be representative of omnivorous crustaceans in general. Corrections were made for the difference in relative metal abundances between lobster food and zooplankton food. The resultant tissue metal concentrations were apportioned according to the tissue mass distribution of a zooplankter, Meganyctiphanes norvegica, for which data was available (67, 98, 209). Metal abundances in two food-chain related pelagic fishes, tuna and anchovy, were taken from published data (174).

1.5 Model of Pb flux in the NE Pacific

A model for the flux of biologically transported Pb in the upper mixed layer of the NE Pacific was developed using data of this study and information on pelagic biomass, community structure, and production and transit rates of biogenic detritus (127, 128, 178). Input fluxes and residence time of Pb derived from chemical oceanographic studies of these waters (41, 98, 173, 193) were compared with calculated biological removal fluxes and biological removal time to show that biological processes are important in transporting Pb out of the upper layers of the ocean.

Chapter II
DEVELOPMENT AND EXPOSITION OF THE BIOPURIFICATION CONCEPT

2.1 Applications of radioisotopic studies of metal metabolism

Because of concern for pollution by fission products from nuclear reactors and weapons, extensive studies have been made of the uptake and excretion, the metabolism, and the tissue localization in plants and animals of radioisotopes of Ca, Sr, Ba, and other heavy metals (10, 11, 30, 37, 46, 61, 66, 67, 83-85, 98, 116, 118, 128, 133, 139, 146, 147, 154, 159, 175, 176, 181, 189, 198, 199, 204, 209, 217, 231). Such studies are useful to this investigation because radioisotopes follow the same metabolic pathways in organisms as do naturally occurring stable isotopes of the same element.

These investigations have shown that the metabolic pathways of many heavy metals, including Sr, Ba, and Pb, parallel the pathways of Ca and that the localization within organisms of these trace Ca-analogue metals coincides with that of Ca, being chiefly in the skeletons of animals and the cell walls of plants (6, 30, 46, 65, 56, 59, 61, 62, 136, 145, 170, 204, 234). A partial list of these "bone-seeking" metals is given in table 2.1.

Subsequent investigations of the behavior of Ca, Sr, Ba, and Pb in the biomass of a subalpine ecosystem have confirmed that the bulk of Sr, Ba, and Pb is contained within structural Ca reservoirs which may constitute only a minor mass fraction of organisms (60, 62, 168). The distributions of these trace metals thus tends to be covariant with that of Ca. These same investigations have further shown that Sr/Ca, Ba/Ca, and Pb/Ca ratios in major Ca reservoirs of animals accurately characterize

Table 2.1. Partial listing of trace heavy metals physiologically similar to Ca.

Periodic Table Group:	Group IIA	Group IIIA	Group IVA
Elements:	Calcium		
	Strontium	Yttrium	Tin
	Barium	Lanthanum*	Lead
	Radium	(Actinium)**	

* In the Lanthanide Series: Ce, Pr, Nd, Pm, Sm, Eu, Ho, Tm, Lu
** In the Actinide Series: Th, Pa, U, Pu

Data are from Engstrom et al. (61).

these ratios in the total body of organisms.

2.2 Regulation of Sr, Ba, and Pb by Ca selection mechanisms

While geochemical and homeostatic control of Ca produces uniform Ca concentrations in many important ecosystem reservoirs such as seawater, cell fluids, and skeletal substances, the biological occurrence of Sr, Ba, and Pb, which are present in the environment in more variable amounts, appears to be regulated to a large extent by Ca levels rather than by the levels of the individual trace metals. This is true so long as those metals remain only trace constituents of the main Ca reservoir in organisms (11, 46, 61, 146, 159, 204). This appears a consequence of large differences in relative abundances but small differences in biochemical properties between Ca on the one hand and Sr, Ba, and Pb on the other (164). Synergistic effects on metal uptake have been noted when the molar abundance of Sr in nutrient media is about 1% of that of Ca, a situation that occurs in seawater. Radiotracer experiments with algae (118, 181) and gastropods (189) have shown that under these conditions uptake of Ca and Sr depends primarily on their concentration in nutrient media but that variation in the concentration of either metal has a slight inverse influence on the uptake of the other.

2.3 The concept of biopurification in ecosystems

Related studies on metal metabolism have shown that the fraction of available Ca, Sr, Ba, and Pb that is actively transported across primary membranes into the bodies of organisms is different for each metal but remains relatively constant in spite of changes in concentrations of other metals in this group. Relative to Ca the uptake efficiency of Sr, Ba, and Pb sequentially decreases and their elimination efficiency

increases with increasing atomic mass, ease of bond polarizability, degree of bond covalency, and strength of chelate formation (46, 61, 133, 146, 159, 164, 204). These trends parallel the change in physiologic character of those metals as one proceeds from nutritive Ca to non-essential, moderately toxic Sr, and toxic Ba and Pb (17, 147, 154,163, 164, 166). The trend towards biological accumulation of Ca and rejection of Sr, Ba, and Pb by organisms characterizes the active uptake of Sr, Ba, and Pb relative to Ca by all organisms thus far studied (30, 32, 46, 60, 146, 159, 168, 204, 234), except where industrial Pb deposited on surfaces of leaves and fur has circumvented natural mechanisms tending to exclude Pb from the food chain (60, 96). The mechanism whereby higher animals and their precursors discriminate within their food chains against non-nutritive heavy metals in favor of abundant and essential Ca is called biopurification of Ca with respect to Sr, Ba, and Pb (164, 166, 202, 205). This purification process, illustrated by the data of table 2.2, is usually evidenced in organisms by reduced Sr/Ca, Ba/Ca, and Pb/Ca ratios in major biochemical Ca reservoirs of organisms compared to these same ratios in major Ca reservoirs of trophic precursors. While it is true that functional groups of the proteins in soft tissues such as muscle tend to preferentially complex Pb and that such soft tissues frequently form the major mass fraction of animals (147, 154, 163, 230), the mass of Ca,Sr, Ba, and Pb contained in soft tissues of animals having skeletons is usually negligible compared to the amount contained in skeletal reservoirs, so that relative to food chain precursors the overall effect is biopurification of Ca (32, 33, 34, 159, 168, 202).

Table 2.2. Atomic abundances of Sr, Ba, and Pb relative to 10^6 atoms Ca in component reservoirs of a subalpine ecosystem

Metal	Rock	Soil Moisture	Sedge Leaves	Vole
Ca	1,000,000	1,000,000	1,000,000	1,000,000
Sr	16,000	21,000	7,300	2,000
Ba	15,000	3,800	2,000	330
Pb	280	210	54	1.4
Pb*	280	210	9	0.2

* After acid-washing sedge leaves to remove industrial Pb aerosols on plant surfaces. This theoretically reduces the body burden of Pb in the vole by 83%.

Data of Hirao and Patterson (96)

2.4 Processes of metal accumulation in marine biota

In the marine environment, two processes govern the distribution of Ca, Sr, Ba, and Pb in organisms: the physico-chemical process of passive adsorption and the biochemical process of membrane transport. In the former case the mode of metal accumulation is essentially independent of metabolic processes and is abiologic in character. In the latter case metals are accumulated by an active biochemical process. Passive adsorption often results in marked bioenrichment of Sr, Ba, and Pb with respect to Ca whereas membrane transport produces the competing effect of biopurification of Ca with respect to Sr, Ba, and Pb.

2.4.1 Bioenrichment effects associated with passive adsorption

Bioenrichment typically results from the selective binding of metals by polysaccharides in the mucilage of marine plants or glycoproteins in the mucus of marine animals. Sr, Ba, and especially Pb frequently form stronger organo-metallic complexes with functional groups of these biopolymers than do major metals in seawater, such as Ca, Mg, Na, and K. This often results in large enrichments on organism surfaces in the ratio of strong complex-formers relative to weak ones compared to their ratio in seawater. Bioenrichment phenomena are discussed more fully in Chapter V.

2.4.2 Biopurification effects associated with active transport

By analogy with processes in terrestrial ecosystems, marine organisms have regulatory mechanisms which discriminate against metals which inhibit catalysis and transfer processes by binding too strongly to intracellular ligands. Transport of Sr, Ba, and Pb across primary membranes of organisms is less efficient than Ca transport with the consequence that the total

reservoir of Ca is biopurified of Sr, Ba, and Pb. As evidenced in Chapters V through VII, the transport of Ca across primary membranes of organisms generally tends to biopurify absorbed Ca of those metals that are most highly bioenriched relative to Ca as a result of passive adsorption processes, such that Pb is more highly discriminated against than Ba or Sr.

2.5 Simultaneity of bioenrichment and biopurification effects

Passive adsorption processes compete with membrane transport mechanisms to determine gross chemical composition of organisms at all trophic levels of marine food chains. However, the fractional contribution of each process to the total metal reservoir of an organism is basically a function of its surface to mass ratio. This is because passive adsorption effects are limited largely to that fraction of total metals bound on organism surfaces whereas membrane transport effects are confined to that fraction of total metals which has been biochemically processed and stored within tissue reservoirs forming the mass of an organism. Phytoplankton and tuna, as well as kelp and kelp snail, represent pairs of extremes in the spectrum of fractional contributions by both processes to total organism chemistry. In most types of marine plants greater than 95% of the mass of divalent metals, predominantly Ca, is localized as extracellular polysaccharide complexes, whereas in animals a similar fraction resides in highly biopurified skeletal reservoirs. Consequently marine plants tend to show an overall bioenrichment of Sr, Ba, and Pb relative to Ca compared to seawater, the source of these metals, while marine animals tend to show overall biopurification of Ca with respect to Sr, Ba, and Pb compared to trophic precursors. Animals frequently do not show overall biopurification with respect to seawater, however, because their metals have been recycled from bioenriched metal reservoirs at the plant level.

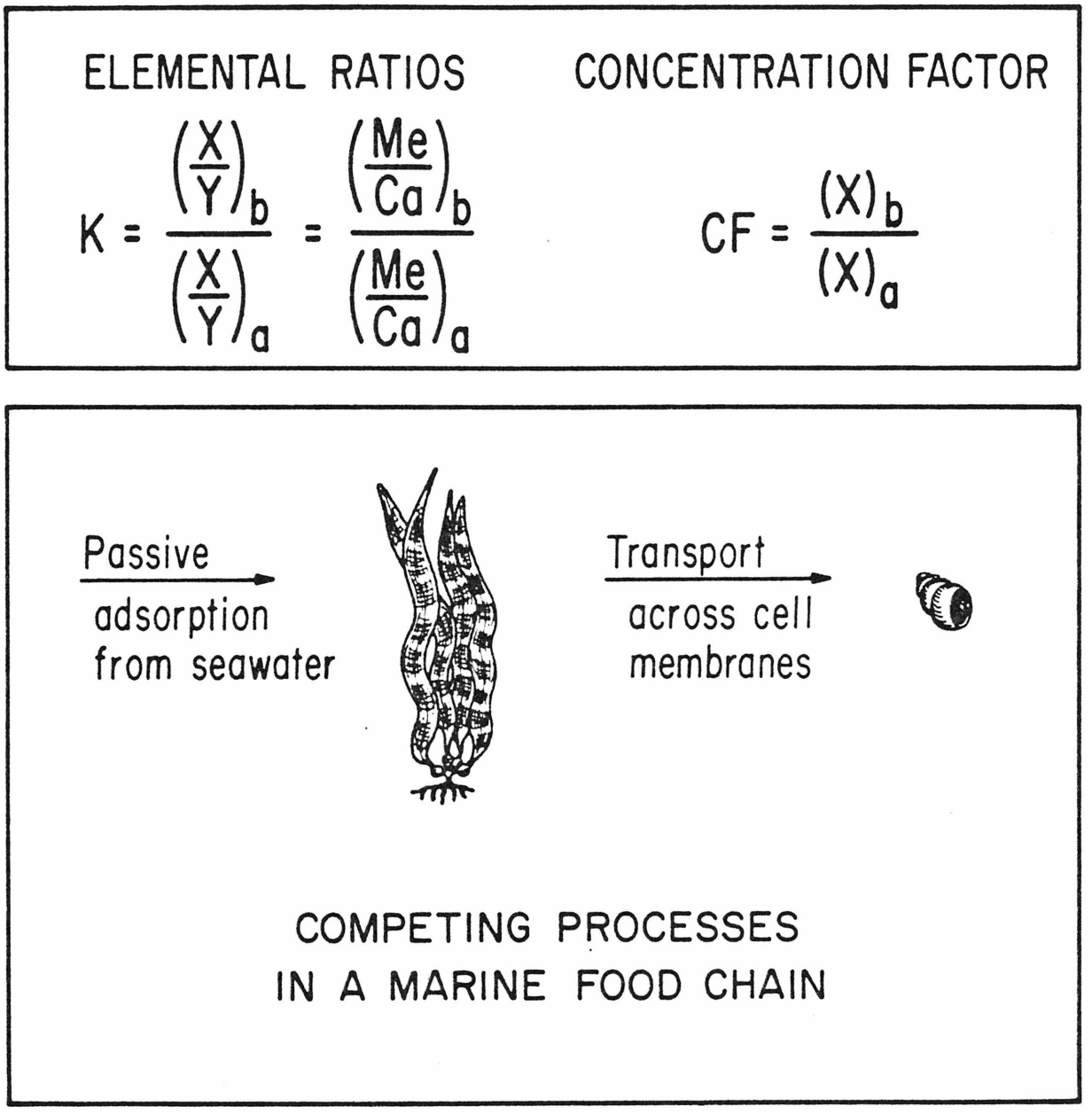

Figure 2.1 Contrasting methods of measuring changes in metal abundance during the transfer of metals through marine ecosystems. Processes of enrichment or purification occurring during the flow of trace metals between major metal reservoirs in ecosystems are most illustratively compared on the basis of fractionation factors, K, rather than so-called concentration factors, CF, because the former allow different components and ecosystems to be compared on the basis of normalized data.

The fractionation factor, K, is defined in figure 2.1 as the ratio of abundances of trace Ca-analogue metals normalized on the basis of corresponding Ca concentrations in the Ca reservoirs being compared. The resultant dimensionless factor is a measure of the magnitude of bioenrichment or biopurification effects resulting from differences in efficiency with which Sr, Ba, and Pb are transferred relative to Ca between major reservoirs of Ca in ecosystems. Comparaing the ratios of Sr, Ba, or Pb relative to Ca in major reservoirs of product organisms with those same ratios in major Ca reservoirs of precursor organisms allows metal distributions in one component of an ecosystem to be meaningfully compared with distributions in other component reservoirs because Ca acts as an internal standard for the metal reservoir of each stage of the ecosystem. This formulation then allows comparisons between different ecosystems to be made on the basis of data normalized to Ca.

Chapter III
COLLECTION, PREPARATION, AND ANALYSIS OF SAMPLES

3.1 Times and locations of sample collections

Kelp and marine invertebrates from the Southern California Bight were collected at the locations shown in figure 3.1.

In late February, 1976, specimens of the kelp Eisenia arborea, the kelp snail Norrisia norrisii, the purple-hinged rock scallop Hinnites multirugosa, the spiny lobster Panulirus interruptus, the pink abalone Haliotis corrugata, and surface seawater were collected at Cortes Banks in 40 m of water. This locality lies within the outer, less polluted portion of the Southern California outer continental shelf (114, 192). By far the most abundant species of brown algae at this site is Eisenia, and the herbivorous gastropods Norrisia and Haliotis are common. At the time of collection specimens of Norrisia were observed to be actively feeding on the Eisenia blades from which they were gathered. Haliotis specimens from Cortes Banks did not seem to be feeding at the time of their collection, although abalones typically show a strong preference for brown algae (2, 28, 48, 131, 132).

In early May, 1976, samples of E. arborea, the giant kelp Macrocystis pyrifera, the kelp snail N. norrisii, the black abalone H. cracherodii, and seawater were collected at Abalone Cove on the Palos Verdes peninsula in 5 m of water. This site lies within the inner, most polluted region of the Southern California outer continental shelf (114, 192). This and other polluted areas are indicated by the stippled zones in figure 3.1. Abalone Cove is adjacent to White's Point sewage outfall, which serves Los Angeles County and is one of the largest sewage outfalls in the world.

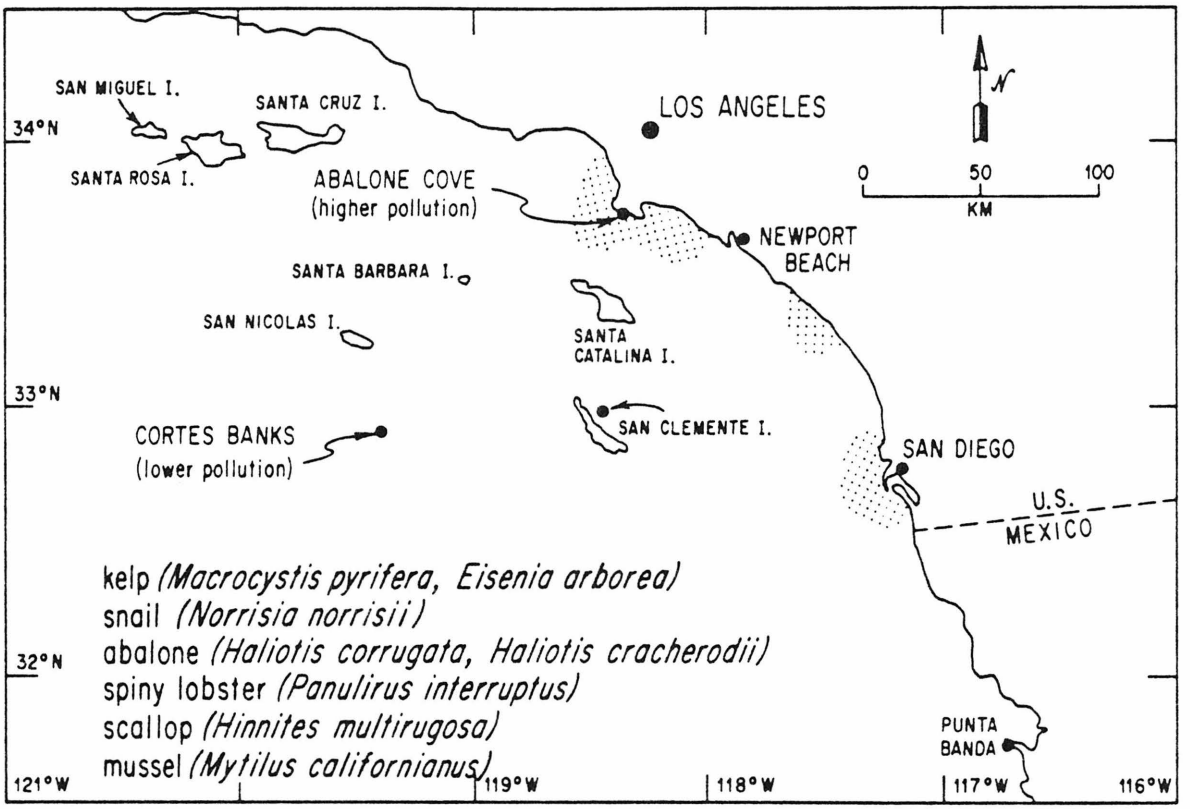

Figure 3.1. Collection localities for organisms used in the study of benthic food chains. Baseline studies indicate that Cortes Banks and Abalone Cove waters approximate the range of Pb pollution effects observed in the Southern California Bight (172, 174, 192). Stippled zones correspond to areas highly affected by input of Pb-rich industrial wastes (192).

At the time of collection both kelp snails and abalones appeared to be feeding. Norrisia specimens were observed to be cutting into and consuming the canopy blades of Macrocystis from which they were collected, while the abalones collected here were clustered about sunken fronds of giant kelp and were actively feeding on the blades. These habits are in accord with known food preferences of each species (48, 131, 132).

A subsequent collection of Macrocystis was made at Abalone Cove in August, 1976. Dr. David Young of the Southern California Coastal Water Research Project provided a specimen of the scallop H. multirugosa that had been collected at this locality within the same time frame.

Supplemental collections of giant kelp, kelp snails, and seawater were also made during August, 1976, near the north side of San Clemente Island in the outer Bight region shown in figure 3.1.

Specimens of the mussel Mytilus californianus were collected in the intertidal zone of the Pacific side of the Punta Banda peninsula, near Ensenada, Baja California, Mexico in February of 1978.

The previous June, 1977, specimens of the giant unicellular alga Valonia ventricosa and surface seawater were collected in 1 m of water near Key Largo, an area of the Florida Keys that is distant from urban centers of Pb pollution. For comparative purposes, Drs. H. Windom and J. Sanders of Skidaway Institute of Oceanography provided specimens of Valonia macrophysa that had been cultured in a growth media containing an unknown amount of the chelating agent EDTA. The EDTA had been added by their supplier as a trace metal stabilizing agent. When the media was later analyzed, it contained 430 ng Pb/ℓ, a very high value, but only 6 µg Ba/ℓ, a typical surface seawater concentration (7, 38). This shows the media was highly contaminated with artifact Pb but was probably not seriously compromised by the addition of pollutant Ba.

To avoid artifact contamination during collection, transport, and processing, samples were collected by divers wearing polyethylene gloves and epoxy-painted weight belts. When a diesel-powered research vessel was used, samples were collected both upwind and upcurrent from it. Seawater samples were collected in specially cleaned and prepared 2-ℓ (169, 171) conventional polyethylene bottles. Abalones and rock scallops were pried from the rocks using a stainless steel instrument. Algae, kelp snails, mussels, and the spiny lobster were hand-collected. Organisms were double-bagged underwater in acid-cleaned polyethylene prior to transfer through the air-sea interface. All samples except Valonia were then placed in dry-ice chests for temporary storage. To prevent contamination during transport to the CIT freezer, where they are stored before processing for analysis, the sample containers were sealed within a third polyethylene bag. Valonia were transported live in clean polyethylene bottles.

3.2 Preparation of plant samples for analysis

Bulk samples of brown algal kelps and cells of the green alga Valonia were prepared by rinsing each sample with a jet of quartz-distilled water (QDW) to remove excess seawater and detrital particles since Pb-rich particles of industrial origin may adhere to algal surfaces in regions of high sewage or other particulate input. The concentration of Pb in these particles has been measured (174), and the adherence of relatively few particles on plant surfaces can greatly increase measured metal concentrations in marine plant materials (15, 26). Usually these particles can be removed by rinsing plant samples with a jet of QDW prior to analysis. Investigations have shown that the rinsing process does not elute divalent metals (except Mg) because unlike alkalies and most other monovalent metals, divalent metals tend to be bound as relatively insoluble

complexes that do not dissociate easily (14, 15, 27, 247).

Table 3.1 shows that the effect of rinsing a fragment of San Clemente Island Macrocystis is to remove a large fraction of the Pb present in an unwashed fragment taken from the same plant. A corresponding increase in the concentrations of alkaline earth metals which accompanies the rinsing process may result from removal of excess water and elution of salts and certain organic constituents. Sr and Ba abundances relative to Ca do not significantly change as a result of rinsing. The difference between the higher Pb/Ca ratio of unrinsed kelp and the lower ratio of the rinsed sample appears to result from the removal of adhered particles because analysis of water-soluble fractions of the surface mucilage, reported later in table 4.1, indicates that it contains very little Ca, Sr, Ba, or Pb.

After rinsing with QDW, kelp blades were shaken to remove excess water and weighed into a clean FEP teflon beaker with a cover.

Valonia samples were prepared in the same manner for bulk analyses. Vacuolar sap was obtained by piercing the cell wall of several "grape-sized" specimens with the fine tip of a silica pipette and withdrawing the fluid. Purity of the sample was indicated by the absence of chloroplasts.

3.3 Techniques of plant tissue analysis

A 1 gram sample of plant tissue was spiked with ^{208}Pb and ^{136}Ba after it had been placed in a beaker. A 30-ml FEP teflon beaker was used for kelp samples, but quartz dissolution ware was used to lower the dissolution blank of Valonia samples, which were known to contain less Pb than kelp. Dissolution was completed by adding 3 ml HNO_3 and 0.5 ml $HClO_4$, then covering the beaker with a quartz watch glass, and digesting for two hours at 80°C in a teflon oven flushed with pure N_2. The temperature was increased to 150°C and the sample was evaporated to dryness uncovered.

Table 3.1. The effect of ultra-clean sample preparation techniques on observed metal concentrations in plant and animal tissues.

Sample	Concentrations in µg/g fw			
	Ca	Sr	Ba	Pb
San Clemente Island kelp (unrinsed)	1200	80	0.87	0.15
San Clemente Island kelp (QDW rinsed)	1450	100	1.15	0.020
Punta Banda *Mytilus* muscle (unshaved)	nd	2.2	0.008	0.056
Punta Banda *Mytilus* muscle (shaved)	180	2.9	0.006	0.025

Kelp were believed to contain enough silica to cause dissolution problems. Silica was therefore deliberately removed by adding 1 ml HF to the cooled residue, covering the beaker, and allowing it to stand overnight at room temperature. The sample beaker was then heated covered for three hours at 60°C; the cover was removed, and the solution was evaporated to dryness at 90°C. The pure white, crystalline residue was dissolved in 1 ml HCl and evaporated to dryness twice. It was finally dissolved in 5 ml of 0.5N HCl. A 10% aliquot was removed for the Ba analysis and spiked with ^{42}Ca and ^{84}Sr. The remaining 90% was used for Pb analysis. The procedure for Valonia was as described except that the HF treatment was omitted because quartz ware was used for digestion of the sample.

After transfer to an FEP teflon separatory funnel, the pH of the Pb aliquot was adjusted to 7 using NH_4OH. 1 ml of 25% ammonium citrate and 2 ml of 2% KCN were added as chelating agents. The Pb was then extracted using 5 ml of dithizone-chloroform. The Pb was then back-extracted into 10 ml of 1N HNO_3; the pH was adjusted to 8, and a second dithizone-chloroform extraction was done without complexing agents to minimize blank corrections. The chloroform solution was washed with 0.02N NH_4OH and then back-extracted into 5 ml of 1N HNO_3. The aqueous solution was washed once with 5 ml of chloroform to remove traces of dithizone and evaporated to dryness in a 30-ml FEP teflon beaker.

The residue of the Pb aliquot was dissolved in a few µl of QDW and loaded onto an outgassed Re filament using a slurry of silica gel and H_3PO_4. The aliqout removed earlier for alkaline earth analysis was evaporated to dryness, dissolved in a minimum volume of QDW, and a portion of the solution was loaded onto an outgassed, oxidized Ta filament.

3.4 Preparation of animal samples for analysis

Dissection of frozen animals was required in order to isolate the muscle tissue with minimal transfer of contamination. The main muscle masses were severed at their point of attachment to the shell using a curved stainless steel spatula inserted between the mantle and the shell. Repeated blows with a stainless steel hammer were sometimes needed to drive the spatula through the frozen muscle of large specimens. Using a stainless steel scalpel, the internal organs were carefully dissected out as a unit and kept frozen for later dissection. Contamination of the muscle tissue by epidermal material whose metal concentrations are high was avoided by shaving away the surfaces of the frozen sample material in successive stages. Cleaned stainless steel instruments, polyethylene gloves, and teflon dissecting platforms were renewed before each subsequent shaving stage. The need for this procedure, which results in a series-dilution of any contamination introduced during the initial dissection, is illustrated in table 3.1 by the comparison between a shaved and an unshaved sample of Mytilus adductor muscle. Although preparation of both samples was carried out in a clean-laboratory, the omission of the primary shaving step resulted in a factor of 2 increase in the Pb concentration of the unshaved specimen but did not appreciably affect alkaline earth concentrations. The higher Pb concentration may originate from passively adsorbed Pb complexed by glycoproteinaceous mucus occurring on surfaces of marine animals (153) such as mussels.

3.5 Techniques of animal analysis

A 1 g sample of fresh muscle or other tissue was spiked with ^{208}Pb and ^{136}Ba in a 30-ml beaker. For most samples the beaker material was FEP teflon, but for the analysis of tuna muscle, which was known to con-

tain an order of magnitude less Pb than most marine animals (45,170), silica dissolution ware was used. 3 ml HNO_3 and 1 ml $HClO_4$ were added, the beaker was covered with a quartz watch glass, and the mixture allowed to digest for 3 hours at 55°C in an atmosphere of pure N_2. The temperature was then raised to 150°C while the solution continued to digest covered. The cover was removed and the solution was evaporated to dryness. The white crystalline residue was dissolved in 1 ml HCl and taken to dryness twice. The residue was then dissolved in 5 ml of 0.5N HCl and a 10% alkaline earth aliquot was removed. This aliquot was spiked with ^{84}Sr and ^{42}Ca, while the remaining 90% was used for Pb analysis.

The Pb procedure consisted of two dithizone extractions, the first using complexing agents and the second without. The amounts of reagents and sample preparation were the same as described in section 3.3 for plant tissues. The 10% alkaline earth aliquot was also treated in the same way as for plant tissues.

3.6 Analytical technique: stable isotope dilution mass spectrometry

Isotope dilution mass spectrometry (IDMS) is a reliable and established method for the standardization of Pb and Ba at the 10^{-9} g/g fw (fresh weight) concentrations that these metals occur in the tissues of marine plants and animals (45, 63, 170, 171). The potential of this technique is realized when it is used in conjunction with ultra-clean collection, transport, storage, and processing techniques specifically designed to exclude artifact contamination at every stage. The latter include the elaborate procedures necessary to control blanks (169). Much time was devoted to minimizing blank contributions from various sources because they are an important source of error. Although this has

curtailed the total number of samples analyzed in this study, it has helped to insure the reliability of the data. Table 3.2 gives the concentrations of Pb and Ba measured in the reagents used in analysis and blanks from labware and handling. Typical Pb blanks were about 0.10 ng for the dissolution, 0.28 ng for the first dithizone extraction, and about 0.17 ng for the second dithizone extraction. Ba blanks were about 1.3, Sr blanks about 5 ng, and Ca blanks about 20 ng for the complete analysis.

Yields for chemical separations were monitored periodically so that proper blank corrections could be made. This was done by adding a known amount of a second isotopic spike after a chemical separation step to a sample previously spiked with an isotopic tracer of the metal to be analyzed. The yield for the preceding operation could then be determined by measuring the ratio of tracer isotopes, knowing the masses of each tracer added, and comparing the measured result with that predicted by a 100% yield. Extraction yields were generally about 90-95%.

The $^{208}Pb/^{207}Pb$ ratio was measured in the mass spectrometer to determine Pb concentration. The expression for computing blank and yield corrected Pb concentrations on the basis of Pb isotope ratios is given as follows:

Total Pb = {[(sample Pb + tracer Pb + dissolution blank Pb)(%Pb aliquot)
+ 1st extraction blank Pb](1st extraction yield)
+ 2nd extraction blank}(2nd extraction yield)
+ loading blank Pb + yield tracer Pb

The $^{136}Ba/^{138}Ba$, $^{88}Sr/^{84}Sr$, and $^{40}Ca/^{42}Ca$ ratios were measured in the mass spectrometer to determine concentrations of alkaline earth metals. The expression used to compute blank and aliquot-corrected concentrations of these metals is given on the next page.

Table 3.2. Typical reagent and measured operational blanks.

Reagent	ng Pb/ml*	ng Ba/ml**
QDW	.0005	.004
HNO_3	.01	.02
$HClO_4$	.02	.07
HCl	.01	.06
HF	.02	.10
HAc	.003	.07
NH_4OH	.0008	.10
$CHCl_3$	.007	
$(NH_4)_3C_6H_5O_7$	.24	
KCN	.02	
Dithizone	.03	

*CIT values

**NBS values

Operation[1]	ng Pb	ng Ba
Dissolution	0.24	0.46 - 1.3
Extraction using complexing agents	0.30	
Extraction without complexing agents	0.20	
Container contamination[2]	0.1 - 2	0.15
Loading blank	0.02	0.01

1) These approximate values vary with amounts of reagents, different reagent batches and individual containers, and duration of exposure time of sample to the various reagents and containers.

2) If HF is used the Pb blank may increase to as much as 2 ng.

For analyses involving no chemical separation steps, the expression is:

Total Ba = [(sample Ba + tracer Ba) + (dissolution blank Ba)](%Ba aliquot)
+loading blank Ba (Rewrite expression for Ca or Sr)

In practice the preceding equations are written for each isotope of the individual pairs measured in the mass spectrometer. The quotient of the two resultant equations equals the measured ratio. The unknown amount of sample isotope is obtained by solving this ratio equation. This result is converted into absolute nanograms of metal on the basis of the known natural isotopic composition of the metal analyzed. Metal concentration is computed by dividing the sample metal mass by the sample weight. Ca concentrations in samples are obtained in a similar manner as Sr and Ba except that Ca is subject to an additional correction because it is measurably fractionated in the mass spectrometer. This correction is obtained between the known and the measured ratios.

3.7 Analytical accuracy and precision

The procedures used in this study to control artifact contamination have evolved from techniques pioneered by geochemists studying the occurrence of ^{204}Pb in rocks and meteorites and are continuously being refined (169, 171). Nevertheless, the greatest source of error in ecosystem studies is artifact contamination. This problem is acute in the case of Pb because industrial Pb pollution is pervasive (169) and because Pb concentrations in crustal materials are orders of magnitude higher than in biological tissues. Marine organisms are especially sensitive to contamination effects because of low Pb concentrations in their environment. This problem is no less severe for Ba because sources of Ba contamination are not well known.

Uncertainties in the procedural blank contribution introduce

Table 3.3. Absolute accuracy and precision of Pb analyses of marine biological materials containing nanogram concentrations of Pb

Absolute accuracy calibration using the CIT Tuna Muscle Reference Standard

Sample size	Standardized Value*	Result**
5.0 g fw	0.0004 µg Pb/g (±30%)	0.0004 µg Pb/g (±30%)

Precision of Pb Analyses

Sample	Results (in ng Pb/g fw) of: Run #1	Run #2
Haliotis corrugata muscle (Cortes Banks)	6.0	6.3
Panulirus interruptus muscle (Cortes Banks)	5.0	5.1
Macrocystis pyrifera slurry (Abalone Cove)	54	55
Eisenia arborea slurry (Cortes Banks)	45	50

* Data of Patterson and Settle (170). Variability expressed as percent represents the range of a series of replicate analyses.

** Variability expressed as percent represents the uncertainty in the blank for a single analysis at this concentration level. This degree of uncertainty is a measure of the reliability of the analysis.

progressively greater error in calculated metal concentration as the ratio of the mass of metal contained in the sample to the mass of blank metal approaches unity. This may be illustrated by considering table 3.3, which shows the results of an absolute accuracy calibration. In this experiment a 5 g sample of the CIT Tuna Muscle Reference Standard (170) was analyzed. The Pb concentration in this reference material has been rigorously standardized by another investigator using the same IDMS technique (171) as that of this study. The 5 g tuna muscle sample was known to contain about 2 ng total Pb on the basis of the previous standardization. The sample was digested in quartz ware and at the same time a parallel blank experiment was run in a separate teflon oven. A total of 4 ng Pb was found in the tuna muscle experiment while total Pb in the blank experiment was 2.5 ng. This blank value was 1 ng larger than the 1.5 ng Pb blank expected on the basis of previously measured reagent and handling blanks. Because of this uncertainty sample Pb in tuna muscle may have represented 1.5 to 2.5 of the 4 ng total Pb found. It is therefore probable that Pb analyses in this study may be in error by as much as 50% at the 10^{-10}g Pb/ g fw concentrations typical of tuna muscle and Valonia vacuolar sap because of inability to control blanks beyond this level. Other investigations in the CIT laboratory have shown that this problem is also prevalent in the case of Ba at similar sample to blank metal mass ratios.

The amounts of sample material analyzed in the tuna muscle experiment and determinations of Valonia vacuolar sap were several times larger than typical plant and animal samples because Pb concentrations in the latter were greater than in the former by an order of magnitude or more. Analytical error did not decrease linearly as Pb concentration in samples

increased because of the diversity of samples and because other sources of error became important. The major source of error in kelp analyses, for example, was not uncertainty in the blank because the ratio of sample Pb to blank Pb was usually about 50. In these samples the major sources of error were uncertainty regarding possible artifact contamination prior to processing for analysis and uncertainty in the water content of kelp tissues due to dehydration during storage and weight changes resulting from the rinsing process. The former was controlled using techniques outlined in preceding sections while the latter is estimated to introduce several percent error in calculated metal concentrations.

Uncertainties in weighing and isotopic equilibration are not significant sources of error in the procedures outlined in sections 3.3 and 3.5 because they have been measured and found to be on the order of several parts per thousand. Biological contamination, such as the presence of diatoms in kelp mucilage, was believed small because of small mass contributions.

As noted earlier, reporting results as concentrations in fresh material introduces error because values are dependent upon water contents which are approximate because of minor dehydration and plasma losses occurring between the time of collection and the weighing of the dissected sample. Although dry tissues are more convenient for analysis, determinations relative to fresh weight give more valuable information regarding the biological significance of metal abundances. Frequently the marked seasonal variations in metal content as a function of dry weight are caused by the accumulation and diminution of storage products and other components and largely disappear when recalculated on the basis of fresh weight (89). Therefore in this study metal concentrations are reported on the basis of fresh weight (fw).

In light of the preceding discussion it is probable that the error associated with Pb analyses may be as much as 50% at concentration levels of 10^{-9}g Pb/g. Error is estimated to diminish to about 5% for Pb concentrations in hard parts at or above the 10^{-8}g Pb/g level but may be as much as 10% in samples of high water content such as kelp, muscle tissue, etc.

Replicate IDMS analyses do not reduce statistical variation between isotopic ratio measurements which vary between one another by several parts per thousand. The most important factor affecting analytical precision is sample inhomogeneity. While this factor was not investigated in depth due to the limited number of samples analyzed, it is probable that tissues containing biogenic granules whose metal content is high may vary significantly within a population of specimens and between tissue samples from a single individual. Mollusc mantle (188), kidney (25, 55), and digestive gland (25) have been found to frequently contain carbonate and phosphatic storage, pathogenic, or excretory products in which Pb concentrations range as high as several hundred μg Pb/g. Normalization of Sr, Ba, and Pb abundances on the basis of corresponding Ca abundance reduces the impact of small sample inhomogeneities on the interpretation of the data because of the tendency for these metals to have covariant abundances in organism subfractions.

Replicate analyses of sample aliquots of kelp, abalone, and spiny lobster tissues shown in table 3.3 indicate that the overall precision of Pb analyses is on the order of several percent at concentrations of 10^{-9}g Pb/g for most samples.

CHAPTER IV

CHARACTERIZATION OF ANALYTICAL RESULTS: PART I. ANALYSES OF MARINE PLANTS PART II. ANALYSES OF MARINE ANIMALS

PART I. ANALYSES OF MARINE PLANTS

4.1 Marine plants analyzed and reporting of results

Specimens of the kelps Macrocystis pyrifera and Eisenia arborea, which are brown algae, and the giant unicellular green alga, Valonia, were collected from waters of different ambient Pb concentration and analyzed for Ca, Sr, Ba, and Pb. The results for brown algae are reported in tables 4.1 and 4.2. The results of Valonia analyses are given in tables 4.3 and 4.4.

Bulk concentration measurements show greater variability than the uncertainty of several percent caused by reporting results as fresh weight because the proportions of water and organic constituents can vary markedly with species, season, geography, and location in the plant (71, 72, 90-92, 155, 239, 244, 247). Plant samples may also contain microscopic mineral particles and organisms, which may not always be removed during cleaning procedures. Plant mucilage, which can form 20% or more of the organic fraction of kelp (155, 177), is lost naturally by either sloughing off (75) or by degradation by marine bacteria (245). In the rinsing process a few weight percent of water-soluble mucilage is also lost. Metal concentrations in this fraction are low, as indicated by data of table 4.1, so little metal is eluted. Metal concentrations in rinsed plant samples may in fact be greater than in unrinsed samples because the latter retain a greater fraction of their initial mass. As a consequence, abundances of Sr, Ba, and Pb normalized on the basis of Ca provide more reliable information on the occurrence of these

metals than bulk concentration measurements.

4.2 Comparison with published analyses of marine plants

Concentrations of Ca, Sr, and Ba reported in tables 4.1 and 4.2 for Macrocystis and Eisenia are in overall agreement with results of some other investigations (16, 45, 156, 184, 239, 242). On the other hand, most reported determinations of Pb in kelp are considerably higher, often by orders of magnitude, than those found in this study. The latter fall at the extreme lower range of reported concentrations, even though some of the samples come from one of the most Pb polluted regions in the world.

The abundance of Ca in cellular subfractions of Valonia has been reported previously (11, 56) and the uptake of Pb by Valonia has recently been studied using electrochemical techniques (179). The results and conclusions of these studies are in good agreement with those of this study.

4.3 Factors affecting observed metal distributions in marine plants

Table 4.1 shows that while metal concentrations may vary by more than a factor of two between Macrocystis plants, the Sr/Ca, Ba/Ca, and Pb/Ca ratios show only small differences between plants and between different tissues of the same plant. This indicates that age is probably not a significant factor in the accumulation of Sr, Ba, and Pb relative to Ca by Macrocystis. Table 4.1 also shows that although the concentration of Ca, Sr, Ba, and Pb are about 2.5 times higher in Cortes Banks Eisenia than in outer shelf Macrocystis from San Clemente Island, Sr/Ca, Ba/Ca, and Pb/Ca ratios are very similar between both species, suggesting that species differences in kelp play only a minor role in the uptake of Sr, Ba, and Pb relative to Ca.

Pb concentrations and, specifically, Pb/Ca ratios in kelps show a high

Table 4.1. Concentrations of Ca, Sr, Ba, and Pb in the kelps *Macrocystis pyrifera* and *Eisenia arborea*

Sample	Locality	Concentration in µg/g fw			
		Ca	Sr	Ba	Pb
Macrocystis blade (QDW rinsed)	Abalone Cove	720	68	0.78	0.053
Macrocystis blade (unrinsed)	" "	1950	145	3.9	0.13
Macrocystis mucilage (H_2O-soluble fraction)	" "	30	0.49	0.006	0.001
Macrocystis apical blade (QDW rinsed)	" "	910	79	0.92	0.019
Macrocystis mature canopy blade (QDW rinsed)	" "	1150	91	1.2	0.035
Macrocystis senescent canopy blade (QDW rinsed)	" "	1050	77	0.85	0.029
Macrocystis blade (QDW rinsed)	San Clemente	1500	100	1.1	0.020
Macrocystis blade (unrinsed)	" "	1200	78	0.87	0.15
Eisenia blade slurry (QDW rinsed)	Cortes Banks	2950	190	2.0	0.050
Eisenia blade fragment (QDW rinsed)	" "	2550	280	2.2	nd
Eisenia blade (QDW rinsed)	Abalone Cove	nd	nd	nd	0.50

Table 4.2. Results of a two-minute 0.1N HCl leach of a 3.5g fragment of Macrocystis pyrifera from Abalone Cove

Reservoir	Masses of metal in µg			
	Ca	Sr	Ba	Pb
Metals released by leach	3.2×10^3	2.3×10^2	2.8	0.17
Metals retained in leached sample	1.5×10^2	1.2×10	0.29	0.034
Total metal in sample (initial)	3.4×10^3	2.4×10^2	3.1	0.20
Percent total metal released by leach	96%	95%	91%	83%
Concentration of metals in sample prior to leach[1] (µg/g fw)	940	66	0.87	0.057
Concentration of metals in separate unleached fragment[2] (µg/g fw)	1000	57	0.69	0.055

[1]The initial mass of each metal in the sample is the sum of the mass of metal released and that retained. Initial concentrations are obtained by dividing this result by the mass of the sample prior to leaching.

[2]This sample was from a different kelp blade of the same plant and is included for comparative purposes.

dependence on ambient concentrations of dissolved Pb. The Pb/Ca ratio of Cortes Banks Eisenia is quite similar to that of San Clemente Island Macrocystis but is a factor of two less than the Pb/Ca ratio of Abalone Cove Macrocystis shown in table 4.1. This correlates with dissolved Pb levels which average twice as high at Abalone Cove as at Cortes Banks (172).

4.4 Influence of foreign mineral inclusions on observed metal concentrations

Eisenia contained 50 ng Pb/g fw at Cortes Banks but 500 ng Pb/g fw at Abalone Cove, as seen in table 4.1. This could indicate a species difference in Pb uptake between the two kelp varieties but is more readily explained by incorporation of foreign mineral grains or aggregates in tissues of the Abalone Cove Eisenia plant. This kind of interference has been described in other investigations and appears to be particularly prevalent in kelps, such as Eisenia or Fucus, which grow near the bottom in turbulent zones of high wave action (15, 26). A residue of insoluble grains was present in the clear solution resulting from the acid dissolution of Abalone Cove Eisenia, but none were observed in digests of Cortes Banks Eisenia, which grew in deeper water, or in acid slurrys of Macrocystis canopy blades. High metal concentrations in holdfasts of Macrocystis have been positively correlated with resistant grains in the sample solution (31). Published analyses of kelp reporting substantially higher Pb concentrations (>150 ng Pb/g fw) than those found in this study (50 ng Pb/g fw) and accumulation of Pb in older parts of the plant and its holdfast may have been influenced by foreign particles adhering to or embedded in the plant.

4.5 Occurrence of alkaline earths and Pb in kelp species

Other investigations have demonstrated that cation exchange between seawater and algin, the major extracellular anionic polysaccharide in kelp,

largely determines the abundance of Sr, Ba, and Pb relative to Ca in brown algae (3, 6, 95, 206, 233). This mechanism by which alkaline earths and Pb are accumulated by kelp produces an overall bioenrichment of Sr, Ba, and Pb relative to Ca in bulk kelp with respect to relative abundances of these metals in seawater. The data of table 4.1 indicate that in going from seawater to kelp, the Sr/Ca ratio increases by a factor of 4, the Ba/Ca ratio increases by 30, and the Pb/Ca ratio increases by about 730. These bioenrichment factors are determined by comparing average kelp data with average concentrations of Ca (410 μg/ml), Sr (8.3 μg/ml), Ba (6 μg/ℓ), and Pb (20 ng/ℓ) in the Southern California Bight (38, 193).

Table 4.2 shows the results of a leaching experiment in which a fragment of Macrocystis was immersed for 2 minutes in 0.1N HCl. This procedure, which is sufficient to liberate metals bound as alginates (89, 200, 233), eluted 83% of total Pb, 91% of total Ba, 95% of total Sr, and 96% of total Ca in the sample.

4.6 Occurrence of alkaline earths and Pb in Valonia species

Concentrations of Ca, Sr, Ba, and Pb in both species of Valonia studied, shown in tables 4.3 and 4.4, are 25 to 100 times lower than those measured in kelp or reported in phytoplankton (143, 144,221, 226). The effective surface to mass ratio of Valonia cells, which are the size of marbles, is much lower than that of multicellular brown algae or microscopic plankton. Metals bound on surfaces of Valonia thus form a proportionally smaller fraction of total metals per unit mass than is typical of most other types of algae. Instead most of the metals in Valonia are contained in the vacuolar sap, which constitutes about 97% of the mass of a mature specimen and which is depleted in Ca relative to seawater by at least a factor of 10. Only a few percent of total metals are associated with the

Table 4.3. Concentrations of Ca, Sr, Ba, and Pb in seawater and *Valonia ventricosa* from the Florida Keys

Sample	Concentration in µg/g of:			
	Ca	Sr	Ba	Pb
Key Largo Seawater	410	8.1	0.006	0.000016
total cell (surface unencrusted)	23	0.12	0.002	0.002
total cell (carbonate encrusted)	150	0.40	0.05	0.003
total cell (dead)	nd	nd	nd	0.007
total cell (leached)	38.9	0.40	0.01	nd*
cell wall	123	0.80	0.05	0.007
vacuolar sap	24	0.22	0.005	0.0004
surface adsorbed metals from unencrusted cell in µg/cm^2	4.3	0.045	0.00034	0.00012
surface adsorbed metals from carbonate encrusted cell in µg/cm^2	13.2	1.2	0.003	0.0008

* Sample lost

Table 4.4. Concentrations of Ca, Sr, Ba, and Pb in seawater and *Valonia macrophysa* cultured in a Pb-polluted media

Sample	Concentration in µg/g of:			
	Ca	Sr	Ba	Pb
Culture media seawater	410	8.1	0.007	0.00043
total cell (surface unencrusted)	68	0.85	0.43	0.006
cell wall	81	0.89	0.12	0.011
vacuolar sap	47	0.53	0.016	0.001
surface adsorbed metals from unencrusted cell in µg/cm^2	2.0	0.022	0.003	0.0006

outer cell wall layer and the film-like layer of protoplasm immediately underlying it (56).

Table 4.3 shows that a two-minute leach of a Valonia cell does not remove as large a fraction of total metals as does leaching of kelp. This is predictable in light of differences between the proportional distribution of alkaline earths and Pb among cellular subfractions of Valonia as compared to kelp.

4.7 Measurement of metals adsorbed onto surfaces of Valonia

It was important to determine the quantities of metals adsorbed per unit area onto Valonia surfaces in order to model phytoplankton. To do this, Valonia ventricosa cells of known dimensions were leached for two minutes in 0.1N HCl. V. ventricosa cells were used rather than cells of V. macrophysa because the former were collected from seawater whose dissolved Pb concentration (16 ng Pb/ℓ) is typical of open ocean surface water (193). The absolute masses of Ca, Sr, Ba, and Pb released by the leaching process were divided by the surface area of the leached cells to determine the concentrations of these metals in $\mu g/cm^2$. The data, reported in table 4.3, show that 0.12 ng Pb/cm^2 desorbs from Valonia cell surfaces under the conditions described. This probably represents the bulk of adsorbed Pb. This determination is significant because it represents the first direct measurement of Pb adsorbed onto the surface of a phytoplankton-related species of primary producer growing in waters whose Pb concentrations are typical of the upper mixed layer of the open ocean.

4.8 Reliability of fractionation factors for Valonia macrophysa

Table 4.4 shows that the concentration of total Pb in the growth media of V. macrophysa was about 440 ng Pb/ℓ, or about 25 times greater than the

seawater in which V. ventricosa grew, whereas Ca, Sr, and Ba concentrations were essentially the same in both media. Due to the presence of an unknown amount of EDTA in the culture media of V. macrophysa, the effective concentration of dissolved Pb could not be readily determined without compromising the small media sample. However this figure is estimated to be about 85 ng Pb/ℓ based on an extrapolation from results showing EDTA inhibits Pb uptake by V. macrophysa by 80% when concentrations of both EDTA and Pb are on the order of 2×10^{-5}M (179). The uncertainty in this estimate diminishes the reliability of fractionation factors measured between seawater and V. macrophysa but does not affect fractionations occurring between cell wall and vacuolar sap.

PART II. ANALYSES OF MARINE ANIMALS

4.9 Marine animals analyzed and reporting of results

Rock scallops, kelp snails, abalones, a mussel, and a spiny lobster were analyzed for Ca, Sr, Ba, and Pb. Concentrations of metals and their abundance relative to Ca in organism subfractions are reported in tables 4.5 through 4.9, while tables 4.10 through 4.12 give distributions of Pb in tissues and organs as the percent of total Pb body burden. Table 4.13 contains these data for tuna. Metal concentrations are reported on the basis of fresh weight and may be in error by several percent due to minor dehydration and plasma losses occurring between the time of collection and the weighing of the sample. Animal tissues tend to be less homogeneous than marine plant tissues so attention is focused on metal ratios.

4.10 Interpretation of relative metal abundances in animal tissues

It is believed that the occurrence of Ca in soft tissues is regulated by homeostatic mechanisms which directly govern Ca. These same mechanisms are also thought to indirectly govern Sr, Ba, and Pb by inefficiently excluding these metals during the metabolic cycle of Ca, as discussed in Chapter II. Changes in the Sr/Ca, Ba/Ca, and Pb/Ca ratios in tissues of specimens of a single species collected from the same locality that correlate with changes in animal body mass are interpreted as signifving that the efficiency of exclusion of Sr, Ba, and/or Pb relative to Ca is probably dependent on growth rate. Differences in the Sr/Ca, Ba/Ca, and Pb/Ca ratios between comparable tissues of different species from the same locality are believed to reflect differences in the composition of their respective foods as well as in physiological factors affecting the way each species processes Sr, Ba, and Pb relative to Ca. On the other hand, vari-

Table 4.5. Concentrations of Ca, Sr, Ba, and Pb in the kelp snail Norrisia norrisii.

Size	Sample	Locality	Concentration in µg/g fw of: Ca	Sr	Ba	Pb
34g	Muscle	Abalone Cove	240	3.6	0.027	0.029
	Aragonite				0.83	0.056
	Calcite				0.79	0.095
36g	Muscle	Abalone Cove	195	2.7	0.041	0.026
	Aragonite				0.75	0.048
	Calcite				0.65	0.093
58g	Muscle	Abalone Cove	140	1.9	0.023	0.019
	Aragonite				0.97	0.030
	Calcite				0.64	0.071
32g	Muscle	San Clemente	135	2.4	0.019	0.014
	Aragonite			1350	2.8	0.014
	Calcite			1100	2.0	0.031
31g	Muscle	San Clemente	115	1.8	0.082	0.049
	Aragonite		370000	1400	1.2	0.021*
	Calcite		350000	1000	0.59	0.032
28g	Muscle	San Clemente	nd	6.5	0.019	0.038
	Gonad		1300	14	0.061	0.044
	Digestive gland		710	110	0.58	0.28
34g	Muscle	Cortes Banks	290	4.8	0.016	0.013
	Aragonite				0.88	0.018
	Calcite				1.1	0.043
27g	Muscle	Cortes Banks	280	4.5	0.015	0.008
	Aragonite				0.53	0.021
	Calcite				0.84	0.049

*Sample possibly contaminated by handling

Table 4.6. Concentrations of Ca, Sr, Ba, and Pb in tissues of the abalones Haliotis corrugata and Haliotis cracherodii

Size	Sample / Locality	Concentration in µg/g fw in: Ca	Sr	Ba	Pb
1600g	H. corrugata Cortes Banks				
	muscle	64	0.98	0.003	0.006
	aragonite				0.070
	calcite				nd*
	buccal mass contents	1600	63	4.4	0.60
	heart	640	9.7	0.52	1.35
	kidney	770	11	0.093	0.051
	gonad	150	1.5		0.009
32g	H. corrugata				
	muscle	75	1.4	0.006	0.020
	aragonite				3.0
	calcite				2.8
250g	H. cracherodii Abalone Cove				
	muscle	97	1.3	0.003	0.009
	aragonite		1500		0.078
	calcite		1350	4.9	0.14
	gonad				0.10
	digestive gland				10
275g	H. cracherodii				
	muscle	66	1.1	0.003	0.009

* Outer calcite layer corroded.

Table 4.7. Distribution of Ca, Sr, Ba, and Pb in the mussel Mytilus Californianus (54 g total weight) from Punta Banda, Baja California, Mexico.

	Pb	Ba	Sr	Ca
Muscle	.025	.006	2.89	179
Gonad	.068	.007	3.56	228
Gut wall	.309	.081	1.78	132
Periostracum	.949	3.29	115.9	29700
Shell	.320	-	-	360000
Gut Conts.	.635	2.39	9.45	960
Mantle	.042	.078	3.73	240
Cross-contaminated muscle	.056	.008	2.18	-

Table 4.8. Concentrations of Ca, Sr, Ba, and Pb in tissues of the purple-hinged rock scallop Hinnites multirugosa

Sample	Locality	Concentration in µg/g fw of: Ca	Sr	Ba	Pb
Muscle tissue	Cortes Banks	160	1.8	0.006	0.004
Shell[1]	" "	nd	nd	1.15	0.145
Digestive gland	" "	710	13	2.4	1.2
Gonad	" "	290	5.0	0.067	0.023
Muscle tissue	Abalone Cove	340	2.9	0.008	0.012
Shell[2]	" "	nd	nd	1.15	0.45
Digestive gland	" "	1100	15	11	9.3
Stomach walls	" "	690	7.5	0.069	0.16

[1]The gross fresh weight of the specimen collected at Cortes Banks was 433g.
[2]The gross fresh weight of the specimen collected at Abalove Cove was 368g.

Table 4.9. Concentrations of Ca, Sr, Ba, and Pb in tissues of the spiny lobster Panulirus interruptus

Sample	Locality	Concentration in µg/g fw of: Ca	Sr	Ba	Pb
Muscle tissue	Cortes Banks	91	0.98	0.008	0.005
Carapace	" "	nd	nd	nd	0.075
Digestive gland	" "	260	5.6	0.078	0.038
Gonad	" "	nd	nd	0.030	0.079

The gross fresh weight of this specimen was 1600 g.

ations in metal ratios between comparably-sized individuals of the same species which come from different localities are believed to reflect environmental variations in the abundances of Sr, Ba, and Pb relative to Ca between the collection sites.

4.11 Determination of true metal distributions in organisms

The results of Ca and Sr analyses of both hard and soft tissues of the animals studied are in good agreement with published analyses of these metals in tissues of similar invertebrates (17, 53, 54, 137, 184, 249). Pb and Ba concentrations reported here are typically orders of magnitude lower than literature values.

Pb and Ba in soft tissues of the animals studied range from several to tens of nanograms per gram fresh weight, being lowest in muscle tissue and highest in visceral organs. In hard tissues Pb is on the order of tens to hundreds of nanograms per gram fw, while Ba is generally several hundreds of nanograms per gram. These levels are far below the commonly accepted range of Pb and Ba concentrations in skeletal material of marine animals. However, it is highly improbable that typical Ba and Pb concentrations in these tissues ever appreciably exceed those reported here because many of the samples of this study were collected from one of the most metal-polluted coastal regions in the world (114, 192). The results of this study therefore provide further documentation to support the conclusions of other investigators who believe that most published data on the occurrence of Pb, Ba, and other ultra-trace metals in marine ecosystem reservoirs are in positive error by orders of magnitude (44, 45, 170, 174).

4.12 Distribution of metals between tissue reservoirs in animals

Only those metabolized metals incorporated in biochemical reservoirs of animals were considered when calculating the internal distributions of Ca, Sr, Ba, and Pb shown in tables 4.10 through 4.12. The common convention of reporting data as bulk concentrations in "total soft parts", viscera, etc., produces deceptively high metal concentrations through the inadvertent inclusion of metals contained in food residues, excretory products, and pathogenic precipitates (12, 33, 65). Moreover, true metal distributions in total organism are often obscured by neglecting to include those metals localized in skeletal hard parts, where the overwhelming bulk of alkaline earths and Pb are usually found. Tables 4.10 and 4.11 illustrate conflicting interpretations occasioned by alternate methods of calculating the distribution of Pb among the various tissues and organs as a percent of total body burden.

4.13 Factors affecting observed metal distributions in marine animals

The data of tables 4.10 through 4.12 show that for the animals studied the skeleton is the principal reservoir for Ca, Sr, Ba, and Pb. Typically more than 95% of total Ca and Sr and 80% of total Ba and Pb are localized in the exoskeleton. As seen in table 4.13, a similar fraction of total metals resides in the skeleton of marine vertebrates. These findings have three implications: first, occurrences of Ca, Sr, Ba, and Pb in tissues of marine animals tend to be covariant; second, the Sr/Ca, Ba/Ca, and Pb/Ca ratios of the skeleton accurately characterize the total reservoir of Ca in the animals studied; and third, physiological and environmental factors affecting the abundances of Sr, Ba, and Pb relative to Ca in skeletal material affect the bulk of biochemically incorporated alkaline earths and Pb. This knowledge may have valuable implications. For example, temporal changes in parameters

Table 4.10. Distribution of Pb in the mussel Mytilus Californianus (54 g total wt) from Punta Banda, Baja California, Mexico.

Tissue	% Body	Pb conc. μg/g fw	% Pb Body Burden[1]	% Pb Body Burden[2]	% Pb Body Burden[3]	% Pb Body Burden[4]
Shell (calcitic)	63	.32	74.6	85.5	-	-
Periostracum	1	.95	3.5	4.0	-	-
Muscle	4	.025	0.4	0.4	3.8	1.8
Mantle	12	.042	1.9	2.0	21.5	8.5
Gills	2	.01*	0.1	0.4	1.3	0.6
Gonad	5.5	.068	1.4	1.6	15.2	6.1
Kidney	3	.05**	0.6	0.8	6.3	2.4
Gut walls	4	.31***	4.6	5.2	51.9	20.7
Gut conts	5.5	.64	12.9	-	-	59.8
[Total Pb conc. (μg/g fw)]			[0.270]	[0.249]	[0.079]	[0.164]

*teleost fish gills ≃ mussel gills (table 4.13)

**abalone kidney ≃ mussel kidney

***value believed elevated as a result of cross-contamination by gut contents

1) Including gut contents and shell

2) In tissues only

3) In total soft parts excluding gut contents

4) In total soft parts including gut contents

Table 4.11. Distribution of lead in tissues of the rock scallop Hinnites multirugosa (433 g total wt) from Cortes Banks.

Tissue	% Body	Pb conc. μg/g fw	% Pb Body Burden[1]	% Pb Body Burden[2]	% Pb Body Burden[3]	% Pb Body Burden[4]
Shell	75	0.14	77	97	-	-
Muscle	11	0.004	0.3	0.4	11	1.4
Gonad	5.1	0.023	0.9	1.1	31	3.8
Mantle	5.6	0.04*	1.3	1.7	48	5.9
Gut contents	2.3	1.2	20	-	-	88
Gills	1.6	0.01*	0.2	0.2	4	0.2
Gut wall	0.2	0.6	0.1	0.1	3	0.4
Kidney	0.2	0.05**	0.1	0.1	3	0.4

[Total Pb conc. (μg/g fresh wt)]···[0.14]····[0.11]·····[0.017]··[0.13]

*est. from bony fish data [2]; mantle ≈ epidermal mucus; gills ≈ epithelial gill tissue.

**est. from abalone from same location [1].

1) Including gut contents and shell

2) In tissues only

3) In total soft parts, excluding gut contents

4) In total soft parts, including gut contents

Table 4.12. Distribution of Ca, Sr, Ba, and Pb in spiny lobster Panulirus interruptus (1685 g total wt) from Cortes Banks.

Organ	% Body[1]	Concentration in μg/g of Ca	Sr	Ba	Pb	Ca	Sr	Ba	Pb
						% body burden incl. gut contents and shell			
Exoskeleton	43.0	246000[2]	3150[2]	2.0[3,4]	0.075	99.9	99.9	99	86.5
Muscle (incl. blood)	50.5	91	0.98	0.008	0.005	0.0	0.0	0.5	8.1
Digestive gland (incl. gut conts.)	4.6	259	5.5	0.078	0.038	0.0	0.0	0.5	5.4
Gills	1.4	1000	4.0	0.050	0.004	0.0	0.0	0.0	0.0
Reproductive system (incl. excretory orgs.)	0.5	300[4]	6.0[4]	0.030	0.079	0.0	0.0	0.0	0.0

1) Value from Bryan and Ward (29)

2) Value from Martin (145)

3) Value estimated from Patterson and Settle (170)

4) Value estimated from scallop data (this work)

Table 4.13. Distribution of lead in organs in tuna (Thunnus alalunga)[1]

Tissue	wt% H_2O	Organ % of body	Pb ($\mu g/g$ fw)	% Pb body Burden
Bone (vertebra)	29	4.6	0.074	56
Scales	25	1.5	0.059	15
Teeth	3	0.005	0.24	0.2
Total skeleton	28	6.1	0.070	70
Muscle	71	73	0.0003	3.6
Liver	64	1.4	0.009	2.1
Spleen	75	0.06	0.019*	0.2
Kidney	75	0.55	0.006	0.5
Caecum wall	65	1.2	0.004	0.8
Gills (epithelium & lamellae)	73	0.5	0.014	1.1
Dermis	44	1.6	0.013	3.4
Epidermal mucus	80	0.03	0.035	0.2
Stomach contents (anchovy)	58	2.1	0.021	7.2
Remainder	-	13	0.005†	10
Whole fish	-	100	0.006	100

*now believed too high - reinvestigation incomplete

†assumed

1) Data of Patterson and Settle (170).

controlling distributions of Pb and Ba in shell material should be preserved in sequential growth layers of shells. Shell material should also record temporal changes in the isotopic composition of soluble Pb in seawater.

Tables 4.5 through 4.9 show that Sr/Ca, Ba/Ca, and Pb/Ca ratios in skeletal material depend on species, size, mineralogy, ambient concentrations of Pb in seawater, and metal concentrations in food. This is true even though the Ca reservoir of shell material is highly biopurified of Sr, Ba, and Pb with respect to food because of physiological and mineralogical factors controlling shell formation (53, 54, 107, 111, 120-123, 188, 189, 236). For example, the Pb/Ca ratio of food particles in scallop digestive gland given in table 4.7 is a minimum 35 times higher than this ratio in kelp food of abalones and kelp snails, as shown in table 4.1. This 35-fold difference in Pb/Ca ratio of food sources correlates with an average 10-fold difference between the Pb/Ca ratio of scallop shell and Pb/Ca ratio of shells of gastropod grazers, which may be seen by comparing the data of table 4.7 with that reported in tables 4.5 and 4.6.

Data of table 4.5 further show that among kelp snails from a single locality there is an inverse correlation between organism mass and Pb concentration in both the outer calcite and inner aragonite shell layers. This observation is supported by a similar relationship between Pb concentrations in the calcite layers of a juvenile and a mature specimen of H. corrugata from Cortes Banks, seen in table 4.6. It should also be noted that there is a marked tendency for Pb concentrations to be higher in the outer calcite layers of kelp snail and abalone shells, while Sr and Ba appear to favor the inner aragonite layer.

Comparison of comparably-sized kelp snails from Cortes Banks and San Clemente Island with specimens from Abalone Cove shows that snails from outer Bight locations have lower Pb concentrations in both shell layers than do the inner Bight specimens. These variations correlate positively with ambient concentrations of Pb in seawater at these sites (178, 193).

Changes in the Pb/Ca ratio of scallop food and of kelp snail food between inner and outer Bight locations, which correlate with respective concentrations of Pb in seawater, also correlate with changes in the Pb/Ca ratio in muscle tissue of scallop and kelp snail between these areas. However, the minimum 35-fold difference between the Pb/Ca ratios of scallop and kelp snail foods is not reflected in the Pb/Ca ratios of their muscle tissue. The Pb/Ca ratio of scallop muscle is quite similar to the Pb/Ca ratio of kelp snail as a comparison of the data of table 4.8 with that of table 4.5 clearly shows.

Sr/Ca, Ba/Ca, and Pb/Ca ratios of Cortes Banks spiny lobster tissues given in table 4.9 correspond closely to the same ratios in comparable tissues from Cortes Banks molluscs. This suggests that Sr, Ba, and Pb are processed in much the same way in crustaceans as in molluscs. Mineralogical differences between the phosphatic carapace of spiny lobsters (153) and the carbonate shell layersof molluscs apparently do not strongly influence Pb concentrations in exoskeleton material. This suggests that physiological factors may exert primary control over Pb concentrations in skeletal substances. Further clarification on this point is clearly needed.

Chapter V

BIOENRICHMENT AND BIOPURIFICATION IN THE MARINE ALGA *VALONIA*

5.1 Modes of metal accumulation in Valonia

The data of table 5.1, which are illustrated by figure 5.1, represent abundances relative to Ca of Sr, Ba, and Pb dissolved in seawater media, bound in *Valonia* cell wall, and contained within the central vacuole of *Valonia*. These distributions are given for two distinct systems which differ from one another by the concentration of dissolved Pb in the media and by the particular species of *Valonia* studied.

Figure 5.1 illustrates the effect of bioenrichment on the cell surface that characterizes the uptake from seawater of Sr, Ba, and Pb relative to Ca. Also illustrated in figure 5.1 is the effect of biopurification of Ca with respect to Sr, Ba, and Pb that results from the active transport of Ca into cell interiors. It should be noted that abundances of Sr, Ba, and Pb relative to Ca change dramatically in going from seawater to cell wall but that the pattern of relative abundances which characterizes cell wall persists after metals are transferred to vacuolar sap. These observations are consistent with a two-stage transport mechanism consisting of passive adsorption of metals from seawater onto cell surfaces followed by absorption of metals into cell interiors, such as has been proposed by other investigators studying the uptake of Cu (141), Zn (85), Cd (47), and Pb (199) by unicellular marine algae including *Valonia* (179).

5.1.1 Bioenrichment effects associated with passive adsorption processes

The bulk of Ca in *Valonia* cell wall is believed to be complexed with

Table 5.1. The effect of Pb pollution on the system: seawater → *Valonia*

Reservoir	Atomic ratios		
	Sr/Ca	Ba/Ca	Pb/Ca
Lower pollution system			
Seawater	9.1×10^{-3}	4.3×10^{-6}	7.5×10^{-9}
bioenrichment factor	0.33	26	1500
Valonia ventricosa cell wall	3.0×10^{-3}	1.1×10^{-4}	1.1×10^{-5}
biopurification factor	0.71	1.8	3.4
Valonia ventricosa vacuolar sap	4.2×10^{-3}	6.0×10^{-5}	3.2×10^{-6}
Higher pollution system			
Seawater	9.1×10^{-3}	5.0×10^{-6}	4.0×10^{-8}
bioenrichment factor	0.56	84	650
Valonia macrophysa cell wall	5.1×10^{-3}	4.2×10^{-4}	2.6×10^{-5}
biopurification factor	1	4.2	6.3
Valonia macrophysa vacuolar sap	5.1×10^{-3}	9.9×10^{-5}	4.1×10^{-6}

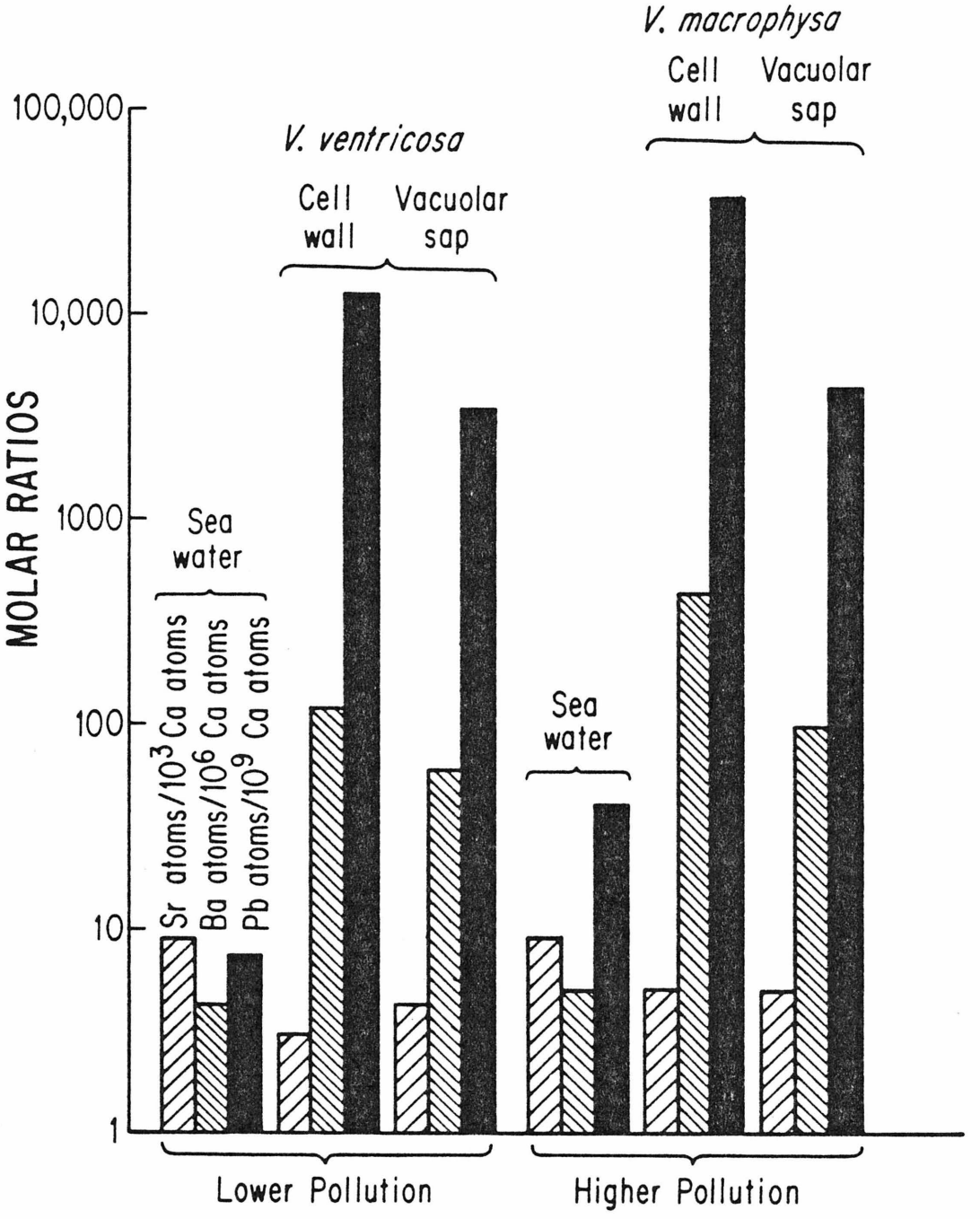

Figure 5.1. Bioenrichment and biopurification effects in Valonia under conditions of different metal concentrations in growth media. Relative bioenrichments in cell wall occurring simultaneously with biopurification of Ca transferred from walls to cell interiors correlate with the cation selectivity of the extracellular polysaccharide pectin. Increases to concentrations of metals in growth media produce proportionate increases relative to Ca in subfractions of Valonia. Note the log scale and the scale factors for the individual ratios measured.

Metal	mequiv. H+ liberated from pectic acid	$\left[\frac{\text{metal}}{\text{Ca}}\right]_{\text{Val}} \div \left[\frac{\text{metal}}{\text{Ca}}\right]_{\text{SW}}$		
		adsorbed metals	cell wall	vacuolar sap
Pb	3.1	720	1500	430
Ba	0.63	5.4	26	14
Sr	0.52	0.53	0.33	0.46
Ca	0.55	1	1	1

Table 5.2 Meq H^+ liberated per ml by addition of metal salts (0.02 meq (0.02 meq/ml) to solutions containing 0.026-0.028 meq half-neutralized polyanion (pectin) per ml are given by the numbers in the second column (94). Increased liberation of H^+ indicates greater affinity of the pectin for the cation. The equimolar conditions for these data are different from field conditions. These second column numbers show that the stabilities of the metal pectates increase in a series Ca>Sr<Ba<<Pb. Numbers in the third column are metals released by a brief 0.1 N HCl leaching experiment, while numbers in the fourth column represent metals in Valonia cell wall. Numbers in the fifth column are for vacuolar sap. Numbers for Valonia subfractions have been normalized to Ca because in the field, pectates in Valonia are predominantly Ca-pectates (56,90); metals in vacuolar sap appear to be derived from metals adsorbed onto the cell wall (table 4.3 and text). The relationship between these numbers which show a similar pattern of depletion of Sr but increasing enrichment of Ba and Pb relative to Ca in going from seawater to Valonia subfractions parallels exactly the relationship of numbers in the second column. Valonia data are compiled from table 4.3.

pectin (56, 248), the major anionic polysaccharide in cell walls of most species of green algae as well as Valonia (71, 90, 93, 248). Compared to seawater, abundances of Sr, Ba, and Pb relative to Ca change markedly when these metals are passively adsorbed onto Valonia cell walls. However, selectivity of cell wall binding agents among cations, indicated by the bioenrichment factors for cell wall given in table 5.1, are similar for both Valonia species and correspond exactly with the sequence of affinities of pectin for Ca, Sr, Ba, and Pb, shown in table 5.2. The stereochemistry of pectin is such that Sr is discriminated against whereas Ba and Pb are preferentially complexed (90). These relationships correlate with observed fractionations occurring between media and cell wall. Using Valonia ventricosa as an example, in going from seawater to cell wall the Sr/Ca, Ba/Ca, and Pb/Ca ratios change by factors of 0.5, 26, and 1500 respectively.

5.1.2 Biopurification effects associated with active transport processes

As seen in table 5.1 and figure 5.1, the change in abundances of Sr, Ba, and Pb relative to Ca in going from seawater to vacuolar sap is similar in kind but generally lesser in degree to that observed in going from seawater to cell wall and corresponds to the sequence of selectivity among cations exhibited by the cell wall polysaccharide, pectin, shown in table 5.2. Thus the source of intracellular metals, such as are contained in vacuolar sap, is most probably the reservoir of metals adsorbed onto cell walls. Otherwise abundances of Sr, Ba, and Pb relative to Ca in vacuolar sap would be similar to their relative abundances in seawater in the same manner that relative abundances of Ca, Sr, Ba, and Pb in soil moisture are seen in table 2.2 to directly correlate with their abundance in terrestrial plants (59, 146, 164, 166, 168).

5.1.3 Source of metabolized metals in *Valonia*

In contrast to the generally large fractionations resulting from passive adsorption of metals from seawater onto cell surfaces, in going from cell wall to vacuolar sap the Sr/Ca ratio is unchanged, the Ba/Ca ratio decreases by a factor of 3, and the Pb/Ca ratio decreases by a factor of 5. These biopurification factors, which are averaged for the two *Valonia* species listed in table 5.1, correlate with the sequence of discriminations against Sr, Ba, and Pb in favor of Ca observed to result from active transfer in all biological systems thus far studied. It is interesting to note that the degree to which Ca is biopurified with respect to Sr, Ba, and Pb during active transport into cell interiors is inversely proportional to the degree of bioenrichment of Sr, Ba, and Pb relative to Ca resulting from passive adsorption. However, because the magnitude of bioenrichment factors for Ba and Pb are much greater than corresponding biopurification factors, compared to seawater abundances overall bioenrichment of the Ba/Ca and Pb/Ca ratios characterizes the total Ca reservoir of *Valonia*.

5.2 Factors affecting metal distributions in algal cells

For purposes of phytoplankton modelling it is necessary to compensate for changes in the distribution of metals between cellular subfractions occurring as a function of cell size. For example, although some 96% of total metals in mature *Valonia* are contained within the vacuolar sap, as cell size decreases the ratio of vacuolar volume to the combined volumes of cell wall and underlying protoplasm also decreases such that in the youngest cells, which have no vacuole, it approaches zero (56). As a consequence, the proportion of extracellular metal-complexing sites relative to intracellular binding sites increases greatly when cell size decreases because of the large increase in surface area per unit mass.

This effect is graphically demonstrated later in Chapter VII by figure 7.1.

It may be inferred that, unlike Valonia, by far the largest fraction of total Ca, Sr, Ba, and Pb in most marine primary producers will be extracellularly rather than intracellularly localized. Carbonate-secreting algae are of course an obvious exception.

This suggests that, while the Pb/Ca ratio of oceanic phytoplankton may be expected to vary over a narrow range because of three-fold variations in the concentration of dissolved Pb in surface waters (193) and small differences in Pb/Ca bioenrichment factors associated with mucilage polysaccharides of algae (cf. tables 5.2 and 6.2), Pb concentrations in bulk phytoplankton may vary by more than an order of magnitude as a result of large differences in surface area per unit mass between 10-μm nannoalgal cells of the open ocean and 300-μm cells found in zones of high productivity.

5.3 Effect on Valonia of variable metal concentrations in growth media

Higher concentrations of Ca, Sr, and Ba reported in table 4.4 for Valonia macrophysa than those reported in table 4.3 for V. ventricosa probably relate to biochemical differences between the species as well as compositional differences between their media, which differ chiefly by total Pb concentration. Comparison of metal ratios in media and cellular fractions for the two systems shown in figure 5.1 shows that a slight increase in the Ba/Ca ratio and a large increase in the Pb/Ca ratio in the media of the higher pollution system produces proportional increases in these ratios in subfractions of Valonia compared to levels observed in the lower pollution system. This effect should be expected if the metal composition of bulk algae is determined primarily by the relative affinity

of extracellular algal polysaccharides for dissolved metals in seawater as the data of this and numerous other studies indicate.

The Pb/Ca ratio of Valonia and of physiologically similar primary producers should be directly proportional to the Pb/Ca ratio of seawater up to the point at which the adsorbent capacity of algal surfaces becomes saturated. Experiments with the phytoplankter Phaeodactylum indicate that this point is not reached until Pb concentrations in the growth media are several orders of magnitude greater than typical seawater concentrations of 15 ng Pb/ℓ (223). Assuming the species composition of plankton remains relatively constant, Pb/Ca ratios of bulk plankton should correlate with concentrations of dissolved Pb in the local environment.

CHAPTER VI

EFFECTS OF TRANSFERS OF Ca, Sr, Ba, AND Pb WITHIN BENTHIC FOOD CHAINS:

I. BIOENRICHMENT AND BIOPURIFICATION IN GRAZER FOOD CHAINS
II. BIOENRICHMENT AND BIOPURIFICATION IN FILTER-FEEDER FOOD CHAINS
III. EFFECTS OF DIFFERENT DEGREES OF Pb-POLLUTION ON FOOD CHAINS
IV. OBSERVATIONS ON THE CHEMISTRY OF Pb IN MOLLUSC SHELLS

PART I. BIOENRICHMENT AND BIOPURIFICATION IN GRAZER FOOD CHAINS

6.1 Benthic grazer food chains studied

Changes in the abundances of Sr, Ba, and Pb relative to Ca in major Ca reservoirs of organisms of different trophic levels were studied in two benthic grazer food chains. One food chain consisted of seawater, kelp, and kelp snail; the other consisted of seawater, kelp, and abalone. Both kelp snails and abalones are known to utilize kelp as their near-exclusive food source (2, 28, 48, 115, 131, 132, 217). Relative metal distributions in the kelp Macrocystis, kelp snails, and black abalone from Abalone Cove were compared with distributions in the kelp Eisenia, kelp snails, and pink abalone from Cortes Banks and with distributions in Macrocystis and kelp snails from San Clemente Island. The relationship of metals in the tissues of the omnivorous spiny lobster to metals in particles contained in its digestive gland is also discussed.

Table 6.1. Bioenrichments and biodepletions in benthic grazer food chains.

Sample	Location	Atomic ratios Sr/Ca	Ba/Ca	Pb/Ca	
Seawater[1]	Cortes Banks	9.1×10^{-3}	4.3×10^{-6}	1.2×10^{-8}	
Bioenrichment factor		3	44	280	
Kelp[2]		2.9×10^{-2}	1.9×10^{-4}	3.3×10^{-6}	
Biodepletion factor		19	240	190	(61)
Kelp snail[3] (abalone)		1.5×10^{-3}	7.8×10^{-7}	1.7×10^{-8}	(5.4×10^{-8})
Seawater[1]	San Clemente Is.	9.1×10^{-3}	4.3×10^{-6}	1.2×10^{-8}	
Bioenrichment factor		4	33	220	
Kelp[2]		3.2×10^{-2}	1.4×10^{-4}	2.6×10^{-6}	
Biodepletion factor		21	200	190	
Kelp snail[3]		1.5×10^{-3}	7.1×10^{-7}	1.4×10^{-8}	
Seawater[1]	Abalone Cove	9.1×10^{-3}	4.3×10^{-6}	2.4×10^{-8}	
Bioenrichment factor		5	74	580	
Kelp[2]		4.4×10^{-2}	3.2×10^{-4}	1.4×10^{-5}	
Biodepletion factor		29	560	340	(220)
Kelp snail[3] (abalone)		1.5×10^{-3}	5.7×10^{-7}	4.1×10^{-8}	(6.4×10^{-8})

Bioenrichment factors for kelp are calculated relative to seawater. Biodepletion factors for kelp snail (abalone) are calculated relative to kelp. Figures in parentheses are for abalone.

1) Data for Ca and Sr from Goldberg (77). Ba data from Chow (38). Dissolved Pb is temporally variable but is estimated to average about 25 ng Pb/ℓ at Cortes Banks and San Clemente Is. but 50 ng Pb/ℓ at Abalone Cove (172).
2) Kelp values are recalculated from table
3) Kelp snail and abalone values are recalculated from tables

6.2 Mode of accumulation of metals by kelp

6.2.1 Comparative metal distributions in kelp and seawater

The uptake of Ca, Sr, Ba, and Pb from seawater by brown algae is characterized by higher concentrations of these metals in fresh kelp than are found in their seawater media, as shown earlier by table 4.1, and by progressively greater bioenrichments of the Sr/Ca, Ba/Ca, and Pb/Ca ratios in total kelp relative to seawater ratios, shown here by the bioenrichment factors in table 6.1.

6.2.2 Observed bioenrichment effects in kelp species

It was noted in table 4.1 that concentrations of Ca, Sr, Ba, and Pb in Eisenia average about 2.5 times higher than concentrations of these metals in Macrocystis. Table 6.1 shows that when concentration data are normalized on the basis of Ca, metal ratios are similar in both species of kelp. Bioenrichment factors given in table 6.1 show that the Sr/Ca ratio in kelp is four-fold higher than in seawater, that the Ba/Ca ratio increases by a factor of 30, and that the Pb/Ca ratio increases by about 750. The affinity of total kelp for Sr, Ba, and Pb relative to Ca is indicated by the magnitude of bioenrichment factors. The sequence of cation selectivity in kelp, $k_{Pb/Ca} >> k_{Ba/Ca} > k_{Sr/Ca} > 1$, corresponds closely to the strengths with which these metals are complexed by algin, listed in table 6.2.

6.2.3 Relation of bioenrichment effects to passive adsorption processes

There is a substantial body of evidence which indicates that the bulk of Ca, Sr, Ba, and Pb is present in brown algae as the result of cation-exchange between seawater and kelp. The occurrence of Ca, Sr, Ba, and Pb at much greater concentrations in kelp than in seawater suggests these metals are present predominantly as bound rather than ionic forms.

Metal	Mequiv. H^+ liberated from M	G	$\left[\frac{\text{metal}}{\text{Ca}}\right]_{Mp} \div \left[\frac{\text{metal}}{\text{Ca}}\right]_{SW}$	% metal liberated by brief 0.1N HCl leach
Pb	2.8	2.4	470	83%
Ba	0.66	1.00	56	91%
Sr	0.53	0.84	38	95%
Ca	0.48	0.43	1	96%

Table 6.2 Meq H^+ liberated per ml by addition of metal salts (0.02 meq/ml) to solutions containing 0.026-0.028 meq half-neutralized poly-mannuronic acid (M) and poly-guluronic acid (G) are given in columns two and three respectively (88,94). Increased liberation of H^+ indicates greater affinity of the poly-anion for the cation. The mucilage of Macrocystis pyrifera is algin which contains about 70% mannuronic acid and 30% guluronic acid (200). The former forms stronger Pb complexes but weaker alkaline earth complexes than the latter. All brown algae thus far examined contain algin as the dominant anionic polysaccharide in their extracellular mucilage, but the ratio of mannuronic to guluronic acid varies with species (88-92, 94,95,177,211). These second and third column numbers show that the stabilities of the metal alginates increase in a series Ca<Sr<Ba<<Pb. The equimolar conditions for these data are different from field conditions. The numbers in the fourth column are normalized to Ca because in the field alginates in M. pyrifera occur predominantly as Ca-alginates (95). The relationship among these numbers, which show an increasing bioenrichment in the series Ca<Sr<Ba<<Pb parallels exactly the relationship among numbers of the second and the third columns. The numbers in the fifth column show that the fraction of metals released by a two-minute leaching with dilute mineral acid is in inverse proportion to the binding affinity for algin, indicated by columns two, three, and four. Data for Macrocystis in columns four and five come from tables 4.1 and 4.2. They vary in detail from sample to sample.

Brown algae generally contain large amounts of extracellular anionic polysaccharides which bind Ca, Sr, Ba, Pb, and other metals (89, 90). The major anionic polymer in brown algae, algin, may constitute up to 40% of the dry weight of the plant (177). The presence of algin in the mucilage of kelps gives these plants a binding capacity of about 0.5 meq/g fw which is much greater than the total meq/g fw of Ca and other divalent metals found in kelp, thereby making it probable that a large fraction of total Ca, Sr, Ba, and Pb in kelp may be bound to this matrix material (90, 151, 206). This inference is confirmed by studies in which cationic dyes and polymer-specific staining tests were used to show that the bulk of Ca in kelp is localized in the extracellular mucilage binding the cells together, is coincident with the localization of algin, and is rapidly exchangeable with other divalent cations (6).

Studies have shown that algin in kelp behaves like a polystyrene-type cation-exchange resin immersed in a dilute solution of metal salts. Metals are bound in the plant according to the preferred sequence shown in table 6.2 and may be exchanged with other metals or H^+ according to the same rapid, reversible, and stoichiometric relationships that characterize exchange reactions in isolated alginates (3, 6, 32, 95, 151, 200, 206, 232, 233). The results of a two-minute 0.1N HCl leach of Macrocystis, which confirm that the bulk of Ca, Sr, Ba, and Pb in this kelp are readily exchangeable with H^+, are compared in table 6.2 with the series of relative binding strengths of algin to show that greater retention of metals by leached kelp correlates positively with binding strengths of algin.

The cation-exchange equilibrium process that appears to control the occurrence of alkaline earths and Pb in kelp is different from other

Table 6.3. Relative metal distributions among tissues of different ages collected from the same Abalone Cove Macrocystis plant

Sample	Atomic ratios		
	Sr/Ca	Ba/Ca	Pb/Ca
Apical blade (juvenile)	3.9×10^{-2}	3.0×10^{-4}	4.0×10^{-6}
Canopy blade (mature)	3.6×10^{-2}	3.0×10^{-4}	5.9×10^{-6}
Basal blade (senescent)	3.4×10^{-2}	2.4×10^{-4}	5.3×10^{-6}

mechanisms of metal sorption in macroalgae. Radiotracer experiments have shown that ^{65}Zn is also passively complexed by algin but progressively accumulates with time in the cell interior as a result of irreversible membrane transport (24, 206). These ionic sorption processes are quite different from the mechanism of particle sorption believed to control the uptake of such fission nuclides as ^{210}Po, ^{239}Pu, and ^{241}Am (72, 110, 280). The reason for this difference is not yet clear but is probably related to differences in seawater speciation (79, 90).

6.2.4 Effect of age on metal distributions in Macrocystis

It was noted in table 4.1 that metal concentrations in blades of different developmental stage but from the same Macrocystis plant do not correlate with age. Table 6.3 shows that normalizing Sr, Ba, and Pb abundances on the basis of Ca results in similar ratios in plant tissues of different age. This indicates that, unlike the case of Zn, the overall sorption of Ca, Sr, Ba, and Pb by brown algae is a time-independent process (24, 95, 206, 233, 239).

6.2.5 Biopurification effects in kelp

Biopurification effects are difficult to study in brown algae because algin typically forms such a large fraction (20-40%) of the organic matter of kelp (155, 177) that passive adsorption effects obscure the physiological mechanisms for processing metals that operate within the microscopic cells. This is probably the reason that neither mortality nor variations in light, oxygen, nutrients, or photosynthetic activity appreciably affect the uptake of alkaline earths and Pb by brown algae (182, 206, 232, 233). By analogy with processes operative in Valonia,

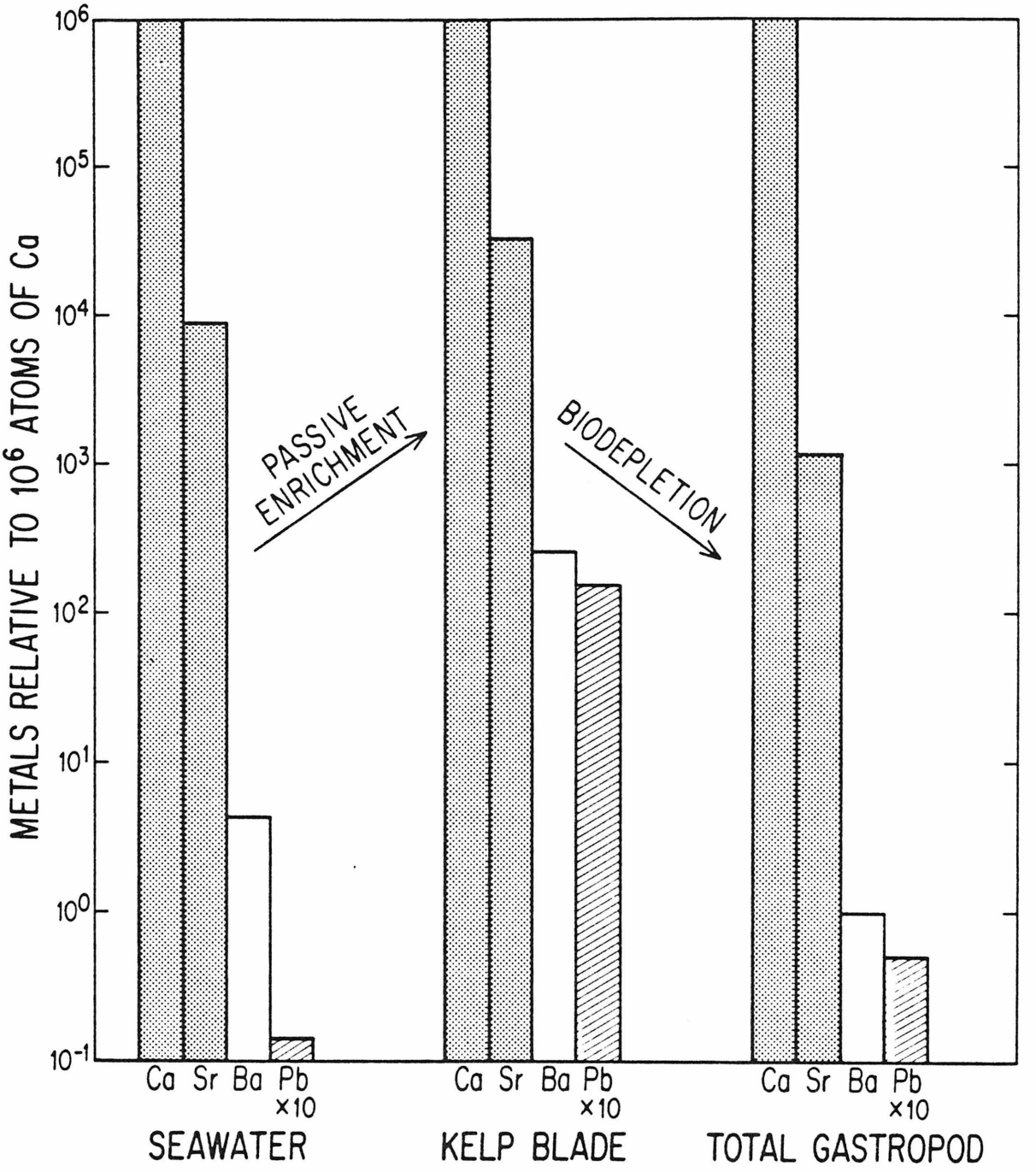

Figure 6.1. Bioenrichment of Sr, Ba, and Pb relative to Ca in total kelp blade followed by biopurification in total kelp snail Norrisia. Relative degree of bioenrichment at the plant level, i.e., Pb>>Ba>Sr>Ca, corresponds exactly to the sequence of cation selectivity of extracellular polysaccharides known to bind the bulk of Ca, Sr, Ba, and Pb in brown algae. Biopurification of Ca in the transfer of metals from plant to consumer gastropod is caused by active membrane processes tending to biopurify Ca of Sr, Ba, and Pb to progressively greater degrees. Note the log scale.

it seems probable that relative metal abundances in the minor intracellular Ca reservoir of kelp are determined primarily by their relative abundances in the source reservoir of passively adsorbed metals and only to a lesser extent by membrane processes tending to biopurify Ca of Sr, Ba, and Pb.

6.3 Biopurification effects within benthic grazer food chains

6.3.1 Source of metals in benthic grazing molluscs

Radiotracer experiments show that food rather than seawater is the immediate source of about 90% or more of metabolized heavy metals in biochemical metal reservoirs of molluscs and crustaceans (1, 10, 11, 18, 75, 83, 84, 98, 116, 139, 147, 176, 209, 231). This may not be true for all marine animals. Scleratinian corals, for example, appear to obtain Ca and Sr directly from seawater. This is indicated by a lack of fractionation of Sr relative to Ca between seawater and coral skeleton which does not correlate with the Sr/Ca ratio of coral food which is lower than that of seawater (80). A significant fraction of Ca, and possibly Sr, absorbed by molluscs may also come from seawater ingested while feeding (11, 153). Studies on metal metabolism by invertebrates indicate that Ca from ingested seawater and the progressively greater fractions of total absorbed Ca, Sr, Ba, and Pb derived from food are transported across walls of the digestive system (11, 46, 133, 154, 164, 204, 234), and only small amounts of Ca, Sr, Ba, and Pb are absorbed across epidermal and gill membranes (11, 42, 147, 153, 175).

6.3.2 Observed biopurification effects

It may be seen from table 6.1 and figure 6.1 that in the transfer of Ca, Sr, Ba, and Pb from kelp to total gastropod, Ca is biopurified with respect to Sr, Ba, and Pb. The degree of biopurification is indicated

by the magnitude of the Sr/Ca, Ba/Ca, and Pb/Ca biopurification factors listed in table 6.1. In total Cortes Banks kelp snail, the biopurification factor for Sr/Ca is 19, but is 240 for Ba/Ca and 190 for Pb/Ca. The close correspondance between the Ba/Ca and the Pb/Ca biopurification factors for total kelp snail shown in table 6.1 indicates that these molluscs process Ba and Pb in much the same manner.

The Pb/Ca biopurification factor for total kelp snail is more than three times greater than that for abalone collected simultaneously with kelp snails at Cortes Banks. Table 6.1 shows that this biopurification factor is 50% greater for kelp snails than for abalone when animals collected simultaneously at Abalone Cove are compared. The species difference in Pb/Ca biopurification factors probably represents a physiological difference in the efficiency with which the two species process Pb relative to Ca rather than a difference in the Pb/Ca ratio of their foods because at the time of collection both kelp snails and abalone from Abalone Cove were observed to be feeding on Macrocystis, their preferred food source (48, 115, 131, 132). Moreover, the composition of kelp particles in the buccal mass of the Cortes Banks abalone, reported in table 4.6, is in good agreement with analyses of Eisenia (table 4.1) upon which Cortes Banks kelp snails were feeding at the time of collection.

It appears that physiological exclusion mechanisms tending to biopurify Ca of Pb operate with increasing efficiency over the life of both kelp snails and abalone. There is a systematic decrease in the Pb/Ca ratio in individual calcite and aragonite shell layers of gastropods which positively correlates with increasing organism size, as is apparent from the data of tables 4.5 and 4.6. This phenomenon is independent of variations in ambient concentration of dissolved Pb between collection sites.

6.3.3 Factors affecting biopurification in grazer food chains

It is probable that the Sr/Ca, Ba/Ca, and Pb/Ca biopurification factors for total gastropod relative to food are the product of sequential fractionations because numerous processes affect the uptake of these metals from food and their deposition in the shell. On the basis of the present data these factors are difficult to isolate and evaluate separately. Some factors influencing biopurification during uptake are discussed now, while treatment of factors affecting relative metal distributions during shell mineralization is deferred until Part IV of this chapter.

It is likely that marine gastropods swallow inadvertently seawater during feeding and may deliberately do so in order to maintain their osmotic balance (11). Compared to metal concentrations in food, seawater is high in Ca but very low in Sr, Ba, and most especially Pb. This may be seen by comparing the Sr/Ca, Ba/Ca, and Pb/Ca ratios in seawater with those ratios in food given in table 6.1. The addition of Ca from seawater tends to purify the reservoir of available Ca by diluting the Sr/Ca, Ba/Ca, and Pb/Ca ratios of available metals in food. This dilution process affects metal ratios to approximately the same degree and occurs prior to the absorption of metals across the gut walls and into the systemic fluids.

It is possible to infer that liberation of adsorbed metals in kelp food by mildly acidic invertebrate digestive juices (pH $\simeq$ 5.5) (153) might tend to diminish available Sr, Ba, and Pb relative to Ca because the data of table 6.2 show that leaching kelp preferentially releases Ca relative to these other metals. However, in the digestive systems of gastropods which feed on brown algae are bacteria whose alginolytic enzymes are capable of degrading the polysaccharide binding the bulk of alkaline earths and Pb in kelp (224). It has been reported that Pb-complexed with

Table 6.4 Bioenrichment and biodepletion in the flow of Ra, Th, U, and Pu through benthic marine food chains.

	Concentration in μg/g fw of Ca	Activity in pCi/kg fw of: ^{226}Ra	^{228}Th	^{238}U	^{239}Pu
seawater	410	0.06	0.0016	1.1	0.0007
macroalgae	1000[1]	8	29[2]	30	0.5-13
molluscs[3]	360000[1]	50	1	30	0.3

1) Average values from this work.

2) Based on the measured bioenrichment factor for ^{232}Th given in the data of Cherry and Shannon (37).

3) Note the strong biodepletions occurring when data is normalized on the basis of Ca, Ra, Th, U, and Pu parallel the metabolic pathways of Ca in organisms and are similarly localized in skeletal tissues (61).

All data from Cherry and Shannon (37) except as noted, Ca abundances in the various reservoirs are given so that the degree of bioenrichment or biodepletion in transfers between trophic levels may be evaluated on the basis of normalized data.

either algin or pectin is absorbed at twice the rate as inorganic Pb species (75), but it is not known how organic complexing affects the uptake of alkaline earths. It is possible that preferential uptake from food of organically complexed Pb relative to Pb leached from food may oppose the Ca-dilution effects previously described.

6.3.4. Biopurification effects in the spiny lobster Panulirus interruptus

The pattern shown in figure 6.1 of bioenrichment of Sr, Ba, and Pb relative to Ca in the transfer of metals from seawater to kelp, followed by biopurification of Ca with respect to these metals in the transfer from plant to gastropod, is paralleled by the sequence of fractionations of Ra, Th, U, and Pu relative to Ca in their flow through mollusc food chains (37). This is illustrated in table 6.4 and is understandable on the basis of parallelisms in biogeochemical pathways between those heavy metals and Ca noted earlier in table 1.1 (61).

Relative metal abundances in the digestive gland of the omnivorous spiny lobster from Cortes Banks are compared in table 6.5 with metal ratios in seawater to show that the Sr/Ca ratio is unchanged, the Ba/Ca ratio increases by a factor of 21, and the Pb/Ca ratio increases by a factor of 2300 in going from seawater to digestive gland. This sequence of bioenrichments observed in the Valonia data of table 5.1 and the kelp data of table 6.1. This may indicate Ba and Pb in spiny lobsters are derived primarily from marine plants. In going from digestive gland particles to total spiny lobster, table 6.5 shows that the Sr/Ca ratio is reduced by 2, the Ba/Ca ratio by 73, and the Pb/Ca ratio by almost 500. This sequence of increasingly greater biopurification factors is closely akin to the sequence of biopurification factors which characterize the flow of Sr, Ba, and Pb relative to Ca from food to molluscs, shown

Table 6.5. Biopurification effects in the spiny lobster Panulirus in relation to food particles of the digestive gland.

Sample	Atomic Ratios Sr/Ca	Ba/Ca	Pb/Ca
Seawater (Cortes Banks)[1]	9.1×10^{-3}	4.3×10^{-6}	1.2×10^{-8}
Bioenrichment factor[2]	1.1	21	2300
Digestive gland contents	9.9×10^{-3}	8.8×10^{-5}	2.8×10^{-5}
Biopurification factor	1.7	73	475
Total spiny lobster[3]	5.8×10^{-3}	1.2×10^{-6}	5.9×10^{-8}

1) Ambient concentrations of dissolved Pb in the region of Cortes Banks is about 20 ng Pb/ℓ (172). Ba is about 6 µg/ℓ (7, 38) and Sr is about 8.1 mg/ℓ (77).

2) Bioenrichments for digestive gland contents are calculated relative to seawater for comparative purposes. Particles in the digestive gland appeared similar to kelp particles in the crop and digestive gland of abalones which may explain the similarity between the above bioenrichment factors and those observed for Cortes Banks kelps. Spiny lobsters are omnivorous and consume animal matter as well as algae.

3) Data for total spiny lobster are recalculated from data of tables 4.9 and 4.12.

by table 6.1 and later by table 6.6, suggesting that biopurification processes in crustaceans and molluscs are similar.

PART II. BIOENRICHMENT AND BIOPURIFICATION IN FILTER-FEEDER FOOD CHAINS

6.4 Benthic filter-feeder food chains studied

Changes in the abundances of Sr, Ba, and Pb relative to Ca in major biochemical Ca reservoirs were studied in two filter-feeder food chains involving pelecypods. One food chain consisted of seawater, particulates, and rock scallop, and the other consisted of seawater, particulates, and mussel. Rock scallops were collected at Cortes Banks and Abalone Cove, while mussels came from Punta Banda, Baja, California, Mexico.

6.4.1 Source of metals in benthic filter-feeders

Scallops, mussels, and other filter-feeders derive their food from a reservoir of suspended organic particles. Under natural conditions this reservoir is composed chiefly of algal detritus but also includes a minor inorganic component. In polluted coastal regions near Abalone Cove, Pb-rich sewage particles constitute the major fraction of suspended organic matter in seawater (33, 34, 172). Fine organic food particles filtered from seawater are segregated into the digestive gland of pelecypods while inorganic particles and large organic particles remain in the digestive tract and are excreted as psuedo-feces (153, 238). Radiotracer experiments with mussels indicate that metals initially accumulated in the digestive gland are the source of heavy metals transported to other tissues (75, 83, 84, 176, 180, 192, 197, 198).

6.4.2 Observed biopurification effects in scallops

As shown in table 6.6, the Sr/Ca ratio of particles contained in the digestive gland of Cortes Banks scallop is about the same as seawater, whereas the Ba/Ca and Pb/Ca ratios of digestive gland contents are higher than seawater by respective factors of 95 and 27000. The Pb/Ca ratio of

Table 6.6. Biopurification effects in scallops under conditions of different ambient metal concentrations in food particles filtered from seawater.

Sample	Location	Atomic ratios Sr/Ca	Ba/Ca	Pb/Ca
Seawater	Cortes Banks	9.1×10^{-3}	4.3×10^{-6}	1.2×10^{-8}
Bioenrichment factor[1]		0.91	95	27000
Digestive gland contents		8.2×10^{-3}	9.9×10^{-4}	3.2×10^{-4}
Biopurification factor		4	1000	4200
Total scallop (shell)		2.0×10^{-3}	9.3×10^{-7}	7.7×10^{-8}
Seawater	Abalone Cove	9.3×10^{-3}	4.3×10^{-6}	2.1×10^{-8}
Bioenrichment factor[1]		0.66	17	76000
Digestive gland contents		6.0×10^{-3}	7.1×10^{-5}	1.6×10^{-3}
Biopurification factor		2	76	6700
Total scallop (shell)		2.0×10^{-3}	9.3×10^{-7}	2.4×10^{-7}

Data are recalculated from table 4.8.

1) Bioenrichment factors for digestive gland contents are calculated relative to seawater but do not have the same significance as in grazer food chains because filtered particulates in the former contain metals derived not only from seawater but also from anthropogenic and terrestrial sources.

Cortes Banks scallop digestive gland contents is about 35 times greater than the Pb/Ca ratio in the kelp food of Cortes Banks gastropods given in table 6.1.

In the transfer of metals from digestive gland to total Cortes Banks scallop, Ca is purified relative to Sr by a factor of 4. Relative to Ba, Ca is biopurified by a factor of 1100 and relative to Pb, by a factor of 4200. The greater biopurification of Ca relative to Ba and Pb compared to the lesser biopurification relative to Sr parallels biopurification effects observed in gastropods and is also characteristic of sequential consumer relationships in terrestrial ecosystems, illustrated earlier by table 2.2 (60, 62, 96, 146, 164, 166, 168). These observations, indicating that biopurification processes operate in similar ways in both marine and terrestrial ecosystems, may reflect fundamental biochemical similarities in the way organisms process Pb, Ba, and Sr relative to Ca.

PART III. EFFECTS OF VARIATIONS IN AMBIENT SEAWATER Pb CONCENTRATIONS ON BENTHIC FOOD CHAINS

6.5 Effects on marine plants of variations in ambient Pb concentrations

As shown in table 6.6 and figure 6.2, the Pb/Ca ratio of Abalone Cove Macrocystis is a factor of two greater than that ratio in kelps from areas less polluted by Pb. The concentration of total Pb at Abalone Cove is temporally variable but averages several hundred ng Pb/ℓ (172). This contrasts with only about 25 ng Pb/ℓ in the waters around Cortes Banks and San Clemente Island. The proportion of particle Pb in seawater at Abalone Cove is about 70-90%, while at the outer Bight collection sites this fraction is about 10% of the total (172). The 10-fold decrease in total Pb in seawater between inner and outer Bight locations, shown in figure 6.2, is largely a decrease in particle Pb since concentrations of dissolved Pb decrease by less than a factor of two. As shown earlier in table 4.1, Macrocystis collected from San Clemente Island contains only 20 ng Pb/g fw compared to 30-50 ng Pb/g fw in Macrocystis collected at Abalone Cove. This difference in Pb concentrations in kelp is proportional to the difference in concentrations of dissolved Pb in seawater between the two regions (25 vs. 45 ng Pb/ℓ) but does not correlate with the difference in concentrations of particle Pb (15 vs. 300 ng Pb/ℓ). This suggests that dissolved Pb is probably more important than particle Pb in the pollution of marine plants. This conclusion is corroborated by studies on the inhibitory effect of "EDTA" on Pb uptake by algae which show that it is the "ionic" species of Pb which is passively adsorbed by algae (179, 199).

6.6 Effects on grazing gastropods of variations in ambient Pb concentrations

Figure 6.2 shows that not only does the two-fold increase in dissolved Pb/Ca which occurs in going from less polluted Cortes Banks to more polluted

Table 6.7. Correlations of metal ratios in kelp with ratios of dissolved metals in seawater at various locations within the Southern California Bight.

		Atomic Ratios		
Location	Sample	Sr/Ca	Ba/Ca	Pb/Ca
Cortes Banks	seawater	9.1×10^{-3}	4.3×10^{-6}	1.2×10^{-8}
	Eisenia	2.9×10^{-2}	1.9×10^{-4}	3.3×10^{-6}
San Clemente Is.	seawater	9.1×10^{-3}	4.3×10^{-6}	1.2×10^{-8}
	Macrocystis	3.2×10^{-2}	2.2×10^{-4}	2.6×10^{-6}
Abalone Cove	seawater	9.1×10^{-3}	4.3×10^{-6}	2.1×10^{-8}
	Macrocystis	3.7×10^{-2}	3.2×10^{-4}	6.0×10^{-6}

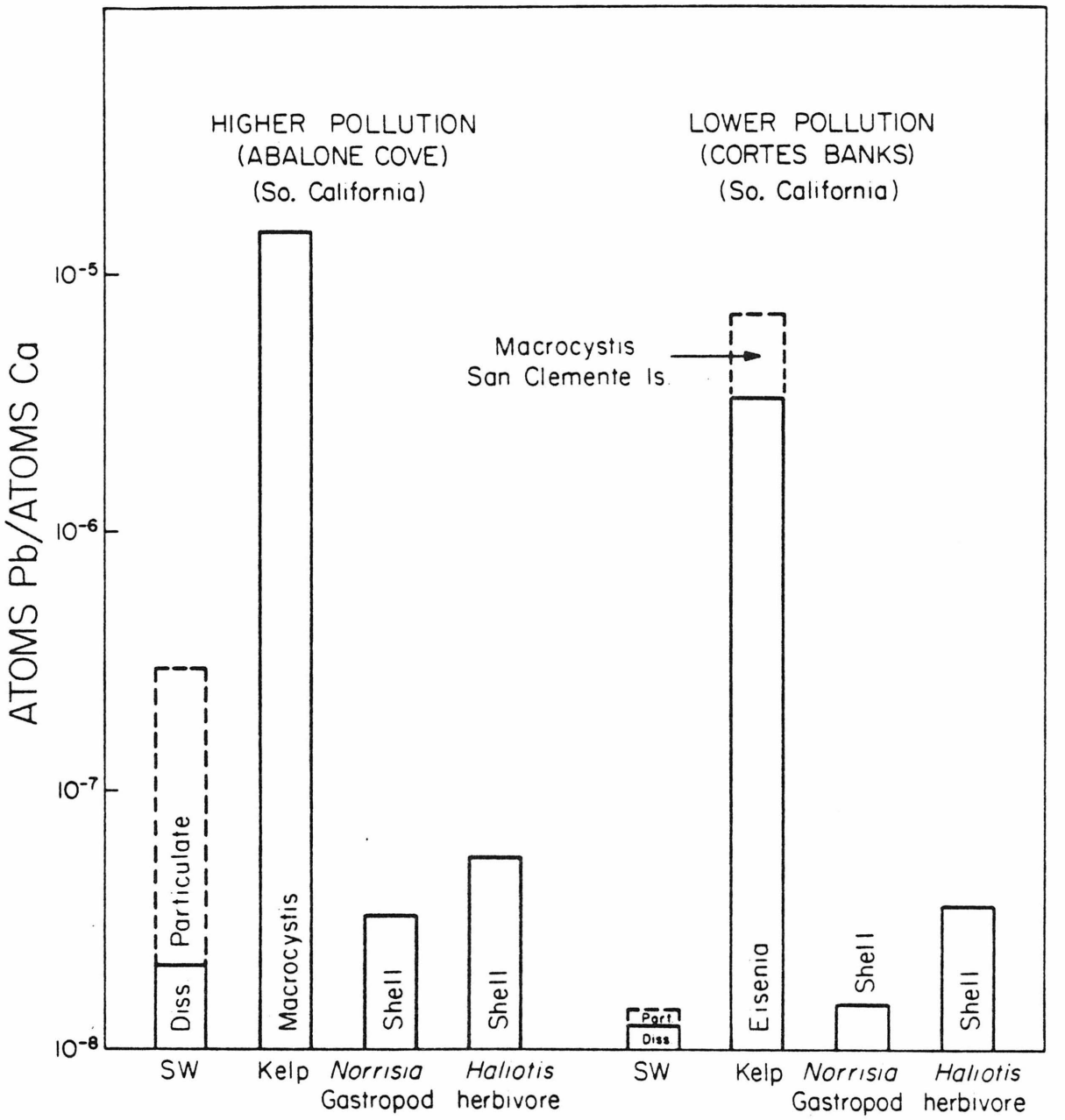

Figure 6.2. Pollution effects caused by simultaneous small increases of dissolved Pb and large increases of particle Pb in seawater. The two-fold increase of dissolved Pb/Ca that occurs in going from the less polluted Cortes Banks region to the heavily polluted Abalone Cove region correlates with two-fold increases in the Pb/Ca ratio of kelp and of gastropod grazer shells. These latter changes are far smaller than the 20-fold difference between particle Pb/Ca at Cortes Banks and Abalone Cove. Figure is based on data of Patterson, Settle, and Glover (172) and tables 4.1 and 4.5. Note the log scale.

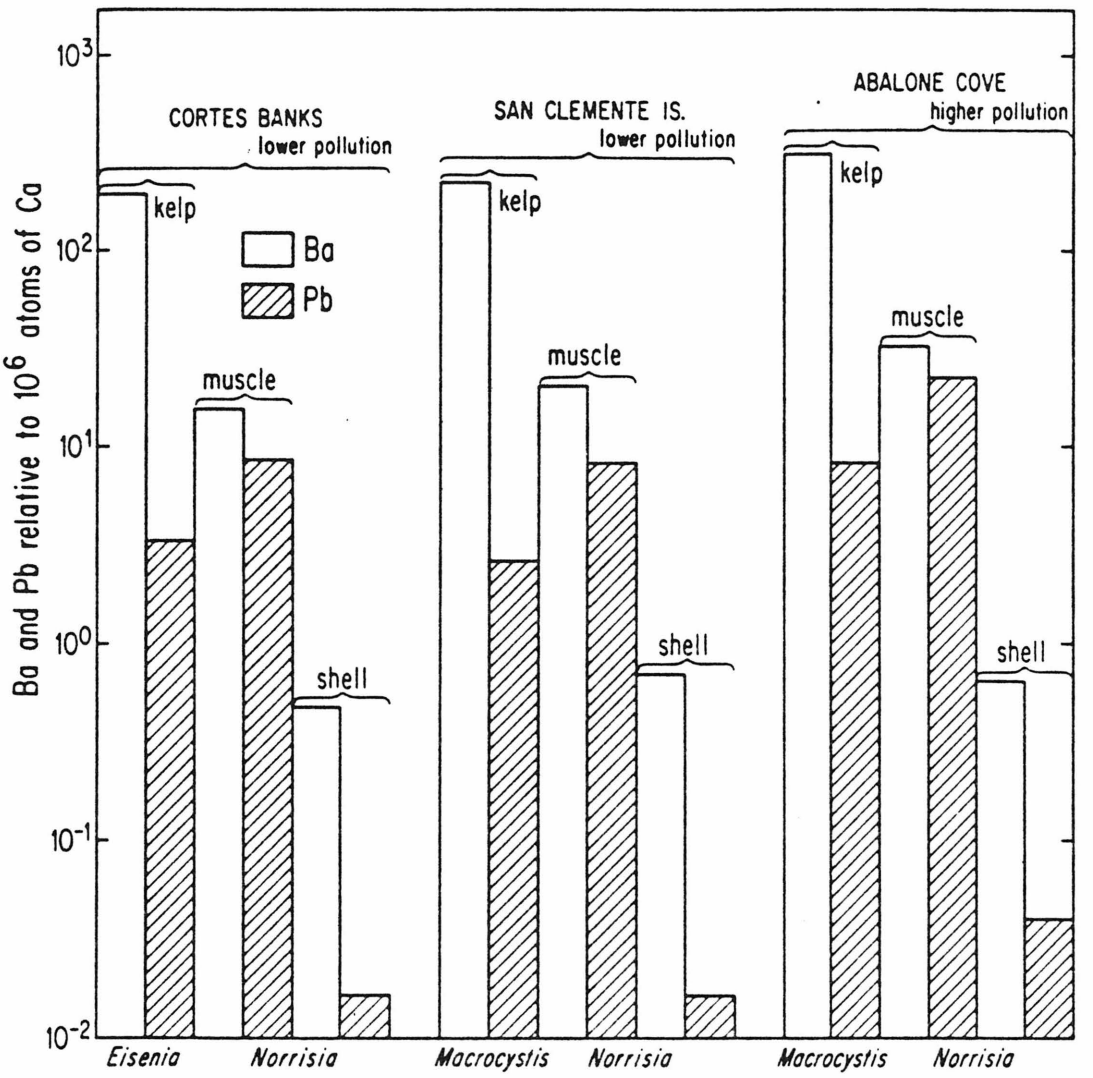

Figure 6.3. The effect of variation in ambient concentrations of Pb in seawater on kelp and the kelp snail Norrisia. Ba concentrations in surface seawater remain uniform but Pb concentrations increase two-fold in going from Cortes Banks to Abalone Cove. These relationships correlate with uniform Ba/Ca ratios in both species of kelp and with increased Pb/Ca ratios in kelp at Abalone Cove. As metals are recycled from plant to consumer, the Ba/Ca ratio of major Ca reservoirs changes very little whereas the Pb/Ca ratio increases in proportion to the change observed in plants in going from zones of lower degree of Pb pollution to a zone of higher pollution.

Table 6.8. The effect of variations in ambient concentrations of dissolved Pb on kelp and kelp snails Norrisia.

	Pb/Ca molar ratios		
	Cortes Banks	San Clemente	Abalone Cove
	lower pollution	higher pollution	
Seawater	1.2×10^{-8}	1.2×10^{-8}	2.1×10^{-8}
Kelp	3.3×10^{-6}	2.6×10^{-6}	6.0×10^{-6}
Norrisia			
calcite layer	2.4×10^{-8}	1.8×10^{-8}	5.2×10^{-8}
aragonite	0.9×10^{-8}	1.1×10^{-8}	2.9×10^{-8}

All Norrisia specimens were of approximately the same size (31-34 g). The concentration of dissolved Pb at each location is temporally variable but averages about 25 ng Pb/l in the outer shelf areas but about 45 ng Pb/l at Abalone Cove. Data are recalculated from tables 4.1 and 4.5.

Abalone Cove correlate with two-fold increases in the Pb/Ca ratio of kelp but also that these differences correlate with similar increases in the Pb/Ca ratio of major Ca reservoirs of gastropods feeding on these kelps. Figure 6.4 shows the effect on the gastropod Norrisia of two-fold increases in the Pb/Ca ratio of kelp but only slight increases in the Ba/Ca ratio is to produce proportionate increases in these ratios in both the hard and soft tissue fractions of the snail. This is true even though the major Ca reservoir, the shell, is about 100 times purer of Ba and Pb than the Ca reservoir of their kelp food. The Pb/Ca ratio of kelp snail shell accurately characterizes the Pb/Ca ratio of its precursor reservoir, brown algae.

6.6 Effects on filter-feeding scallops of variations in ambient Pb levels

The composition of detritus forming the food source of filter-feeders such as scallops is more variable than the kelp food of Norrisia snails. This is especially true in areas like Abalone Cove, where temporal variations in the composition and quantity of sewage discharges produces a more heterogeneous reservoir of detritus than would normally be found at Cortes Banks. Analysis of particles contained in scallop digestive gland, given in table 4.7, indicates that Ba/Ca and Pb/Ca ratios of these particles are about a factor of five higher at more polluted Abalone Cove. The higher Ba/Ca ratio of digestive gland particles is not replicated by correspondingly higher ratios in either muscle tissue or shell material of Abalone Cove scallop compared to Cortes Banks scallop, leading one to conclude that ambient Ba/Ca ratios of food particles are much the same at both locations. This inference agrees with what is known regarding the minimal levels of Ba pollution at most areas within the Southern California Bight with the exception of drilling tracts. On the other hand, the five-fold increase

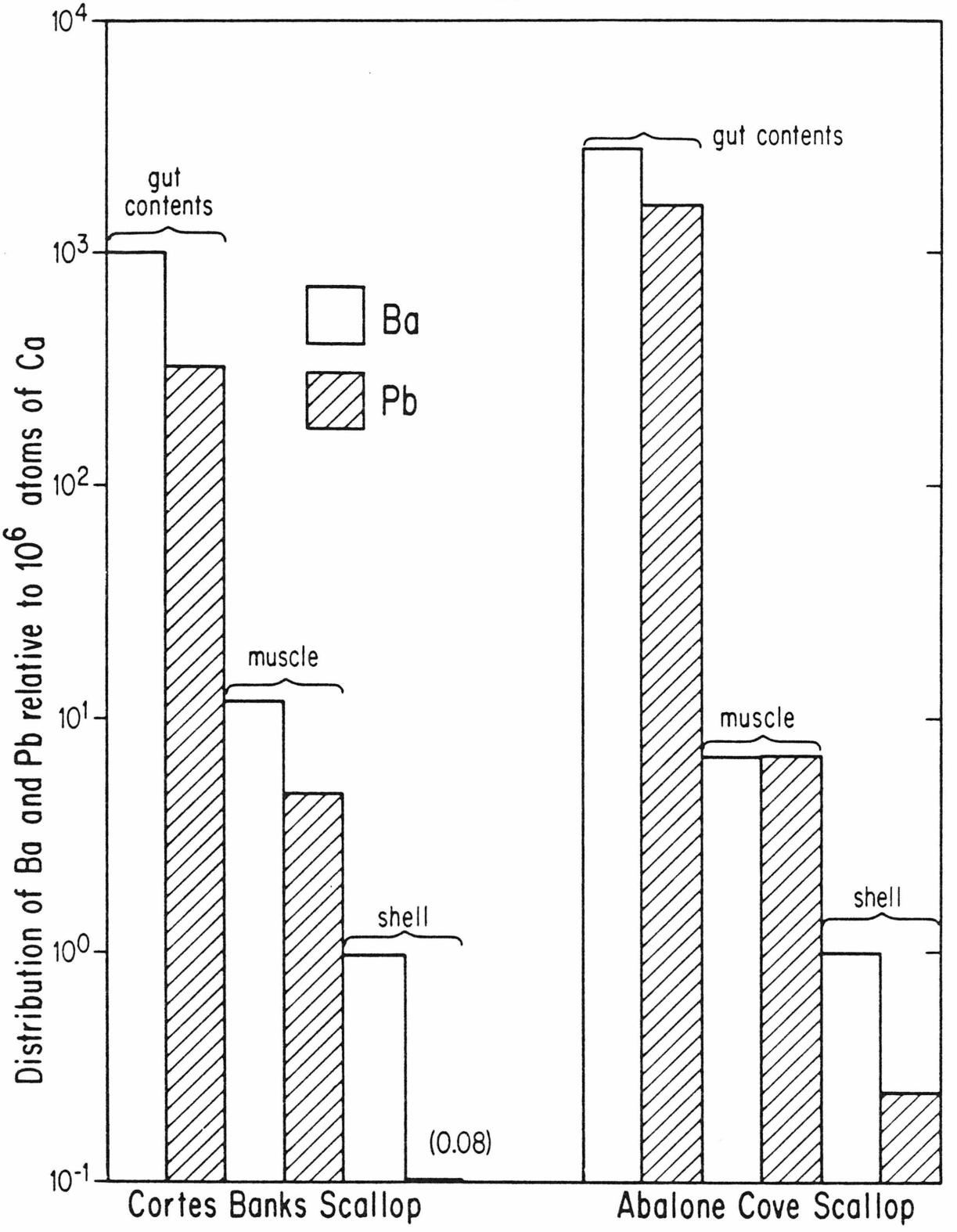

Figure 6.4. Biopurificiation effects in scallops under conditions of different relative metal abundances in food. The 5-fold increase in Pb/Ca of detrital food particles at Abalone Cove compared to Cortes Banks correlates with a 3-fold increase in the Pb/Ca ratio of shell but only a 50% increase in muscle. Note the log scale.

in the Pb/Ca ratio of food particles at Abalone Cove does correlate with an observed 20-fold increase in the concentration of particle Pb at this location (172) and with a three-fold increase in the Pb/Ca ratio of scallop shell, as shown in figure 6.5. The Pb/Ca ratio of scallop muscle also increases but by only 50%. Thus the Pb/Ca ratio of detritus is most accurately characterized by the ratio in shell material even though the latter is more than 1000 times purer of Pb than the Ca reservoir of the former.

PART IV. OBSERVATIONS ON THE CHEMISTRY OF Pb IN MOLLUSC SHELLS

6.8 General observations

In order to determine how differences in ambient Pb concentration in seawater, growth rate, and shell mineralogy affect the concentration of Pb in mollusc shells, shells formed under relatively uniform conditions should be analyzed (53, 54, 137, 259). Mussels seemed unsuitable for study because of the heterogeneity of microenvironments and metal uptakes in rocky intertidal zones (180). Continually submerged rock scallops, although inhabiting a more homogeneous biotope than mussels, have a largely monomineralic shell, while abalones, despite their large size and two-layer calcite-aragonite shell, proved difficult subjects for study because of the tendency for the outer shell layer to become encrusted and corroded. The kelp snail Norrisia was found to be an excellent subject for study because it not only possesses a shell with an inner aragonite layer and an outer calcite layer that resists colonization by sessile organisms but also its food source, kelp, is homogeneous with respect to distributions of Sr, Ba, and Pb relative to Ca, as was shown in Part I of this chapter.

Separate aragonite and calcite shell fractions of kelp snails and abalones, identified by x-ray powder diffraction analyses reported in table 6.9, were analyzed in order to relate Pb concentrations in shell layers to physiological and environmental parameters. Samples were taken from the last formed portions of the shell near the posterior margin by breaking off the outermost few millimeters and mechanically separating the individual shell layers. It was assumed that by taking samples in this manner only a relatively short period of time in the recent past was represented and that any effects of variation in temperature and salinity were covariant within

Table 6.9. Results of X-ray powder diffraction analyses of shell layers of kelp snail and abalones.

Species	Shell fraction	Mineralogy
Norrisia norrisii	inner nacreous layer	Aragonite[1]
	outer prismatic layer	Calcite
Haliotis cracherodii	inner nacreous layer	Aragonite[1]
	outer prismatic layer	Calcite
Haliotis corrugata	inner nacreous layer	Aragonite[1]
	outer prismatic layer	Calcite

1) All aragonite samples displayed a few unidentified peaks believed to correspond to Mg impurities.

Analyses were performed by C. Kendall

individual shell layer samples from a given locality. Since all samples were taken from shell margins, the time interval represented by each sample is approximately bounded at one point by the time of collection. However, until shell preparation techniques are developed which allow the contamination controlled study of Pb in sequential growth increments of individual shell layers, it is difficult to insure that the same length of time is represented by each sample. Because of these uncertainties, figure 6.5 and the supporting data of table 6.10 may be regarded as representing the probable distribution of Pb between shell layers. It is likely that future research may modify the magnitude of the slope of the curve describing the partitioning of Pb between calcite and aragonite in shells of kelp snails, shown by figure 6.5, but will probably change neither the sign of the slope nor its sense of curvature.

In this section some effects of mollusc size, ambient environmental Pb concentrations, and possible physiological factors influencing the concentration of Pb in, and its distribution between, adjacent mineralogically distinct shell layers of kelp snails and abalones are discussed. The kelp snail Norrisia norrisii is the only gastropod species which was collected at more than one locality, so it was studied in greatest detail. In the ensuing discussion it is assumed that growth rates of this gastropod are approximately the same at all locations, so that equivalently-sized specimens from different localities are interpreted to be of approximately the same age (2).

Frequent reference will be made to the curve of figure 6.5 which represents data of table 6.10.Three salient features of this curve should be borne in mind. First, Pb concentrations in both the calcite and the aragonite layers of snails from a particular locality decrease with increasing organism mass, so that the oldest largest kelp snails tend to have

Table 6.10. The partitioning of Pb between shell layers of gastropods

Size	Species	Location	Pb conc. ng/g Calc	Arag	Calc/Arag Atomic ratio
34	Norrisia norrisii	Abalone Cove	95	56	1.7
36	N. norrisii	Abalone Cove	93	48	1.9
58	N. norrisii	Abalone Cove	71	30	2.4
34	N. norrisii	Cortes Banks	43	18	2.4
27	N. norrisii	Cortes Banks	49	21	2.3
32	N. norrisii	San Clemente	31	14	2.2
1600	Haliotis corrugata	Cortes Banks	nd	70	
32	H. corrugata	Cortes Banks	2800	3000	0.9
250	H. cracherodii	Abalone Cove	140	78	1.8

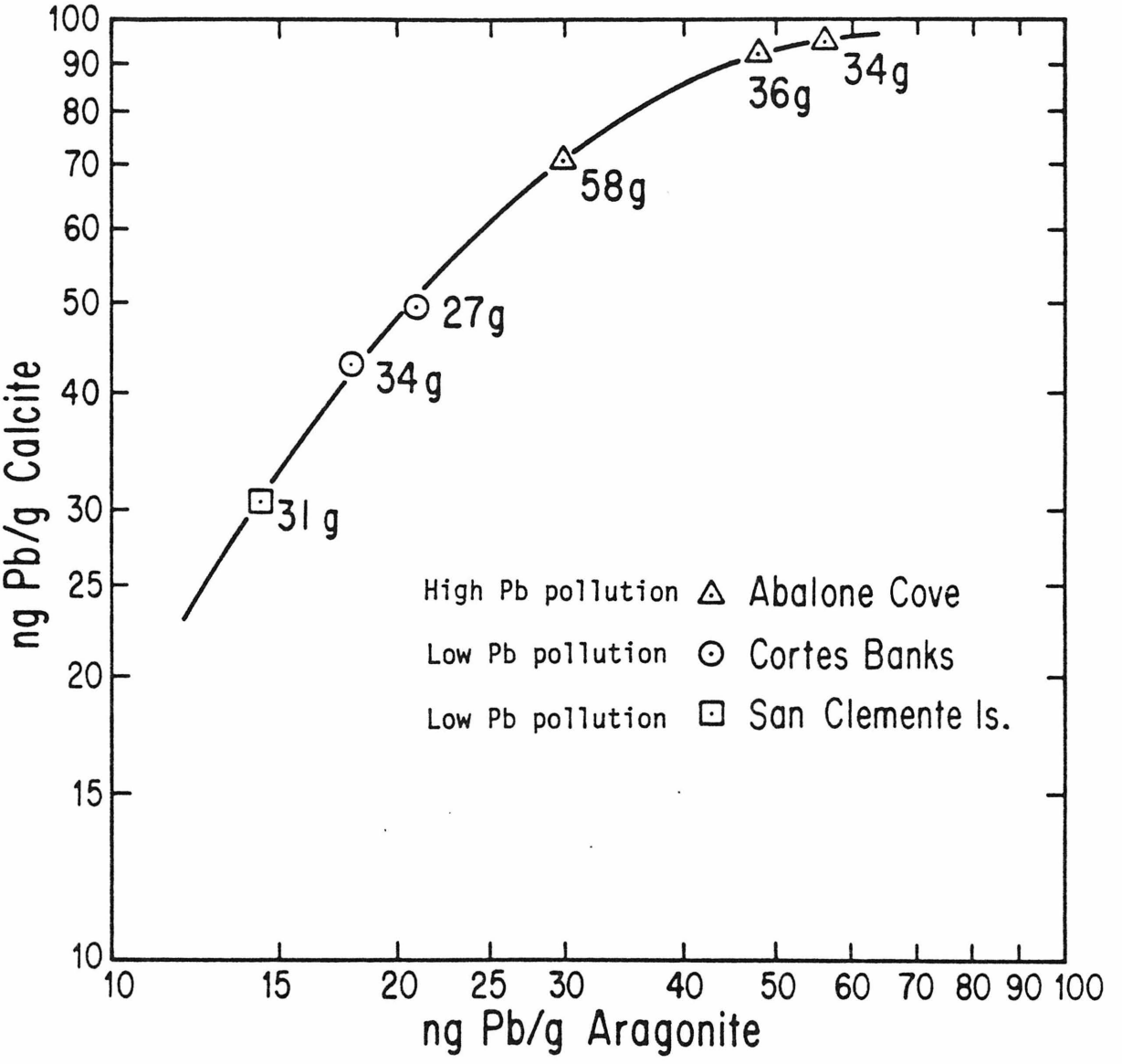

Figure 6.5. Distribution of Pb between shell layers of the kelp snail Norrisia in relation to shell mineralogy, size, and location. Pb concentrations in both shell layers of snails from lower pollution sites are less than in shells of comparably sized specimens from more polluted Abalone Cove. Pb concentrations in recently formed portions of both shell layers of older larger specimens are less than in younger specimens from the same locality. The partitioning of Pb between shell layers changes as a function of concentration. Note log scales.

lower Pb concentrations in each shell layer than do younger smaller snails. Second, Pb concentrations tend to be higher in the calcite layer than in the adjacent aragonite layer. Third, the ratio of Pb concentration in calcite to Pb concentration in coexisting aragonite decreases as the concentration of Pb in both layers increases. A satisfactory explanation of the occurrence of Pb in gastropod shells must resolve the problems posed by these three considerations. In subsequent sections each of these aspects will be treated individually and a concluding section deals with them collectively.

6.9 Effects of growth rate on Pb concentrations in gastropod shells

The data of table 6.10, illustrated by figure 6.5, show that the Pb concentration in both the inner aragonite layer and the outer calcite shell layer of kelp snails from the same locality is higher in younger smaller snails than in larger older ones. Table 6.10 shows that this also appears to be true for abalones, which have a similar shell structure. A decline in growth rate with age has been documented in abalone and other gastropods (217), and the inverse relationship between Pb concentration in shell layers and mollusc body mass positively correlates with this decline in growth rate, as is seen from figure 6.5. A 4% decrease in Sr content of last formed portions of the outer prismatic calcite layer in the mussel Mytilus californianus has been correlated with an increase in shell size among specimens collected from a single locality at the same time (53), and a 10-fold reduction in the width of growth layers of the pelecypod Crenomytilus, interpreted as representing a 10-fold decrease in growth rate, has been correlated with a 20% decrease in the Sr content of its shell (249). These findings parallel the trend of Pb in kelp snails and abalone. Sr and Ba data for kelp snails, reported in table 4.5, do not appear to correlate with size.

The reason for this is not yet clear, but the existence of such phenomena indicates there may be a physiologic difference between the way alkaline earths are processed and biochemical mechanisms controlling Pb.

It is surmised that the reason why the Pb/Ca ratio of individual shell layers of young kelp snails and abalones more closely approaches the Pb/Ca ratio of their food than do corresponding ratios of larger neighbors is that mechanisms tending to biopurify Ca of Pb are either less well developed or work with lower efficiency in faster-growing juveniles than in slower-growing mature specimens

If Pb concentrations in mollusc shells are dependent in part on growth rate, it should be possible to detect seasonal variations in Pb concentration between sequential growth increments of each shell layer caused by the dependence of growth rate on temperature, such as is observed for Sr in mussel shells (53). These cyclical variations should be most pronounced in organisms from kelp bed canopies or shallow water, where temperature conditions are likely to be most variable.

6.10 Observed partitioning of Pb between layers of gastropod shell

Figure 6.6 illustrates the tendency for Pb to be preferentially partitioned into the calcite layer relative to the aragonite layer of kelp snails. From a purely mineralogical point of view this distribution is anomalous because the aragonite lattice permits easier substitution of Sr, Ba, or Pb at Ca sites than does the lattice of calcite. The data of table 4.5 indicate that the distribution of Sr and Ba between shell layers is different from Pb because these metals tend to be preferentially partitioned into the layer containing aragonite. The fact that Pb behaves differently suggests that the distribution of Pb is subject to stronger biochemical control than Sr or Ba and that studies on the partitioning of Pb between coexisting calcite

and aragonite in inorganic systems are probably not relevant for biological systems such as this.

Preferential partitioning of Pb into the calcite shell layer of molluscs has been described previously (135) and is of a type of behavior experimentally observed to be related to the presence of certain organic compounds found in molluscan extrapallial fluid, the solution which supplies shell constituents at the site of shell formation (50, 120, 123).

6.11 Experimental observations on metal partitioning in mollusc shells

Laboratory experiments have shown that common organic constituents of extrapallial fluid such as glycogen, malate, and especially citrate, form complexes with Ca^{++}, Zn^{++}, and Pb^{++}, as well as other divalent metals (120, 123, 205). In $Ca(HCO_3)_2$ solutions containing $CaCl_2$ and Na-citrate in the various proportions that are found in extrapallial fluids, the Zn distribution coefficient for calcite relative to the solution has been shown to range between 1 and 20, while the distribution coefficient between coprecipitated aragonite and the solution ranges between 0 and 4 (121, 122). In general, then, Zn tends to be preferentially fractionated into the calcite phase of systems containing coexisting calcite and aragonite precipitated from solutions containing organic chelating agents such as those mentioned above. It should be expected that a similar relationship would hold for Pb because the Pb-citrate stability constant ($k = 10^{5.74}$) is an order of magnitude greater than the Zn constant ($k = 10^{4.71}$) (205). Organic complexing is probably not important in controlling the distributions of Sr and Ba because the stability constants of their citrate and malate complexes are lower than that of Ca (205). The data of table 4.5 agree favorably with these predictions.

6.12 A hypothesis to explain Pb partitioning in gastropod shells

Table 6.10 shows that in both kelp snails and abalones the ratio of Pb in the calcite layer to Pb in the aragonite layer decreases with increasing total Pb concentration. In the juvenile abalone from Cortes Banks this ratio is less than one, which means that the partitioning of Pb typical of mature specimens probably represents an inversion of the initial partitioning. A similar cross-over is predictable from the trend toward incorporating a greater fraction of total Pb into the aragonite layer of Norrisia as the concentration of total Pb in the shell increases, shown in figure 6.5. If the distribution of Pb in the shells of a group of gastropods collected from the same locality at the same time is only a function of growth rate, which controls total Pb concentration in the shell, and organic complexing effects, which control partitioning of Pb between shell layers, the faster growth rate of younger gastropods should always result in a greater fraction of total Pb being incorporated into the mineralogically unfavorable calcite layer relative to the structurally favorable aragonite layer when compared to mature, slower-growing neighbors. This is because the partitioning mechanisms of younger individuals should work less efficiently under the stressed conditions of rapid growth. Yet the partitioning observed in the juvenile abalone shell seen in table 6.10 contradicts this hypothesis.

It may be argued that an environmental factor such as temperature might indirectly influence physiological factors so as to produce the observed change in partitioning. In support of this, it has been shown that the influence of mineralogy on mollusc shell chemistry is temperature-dependent: for mussels growing at temperatures greater than 10°C, Sr is slightly higher in the outer calcite layer than in the inner aragonite layer because Sr levels in calcite are directly proportional to temperature while this relationship is reciprocal in aragonite (53, 249). There

may be such an effect for Pb as well, but this explanation does not suffice to explain the 30% decrease in the ratio of Pb in calcite to Pb in aragonite seen in the data of table 6.9 when the size of kelp snails collected simultaneously at Abalone Cove decreases from 58g to 34g. The decrease in this ratio, illustrated by figure 6.6, appears to depend upon total Pb concentration in the shell as well as other factors because the proportions of Pb in the calcite layer to Pb in the aragonite layer in groups of differently sized kelp snails from other localities fall on the same smooth curve. The fact that this ratio is about 30% less in kelp snails from Abalone Cove than in comparably-sized kelp snails from either Cortes Banks or San Clemente Island, as may be seen in table 6.10, indicates that the higher ambient concentrations of Pb in seawater at Abalone Cove affect not only Pb concentrations in each shell layer but also affect physiological mechanisms for partitioning Pb between shell layers.

A possible explanation of Pb distributions in gastropod mollusc shells arises if one considers that Pb may not be present entirely as a mineralogical lattice substituent. This is not to suggest that Pb is present in a separate mineral phase as it is an ultra-trace constituent. It is also unlikely that chemical variations between the organic matrices separating individual crystallites of each shell layer exert active control over variation in the calcite to aragonite Pb concentration ratio. Although such differences are probably responsible in part for the relative enrichment of Pb in the calcite layer generally observed, invoking this explanation would seem to require a progressive biochemically-controlled change in the structure of the organic matrix characteristic of each shell phase that is induced by increasing Pb concentrations. This is improbable and conflicts with findings indicating the primary function of organic matrices are to

act as a template for mineralization (50, 236). Yet, organic matrices may play a crucial role in the localization of Pb in shells. Because of the affinity of Pb for the organic ligands present in molluscan extrapallial fluid (50, 120, 123) and in the organic matrix (86, 236), one must consider the possibility that Pb may tend to be localized as complexes with acidic groups of glycoproteins forming the organic shell matrix.

The chemical constitution of the organic membranes separating crystallites in each shell layer is similar to that of the periostracum, the organic covering formed by the mantle edge and which physically isolates the shell from seawater. Pb concentrations in mollusc periostracum were reported in tables 4.6 and 4.9 to average about 1 µg Pb/g fw in the hydrated state that results from their contact with seawater; the Pb concentration in the dehydrated organic fraction is about 2.5 µg Pb/g. This Pb could result from surface adsorption from seawater, but this has been evaluated using ^{210}Pb, ^{85}Sr, and ^{45}Ca tracers and found to be negligible compared to mantle-controlled deposition (116, 189). It therefore seems that Pb in the periostracum is a residue representing metabolized Pb that has been excluded from incorporation into the other shell phases. Assuming dehydrated periostracum Pb concentrations of 2.5 µg Pb/g approximate concentrations in the matrix of the shell, observed concentrations of tens of nanograms Pb per gram and more in mollusc shell reported in tables 4.5 through 4.8 are consistent with the view that a large fraction of total Pb in shell material is localized in the matrix because the matrix constitutes several weight per cent of most mollusc shells (86).

It is theorized that at concentrations below those at which physiological partitioning mechanisms are by-passed, Pb tends to be excluded from the crystal lattice of both shell layers during calcification and

instead is complexed by ligands in the organic matrix and periostracum, which serve as receptacles for structurally incompatible cations. The fraction of total Pb that is present in the mineral grains is partitioned between the mineral phases in accordance with experimental results (120, 123) indicating that complexing of Pb by organic ligands in the extrapallial fluid produces enrichment of Pb in the calcite layer relative to the aragonite layer. However, when the quantity of Pb transported to the site of mineralization is such that available Pb-complexing ligands of the extrapallial fluid and/or the organic matrix are saturated, the excess Pb behaves like Sr and Ba, which tend to partition between shell layers according to isostructural affinities. The ligand saturation effect may result when either ambient concentrations of Pb are sufficiently high, as may occur in Pb-polluted waters, or the efficiency of membrane exclusion is sufficiently low, as in rapidly growing young individuals, that equivalent amounts of Pb enter the systemic fluids of molluscs.

According to this theory, the effect of more efficient membrane transport of Pb by fast-growing young molluscs, typified by the Cortes Banks juvenile abalone of table 6.10, is to allow more Pb to pass into systemic fluids than can be complexed by available ligands. Ligand saturation occurs and Pb enters the aragonite lattice as a Ca substituent. This same effect may be artificially induced when Pb concentrations in seawater are sufficiently high that abnormally large amounts of Pb enter the system of slower-growing older molluscs that under natural conditions would efficiently exclude the excess Pb, as at Abalone Cove where snails have higher Pb levels and a higher proportion of total Pb in the aragonite layer than do comparably-sized snails from sites of lower ambient Pb concentrations in seawater.

If this hypothesis is correct, the difference in the calcite to aragonite Pb concentration ratio between Abalone Cove kelp snails and comparably-

sized kelp snails from either Cortes Banks or San Clemente Island is a measure of the extent to which natural physiological mechanisms of Ca biopurification are being altered by Pb whose isotopic composition defines it as anthropogenic (40, 43, 172, 173) and which occurs at concentrations considered typical, if not natural, in coastal marine and estuarine environments.

6.13 Paleoecological applications

The Pb/Ca ratio of present-day mollusc shells is about 1000 times lower than the Pb/Ca ratio of recent coastal marine sediments (34, 45, 174) and about 10,000 times lower than the crustal ratio (17). Because Pb concentrations in coastal waters of the Northern Hemisphere have been raised by roughly an order of magnitude during historical time as a result of man's technology, the Pb/Ca ratio of unaltered fossil mollusc shells should be about 100,000-fold lower than crustal ratios (41, 149, 173, 193). The Pb/Ca ratio in mineral layers of fossil mollusc shells should thus be a sensitive indicator of diagenetic alteration. The effect of equilibrating the original Pb in the shell with an external Pb reservoir would be to increase the Pb/Ca ratio of each shell layer. Easier substitution of Pb for Ca into the structurally favorable aragonite layer relative to the calcite layer during diagenesis would tend to obscure the original physiological partitioning of Pb between shell layers, which table 6.9 and figure 6.6 indicate generally favors the calcite layer.

Pb in marine mollusc shells is derived largely from soluble fluvial Pb coming from denudation of adjacent continental drainage basins (41) and, in present-day molluscs, from isotopically distinct, solubilized industrial aerosol Pb (40, 43, 44, 101, 149, 173), which is incorporated into the plant material and organic detritus forming the food of most molluscs. Temporal

changes in isotopic composition of Pb and covariant increases in Pb concentration in shells within a stratigraphic sequence should uniquely correlate with similar historical changes in the character of soluble Pb in seawater that are due to increased Pb production from known point sources (43, 62, 149, 205). For purely isotopic studies it is not necessary that the shells studied be of the same species, but only that they preserve their original Pb isotopic composition acquired during their formation. However, the mussel Mytilus may be a good subject for study because it is very common in Pleistocene terrace deposits and Indian kitchen middens (53) and is widely distributed throughout the world. It may even be possible to trace shifts in continental drainage patterns between isotopically distinct provinces (41) using mollusc shells that have retained their original isotopic imprint. Lack of appreciable diagenetic alteration should be deducible from the Pb/Ca ratio of individual shell layers.

CHAPTER VII

BIOENRICHMENT AND BIOPURIFICATION IN A MODEL PELAGIC FOOD CHAIN

7.1 Transfers of Sr, Ba, and Pb relative to Ca in a model pelagic food chain

7.1.1 Food chain model of metal transfers within the pelagic food web

In the food chain model of the pelagic system, the webbed feeding relationships and multiplicity of organisms typical of the open ocean is generalized to a linear model in which matter produced by phytoplankton photosynthesis is transferred in approximately three to five stages from the primary producer level through successive consumers, beginning with microcrustacean herbivores that prey on phytoplankton and ending with large carnivorous fishes (58, 82, 187, 191). In the system modeled here matter considered to be recycled between sequential compartments consisting of seawater, phytoplankton, zooplankton, anchovy, and tuna. This model food chain is depicted by figure 7.1 and in a shortened version by figure 7.2.

Radiotracer experiments have shown that, although some metal is absorbed across epidermal and gill membranes, 90% or more of most heavy metals in crustaceans and fish comes from their food and is absorbed along with Ca across walls of the digestive system (10, 11, 46, 83, 84, 98, 153, 175, 176, 209). The food chain relationships of figures 7.1, 7.2, and 7.3 probably are good representations of the stages through which Ca, Sr, Ba, and Pb are transferred during their flow through the pelagic food web. It should be noted that this model does not depend on the absolute masses nor the fluxes of individual metals because it is their movement relative to Ca which is studied. Processes tending to enrich or purify the Ca reservoir of each trophic compartment with respect to Sr, Ba, and Pb may be recognized by comparing the metal ratios of precursor reservoirs with those of subsequent product reservoirs. Both observed Sr/Ca, Ba/Ca, and Pb/Ca ratios

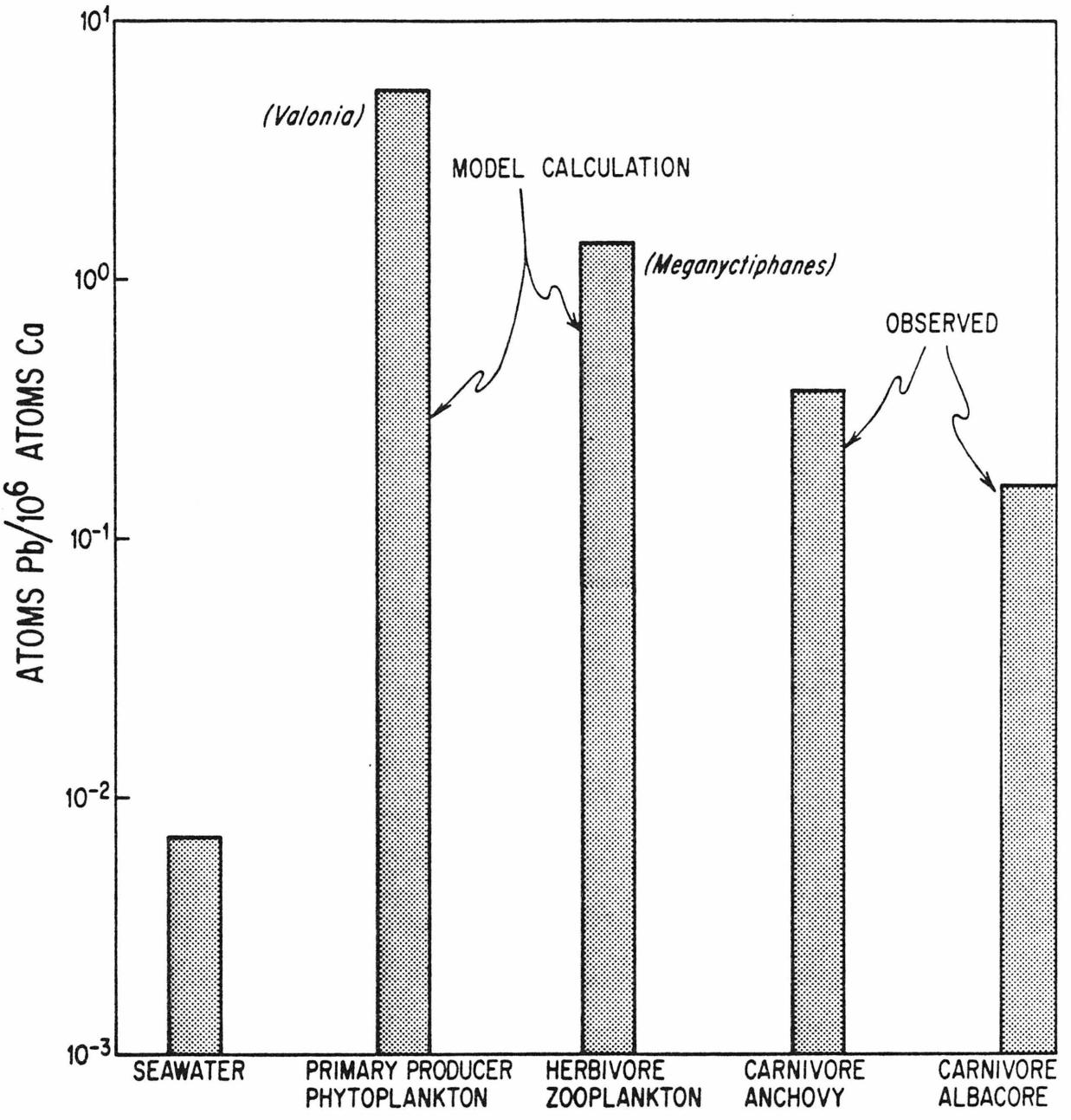

Figure 7.1. Bioenrichment of Pb relative to Ca followed by biopurification of Ca in a generalized pelagic food chain. Passive adsorption of Pb onto surfaces of primary producers results in large bioenrichments relative to Ca. Initial bioenrichment effects are greater than the efficiencies of sequential membrane processes tending to purify Ca of Pb in transfer to higher food chain levels with the result that the Pb/Ca ratio remains greater than the seawater ratio even at the highest carnivore stages.

Table 7.1. Bioenrichment and biodepletion in pelagic food chains: comparison of observed values with model predictions

Reservoir	Molar Ratios			
	Sr/Ca	Ba/Ca	Pb/Ca lower pollution	Pb/Ca higher pollution
Seawater[a]	9.1×10^{-3}	4.3×10^{-6}	7.5×10^{-9}	2.4×10^{-8}
Phytoplankton[b]	1.6×10^{-1}	1.6×10^{-3}	1.8×10^{-5}	9.9×10^{-5}
Valonia model[c]	4.0×10^{-3}	2.8×10^{-4}	0.6×10^{-5}	5.6×10^{-5}
Zooplankton[d]	1.6×10^{-2}	3.7×10^{-4}	2.0×10^{-5}	
Meganyctiphanes model[e]	nd	nd	1.3×10^{-6}	
Anchovy[f]	1.0×10^{-3}	1.3×10^{-5}	3.7×10^{-7}	
Tuna[f]	1.9×10^{-3}	3.4×10^{-6}	1.6×10^{-7}	

a) Concentration of Pb in minimally polluted open-ocean surface seawater is estimated at 15 ng Pb/ℓ (193); in highly polluted water, 50 ng Pb/ℓ.

b) Data from Martin *et al.* (144). In this study the data fall into two distinct populations as regards Pb concentrations and Pb/Ca ratios. The higher pollution group came almost exclusively from north of Punta Eugenia, Baja California, Mexico and had mean Pb concentrations of 922 ± 372 ng Pb/g fw and mean Pb/Ca ratios of $9.9 \pm 3.0 \times 10^{-5}$ ($n = 10$). The lower pollution group had Pb concentrations of 182 ± 61 ng Pb/g fw and Pb/Ca ratios of $1.8 \pm 0.6 \times 10^{-5}$ ($n = 10$). Concentrations are recalculated from dry weight assuming 90% water content.

c) Surface adsorption data from *Valonia* data in tables 4.3 and 4.4 . Sr/Ca and Ba/Ca values are averages.

d) Hawaii zooplankton data of Martin and Knauer (143).

e) Based on data of table 4.12. This model zooplankter shows a lower Pb/Ca ratio than observed in bulk zooplankton in part because bulk zooplankton analyses include ingested particulates (65).

f) From Patterson and Settle (170).

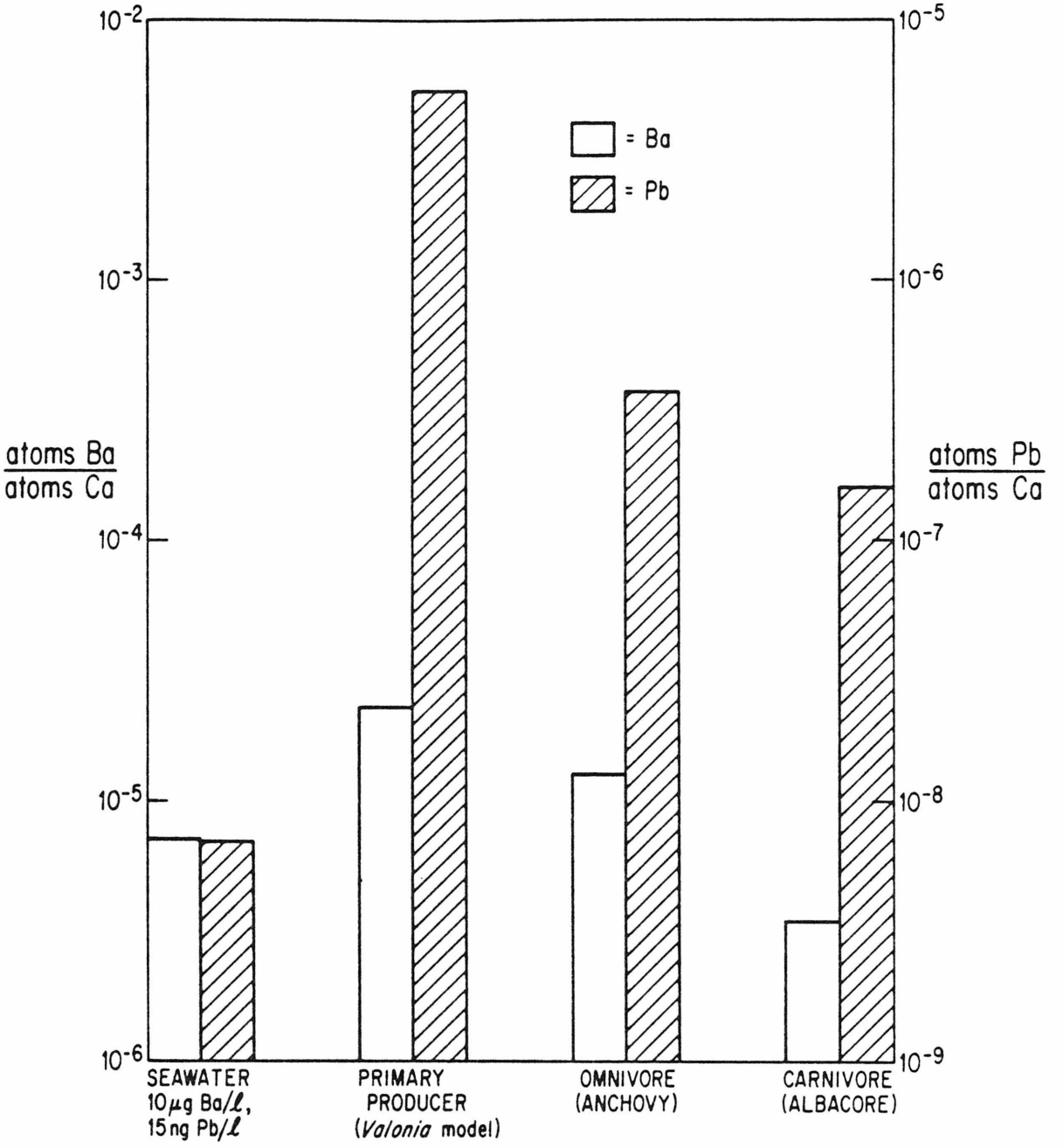

Figure 7.2. The flow of Ba and Pb relative to Ca in a model pelagic food chain. Relative to Ca both Ba and Pb are bioenriched at the primary producer level but are sequentially excluded by mechanisms tending to biopurify Ca during tranfers of metals to successively higher trophic levels. Note the difference in log scales for Ba/Ca and Pb/Ca. Figure is based on data of tables 4.3, 4.13, and 7.4.

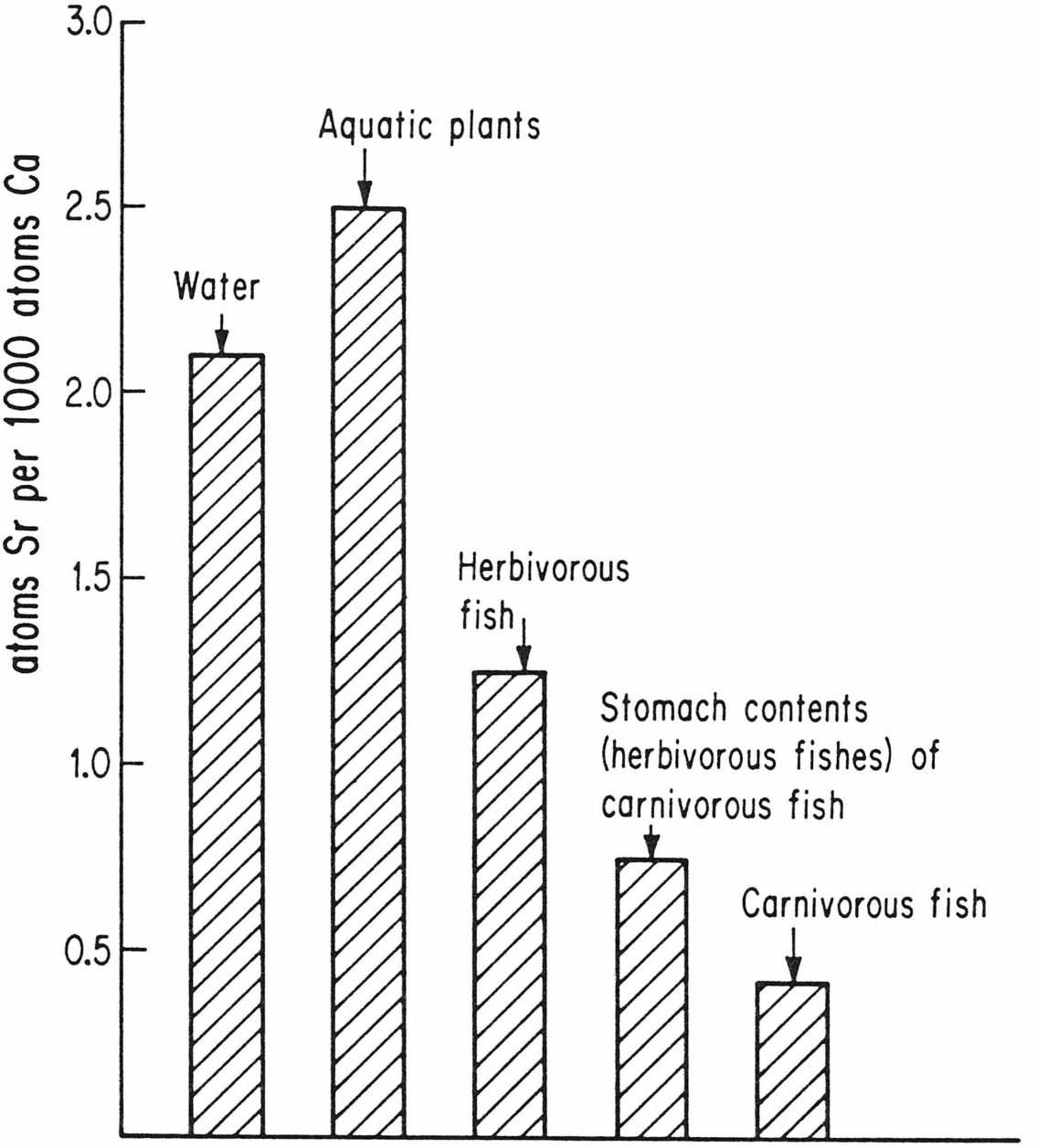

Figure 7.3. Bioenrichment of Sr relative to Ca followed by biopurification in a freshwater food chain. Bioenrichment effects at the plant level due to passive adsorption characterize the flow of metals in aquatic food chains. Biopurification of Ca with respect to trace impurities which occurs during transfers from plants to successive consumer animals has been observed in all terrestrial and aquatic food chains thus far studied. Data from Ophel and Judd (159). Note the linear scale.

as well as those predicted by the model are given in table 7.1.

7.1.2 Formulation of the model of the pelagic food chain

Ratios of metals passively adsorbed onto the surface of Florida Valonia, reported in table 4.3, were used to model relative metal abundances in phytoplankton, shown in table 7.1 and in figure 7.1. The good agreement between observed Pb/Ca ratios in mixed phytoplankton from offshore zones of low pollution (1.8×10^{-5}) (144) and the Pb/Ca of adsorbed metals on Valonia (0.6×10^{-5}), as shown in table 7.1, probably result because Pb concentrations in their respective nutrient media were similar and because polymer selectivity for Pb relative to Ca has been found to be essentially independent of the detailed structure of extracellular matrix polysaccharides which bind Pb (126, 151, 200). The latter point was discussed in section 4.1 and is important in understanding why Pb frequently behaves in a different manner than Sr and Ba when organic complexing is involved. In section 4.1 it was noted that complexing of alkaline earth metals by algal polysaccharides is very sensitive to stereochemical effects. It has been found that differences in the degree of esterification of pectins changes their ion-exchange properties with respect to alkaline earth metals (90, 126). There is evidence based on structural investigations of chelate complexes of uronate polymers, such as pectate and alginate, that the binding sites and mechanisms of chelate formation are not the same between alkaline earths and Pb (88-90, 126, 200, 211, 212). Such differences probably explain why predictions based on Valonia studies do not agree with observed Sr/Ca and Ba/Ca ratios in phytoplankton, as shown in table 7.1, nor with Sr and Ba data of some published studies (143, 221, 226). Investigations of the occurrence of alkaline earths in phytoplankton (221) confirm that selectivity for these metals parallels the selectivity of polystyrene-type cation-exchange

resins in that the affinity of bulk zooplankton for alkaline earths decreases in the series: Ra > Ba > Sr > Ca. This indicates that passive adsorption processes probably play an important role in controlling the occurrence of alkaline earths as well as Pb in phytoplankton.

The abundance of Pb relative to Ca in zooplankton was modeled using data from this and other studies (98, 143, 246) because artifact contamination (33, 143) and the widespread occurrence in zooplankton digestive tracts of ingested sediments and food particles (12, 65) has caused investigators to suspect the significance of trace metal data for bulk zooplankton. Published analyses of Sr and Ca may be more reliable since these metals are less susceptible to effects of artifact and biological contamination because of their generally high abundances in biota.

The model Pb/Ca ratio of zooplankton was computed by assuming that the Pb/Ca ratio of spiny lobster tissues, when corrected for differences between the Pb/Ca ratio of spiny lobster food particles and the ratio of adsorbed metals on Valonia, approximated the Pb/Ca ratio of zooplankton tissues. The Pb/Ca ratios of individual tissues were combined according to the distribution of Ca among the tissues of the euphausiid Meganyctiphanes norvegica (98). A factor based on Valonia studies was introduced to compensate for adsorption of Pb onto the chitinous exoskeletons of zooplankton (246). For this calculation zooplankton were considered to be cylinders having a volume of 0.16 mm^3 (139) and a diameter to height ratio of 0.25 (134). Table 7.2 shows the steps taken in calculating the total body Pb/Ca ratio in the model zooplankton, which is given in table 7.1 and illustrated by figures 7.1 and 7.2. Sr/Ca, Ba/Ca, and Pb/Ca ratios measured in bulk zooplankton are given in table 7.1 also.

Abundances of Sr, Ba, and Pb relative to Ca in fish are represented in the model by published IDMS analyses of a tuna and of an undigested anchovy

Table 7.2. Composition of a model zooplankter based on the euphausiid Meganyctiphanes norvegica.

Tissue	% body weight	Ca	Pb	Ca	Pb
		μg/g in tissue		μg in 1g zooplankton	
exoskeleton	35.5	16,400[2]	0.075	5822	0.027
viscera	5.3	259	0.038	14	0.002
muscle (incl. hamemolymph)	59.2	91	0.005	54	0.003
				5890	0.032
contribution of surface adsorbed Pb[3]					0.006
					0.038

Tissue concentrations from data on Panulirus (table 4.10) except as noted below.

1) Data of Heyraud et al. (98) on the euphausiid Meganyctiphanes norvegica.

2) Data of Martin and Knauer (143) adjusted for 20% water content (145).

3) Calculated by applying Valonia surface adsorption data to the calculated surface area of 1g of copepods whose average volume of 0.16 mm^3/individual (139) was considered to be a cylinder having the dimensional relations: diameter/height = 0.25. Because the work of Yoshinari and Subramanian (246) has shown that Pb is not strongly sorbed by chitin, the Valonia areal adsorption value was reduced from 0.12 ng Pb/cm^2 to 0.06 ng Pb/cm^2. In this case surface adsorbed Pb constitutes 16% of total zooplankter Pb; if the higher value is used, passively adsorbed Pb constitutes 27% of the total body burden of 44 ng Pb/g fw.

contained within the stomach of the tuna (174).

7.1.3 Comparison of model predictions with observed data

Figure 7.1 depicts the flow of Pb relative to Ca through successive trophic levels of a model pelagic food chain. The initial 700-fold bioenrichment of Pb relative to Ca resulting from the transfer of these metals from seawater to phytoplankton surfaces is followed in the model by sequential biopurification of Ca with respect to Pb in successive transfers between consumer organisms. In going from primary producer to total tuna, the Pb/Ca ratio decreases by 30-fold due to biopurification processes.

The pattern of fractionations of Pb relative to Ca in the model is paralleled by a similar pattern of fractionations in observed data, as shown in table 7.1. The increase in the Pb/Ca ratio in going from seawater to phytoplankton and the subsequent decrease in this ratio at successive consumer levels observed in both the model and in biota is paralleled by covariant fractionations of Ba and Sr relative to Ca which the data of table 7.1 indicate are more moderate in degree for Ba and Sr than for Pb. Biopurification of Pb relative to Ca in the transfer from phytoplankton to zooplankton, predicted by the model food chain of table 7.1 and figure 7.1, is not replicated in the observed data, shown also in table 7.1. This is probably because bulk zooplankton analyses tend to reflect the composition of particles in zooplankton digestive tracts (65, 213). However, since metabolic processes are broadly similar between crustaceans (220), decreases in the Sr/Ca, Ba/Ca, and Pb/Ca ratios of total spiny lobster relative to these ratios in digestive gland particles, shown earlier in table 6.4, suggest that biopurification mechanisms operate at the zooplankton level as well.

The model is corroborated by a study of the flow of Sr relative to Ca

Table 7.3. Bioenrichment and biodepletion of Ra, Th, U, and Pu relative to Ca in pelagic marine food chains

Reservoir	Conc. in µg/g fw Ca	Activities in pCi/kg fw of: ^{226}Ra	^{228}Th	^{238}U	^{239}Pu
seawater	410	0.06	0.0016	1.1	0.0007
phytoplankton	1000[1]	100	30	11	0.3[2]
zooplankton	2000[1]	6	9	6	1.6
fish	7200[3]	5	-	0.07[4]	0.1-0.2[5]

[1]Data of Martin and Knauer (143) for zooplankton collected near Hawaii.

[2]Possible low value; macroalgae fall in the range 0.5-13.

[3]Data of Patterson and Settle (170) for tuna.

[4]Muscle tissue value; Ca in fish muscle is about 25 µg/g fw (170).

[5]Bone value. Pu is a bone-seeking metal like Pb (61) and is localized primarily in the skeleton (231). The corresponding Ca concentration in bone is 115,000 µg/g fw (170).

Except as noted above, data are from Cherry and Shannon (37).

in the freshwater ecosystem of figure 7.3 (159). Bioenrichment at the plant level relative to nutrient media, followed by biopurification at subsequent consumer levels relative to trophic precursors, is also paralleled by the same pattern of fractionations relative to Ca in the flow of Ra, Th, U, and Pu through pelagic food webs (37), seen in table 7.3.

It appears that in pelagic food chains, metals which are physiologically similar to Ca, such as Sr, Ba, Ra, Pb, Th, U, and Pu (61), behave in a like manner during transfers between trophic levels and that the bioenrichment and biopurification effects observed in tables 7.1 and 7.3 are much the same as are found in the benthic systems studied. This indicates that bioenrichment of these metals relative to Ca at the primary producer level followed by biopurification of Ca with respect to those same metals as a result of transfers between major Ca reservoirs of sequential consumers is a fundamental biogeochemical process in marine ecosystems.

7.1.4 Factors affecting biopurification in pelagic food chains

It is interesting to note in table 7.1 that while the Sr/Ca ratio of tuna is reduced to below the seawater ratio, the Ba/Ca ratio of tuna approximates the seawater ratio and the Pb/Ca ratio of tuna remains 5-fold above the corresponding seawater ratio. These relationships contrast with those observed in natural terrestrial ecosystems, wherein the Sr/Ca, Ba/Ca, and Pb/Ca ratios of top carnivores are lower than corresponding ratios in nutrient soil moisture by sequentially greater factors (68, 164, 168). Moreover, the 30-fold Pb/Ca biopurification factor between algae and total tuna in table 7.1 is about an order of magnitude smaller than the biopurification factor measured between plants and top carnivores in terrestrial ecosystems (62, 96, 164, 166, 168). These differences may be attributable to several

Table 7.4. Observed atomic ratios of metals in the tissues of the tuna Thunnus alalunga.

Sample	Atomic ratios Sr/Ca	Ba/Ca	Pb/Ca
Seawater	9.1×10^{-3}	4.3×10^{-6}	7.1×10^{-9}
Bioenrichment factor	0.23	20	580
Epidermal mucus	2.1×10^{-3}	8.5×10^{-5}	4.1×10^{-6}
Epidermis	2.7×10^{-3}	6.1×10^{-5}	3.5×10^{-6}
Muscle	1.0×10^{-3}	8.9×10^{-5}	3.4×10^{-6}
Kidney	2.9×10^{-3}	6.1×10^{-5}	1.0×10^{-5}
Spleen	3.0×10^{-3}	1.8×10^{-5}	1.8×10^{-5}
Liver	2.0×10^{-3}	2.1×10^{-4}	1.2×10^{-5}
Bone	1.9×10^{-3}	2.5×10^{-6}	1.3×10^{-7}
Teeth	1.7×10^{-3}	2.9×10^{-6}	2.4×10^{-7}
Gill filaments	4.0×10^{-3}	1.6×10^{-5}	1.8×10^{-7}

Recalculated from the data of Patterson and Settle for Thunnus alalunga (170).

1) Calculated between epidermal mucus and seawater.

considerations. Initial enrichment at the primary producer level by passive adsorption and selectivity effects of chelating polysaccharides on algal surfaces may explain why in biota abundances of Sr, Ba, and Pb relative to Ca do not closely correspond to relative abundances of these metals in seawater. It would also seem probable that the addition of Ca from seawater swallowed with food would increase the reservoir of Ca available to marine consumers and thus enhance the biopurification of Ca believed to be caused by differential permeability of digestive system membranes which favor Ca relative to Sr, Ba, and Pb (17, 164, 204). Moreover, this effect should be most pronounced in marine fish, which are hypo-osmotic regulators and swallow from 50 to 200 ml of seawater per kilogram fish per day (11). Observed biopurification factors, given in table 7.1, that are much less than would be predicted on this basis suggest that processes operate in marine food chains which tend to damp the overall biopurification of Ca.

Table 4.13 shows that only about 70% of total Ba and Pb in tuna is contained in the skeleton compared to about 95% in the molluscs studied. A larger fraction of total Ba and Pb in tuna is contained in muscle, epidermis, and mucus than is the case with invertebrates, for example (33, 174). Table 7.4 shows that Sr/Ca, Ba/Ca, and Pb/Ca ratios in surface mucus, epidermis, and muscle of tuna are very similar to each other and are quite distinct from corresponding ratios in seawater. Fractionations occurring in the transfer of metals from seawater to fish mucus are similar in character to those associated with surface adsorption processes of Valonia discussed in Chapter V. For example, Sr/Ca decreases by a factor of 4 in going from seawater to tuna mucus whereas Ba/Ca and Pb/Ca ratios are bioenriched by factors of 20 and 580 respectively. These ratios do not significantly change in going from mucus to epidermis and from epidermis to muscle. This suggests that Ba and Pb may be absorbed

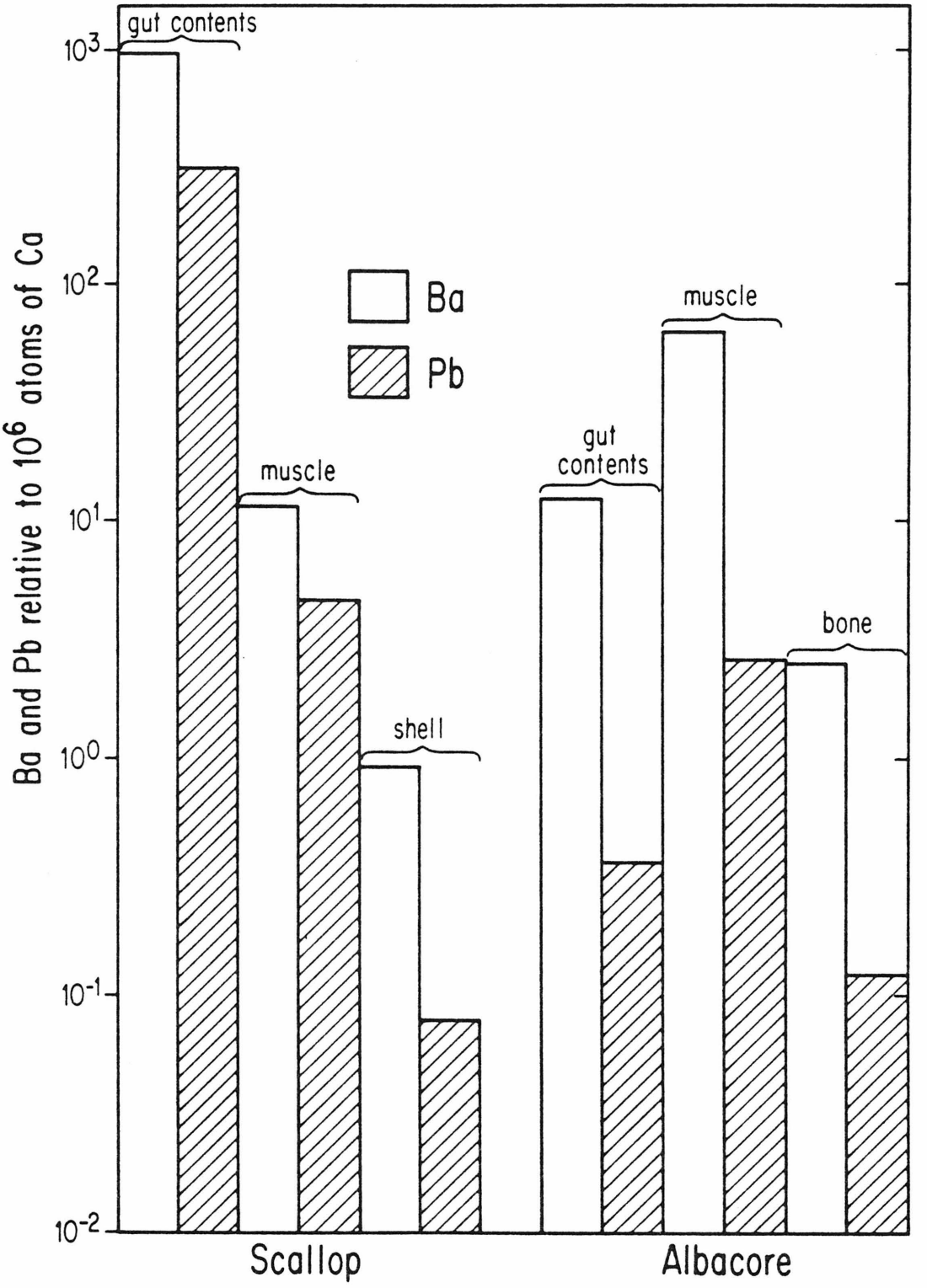

Figure 7.4. Biopurification effects in scallop and albacore in relation to their food sources. This figure shows the greater overall efficiency of Ca biopurification in scallops compared to tuna. Lesser efficiency of Ca biopurification in tuna, despite a precursor food chain, may be indicative of significant absorption of Ba and Pb from bioenriched reservoirs of epidermal mucus. Note the log scale. Figure is based on data of tables 4.8 and 4.13 and work of Patterson and Settle (170).

into muscle and systemic fluids from bioenriched reservoirs localized in epidermal mucus. Absorption of Ba and Pb across epithelial membranes may compete with the efficiency of alimentary absorption processes in biopurifying Ca of Ba and Pb. This could explain why observed biopurifications in tuna shown in figure 7.4 are less efficient than in scallops.

Other factors influential in damping overall biopurification effects in pelagic food chains may be related to feeding patterns. Compared to nutrient-impoverished regions like the open-ocean, the initially larger size and frequently colonial nature of phytoplankton from upwelling regions of high productivity gives rise to an herbivorous microcrustacean population whose large size allows direct consumption by carnivorous fishes without the mediation of a microcarnivore subchain (162). Food chains in high productivity zones thus tend to be shorter than those of nutrient-poor zones. Moreover, during a bloom anchovies often feed directly on globular masses of phytoplankton, and tuna may consume large euphausiids and other zooplankton (191, 219). The general shortening and frequent "short-circuiting" of trophic links in regions of high productivity results in Ca reservoirs of consumers being less biopurified than if there had been more trophic precursors.

7.2 The effect of Pb pollution on the pelagic food web

At present anthropogenic aerosols release about 10 times more Pb into the dissolved phase of North Pacific seawater than was present in prehistoric times (34, 41, 173, 205). This excess Pb enters the pelagic food chain by passive adsorption onto phytoplankton surfaces, as described in Chapter VI. Only minor amounts of Pb appear to be sequentially introduced into the food chain at higher trophic levels because most trace heavy metals are recycled through successive consumers in a similar fashion to organic matter (11,

147, 175). Physiological processes tending to exclude Pb in favor of Ca operate on a fractional basis so long as Pb remains a trace constituent in the reservoir of available Ca (62, 164). For example, the alimentary absorption efficiency for Pb tends to remain constant over a wide range of Pb concentrations in food (62, 164). In marine animals this is indicated by similar Pb/Ca biopurification factors among specimens of the same species collected from waters of different Pb concentration, seen in tables 6.1 and 6.5. Hence the present 10-fold Pb pollution factor is transferred through the pelagic food web as a constant incremental factor (33, 34), rather than increasing during the process of recycling organic matter as is commonly supposed. This is supported by results of this study, illustrated in figure 6.2, which show that Pb pollution effects in benthic grazer systems are transmitted in this fashion.

The impact of present industrial Pb pollution should not be expected to be geographically uniform. The bulk of anthropogenic Pb is transported to the sea as aerosols whose distribution is controlled by the circulation characteristics of the lower troposphere (193). The input of industrial Pb into the North Atlantic from the North American continent is believed to be several times larger than the input of Pb into the North Pacific from the Asian continent (193). Neglecting species differences, the Pb/Ca ratio of organisms from the North Atlantic should be proportionally higher than organisms from equivalent trophic levels of North Pacific food webs. Furthermore, organisms from the Southern Hemisphere should show substantially lower Pb/Ca ratios at all trophic levels than comparable organisms from the Northern Hemisphere, where the bulk of Pb aerosols originates, because the Coriolis effect precludes large-scale transport of aerosol-laden air masses across equatorial boundaries (62, 173, 193).

Chapter VIII
MODEL OF LEAD FLUX IN THE NORTH-EAST PACIFIC

8.1 Observed fluxes of Pb to the ocean

It has been calculated that some 80% of the 10^5 tons Pb/yr presently dispersed into the atmosphere as industrial aerosols is eventually deposited on the sea surface (173, 193). Atmospheric concentrations of 1 ng Pb/m^3 over the North Pacific (43) correlate with aerosol deposition fluxes of 60 ng Pb/cm^2-yr (1500 ng Pb/m^2-day) (193). The present aerosol flux to the sea exceeds the prehistoric flux of fluvial soluble Pb (3 ng Pb/cm^2-yr) by a factor of 20 (the prehistoric aerosol flux was negligible) and exceeds by a factor of 12 the present fluvial input of soluble Pb (5 ng Pb/cm^2-yr) (41, 173, 193). The latter includes a component derived from the "washout" of industrial Pb aerosols deposited on surfaces of the terrestrial biosphere. Present rates of Pb input from the atmosphere correlate with concentrations of 10-15 ng Pb/ℓ in the dissolved phase of NE Pacific seawater, which decrease to 5 ng Pb/ℓ in coastal zones of high biologic productivity (193).

8.2 Isotopic evidence for short residence times of Pb in surface waters

Geographical inhomogeneities in the isotopic composition of authigenic Pb in pelagic sediments, reflecting the isotopic composition of correlative continental source regions, indicate a short residence time of Pb in the upper layers of the ocean (41, 165, 193). Otherwise Pb that was originally soluble would have become isotopically homogenized with Pb from other source regions through rapid horizontal advection and diffusion processes. Radiotracer studies likewise indicate that many trace metals

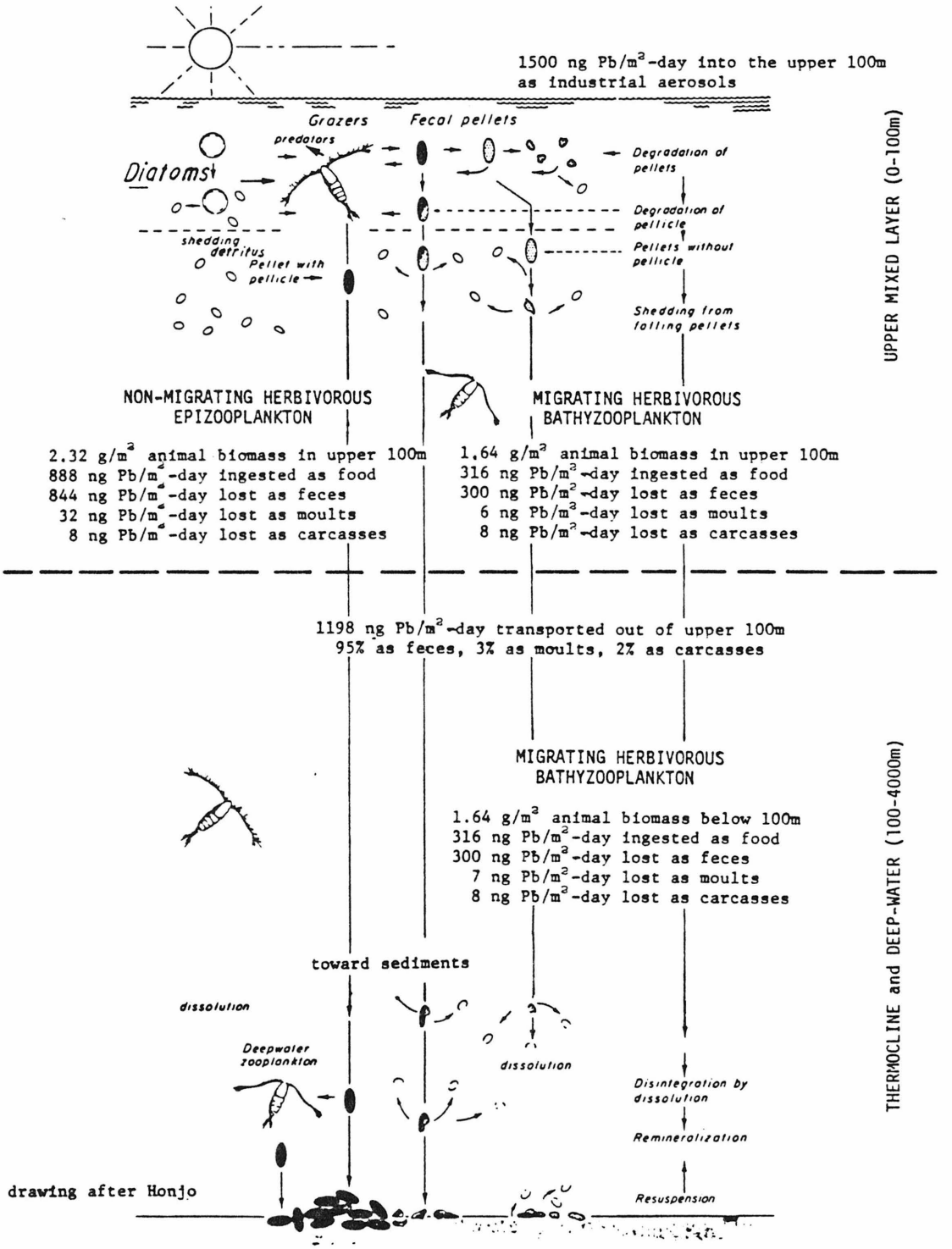

Figure 8.1. Model of Pb flux through the upper 100m of the NE Pacific. The model indicates that 80% of the daily input of industrial aerosol Pb is transported out of the upper mixed layer as a result of zooplankton metabolic activity. 95% of biological Pb transport is by fecal pellets. Non-migrating microcrustaceans are estimated to account for 80% of the fecal pellet flux through the upper 100m. Figure from (5).

are transported to great depth on a time scale that is rapid compared to mixing of water masses (9, 50, 130, 152, 157, 160). Biological transport mechanisms are often postulated to account for these observations (41, 157, 160) because they act on faster time scales and over greater distances than advection, turbulent diffusion, transport along density gradients, precipitation, or other purely physical or chemical processes.

8.3 Biogenic sources of vertical Pb flux in the upper mixed layer

Most investigators agree that Pb-rich detritus is the major vehicle for material transport of Pb out of the surface mixed-layer of the ocean. This detritus has basically only two sources, phytoplankton and zooplankton, because the combined biomasses and productivities of the higher trophic levels of the pelagic marine ecosystem are too small to generate significant quantities of detritus (17, 51, 58, 82, 183, 186, 192, 218). Zooplankton fecal pellets, moults, carcasses, and shells of pteropods, as well as foraminifera, diatoms, and coccolithophorids have all been considered as vehicles for transporting Pb and other trace metals out of the surface layer (36, 68, 102, 103, 104, 142, 213, 235).

While it is likely that all forms of organic matter are partially responsible for transporting Pb out of surface waters, an effective mechanism must provide a sufficiently rapid rate of transfer to satisfy the isotopic relationships outlined in section 8.2. Several studies have shown that the sinking of a fraction of either the biomass of phytoplankton or of algal detritus is too slow to satisfy the geochemical time constraints (102, 134, 160). Furthermore, profiles of particulate organic carbon concentration typically show a drastic decrease below the upper few hundred meters (161, 219). This indicates that phytoplankton and algal detritus tend to remain suspended above the thermocline layer. On

the other hand, zooplankton consumers of phytoplankton generally have such high metabolic rates, food requirements, and rates of detritus production (178, 220) that transport of Pb by large, dense particles of zooplankton detritus can satisfy the geochemical constraints reviewed in section 8.2.

8.4 Model of Pb flux through the upper mixed layer

In the following model, fluxes of Pb through the upper mixed layer of the NE Pacific were calculated by considering plankton biomass and community structure, daily zooplankton rations of phytoplankton, the dependence of phytoplankton Pb concentrations on cell size, the production and the transit rates of zooplankton detritus, as well as particle resistance to degradation. This model is based in part on detailed studies of the structure and the detritus production of the plankton community in the Black Sea (178). Plankton structure in this water mass is similar to that in temperate and subarctic ocean waters in that a few highly productive species are dominant (153). Data on the rate of detritus formation by the herbivorous zooplankton population in the Black Sea (178) were normalized to an accepted value for the average zooplankton abundance in the NE Pacific (127, 128). These data, which are shown in table 8.1 and figure 8.1, agree well with other measurements of zooplankton biomass in the same region (117).

8.5 Model of Pb concentrations in phytoplankton

Pb concentration in phytoplankton, shown in figure 8.1 as a function of size, are predicted from Valonia sorption studies and analyses of cellular subfractions, reported in Chapter IV, Part I, and discussed in Chapter V. It was assumed that the quantity of intracellular Pb remains

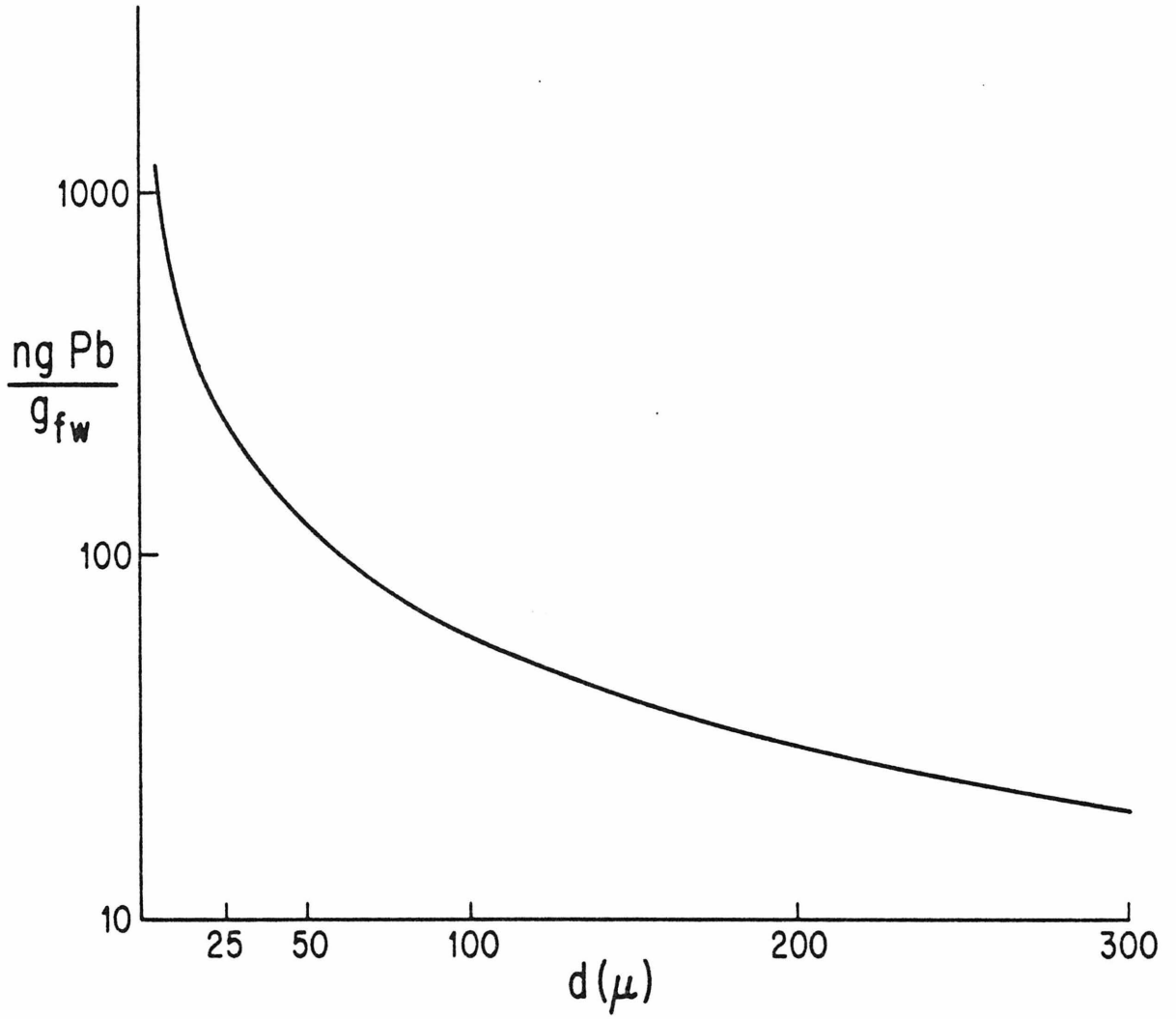

Figure 8.2. Model Pb concentrations in phytoplankton based on studies of Valonia ventricosa. Pb concentrations are plotted as a function of phytoplankton cell diameter in microns (μ) to show how the higher surface area per unit mass of smaller cells produces exponentially greater Pb concentrations in bulk phytoplankton. This model calculation assumes all the Pb is present as the result of passive adsorption onto algae surfaces. The figure is based on data of table 4.3. Note the log scale for Pb concentrations.

relatively constant over the size spectrum of marine phytoplankton (10-300 μm) and that it constitutes only a very minor fraction (<1%) of total Pb in phytoplankton because Pb has not been detected in appreciable amounts in either Valonia vacuolar sap or in the silica frustules of diatoms (143), the major pelagic primary producers. Variations in phytoplankton Pb concentration were assumed instead to result mainly from changes in adsorbent surface area per unit mass. The large increase in calculated Pb concentration with decreasing cell size, shown in figure 8.1, reflects the greatly increased surface/mass ratio of smaller cells. The calculated Pb concentration of a particular size cell was determined by applying the measured adsorption of Pb per unit area for Valonia, reported in table 4.3, to the surface area of 1 g of spherical cells having a density of 1.1 g/cm^3 (56). It was thought that because the ambient concentration of dissolved Pb in the open ocean (15 ng Pb/ℓ) (193) is nearly the same as the water in which Florida Valonia grew, the surface concentration of 0.12 ng Pb/cm^2 that was measured on the surface of Valonia cell wall was probably representative for adsorbed Pb in phytoplankton too. While the presence of perforations and other structures in real phytoplankton would tend to increase the effective surface area per unit mass beyond that of the spherical model, this effect was counterbalanced to some extent by using surface adsorption data from mature Valonia specimens whose thicker cell walls probably contain more leachable Pb than do the thinner walls of small cells.

The model predicts that in well-mixed seawater the largest Pb concentrations will be associated with the smallest cell sizes and the converse. As figure 8.2 shows, the size distribution of cells in bulk plankton samples is probably a very important factor in interpreting data for Pb in phytoplankton. On the basis of the model presented in figure 8.2, open

ocean nannoplankton (10 μm diameter) are predicted to have Pb concentrations of 700 ng Pb/g fw, whereas in coastal or upwelling areas, where microflora (25-50 μm diameter) replace the nannoflora of the open ocean (162), Pb concentrations should be closer to 200 ng Pb/g fw. These estimates agree well with some recent observations (143, 144). However, it should be remembered that in a specific locality, the concentration of Pb in bulk phytoplankton will be a function not only of cell size but also of ambient dissolved Pb concentration and species composition of plankton.

8.6 Zooplankton metabolism and vertical Pb transport processes

When Pb-rich phytoplankton are consumed by zooplankton, Pb in the food is not entirely retained. Ingestion leads to a division of food-Pb into an assimilated fraction (5%) and a fraction voided as feces (95%) (139). A portion of the assimilated fraction is eventually excreted as metabolic wastes and additional Pb is discarded in the form of exoskeletal moults and seasonal egg production until the animal dies. Fluxes of Pb from the various forms of zooplankton detritus, including carcasses, have been tabulated in table 8.2 and are shown in figure 8.1.

The total contribution of zooplankton to the downward transport of Pb was obtained by combining the partial contributions from different forms of zooplankton detritus, as done in table 8.1. Daily zooplankton rations of phytoplankton were first multiplied by Pb concentrations, estimated from figure 8.1, for the discrete size range of phytoplankton consumed by the various members of the zooplankton community. The product thus represents the daily intake of Pb by the zooplankton biomass. The figure for daily Pb intake was then multiplied by a Pb assimilation factor, inferred by analogy with measured zooplankton assimilation effi-

Table 8.1. Estimation of rates of daily Pb ingestion by members of the herbivorous zooplankton community.

Herbivore Group	Wt % Community[1]	Partial Wt[2]	Phytoplankton Feeding Size in Microns[1]	Est ng Pb/g in Food[3]	Daily Ration % Body Wt	ng Pb Ingested per day
Non-migrating Epiplankton						
1) Nauplii	4.2	0.10	15-50	400	140	56
2) I-III Copepodites	23.3	0.54	7-50	700	115	435
3) IV-VI *Paracalanus*	40.5	0.94	20-30	300	45	127
4) *Oikopleura*	27.2	0.63	7-25	700	60	264
5) IV-VI *Calanus*, *Psuedocalanus*	4.5	0.10	15-60	400	15	6
6) Mollusc larvae	0.3	0.01	15-60	400	5	0.2
Total biomass		2.32 g fw				888
Migrating Bathyplankton						
1) Nauplii	4.4	0.07	15-60	400	20	6
2) I-II Copepodites	10.1	0.17	15-60	400	65	44
3) IV-VI *Paracalanus*	3.6	0.06	30-50	200	25	3
4) *Oikopleura*	8.2	0.13	30-50	200	30	8
5) IV-VI *Calanus*, *Psuedocalanus*	72.7	1.19	15-60	400	120	571
6) Mollusc larvae	0.9	0.02	30-50	200	3	0.1
Total biomass		1.64 g fw				632

1) Zooplankton community structure and detritus production rates from Petipa *et al.* (178).
2) Biomasses derived from NE Pacific data of Kuenzler (127, 128).
3) Pb concentrations based on *Valonia* data (this work).

It is assumed that open-ocean zooplankton consume the smallest particles in the feeding spectrum because the range of open ocean nannoflora averages only 10-25 μm (102, 130, 203) and particle size distribution in the sea is skewed toward the smaller particles (130).

Table 8.2. Partial elimination rates by various forms of zooplankton detritus.

Group[1]	ng Pb ingested/day[2]	dietary adsorption factor[3]	ng Pb ingested/day lost as feces[4]	moults[5]	death[5]	soluble excretion[6]	eggs[7]
Epizooplankton 2.32 g/m^2	888	0.05	844	32	8	4	<1
Bathyzooplankton 1.64 g/m^2	632	0.05	600	13	16	3	<1
Total	1520		1444	45	24		

1) Data of Kuenzler (127, 128) for NE Pacific.

2) Determined from table 32.

3) From Patterson (164).

4) Feces provides about 95% of the observed elimination of the Ca-analogue elements Pb (16), Np (83, 84).

5) About 90% of the Pb, Np, and Pu in crustaceans is localized in the exoskeleton (table 17, refs. 83, 84, 231) which comprises approximately 50% of microcrustaceans by mass (10, 98, 158, 220). In epiplankton the rate of moult production is twice as great by mass as the rate of carcass production; in bathyplankton moult production is only 0.4 as great by mass as carcass production (178). Therefore assuming all lead is contained in the exoskeleton, in epiplankton 4 times as much lead is released by moult production as by death, whereas in bathyplankton death accounts for 1.2 times as much as moulting.

6) Daily soluble excretion accounts for about 10% of the elimination of absorbed Ca (10 and Po (209). This lead is not transported out of the mixed layer.

7) Egg production during the season accounts for only 2% of the mass of fecal pellet production (209).

ciencies for physiologically similar radionuclides (10, 84, 98, 231), so that the assimilated and excreted fraction of ingested Pb could be determined. The assimilated fraction was apportioned between tissue masses according to the observed partitioning of Pb-analogue radionuclides in zooplankton (10, 84, 98, 231). The plankton were assumed to be in steady-state with respect to Pb-accumulation, so that when the daily flux of Pb to the various tissue masses is combined with information on the rate of detritus formation, partial elimination rates of Pb from these tissues results. These data are shown in tables 8.1 and 8.2 and are depicted in figure 8.1.

8.7 Comparison of model predictions with observed data

As shown in figure 8.1, the metabolic activity of the zooplankton biomass is estimated to remove some 1200 ng Pb/m^2-day from the upper mixed surface layer of the NE Pacific. This represents 80% of the measured input flux for this region (193). The partial elimination rates show that fecal pellets account for about 95% of the vertical mass flux of Pb. Only minor amounts of Pb appear to be transported by moulted exoskeletons, carcasses, and zoogenic detritus.

Although radiotracer studies (10, 84, 98, 231) indicate that the bulk of biochemically incorporated Pb is contained in zooplankton exoskeletons, the quantities of Pb associated with moulted carapaces appears very small by comparison with fecal pellets because the daily mass production of moults amounts to only 10% of that of Pb-rich fecal pellets (178, 209), even when estimates of surface adsorbed Pb are included, as in table 7.2. By contrast, fecal pellet release provides about 85 to 95% of the observed elimination from crustaceans of many metals, including Pb (16), Po (98), Np (84), Pu (231), Zn (207-209), and Cd (10). Daily

egg production during the reproductive season is a negligible factor, being only about 2% of the mass of fecal pellet production, and daily soluble excretion, which does not involve appreciable Pb transport, amounts to only about 10% of the total body burden of Pb on the basis of euphausiid data (10, 209). The contribution of carcass production to Pb transport also appears to be insignificant by comparison with either fecal pellets or moults (178) since the lifetime of a crustacean zooplankter is generally a year or more (58) and its body burden of Pb is small.

In contrast to other forms of biogenic detritus, surface-formed zooplankton fecal pellets containing well-preserved phytoplankton debris are found in abundance in ocean floor sediments and particle traps at depths up to 5000m (13, 102-104, 196, 213, 235). Feces have been found to account for 99% of the vertical mass flux through the upper 400m of the ocean in areas where they form only about 4% of suspended particles (13). At some stations it has been estimated that more than 90% of phytoplankton remains in ocean floor sediments has been transported by accelerated communal sinking as zooplankton fecal pellets (102, 104).

8.8 Factors affecting fecal pellet fluxes of Pb

Measured fecal pellet sinking rates averaging 150m/day (68, 69) suggest that most of the fecal pellet mass is transported out of the upper 100m mixed layer in less than one day and before bacterial degradation of the casing initially protecting voided feces from dissolution during transport can release large quantities of Pb back into the mixed zone (36, 64, 69, 102, 104, 196, 208, 210, 228). Incorporation of siliceous diatom

frustules, carbonate coccoliths, and ingested mineral grains increases fecal pellet density and results in observed sinking rates that are comparable to the high sinking rates of exoskeletal moults (68, 69). The latter often sink at rates of several hundred meters per day.

The efficiency of Pb transport by zooplankton fecal pellets may be increased by the release of pellets in deeper waters during the vertical migration of larger zooplankton, and it has been suggested that reingestion of fecal pellets by deeper-feeding coprophagous zooplankton may act as a second-stage transport mechanism (64).

8.9 Improving the predictive ability of the model

Although the model agrees well with conclusions of recent mass flux studies emphasizing the importance of zooplankton fecal pellets in vertical mass transport (13, 102-104, 213, 235) and satisfies the geochemical time constraints outlined in section 8.2 (41, 160), adjustments must be made to accommodate variations between this general model and local conditions if this model is to have predictive ability at specific stations. For example, the model is highly sensitive to variations in zooplankton abundance, which may be extreme in any given area even though biomass distribution is much less patchy in the open ocean than in coastal zones of high productivity (124, 229). The model is also sensitive to geographical and temporal variations in Pb input fluxes as well as changes in plankton composition. Pb concentrations in phytoplankton generated by the *Valonia* model fall at the lower end of the range observed in mixed phytoplankton samples, as seen in table 7.1 (143, 144). If these latter findings are reliably confirmed an upward revision of Pb concentrations will need to be made in the model. Some adjustment in Pb concentration predicted for a particular size fraction of phytoplankton is obtained by

modifying the assumption of cell sphericity. This may also be accomplished by correcting phytoplankton Pb concentrations for any difference between ambient dissolved Pb concentrations in seawater at particular stations and that in the media in which Valonia used in this study were grown (16 ng Pb/ℓ). Both of these changes propagate through the model as constant incremental factors affecting Pb concentrations and fluxes. However, whereas the former has no effect on the Pb/Ca ratio in ecosystem reservoirs of Ca, the latter affects this ratio at the primary producer and at all subsequent trophic levels.

Future studies may show Pb concentrations and Pb/Ca ratios associated with phytoplankton and successive consumer organisms are actually lower during periods of high productivity than would be predicted on the basis of cell size and average local concentrations of Pb in seawater. At these times Pb is removed from the mixed layer by adsorption onto phytoplankton and subsequent fecal pellet transfer at a faster rate than in periods of low productivity (193). This depletes available seawater Pb and produces lower Pb/Ca ratios in organisms and lower Pb concentrations in phytoplankton than can be accounted for on the basis of larger cell size during bloom cycles. Pb fluxes on the other hand should increase during times of high productivity.

8.10 Biological removal time of Pb from the upper mixed layer

A calculation was made to assess the effect of biota on the residence time of Pb in surface ocean waters. This is shown in table 8.3. In this table the total zooplankton contribution to the removal time of mixed-layer Pb, designated by R (zooplankton removal time), is the mass of Pb in the water column of the upper mixed layer, C, multiplied by the reciprocal of the rate constant for removal of Pb by zoogenic detritus,

Table 8.3. Calculation of the biological removal time of Pb in the upper mixed layer of the NE Pacific.

The zooplankton removal time may be calculated using the expression[1]:

$$R_z = C/M_zE_z$$

where R_z = zooplankton removal time

C = mass of lead in upper 100 m

M_zE_z = zooplankton removal rate

M_z = zooplankton biomass in upper 100 m of water column

E_z = sum of zooplankton-mediated pathways of lead removal

Pb reservoir[2] of upper 100 m $C = 15 \text{ ng Pb/}\ell \times 10^3 \text{ l/m}^3 \times 10^2 \text{ m} = 1.5 \times 10^6 \text{ ng Pb/m}^2$

Zooplankton removal rate[3] $M_zE_z = 1.2 \times 10^3 \text{ ng Pb/m}^2\text{-day} \times 365 \text{ days/yr} = 4.4 \times 10^5 \text{ ng Pb/m}^2\text{-yr}$

Zooplankton removal time $R_z = 3.4$ yr

1) After Small *et al.* (209) and Heyraud *et al.* (98).

2) Pb concentrations in surface layer from Schaule and Patterson (193).

3) From table 8.1

given by $M_z E_z$. The equation is thus: $R_z = C/M_z E_z$, where M_z is the zooplankton biomass in the mixed layer and $E_z = \Sigma E_i$, the sum over individual pathways of Pb transport by zooplankton detritus (10, 36, 98, 209). The term C is computed by assuming an average depth of 100m for the NE Pacific mixed layer and using recent measurements of 15 ng/ℓ for dissolved Pb in these waters (193). The depth of the mixed layer varies laterally and temporally from location to location as do Pb concentrations, but both values are sufficiently reliable in view of other uncertainties in the model, such as the figure for average zooplankton biomass in the region (127, 128) and Pb elimination rates calculated in tables 8.1 and 8.2.

The biological removal time for Pb estimated in table 8.3 on the basis of the data referred to above is 3.4 years. This value agrees well with independent estimates based on ^{210}Pb studies (9, 152, 194) and mass balance calculations using stable Pb input fluxes (41, 149, 193) that suggest the residence time of Pb in NE Pacific surface waters is about 2.5 years. The concordancy in estimates of the residence time of Pb obtained by purely geochemical studies and the removal time of Pb calculated by considering biological factors implies that zooplankton metabolic processes are important in controlling the occurrence of Pb in the surface layer of the ocean. It appears that the residence time of Pb in the upper mixed layer is largely determined by the removal rate of Pb contained in fecal pellets.

It may be inferred from these findings that anthropogenic perturbations in the natural geochemical cycle of Pb propagate rapidly through the marine hydrosphere because fecal pellet transport mechanisms operate on a time scale of days, which appears instantaneous when compared with the estimated 1000 year residence time of deep seawater. In temperate waters it appears the majority of fecal pellets disintegrate around 300m, a

region of both a particle and a dissolved Pb maximum (193). Differentially disintegrating fecal pellets continuously release Pb over the entire depth range of the ocean. The dissolution of biogenic mineral particulates supplements Pb released by bacterial decomposition of substrates comprising the organic fraction of fecal pellets. These remineralization processes affect Pb concentrations throughout the water column and make it improbable that natural prehistoric concentrations of Pb in seawater exist anywhere in the world today.

Chapter IX
CONCLUSIONS

9.1 Impact of industrial Pb aerosols on the marine biosphere

The techniques and procedures of collection and analysis utilized in this study to overcome problems of artifact contamination in samples containing nanogram amounts of Pb have resulted in measured metal concentrations that are often orders of magnitude lower than reported values widely accepted as typical in marine biota. The discovery that soft tissues of marine invertebrates contain only nanogram concentrations of Pb and hard parts hundreds of nanograms per gram, rather than microgram Pb concentrations as is commonly but erroneously believed, indicates that the reliability of most published analyses of Pb in marine biota must be viewed with extreme caution and that the Pb reservoir in the marine biomass is probably about ten times smaller than was previously thought. The susceptibility of the marine ecosystem to effects resulting from the annual aerosol input of 10^5 tons Pb/yr (193) has thus increased by a corresponding ten-fold factor.

9.2 Role of passive adsorption in marine metal transfer processes

Dissolved Pb, which forms about 90% of total Pb in most marine waters (193), enters food chains through algal sorption processes and is transferred through successive consumer animals. In regions of high concentrations of suspended particles some particle Pb may adhere to algal surfaces, as in zones of high sewage input. However, most particle Pb enters the food chain through filter-feeders and other detritovores.

The bulk of Pb in the biomass of marine algae occurs as a result of effi-

cient competition with Ca for available binding sites of acidic polysaccharides found in algal mucilages. Pb/Ca ratios measured in marine phytoplankton (143, 144) and in kelp agree closely with each other and with the measured ratio of Pb and Ca adsorbed onto the surface of Valonia, a phytoplankton related species of primary producer. Moreover, Pb concentrations measured in phytoplankton agree closely with values predicted from a model based on Valonia surface adsorption experiments in which it is assumed that all Pb present in phytoplankton results from passive surface adsorption.

Brown algae in this study are distinguishable geochemically from the green alga Valonia on the basis of Sr/Ca, Ba/Ca, and Pb/Ca bioenrichment factors for algae relative to seawater. Bioenrichment factors for kelp are generally greater and increase in a sequence different from that of Valonia. Distinct patterns of relative metal abundances observed in both classes of algae correspond closely with selectivities of major anionic matrix polysaccharides constituting the extracellular mucilage of each algal class. Adsorption selectivities of these extracellular polysaccharides appear to determine relative abundances of intracellular Ca, Sr, Ba, and Pb in Valonia as a result of these metals being derived from reservoirs of adsorbed metals residing on Valonia cell wall surfaces.

Passive adsorption of metals from seawater onto organism surfaces constitutes a fundamental biogeochemical difference between marine and terrestrial ecosystems. Large initial bioenrichments of the Pb/Ca ratio in algae relative to seawater and corresponding high Pb concentrations in bulk algae samples characterizes the transfer of Pb from seawater to the primary producer level of marine food chains. It is probable that understanding this mode of metal accumulation in marine plants will yield valuable insights into similar mechanisms of metal accumulation in other

systems, both fossil and extant.

9.3 Role of active transport in marine metal transfer processes

In consumer organisms the bulk of biochemically processed Pb shares coincident localization with Ca in the skeleton as the result of inefficient exclusion of Pb by mechanisms designed to directly regulate Ca metabolism. Pb is thus similar to Sr and Ba because all three metals parallel the metabolic pathways of Ca in organisms. However, natural mechanisms tending to biopurify Ca with respect to Sr, Ba, and Pb deplete these latter metals while absorbing Ca during transport of metals across primary membranes.

Preferential permeability of membranes to Ca relative to Sr, Ba, and Pb acts as a natural mechanism of biopurification at every stage of marine food chains. The results of this study suggest that the efficiency of this mechanism is inversely proportional to growth rate such that the major Ca reservoir of older molluscs tends to be purer of Pb than that of younger specimens from the same locality. In sequential transfers between consumer levels of food chains, product organisms tend to be purer of Pb relative to Ca than trophic precursors. Consequently the process of recycling organic matter by successive transfers between food chain levels does not act as a continuous extraction process in which Pb becomes most concentrated in the oldest organisms at the highest trophic levels, as is commonly but erroneously believed. It appears instead that the initial bioenrichment of Pb relative to Ca at the primary producer level is followed by sequential biodepletions of Pb relative to Ca in subsequent transfers to consumer organisms at successively higher trophic levels of marine food chains. With minor modifications this progression also accurately describes the flow of Sr and Ba through major Ca reservoirs

of marine food chains.

9.4 Effects of Pb pollution on benthic food chains

The effects of increased concentrations of anthropogenic Pb in the seawater media of organisms was investigated in mollusc grazer and filter-feeder food chains. In near-shore regions subject to different levels of anthropogenic Pb input, the Pb/Ca ratio of the kelp food of grazers and the Pb/Ca ratio of the particles in the digestive system of filter-feeders correlate with differences in ambient environmental Pb concentrations. Even though a 100-fold reduction in the Pb/Ca ratio occurs in the transfer of these metals from food to total animal, grazing and filter-feeding molluscs from regions of higher ambient Pb concentrations reflect their increased Pb exposure relative to specimens from regions of lower Pb pollution in the form of higher Pb/Ca ratios in tissues. This is because the flow of Ca is from bioenriched marine plant reservoirs to consumer animals. Variations in ambient environmental Pb concentrations are most accurately preserved in skeletal tissues of animals. The Pb/Ca ratio of skeleton also reflects effects of variations in species, size, and shell mineralogy of specimens collected from a single locality.

There is some preliminary evidence to suggest that in Pb-polluted regions the natural physiological processes of partitioning Pb between shell layers of molluscs may become perturbed by the incorporation into the lattice of crystallites in the aragonite shell layer of Pb that under natural conditions is believed to be localized in the organic shell matrix. If this tentative conclusion is supported by results of future research, it will provide the first documentation that physiological mechanisms tending to biopurify Ca of Pb are being impaired by anthropogenic Pb occurring in seawater at concentrations considered "typical"

and "normal", if not natural.

9.5 Effects of Pb pollution on pelagic food chains

Anthropogenic aerosols present in the atmosphere at concentrations of 1 ng Pb/m^3 release at least 10 times more Pb into the open ocean than was present in prehistoric times. This excess Pb is initially introduced at the primary producer level, and little Pb is sequentially introduced into the food chain thereafter because absorption of Pb from seawater is a negligible route of entry compared to food. Hence the 10-fold differential between the natural prehistoric Pb/Ca ratio in seawater and the higher Pb/Ca ratio typical of present-day marine waters represents a constant Pb-pollution factor that is incorporated into the Pb/Ca ratio of organisms at every trophic level of the marine ecosystem. Thus an average 90% of the Pb in the marine biomass is a by-product of Pb technology.

9.6 Fecal pellet model of Pb transport in surface ocean waters

Plankton biomass, community structure, prey size, Pb concentrations, and detritus production rates were used to model biological fluxes of Pb through the surface mixed layer of the NE Pacific. This model shows that zooplankton metabolic processes are important in vertically transporting Pb out of these waters and towards the sediments. Model calculations show that algal cells, moulted exoskeletons, and zooplankton carcasses are quantitatively minor modes of Pb transport. Instead it appears fecal pellets containing Pb-rich phytoplankton remains account for about 95% of the Pb removed from the upper mixed layer by biological mechanisms. This conclusion agrees with recent mass flux measurements that emphasize the importance of fecal pellets in vertical trace metal transport. Such a mechanism also satisfies time constraints imposed by geochemical mass

balance and isotopic studies.

It is important to note that while Pb appears to be removed from surface waters primarily through fecal pellet transport, this does not mean that Pb contained in surface-formed fecal pellets is quickly removed to the sediments. On the contrary, differentially disintegrating fecal pellets continue to release Pb over the entire depth range of the ocean. Pb liberated by bacterial decomposition of organic substrates in feces and that resulting from the dissolution of biogenic mineral particles affect Pb concentrations throughout the water column, making it improbable that natural Pb concentrations in seawater exist anywhere in the oceans today.

9.7 A final perspective

The findings reported in this work are fundamental to a proper recognition of processes affecting the biogeochemical cycle of Pb, its relationship to the biogeochemical cycles of alkaline earth metals, and the extent of Pb pollution in the marine biosphere. They explain, corroborate, and extend results and conclusions of other reliable investigations. By delineating some important mechanisms controlling the behavior of Pb in marine ecosystems, this study contributes toward a fuller understanding of the nature and magnitude of biological Pb reservoirs and of nutrient pathways linking these reservoirs along which Pb is transferred.

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APPENDIX I

Chapter 2 Transport of Pollutant Lead to the Oceans and Within Ocean Ecosystems

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Transport of Pollutant Lead to the Oceans and Within Ocean Ecosystems

C. Patterson, D. Settle, B. Schaule, and *M. Burnett*

Extent of Industrial Lead Pollution

Concentrations of the toxic metal lead are about 12 μg/g within the earth's crust (Chow and Patterson, 1962), and were ~.004 μg/g wet weight or less within the earth's biomass during neolithic times (Appendix 2A, Note 1). The mining of lead from the earth and dispersal of it as aerosols in smelter fume and auto exhausts has been carried out on such a massive scale during the past century (~130,000,000 tons lead smelted, ~10,000,000 tons lead alkyls burned) that neolithic natural reservoirs and fluxes of lead (~10,000 tons lead in biosphere, ~10,000 tons/yr soluble lead added to oceans, 1000 tons/yr aerosol Pb transferred to oceans) in the biosphere have been completely overwhelmed (Appendix 2A, Note 2). Today the amount of lead mined each year in the world and dispersed by industrial activity (3,000,000 tons/yr) is about 500 times larger than the total amount of lead now circulating annually within the growth cycle of the earth's biosphere (~5000 tons lead/yr). This has elevated by contamination the average concentration of lead in the biosphere by an estimated factor of >20 to ~0.1 μg/g wet weight (Appendix 2A, Note 1). The lead pollution factor is greater for humans ($>10^2$) than for most other organisms in the biosphere because their food chains are contaminated by a large number of additional inputs from industrial sources other than smelter fume and gasoline exhausts (pesticides, coatings, bearings and alloys in food machinery, paints, glazes, food can solder, paper and cloth pigments, pipe cutings, etc.) (Appendix 2A, Note 3). The health hazard to virtually all humans by environmental lead pollution in the form of central nervous system disfunctions, although not yet widely appreciated, is a serious matter (Elias, Hirao and Patterson, 1975), and merits considerable effort in the evaluation of the problem.

Knowledge of the extent and effects of industrial lead pollution of the oceans should have a high priority, even though humans receive much of their lead pollution from interactions with their environment on land. The lead pollution factor for seafood probably ranges from an extimated minimum of ten to an observed ten thousand (Appendix 2A, Note 4). It is important to know both the extent of lead pollution in marine organisms that provide food for humans and the manner in which the pollution was transferred to the organisms. It is perhaps even more important to know what the effects of this massive injection of industrial lead into the oceans at rates far above natural levels has upon the existence of marine life. The oceans are a fundamental unit of the world

ecosystem in which humans live, and we had better find out the ramifications of dumping prodigious quantities of toxic metals such as lead into them. Deleterious effects may be prolonged by release of lead from polluted nearshore sediments and terrestrial soils.

Atmospheric Transport of Industrial Lead to the Seas

The atmosphere is a major route for the transport of heavy metals to the open oceans. Aerosols account for about one third of the industrial lead added to the oceans, as shown by the estimates listed in Table 2-1. About 40,000 tons of these aerosols are added annually by dry deposition and washout from the atmosphere, while about 60,000 tons are added by rivers and sewers as storm runoff from paved surfaces which collected the aerosols on land.

The amount of lead aerosols introduced to the oceans by dry deposition can be estimated from two types of data. The observed atmospheric concentration of lead over the open ocean can be combined with an estimated transfer velocity to yield a deposition rate. A 1 ng Pb/m^3 concentration in the mid-Pacific atmosphere has been observed by Chow, Earl and Bennett (1969). A 3.4 ng Pb/m^3 concentration in the North Atlantic has been observed by Duce, Hoffman, Ray, Fletcher, Wallace, Fasching, Piotrowicz, Walsh and Hoffman (1976). The North Atlantic is much more polluted than oceans in other regions of the earth and an average value over all the oceans of 1.8 ng Pb/m^3 can be found by giving the mid-Pacific value of Chow, Earl and Bennett (1969) a weight of 2 and the North Atlantic value of Duce, Hoffman, Ray, Fletcher, Wallace, Fasching, Piotrowicz, Walsh and Hoffman (1976) a weight of 1. Elias, Hirao, Hinkley, and Patterson (1975) measured a transfer velocity of 0.10 cm/sec for lead aerosols on horizontally oriented plastic surfaces in the Yosemite subalpine region. They also

Table 2-1
Approximate Lead Input for Total Oceans
(In Tons/Year)

Industrial Inputs	
Aerosols (gasoline)	37,000
Aerosols (smelters and forest fires)	3,000
Rivers and Sewers (soluble, mainly from aerosols)	60,000
Rivers and Sewers (solids)	200,000
Neolithic Inputs	
Aerosols	1,000
Rivers (soluble)	13,000
Rivers (solids)	100,000

measured transfer velocities of 0.023 cm/sec for lead aerosols on vertically oriented surfaces in this same region. These investigators believe that the observed large deposition velocities of lead-containing aerosols on horizontal surfaces at this remote site result from on-site agglomeration of Aitkin sized lead-rich particles with much larger non-lead particles. These investigators as well as other (Duce, Turekian, private communication) believe that seaspray-generated micron-sized salt particles may serve to scavenge Aitkin-sized lead-rich particles from the marine air to the sea surface by gravitational settling. It is therefore likely that the measured transfer velocity for lead aerosols on horizontal surfaces in the remote alpine region can be related to mid-ocean regions because the lead particles had traveled hundreds of kilometers through the atmosphere before deposition. However, one is forced to assume, according to experiments of Vittori (1975), that the particle deposition flux on water is about one fourth that on dry surfaces. Therefore, the resulting transfer velocity for lead aerosols of 0.025 cm/sec can be combined with an atmospheric lead concentration of 1.8 ng Pb/m^3 to give a mid-ocean lead aerosol deposition rate of 4.5×10^{-17} g/cm^2 sec. This calculates to 5000 tons Pb/yr added to the world's oceans by dry deposition.

Duce, Hoffman, Ray, Fletcher, Wallace, Fasching, Piotrowicz, Walsh and Hoffman (1976) used a lead dry deposition velocity of 0.25 cm/sec calculated from the data of Cambray, Jeffries and Topping (1975) and multiplied this by their observed 3.4 ng Pb/m^3 concentration at Bermuda and obtained a deposition rate of 8.5×10^{-16} ng Pb/cm^2 sec (part of their model 3). If this deposition is extended to the entire oceans, a value of 100,000 tons Pb/yr results, which is considerably higher than the first calculated value mainly because a larger deposition velocity was used.

Additional inputs of Pb aerosols by dry deposition to the oceans can be estimated by considering the concentrated inputs from near-urban coastal waters as a special case. Studies of atmospheric input to the Souther California Bight by Huntzicker, Friedlander, and Davidson (1975), and by Patterson and Settle (1973), may be summarized as follows. Depositions on horizontal teflon plates were about 45 ng Pb/cm^2/day at urban locations, and about 1.4 ng Pb/cm^2/day at an island location at the outer periphery of the Bight. These and additional data can be combined to yield an average deposition rate of 7 ng Pb/cm^2/day by dry deposition on areas of the Bight. If this rate on plastic surfaces is reduced by a factor of one fourth to account for deposition on water, the rate becomes 1.7 ng Pb/cm^2/day. If we enlarge this strip of urban coastal water to include all such waters 40 km wide surrounding North America, Europe and within the Mediterranean Sea (about 1×10^6 km^2), a deposition of 6000 tons Pb/yr is estimated.

These two estimates of the input of lead aerosols by dry deposition to the

oceans sum to a total magnitude of about 10,000 tons Pb/yr. To this input, contributions by precipitation must be added. There are no reliable published measurements of lead in marine rain at present, and this includes the data of Cambray, Jeffries, and Topping (1975). We can estimate a value from the observed reliable concentration measurements made in recent firn in northern Greenland (Murozumi, Chow, and Patterson, 1969). Snow is at least as efficient a scavenger of lead from the atmosphere as rain in remote regions because lead is contained in Aitkin-sized particles and the outer portions of the snowflake contain large numbers of such particles, while raindrops, because of smaller surface-to-mass ratios, may contain smaller amounts of these particles. Recent preliminary observations show that reliable lead concentrations are approximately equal in snow and rain precipitated at the same time of the year in high mountains (Elias, Hirao, Hinkley and Patterson, 1975). Northern Greenland firns today average about 0.2 μg Pb/kg by weight and if this concentration is assigned to ocean rain, an input of about 60,000 tons Pb/yr is obtained. The input by rain is probably considerably smaller, say half, or 30,000 tons Pb/yr because the observed lead in Greenland firn is the sum total of equal contributions by precipitation and by dry deposition on fallen snow. The combined input of lead to the oceans by dry deposition and precipitation is therefore probably about 40,000 tons Pb/yr.

Another method of estimating total lead aerosol inputs to the oceans is to consider the mass transport of particles from land to sea estimated by Hidy and Brock (1971), Robinson (1976), Prospero (1976) and Goldberg (1971). These estimates vary from 500×10^6 tons of mostly silicate particles (Goldberg, 1971) for deposition in the Pleistocene to 100×10^6 tons of mostly nonsilicate anthropogenic particles (Robinson, 1976) at present times. The Pb/silicate ratio is observed to be about 100 times above natural levels in air particles at remote locations in North America (Elias, Hirao, Hinkley, and Patterson, 1975), in northern Greenland (Murozumi, Chow, and Patterson, 1969), and over the Atlantic ocean (Duce, Hoffman, Ray, Fletcher, Wallace, Fasching, Piotrowicz, Walsh and Hoffman, 1976). This lead/silicate enrichment can be applied to about 100×10^6 tons silicate deposition to the oceans per year. From this flux one can estimate an input of 120,000 tons of lead to the oceans each year by both dry deposition and washout. Of the two estimates for total lead input to the oceans, the 120,000 tons calculated from silicate dust is the least accurate and is probably high according to the more accurate 40,000 tons figure.

Duce, Hoffman, Ray, Fletcher, Wallace, Fasching, Piotrowicz, Walsh and Hoffman (1976) have used the data of Cambray, Jeffries and Topping (1975) in their model 2 to estimate the total input of lead by rain plus dry fallout at their Bermuda site. If we use their Bermuda calculations to apply to all the oceans (model 2 type) an input of 260,000 tons Pb/yr is obtained. This estimate is probably much too high because it requires a high mid ocean concentration of lead in rain of 0.9 μgPb/kg. Even if the model 1 Bermuda calculations of Duce,

Hoffman, Ray, Fletcher, Wallace, Fasching, Piotrowicz, Walsh and Hoffman (1976) are used to apply to all the oceans, an input of 130,000 tons Pb/yr is obtained, which still seems high since it requires a high mid-ocean concentration of 0.4 μg Pb/kg rain. Patterson and Settle (1973) observed an average reliable concentration of 5 μg/kg in four rains falling in the Los Angeles, California, basin where the yearly atmospheric concentration of lead averaged about 3000 ng Pb/m^3 throughout the 5000 km^2 area of the basin. However, it was observed (Elias, Hirao, Hinkley and Patterson, 1975) that rain falling in the Yosemite subalpine study area contained a reliable concentration of 10 μg Pb/kg where atmospheric lead concentrations average 25 ng Pb/m^3. It is believed that high lead concentrations in this type of remote region rain result from downstream precipitation of urban lead generated upstream. Los Angeles basin rain may contain less than average urban amounts of lead because the rain is precipitated from low-lead Pacific air masses before the air becomes heavily polluted. It seems unlikely that the concentration of lead in rain over the oceans would be as high as 1/10th of that in urban rain when atmospheric concentrations of lead over the oceans are 1/1000th of those in urban areas. It therefore seems rather unlikely that atmospheric inputs of lead to the oceans of 130,000 tons/yr or 260,000 tons/yr are realistic because the inputs are calculated by models that require high concentrations of lead in rain.

From the standpoint of mass balance, industrial sources can probably provide an aerosol input of 40,000 tons Pb/yr. About 400,000 tons of lead are burned as alkyls in automotive fuels each year (*Minerals Yearbook*, 1973, plus estimated non-U.S. production) of which approximately one third is widely dispersed in the atmosphere as submicron-sized aerosols. To this must be added contributions of lead aerosols from base metal smelting which amount to about 10,000 tons Pb in aerosols each year. The reentrainment of industrial lead in the form of aerosols by forest fires is also significant. Robinson (1976) estimates that the world mass of forest fire aerosols is about half the world mass of anthropogenic aerosols generated yearly. Elias, Hirao, HInkley and Patterson (1975) estimate that about one kg/km^2/yr industrial lead aerosol input to their study area is deposited on conifer needles and bark. About 75% of the lead on needles is washed off by rain, but most of the lead on bark and surface litter is retained. Their study area was only 15% forested, so it was not anomalous in this regard to rural North America. One can estimate that between 1000 and 10,000 tons Pb/yr are introduced into the atmosphere by forest fires and all of this is reentrained industrial lead originally deposited on foliage by dry deposition.

The potential source of industrial lead aerosols for ocean transport amounts to about 150,000 tons Pb/yr. At the present time reliable mass balance inputs of such industrial aerosols by dry deposition and by precipitation to a defined basin have been made only for one remote terrestrial site (Elias, Hirao, Hinkley and Patterson, 1975) and those studies indicate the following lead inputs

($kg/km^2/yr$): 1.3 by dry deposition; 1.5 by snow; and 1.2 by rain. The total industrial origin of these aerosols is indicated by isotopic relationships, chemical composition, and mass balance considerations. This rate of deposition, extended to the land surface of North America, Europe and Asia, would require 400,000 tons of industrial aerosol lead per year, a figure 2.7 times larger than the potential source. This suggests that the deposition rate at the Yosemite subalpine study area is about 3.6 times higher than the average for the total land mass in the northern hemisphere.

It is highly probable that industrial lead emissions have brought about at least a tenfold enrichment of lead in tropospheric aerosols in remote land, polar, and mid-ocean regions. A 2500-fold enrichment of lead above crustal silicate values observed at the South Pole in tropospheric aerosols is believed to be partially anthropogenic (Zoller, Gladney and Duce, 1974). Volcanic emanations are also believed responsible for part of the enrichment (Zoller, Gladney and Duce, 1974). Hundredfold enrichments of Pb above crustal silicate values have been observed in mid-Atlantic marine aerosols, and variations in these enrichments correlate with downstream air trajectories from urban regions generating large amounts of industrial lead aerosols, indicating anthropogenic causes of part of the enrichments (Zoller, Gladney and Duce, 1974). Wave-generated aerosols enriched in microlayer heavy metal constituents, are held responsible for some of the observed lead enrichment (Zoller, Gladney and Duce, 1974). Hirao and Patterson (1974) observed 100-fold enrichments of Pb above crustal silicate values in aerosols and snow at the Yosemite subalpine study area in 1973. 90% of this enrichment was conclusively shown to be anthropogenic on the basis of isotopic tracers, because, fortunately, the isotopic composition of lead in California mountain snow collected in 1962 had been measured by Patterson and colleagues (Chow and Johnstone, 1965). The Pb^{206}/Pb^{207} ratio in California mountain snow changed from 1.144 in 1962 to 1.183 in 1973. This change coincides with the change observed by Chow, Snyder and Earl (1975) in both gasolines and lead-rich urban aerosols derived from gasoline in San Diego during this same time period. The isotopic change was brought about by the introduction of increased amounts of Missouri lead into the U.S. pool of industrial leads. These isotopic tracers are insensitive to more than an order-of-magnitude effect, so that only part of the observed 100-fold enrichment can be ascribed by the isotopic method to anthropogenic causes.

A greater than 100-fold enrichment of lead above crustal silicate values in present-day firn in Greenland near 80° north latitude has been demonstrated by Murozumi, Chow and Patterson (1969). Here the entire two-order-of-magnitude change has been definitely ascribed to antropogenic causes, since an increase of this size with time was observed in dated layers of ice. This interpretation rests on the significance of an extremely low lead concentration ($<1 \times 10^{-12}$ g Pb/g ice) observed by Murozumi, Chow and Patterson (1969) in a block of 3000-year-old ice cut out under ultra-clean conditions from the edge of the ice

sheet and dated by C^{14}. This snow was deposited in the interior, beyond the ablation zone, 3000 yrs ago. The concentration of silicate dust in this ice was about the same as that in the interior firn sampled for more recent lead concentrations, where the 1965 layer contained 210×10^{-12} g Pb/g ice. This can be regarded as conclusive evidence that the entire ~100-fold lead enrichment over crustal silicate values in tropospheric aerosols is anthropogenic.

It is improbable that volcanic emanations can account for the ten-fold enrichment of lead above crustal silicate values in tropospheric aerosols, which some investigators suppose is a natural enrichment underlying a further anthropogenic ten-fold enrichment. The amount of lead contributed to the atmosphere by volcanic gases seems to be less than a 100-ton standing crop out of a 5000-ton total troposphere standing crop of lead. The latter figure is obtained from the 2 ng Pb/m^3 open ocean atmosphere concentration. The 100-ton upper limit number can be estimated in two ways.

One is to consider the total volume of lava emitted by volcanoes above sea level each year and to consider that the fraction of the lead in the lava that is volatilized may be about 10%. Figures which can be used are 0.5 km^3 lava/yr (Verhoogan, 1946; Sapper, 1927), 12 μg Pb/g concentration, 10% volatilization of lead, and an atmospheric residence time of two weeks. The amount of lead which can be introduced into the atmosphere by this process is limited by mass imbalances that would be created between the sedimentary cycle and the igneous cycle after a prolonged time of operation if the fraction of lead volatilized is too great.

The second method is based upon recent determinations of the concentrations of lead in two samples of volcanic fume from Hawaii (Cadle, Wartburg, Pollock, Gandrud, and Shedlovsky, 1973). These also yield an average crop of about 100 tons. In one instance the lead/sulphate ratio was 3.2×10^{-5} and in the other instance it was 7.8×10^{-4}. The following figures were used: a half km^3 of lava issued above sea level world-wide per year, the fraction of lava which is gaseous fume was set equal to half percent (G. Macdonald, private communication), 80% of the fume was assigned to sulphate (Anderson, 1975), and a two week atmospheric residence time was assumed. Cadle's lead values are upper limits because of possible high errors due to lead contamination effects.

At the present time we cannot estimate the mass balance input of anthropogenic and natural lead aerosols to the ocean from the difference between the total generated production of lead aerosols and the total deposited on land because the rates of generation of natural lead aerosols from volcanic and soil sources, and rates of land deposition are not known with sufficient accuracy. Considering the greater plane projected surface areas of the oceans compared to land, the apparently great significance of precipitation removal mechanisms, and the large dispersal range of industrial lead aerosols, it seems highly probable that an appreciable fraction of the annual 150,000 tons of industrial lead aerosol production is added to the seas. That is, a yearly input to the seas of

40,000 tons of industrial lead associated with aerosols seems possible on a mass balance basis.

As shown in Table 2-1, it is estimated that the present annual rate of atmospheric lead addition to the oceans exceeds the former neolithic rate by a factor of about 40, that the increase is probably due to industrial additions, and that the major impact is on the concentration of freely available lead in the mixed zone.

Dissolution of Anthropogenic Particle Lead in Seawater

In order to measure the dissolution fluxes of particle lead, knowledge of the speciation, distributions, and rates of dissolution must be obtained. Until 1975 knowledge of the speciation and concentration of lead in seawater was not reliable despite decades of measurement (Participants of the Lead in Seawater Workshop, 1974). Contamination of seawater with industrial artifact lead has been widespread in the past during collection, handling, and analysis, yielding data that were wrong on the high side. The work by Tatsumoto and Patterson (1963a, 1963b) and Chow and Patterson (1966) which gave the proper perspective of the occurrence of lead in seawater may have been adversely affected by artifact contamination during collection. True concentrations of lead in mid-ocean waters are probably less than those present in laboratory distilled waters (the latter range from 5 to 50 ng Pb/kg.

Investigators have published theoretical estimates of the speciation of lead in seawater based on stabilities of common possible soluble complexes; however, the interaction of dissolved lead with both living and inanimate particles may be the most important factor determining the practical aspects of lead speciation in seawater.

Lead concentrations in coastal waters near urban regions range from 25 ng Pb/kg in surface samples of ordinary coastal water to 150 ng Pb/kg in waters highly polluted with sewage (Patterson, Settle and Glover, 1976). Studies of lead in sewage effluent from Los Angeles show that virtually all the lead in sewage is contained in the particle phase before it enters the ocean but that about 11% is made freely available within a day by cation exchange when the sewage is mixed with seawater. Essentially no more dissolved lead is released from the sewage particles even after weeks of exposure (Patterson, Settle and Glover, 1976). Observers have studied the displacement of other heavy metals in particles by exchange with cations in seawater (Johnson, Cutshall and Osterberb, 1967; Kharkar, Turekian and Bertine, 1968; Evans and Cutshall, 1973). Investigations carried out by European workers on the dissolution of lead from particles into seawater along the shores of the North Sea are unreliable because of analytical error. Lead in the filtrate of seawater passed through a 0.4μ cellulose acetate filter or extracted by dithizone in chloroform from untreated seawater

has been designated freely available lead. The amounts of lead measured by these two methods are nearly identical for parallel aliquots of seawater (Patterson, Settle and Glover, 1976). As the proportion of sewage lead in seawater increases, the fraction of freely available lead decreases. For example, surface seawater containing ~200 ng Pb/kg total was found to contain 29% freely available lead, while surface seawater containing 110 ng Pb/kg was found to contain 42% freely available lead (Patterson, Settle and Glover, 1976). It is believed that in areas of high sewage pollution (>50 ng Pb/kg) most of the particle lead in the waters is associated with sewage, not plankton.

Total lead concentrations appear to decline to 30 ng Pb/kg levels before contributions of plankton particle lead become significant. Single measurements of total lead (determined after evaporation and dissolution with aqua regia) in surface waters from the Straits of San Juan de Fuca and from outside the Southern California Bight showed 24 ng total Pb/kg at the first location and 25 ng total Pb/kg at the second. However, the proportion of particle lead ranged from 90% at the first location to less than 3% at the second (Patterson, Settle and Glover, 1976; Schaule, unpublished). Surface waters off La Jolla showed 36 ng total Pb/kg during a dinoflagellate phytoplankton bloom and 16 ng total Pb/kg when the water was unusually clear. About one third of the lead associated with the phytoplankton was contained in chiton (Patterson, Settle and Glover, 1976). Some total reported lead concentrations in seawaters are listed in Table 2-2.

The isotopic compositions of total leads in coastal waters indicate in some instances that more than one kind of industrial lead was present in the waters and that they were not well mixed (Patterson, Settle and Glover, 1976). The

Table 2-2
Measured and Estimated Total Leads in Seawater, (Aqua Regia Dissolution)

Location	*Date*	*Depth (m)*	*ng Pb/kg*
Los Angeles (above JWPCP outfall)	April 28, 1975	0.2	230
Los Angeles (above JWPCP outfall)	March 25, 1974	0.2	(200)[a]
Los Angeles (above JWPCP outfall)	March 25, 1974	7	(330)
Los Angeles (above JWPCP outfall)	March 25, 1974	30	(1300)
Los Angeles (above JWPCP outfall)	April 9, 1973	0.2	(110)
La Jolla (5 km west of Scripps Pier)	Jan. 13, 1976	0.2	36
La Jolla (5 km west of Scripps Pier)	Nov. 1, 1972	0.2	(16)
San Juan de Fuca Straits	July 29, 1975	0.2	24
50 miles southwest of Los Angeles[b]	Feb. 29, 1976	0.2	25

Source: Patterson, Settle and Glover (1976).

[a]Concentrations in parentheses are not observed, but are calculated by multiplying measured concentrations of aliquots poured from carboys by 1.5 to correct for wall adsorption.

[b]Schaule, unpublished.

Pb^{206}/Pb^{207} ratio of total lead in coastal surface seawater collected near Los Angeles was 1.194 (concentration ~100 ng Pb/kg) and a day later was 1.188 (concentration ~70 ng Pb/kg) near La Jolla. A difference of 0.2% between values of this ratio is significant. It is believed that the above difference shows a lack of mixing of contributions from two different sources of lead pollution: one was a pulse of rain storm runoff of gasoline lead (Pb^{206}/Pb^{207} observed to be ~1.197) from paved surfaces added to Los Angeles waters and the other component was sewage lead (Pb^{206}/Pb^{207} estimated to be ~1.188) from San Diego added to La Jolla waters.

Interaction of Seawater Lead with Marine Organisms

Contrary to common opinion, lead is not necessarily concentrated in higher organisms as it is transported to the higher ends of food chains. In marine ecosystems there are two different processes competing with each other in the transport of lead along food chains: passive absorption of lead from seawater by chelating agents on organism surfaces to form stable lead complexes (enrichment process); and inefficient active transport of lead across cell membranes (depletion process). To distinguish between these opposing effects in marine organisms, it is important for mass-balance, morphologic distributions of lead among different tissues of the various organisms in food chains to be worked out. The enrichments or depletions of lead should not be expressed in such simple terms as concentrations of lead in water or in wet, dry, or ashed tissues. The actual enrichment or depletion of lead is better expressed as a change relative to the more or less fixed bulk of calcium, which is an abundant nutritious metal that flows easily along food chains, and is biochemically similar to lead in many respects. It is revealing to study the biodiminutions and bioamplifications of both barium and lead, since barium is not yet a serious pollutant in the marine environment, and observed differences between the distributions of barium and lead in food chains give clues regarding lead pollution effects (Hirao and Patterson, 1974).

Lead, barium, strontium and calcium have been studied by isotope dilution clean-laboratory techniques in a seawater-kelp-gastropod food chain (*Macrocystis pyrifera*, and *Norrisia norrisii*) (Burnett, unpublished). The data indicate that Sr, Ba, and Pb are enriched relative to Ca in going from seawater to total kelp blade, Sr/Ca increasing by 8, Ba/Ca increasing by 20, and Pb/Ca increasing by 2000. A major fraction of kelp consists of an alginic acid-rich binding matrix, holding the kelp-blade cells together. The strengths of alginic acid-metal complexes have been determined (Haug, 1961) and the relative stabilities of those complexes are $Pb \gg Ba > Sr > Ca$. The binding matrix is in contact with seawater during and after growth, and it is believed that the relative enrichments cited above, which parallel the relative strengths of the metal complexes, show passive adsorption of Pb, Ba, Sr, and Ca from seawater by the alginic acid-rich matrix.

The gastropod that was studied feeds on kelp, cutting down into and consuming the blade material (Leighton, 1971). Using the composition of total kelp blade as gastropod food, Sr, Ba and Pb are depleted relative to Ca in going from kelp to total gastropod, Sr/Ca decreasing by 70, Ba/Ca decreasing by 600, and Pb/Ca decreasing by 150. Analyses of separate organs of the gastropod indicate differences in metal depletions. The biodiminutions cited above refer to shell material, but they also apply to total gastropod because the bulk of metals in the gastropod are in its shell. The situation is different for muscle tissue, Sr/Ca decreasing by 10, Ba/Ca decreasing by 10 and Pb/Ca decreasing by 2, in going from kelp to gastropod muscle.

Comparison of the distributions of alkaline earths and lead in tuna and terrestrial animals provide insight into lead pollution in marine animals. Ninety-five percent of the Ca and Sr, and 75% of the Ba and Pb in tuna are contained in the skeleton (Patterson and Settle, 1976). These distributions, when compared to those in a terrestrial carnivore (*Martes americana*) (Elias, Hirao, Hinkley and Patterson, 1975), are nearly the same. The surface of the fur of the terrestrial carnivore contains large amounts of dry deposition aerosol lead. A tuna was observed to contain unusually high concentrations of lead in epidermal mucus which is believed to originate from artifact contamination by fishermen (Patterson and Settle, 1976).

Although the morphological distributions of the alkaline earths and lead are nearly identical in these terrestrial and marine carnivores, the concentrations are quite different. The Ba/Ca and Pb/Ca ratios in tuna are about one tenth of those in marten because much smaller amounts of Ba and Pb are associated with the Ca in seawater than with the Ca in terrestrial soils, which is shown in Table 2-3. This does not mean that there is a greater biodiminution of Ba and Pb in albacore than in marten. The total biodiminution is a factor of 1000 for both Ba and Pb relative to Ca in going from rock to carnivore in the marten food chain (Elias, Hirao, Hinkley and Patterson, 1975). In albacore, Ba/Ca is

Table 2-3
Concentrations (μg/g) of Elements at Lowest and Highest Trophic Levels of a Marine and a Terrestrial Food Chain

	Ca	*Sr*	*Ba*	*Pb*[a]
Seawater[b]	400	8.1	0.03	0.00002
Albacore (body burden-wet)	8800	36.	0.1	0.008
Wall Rock[c]	12000	510	1100	22.
Marten (body burden-wet)[c]	15000	9	1	0.15

[a]Surface lead deposited on the organism has been excluded from these values.

[b]Goldberg (1963).

[c]Elias, Hirao, Hinkley and Patterson (1975).

biodiminished only by a factor of 10 in going from seawater to fish, while Pb/Ca is bioamplified by a factor of 10. This marked contrast in the food chain transport of lead between albacore and marten may be due to passive adsorption effects in the marine ecosystem.

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Appendix 2A

1. Ninety-five percent of the earth's biomass resides in forests (Bowen, 1966). Most published data regarding lead in trees are either erroneous because of laboratory contamination or apply only to excessively polluted urban foliage. A careful mass distribution study of lead in a forested ecosystem has been carried out at a remote subalpine location within Yosemite National Park (Elias, Hirao, Hinkley and Patterson, 1975). Eighty-five percent of the lead in that ecosystem was contained in igneous minerals in the thin soil lying on bedrock, 15% of the lead was in soil humus, and 0.3% of the ecosystem lead was in the total biomass. Ninety-eight percent of the biomass consisted of coniferous trees which contained an average of 0.09 μg Pb/g. The average lead concentration of the remaining 2% of the biomass was about 0.3 μg/g. Ninety-five percent of the lead in the biomass of the ecosystem was therefore found to reside in trees. About 85% of the lead in the trees was located on a thin outer layer of bark while 10% of the tree lead was located in foliage (mainly on needle surfaces). The mass distribution of lead in this remote subalpine ecosystem indicates that most of the lead in the biosphere of the northern hemisphere is contained in the outer bark of trees. In the subalpine ecosystem that was studied more than 90% of the tree lead is surficial, and it originated from industrial aerosols, as was shown by aerosol deposition measurements and chemical washing experiments. A major fraction of the remaining 10% is also industrial because isotopic and mass balance data indicate that much of the lead in soil humus originated from industrial aerosols, and the deposition of industrial lead aerosols on foliage is so excessive that the magnitude of foliar uptake of industrial lead probably approaches root uptake. Therefore the neolithic concentration of lead in trees (earth's biosphere) was equal to or less than about 0.004 μg/g, or considerably less than one tenth the present concentration of 0.09 μg/g. Since half of the earth's biomass consists of tropical forests and the concentration of lead in the bark of these trees has not been measured, the concentration of lead in the earth's biomass is still somewhat uncertain.

2. The contamination of the earth's biosphere by industrial lead from smelter fumes is a process that extends about 4000 years back in time, involving the primary smelting of about 70,000,000 tons of lead before A.D. 1850 (Elias, Hirao and Patterson, 1975). The per capita production of lead within the Roman Empire was about one fifth of that within today's industrialized nations. People within ancient civilizations ingested industrial lead at rates comparable to those of today because of various common practices, such as putting lead-rich additives in green wine to inhibit souring, a process that began before 600 B.C. and continued until A.D. 1850.

3. Studies of metal distributions in food chains within the Yosemite subalpine ecosystem (Elias, Hirao, Hinkley and Patterson, 1975) indicate that lead is

naturally biodiminished relative to calcium by a factor of about 1000 in going from rock to herbivore and carnivore in the absence of lead pollution. Lead in the typical U.S. person is biodiminished relative to calcium only by a factor of about 2.5 from rocks that supply metals to human foods, indicating a typical lead contamination level about 400 times above neolithic natural levels (Elias, Hirao and Patterson, 1975).

4. The concentration of freely available lead in surface seawaters has been elevated by industrial lead pollution by about 10, according to estimates of the increased input of soluble-type lead to the oceans (10,000 tons/yr neolithic input from rivers, vs. 40,000 tons/yr atmospheric industrial input plus 60,000 tons/yr industrial lead from aerosols deposited on pavements and roofs and washed into the oceans by storm runoff and rivers). Aerosol lead from gasoline exhausts is much more reactive than lead in natural silicate clays, so that it contributes most of the freely available lead in seawater. The residence time of freely available lead in surface water is very short. According to inferences from lead isotope areal patterns in sediments (Chow and Patterson, 1962), the residence time should be less than a year. This means that freely available lead concentrations in surface waters responded quickly to increased industrial inputs.

The absolute concentration of lead in albacore muscle is exceedingly small (0.0003 μg Pb/g wet weight), and in the process of preparing this tissue for human consumption, excessive contamination factors are involved (grocery store tuna is 0.5 μg Pb/g wet weight) (Chow, Patterson and Settle, 1974). Since it is probable that lead concentrations in the upper waters of the oceans have been elevated by a factor of 10 by industrial lead pollution, the overall lead pollution factor for tuna muscle, a widely consumed human food, is about 10^4.

APPENDIX II

THE SOUTHERN CALIFORNIA BASELINE STUDY AND ANALYSIS FINAL REPORT

Volume III Chapter 4.4

Sections 4.4.4 and 4.4.5

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STANDARDIZATION OF REFERENCE SAMPLES FOR CERTAIN TRACE METALS

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4.4.4 Barium and Lead Analyses of Biological Material

4.4.4.1 Sample Collection and Preparation of References

Kelp and abalone were collected at two different locations in the Southern California Bight. In late February, specimens of the kelp Eisenia arborea and the pink abalone Haliotis corrugata were collected at the south end of Cortes Banks at a depth of 40 m in an area where E. arborea was the dominant macrophyte and H. corrugata the dominant prosobranch gastropod. This region lies within the outer continental shelf, and in an area of natural oil seeps. In early May, samples of the giant kelp Macrocystis pyrifera and the black abalone H. cracherodii were collected at Abalone Cove on the Palos Verdes peninsula in 5 m of water, an area where both are the predominant species of their kind. This region lies within, at present, the most polluted portion of the Southern California Outer Continental Shelf. It is adjacent to the White's Point sewage outfall, which serves Los Angeles County.

To avoid contamination during collection, transport, and processing, all samples were collected upwind and upcurrent of a diesel-powered research vessel by divers wearing

polyethylene gloves and epoxy-painted weight belts. Abalone were pried from the rocks using a stainless steel tool, while kelp blades were hand-gathered. The samples were double-bagged in acid-cleaned polyethylene beneath the sea surface prior to transfer through the air-sea interface, whereupon they were placed in dry ice chests for storage. To prevent contamination during transport to the CIT freezer, where they are stored before processing for analysis, the sample containers were sealed in another plastic bag.

The largest of the abalone collected at Cortes Banks was chosen for standardization analysis. This single specimen constituted the BLM-CB H. corrugata reference. It was a heavily encrusted mature adult, weighing 1650 g. The two abalone from Abalone Cove were much smaller, weighing ∿225 g each, unencrusted, and believed to represent adolescents or young adults. A single abalone constituted the BLM-AC-A abalone reference, while the BLM-AC-B abalone reference was prepared from a second specimen.

Dissection of frozen abalone was required in order to isolate the muscle tissue with minimal transfer of contamination. The main muscle was severed at its point of

attachment to the shell with a curved, stainless steel spatula inserted between the mantle and the shell. Repeated blows with a stainless steel hammer were needed to drive the spatula through the frozen material. Using a scalpel, the internal organs and head were carefully dissected out as a unit and kept frozen for later dissection. Contamination of the muscle tissue by epidermal material, whose metal concentrations are high, was avoided by shaving away the outside surfaces of the frozen material in successive stages. Cleaned stainless steel dissecting instruments, polyethylene gloves, and teflon dissecting platforms were renewed before each later shaving stage. While one sample of muscle was desiccated prior to aliquoting, it was preferable to cut the frozen muscle into aliquots before desiccation to promote quicker drying. In vertical cross section, blood vessels are larger and more numerous near the foot-surface of the muscle. All outer portions of the muscle were cut away, leaving only the central core for analysis.

Each aliquot of a given abalone reference consists of a number of randomly chosen small pieces of vacuum-dried muscle from a single specimen contained within an ultra-

cleaned plastic bottle, which, in turn, is sealed within an acid-cleaned plastic bag. The water content of the muscle is given on the bottle label. The abalone reference should be stored frozen to prevent putrefaction.

Kelp samples were prepared by rinsing each blade with a jet of QDW to remove seawater and grossly large adherent particles. The Macrocystis blades, which lost a few percent of their volume by exuding a mucilaginous liquid during this cleaning, were shaken to remove excess QDW and weighed into a clean FEP teflon beaker with cover. No similar loss was observed during the preparation of the Eisenia reference.

Loss of adsorbed Pb during the rinsing process was insignificant as the mucilaginous liquid was found to contain only 1-2 ng Pb/g fw.

A 25% slurry of wet kelp in concentrated HNO_3 was made so that homogeneous aliquots could be distributed. The slurry was prepared by heating a 1 to 3 weight mixture of kelp and HNO_3 in the FEP teflon beaker in an atmosphere of pure, dry N_2 at 100° for 3 days until a transparent yellow liquid was obtained. The only remaining solids consisted of numerous tiny, very short filaments of either pseudo-cellulose or silica needles, which were readily divided among various aliquots.

The slurry was poured into ultra-cleaned FEP bottles that had been refluxed with ultra-pure concentrated HNO_3, but which were not rinsed with dilute acid. These were sealed within acid-cleaned plastic bags. The weight fraction of equivalent wet kelp in the acid slurry is given on the bottle label. The kelp is not completely oxidized in the slurry, and an acid dissolution procedure must be completed on this reference when it is analyzed. Presently available information indicates that the concentrated acid will not leach significant amounts of Pb or Ba from the bottle within a year. Do not add water to the reference in the bottle. If the slurry is diluted with water in the bottle, serious contamination from bottle walls will probably result, both immediately and upon standing.

4.4.4.2 Methods of Analysis

4.4.4.2.1 Analysis of Plant Tissue

A 2 g aliquot of the partially dissolved slurry was spiked with Pb^{208} and Ba^{136} in a 30-ml FEP beaker. The dissolution was completed by adding 0.5 ml $HClO_4$, digesting covered for 2 hours at 80°C in an atmosphere of particle-free dry N_2, and then increasing the temperature to 150°C and evaporating

to dryness uncovered. HNO_3 was added drop-wise to prevent charring during the fuming of $HClO_4$. Marine algae contains enough silica to cause dissolution problems, and it should be deliberately removed. One ml HF was added to the cooled residue and the beaker was covered and allowed to stand overnight at room temperature. The beaker was then heated (covered) for three hours at 60°C. The cover was removed, and the solution was evaporated to dryness at 90°C. The pure white crystalline residue was dissolved in 1 ml HCl and evaporated to dryness twice, then dissolved in 5 ml of 0.5 N HCl. A 10% aliquot was removed for the barium analysis; the remaining 90% was used for the lead analysis.

The Pb aliquot was transferred to an FEP separatory funnel and adjusted to pH 7 with NH_4OH. 1 ml 25% ammonium citrate and 2 ml 1% potassium cyanide were added, and the lead was extracted with 5 ml of dithizone-chloroform. The Pb was then back-extracted into 10 ml of 1 N HNO_3, the pH was adjusted to 8, and a second dithizone-chloroform extraction was done without complexing agents to minimize the blank correction. The chloroform solution was washed with 0.02 N NH_4OH and then back-extracted into 5 ml 1 N HNO_3. The aqueous solution was washed once with 5 ml chloroform and

then evaporated to dryness in a 30-ml FEP beaker. The residue was dissolved in QDW and loaded onto an outgassed rhenium filament with a slurry of silica gel and H_3PO_4. The Pb^{208} to Pb^{207} ratio was measured in a mass spectrometer to determine the Pb concentration. Typical lead blanks were about 0.15 ng for the dissolution, 0.28 ng for the 1st dithizone extraction, and 0.17 ng for the 2nd extraction. Extraction yields were 90 to 95%.

The aliquot removed for Ba analysis was evaporated to dryness and dissolved in a minimum amount of QDW. A portion of the solution was loaded onto an outgassed oxidized tantalum filament. Ba blanks were about 0.4 ng for the complete analysis. The Ba^{138} to Ba^{136} ratio was measured in a mass spectrometer to determine Ba concentration.

4.4.4.2.2 Analysis of Animal Tissue

A 0.3 g aliquot of dried abalone muscle, equivalent to approximately 1 g fresh weight, was spiked with Pb^{208} and Ba^{136} in a 30-ml FEP beaker. Three ml HNO_3 and 1 ml $HClO_4$ were added, the beaker was covered, and the mixture allowed to digest 3 hours at 55°C in an atmosphere of pure, dry N_2. The temperature was then raised to 150°C, the solution was digested (covered) for 3 hours, the cover was removed, and

the solution was evaporated to dryness. One ml HF was added to the cooled residue, it stood covered overnight, and was then digested covered at 55°C for 2 hours. The cover was removed and the solution was evaporated to dryness at 150°C.

The white, crystalline residue was dissolved in 1 ml HCl and taken to dryness twice. The residue was then dissolved in 5 ml of 0.5 N HCl and a 10% aliquot was removed for Ba analysis. The remaining 90% was used for lead analysis. The lead analysis consisted of two dithizone-chloroform extractions, the first with complexing agents and the second without complexing reagents. The amounts of reagents were the same as those used in the analysis of plant tissue described above, as was the measurement of the Pb^{208} to Pb^{207} ratio in the mass spectrometer. Blanks and yields were also approximately the same as those for the plant tissue.

The 10% Ba aliquot was treated in the same way as the Ba aliquot of plant tissue. Blanks were also approximately the same as for plant tissue.

4.4.4.3 Results

Since the kelp references were acid slurries of digested material, it is probable that they were homogeneous. Duplicate analyses of these references resulted in differences in Pb and Ba values that were within experimental error of the method. Results are listed in Table 4.4-9.

Concentrations of Pb in abalone muscle appeared to be uniform within an individual, and differences in Pb values of duplicate analyses of these references were within experimental error of the method. The standardized values of Pb in abalone muscle are listed in Table 4.4-10. Results of Pb and Ba determinations for these references in laboratories analyzing field samples are compared with standardized values in Table 4.4-11.

A standardized value for Ba in abalone muscle has not yet been determined because repeated analyses gave scattered results. It is not yet known whether the scatter is due to sample heterogeneity or faulty analytical technique. Observed concentrations of Ba in various pieces of abalone muscle are listed in Table 4.4-12.

Table 4.4-9. Standardized Pb and Ba concentrations in kelp references.

Sample	Run #	ng Pb/g fw	ng Ba/g fw
Cortes Banks (lower pollution)			
BLM-CB *Eisenia* (0.3071 g kelp/g soln)	1	50	2000
"	2	45	2100
Abalone Cove (higher pollution)			
BLM-AC *Macrocystis* (0.2591 g kelp/g soln)	1	54	780
"	2	55	800

Table 4.4-10. Standardized Pb concentrations in abalone references.

Sample	Run #	ng Pb/g fw
Cortes Banks (lower pollution)		
BLM-CB, H. corrugata muscle (69% H_2O), many veins)	1	6.3
BLM-CB, H. corrugata muscle (69% H_2O), few veins)	2	6.0
Abalone Cove (higher pollution)		
BLM-AC, H. cracherodii "A" (71% H_2O)	1	8.8
"	2	9.5
BLM-AC, H. cracherodii "B" (71% H_2O)	1	8.5
"	2	10.5

Table 4.4-11. Comparison of standardized values with AA determinations of Pb and Ba concentrations in organism references.

Reference	ng Pb/g fw			ng Ba/g fw	
	Standardized Value	Martin	Betzer	Standardized Value	Martin
BLM-CB-E	48	54	90	2000	1100
BLM-AC-M	55	49	150	790	200
BLM-CB-Ab	6.2	3	<3	2 to 37	<100
BLM-AC-Ab-A	9.1	3	-	5 to 47	<200

Table 4.4-12. Concentrations of Ba measured in different pieces of abalone muscle from three abalone references* by separate analyses (ng Ba/g fw).

Run #	BLM-CB	BLM-AC-A	BLM-AC-B
1	(37)	7	4
2	(36)	(47)	5
3	7	(16)	6
4	(16)	5	(13)
5	2		(12)

*Described in Table 4.4-10

( ) Values not acceptable because Ba came from lid and walls of dissolution beaker before contamination from this source was controlled.

4.4.4.4 Significance of Data

Erratic results were found during the past several years with IDMS analyses of Ba in animal tissue at concentrations below 10 ppb. Modifications of the analytical procedure were made which apparently solved the problem. These included: installation of a protective shield in the analytical balance to prevent contamination by particles of Ba-rich paint which were discovered falling into the samples during weighing; fabrication of a Ba-free type of insulator which supported the sample filaments in the mass spectrometer source to replace the old type found to contain 4% Ba; adding Ba isotope tracer to the sample before dissolution to insure better isotopic equilibration; and accepting only those analyses in which the proportions of isotope tracer Ba to sample Ba were nearly equal.

After six analyses of Ba in abalone muscle had been carried out, the procedure was modified further to end with two evaporations to dryness with HCl, which were designed to insure complete dissolution of any solid $BaSO_4$ that might have formed and incorporated nonrepresentative proportions of tracer and sample Ba during acid decomposition of the samples. The scatter of Ba concentration data shown in

Table 4.4-12 can be due to sample heterogeneity, undisclosed sources of contamination in the laboratory, or lack of isotopic equilibration. One factor seems to argue against gross sample heterogeneity: concentrations of Ca are 69±5 ppm fw, and concentrations of Sr are 1.0±0.05 ppm fw in the same pieces of muscle in which Ba is supposed to range from 2 to 47 ppb fw. It is possible that Ba concentrations may actually vary by a factor of two within the muscle of one abalone, and differ from one abalone to another, even though Ca and Sr concentrations do not, because Ba concentrations are very much smaller than those of Ca and Sr, and Ba is not conserved in seawater, while Ca and Sr are.

The BLM required method of analysis for Ba in field samples of marine plants and animals does not appear to be adequately sensitive, as shown in Table 4.4-11, despite the lack of a standardized Ba concentration in the abalone muscle reference. It is probable that many Ba analyses of field samples of plants and animals carried out by this method are not acceptable for baseline evaluation purposes.

Although the BLM-required method may seem to be adequately sensitive for the analysis of Pb in field samples of marine plants, different field sample analysis laboratories find quite different Pb results on the same plant samples. It is likely that an AA method which uses a preconcentration step would produce a much smaller scatter of results among different laboratories. This would have the additional advantage of providing an AA method that might also be adequate for marine animals, since comparisons in Table 4.4-11 indicate that the BLM-required method for the analysis of Pb in marine animals is not sufficiently sensitive, and many Pb analyses of field sample animals by the method are probably not acceptable for baseline evaluation purposes.

4.4.5 Evidence Showing That in Seawater Dissolved Lead May Be More Important Than Particle Lead in the Pollution of Marine Ecosystems.

The average concentrations of Pb and Ba in two dry sediment references (8 ppm Pb, 800 ppm Ba) are about the same as those found in dry particle material filtered from outer shelf surface waters (11 ppm Pb, 360 ppm Ba). However, in seawater the effect of particle Pb may be less than that of dissolved Pb.

When assessing possible pollution effects, it is necessary that bioamplification and biodiminution processes in food chains be considered. In marine ecosystems, there are two different processes competing with each other in the transport of lead along food chains: passive adsorption of lead from seawater by chelating agents on organism surfaces to form stable lead complexes (enrichment process); and non-efficient active transport of lead across cell membranes (depletion process). The actual enrichment or depletion of barium and lead is better expressed as a change relative to the more or less fixed bulk of calcium, which is an abundant nutritious metal that flows easily along food chains and is biomorphologically similar to barium and lead in many respects. Data for the food chain consisting of seawater-kelp-gastropod are presented in Table 4.4-13.

Sr, Ba, and Pb are bioamplified relative to Ca in going from seawater to kelp, but are biodiminished relative to Ca in going from kelp to gastropod. Sr/Ca is first increased by 3, Ba/Ca by 60, and Pb/Ca by 1600. A major fraction of kelp consists of an alginic acid-rich binding matrix, holding the kelp blade cells together. The strengths of alginic acid-metal complexes have been determined (References 6 and 13) and the relative stabilities of those complexes are: Pb>>Ba>Sr>Ca. The binding matrix is in contact with seawater during and after growth and the relative enrichments of Pb, Ba, and Sr cited above, which parallel the relative strengths of the metal complexes, result from passive adsorption of Pb, Ba, Sr, and Ca from seawater by the alginic acid-rich matrix. The gastropod Norrisia feeds on kelp, and Sr, Ba and Pb are then depleted relative to Ca in going from kelp to gastropod, Sr/Ca decreasing by 30, Ba/Ca by 260, and Pb/Ca by 330 (References 2 and 12).

The above relations show the progress of dissolved Ba and Pb through a marine food chain, but Pb pollution in dissolved form can be accumulated differently by different species of plants and animals. The concentration of total Pb in near-surface seawater averages about 100 ng Pb/l at highly polluted Abalone Cove, and 20 ng Pb/l at less

polluted Cortes Banks. The proportion of particle Pb is about 70% of the total at Abalone Cove, and about 5% of the total at Cortes Banks. The decrease in the concentration of pollution Pb in seawater in going from Abalone Cove to Cortes Banks is characterized by a pronounced decrease in the concentration of particle Pb, but the concentration of dissolved Pb apparently does not decrease very much.

Macrocystis collected from a less polluted location analogous to Cortes Banks contained only 30 ppb Pb fw compared to 50 ppb Pb fw at Abalone Cove. This modest difference in Pb concentration in the kelp is consistent with a modest difference in dissolved Pb in seawater. However, another species of kelp, Eisenia, contained 50 ppb Pb fw at Cortes Banks but 500 ppb Pb fw at Abalone Cove. This indicates that the difference in the concentrations of dissolved Pb at the two locations might not be so modest, or that there is a species difference in response to changes in Pb concentrations in seawater.

The concentration of Pb in the muscle of abalone collected at the highly polluted and less polluted locations differed by less than a factor of 2. Again, this might be a reflection of the more modest difference between dissolved Pb concentrations at the two locations, rather than the extreme difference in particle Pb at the two locations. An alternate

explanation of this great disparity is incomplete elution of adhered particulate matter during the rinsing process. This is suggested by the fact that an unrinsed 150 mg dw fragment of Macrocystis from Abalone Cove was found to contain 130 ng Pb/g fw, while a similar 150 ng dw rinsed fragment contained 55 ng Pb/g fw, the same as the slurry. That this difference is not due to the attrition of mucilage observed during rinsing is evidenced by analysis of this eluted water-soluble fraction of mucilage which indicated 1 - 2 ng Pb/g fw.

Table 4.4-13. Bioamplification and biodiminution of Ba and Pb in a marine food chain (atoms of metal per 10^6 atoms Ca in each substance).

Metal	Seawater	Kelp (M. pyrifera)	Gastropod (N. norrisii)
Ca	1000000	1000000	1000000
Sr	8500	30000	1000
Ba	4	260	1
Pb	.01	16	.05

Total Pb was determined in waters from a station in a less polluted region similar to Cortes Banks. Twenty-seven ng Pb/kg were found at 0.2 m, while 4 ng Pb/kg were found at 1000 m. The first value is close to other results from the Southern California Bight (Patterson, Settle, Schaule, and Burnett, 1976). The deepwater value, however, is the lowest ever measured in the Pacific. IDMS measurements of particle Pb, determined either by filtration, or by difference from total Pb and freely available Pb, did not vary significantly with depth, being ~1 ng Pb/kg at 0.2 m, and 2 to 3 ng Pb/kg at 1000 m. This indicates that particle Pb

s an insignificant fraction of total Pb in open surface aters, but constitutes the major portion of total Pb at epth. This extreme variation in the proportion of particle b with depth suggests that it is essential to measure total b in the euphotic zone of seawater, and that particle Pb eterminations are much more significant in deep than sur- ace waters. These relationships may hold only in polluted helf waters, however, since it has been found (B. Schaule, npublished) that in the open ocean, particle lead is a small raction of total lead in deep waters.

otal Ba in BLM station 42 waters was determined by isotope ilution techniques: 6000 ng Ba/kg were found at the sur- ace (0.2 m) and 16,000 ng Ba/kg at 1000 m depth. Particu- ate Ba therefore represents 1 to 3% of total Ba at the urface. The 25% acetic acid leach used in the BLM analyt- cal method dissolved about 90% of the particle Ba, and ore than half the particle Pb in surface waters in agree- ent with Bruland's findings. It is probable that the bomb reatment used in the BLM analytical method dissolves the emainder of the particle Ba and Pb, although this was not onfirmed because of filter blank scatter.

APPENDIX III

Comparative Distributions of Alkaline Earths and Pb among Tissues of Marine Plants and Animals

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Lead, barium, strontium, and calcium have been studied by isotope dilution, clean-lab techniques in both a marine and a terrestrial ecosystem. Analyses for Pb and Ba are difficult since their concentrations range down to the ng/g level in plant and animal tissue.

The accuracy of Pb and Ba analyses at ng/g levels is dependent upon the ability of the analyst to avoid positive errors introduced by contamination during collection, transport, and processing of samples, so that elaborate precautions must be taken. The laboratory requirements and procedures necessary to perform analyses at this level have been detailed elsewhere (Patterson and Settle, 1975) but include the use of a positive pressure laboratory with an independent source of filtered air, highly purified reagents and water, and specially cleaned quartz and FEP teflon ware for chemical operations. The magnitude of the blank contribution from air exposure, container walls, and each reagent must be known with certainty. The yield for each step in a chemical separation procedure must be measured to accurately modify the blank contribution at any given stage caused by a yield of less than 100% in each preceding step. Straight blank subtraction rather than yield corrected blanks introduces negative error in a relative analytical method such as atomic absorption and positive errors in an absolute analytical method such as stable isotope dilution (Patterson and Settle, 1975).

Measures taken at the CIT laboratory to insure the integrity of the sample include the requirement that marine samples be collected upwind and upcurrent of a diesel-powered

research vessel by divers wearing polyethylene gloves and epoxy-painted weight belts. Samples are double-bagged in acid-cleaned polyethylene beneath the sea surface prior to transfer through the air-sea interface, whereupon they are placed in dry ice chests for temporary storage. To prevent microgram level contamination during transport to the CIT freezer, where they are stored prior to analysis, the sample containers are sealed in another plastic bag.

Dissection of frozen samples is required in order to isolate the muscle tissue with minimal transfer of contamination. Contamination of the muscle tissue by epidermal material, whose metal concentrations are relatively high, is avoided by shaving away the outside surface of the frozen materials in three successive stages; ultra-clean stainless steel instruments, untalced polyethylene gloves, and teflon dissecting platforms are renewed before each later shaving step.

For most marine plant and animal tissue analyses a sample aliquot equivalent to approximately 1 g fresh weight is placed in a 30 ml FEP teflon beaker, spiked with isotopic tracers, and subjected to a HNO_3 - $HClO_4$ dissolution-oxidation procedure in an all-teflon oven flushed with purified N_2. Some marine samples contain enough silica to cause dissolution problems, and it must be deliberately removed with an HF treatment. The sample residue is converted to the chloride form by evaporation to dryness with excess HCl. This is done to break down insoluble precipitates such as $CaSO_4$, and to insure isotopic equilibration of Ba. The sample is redissolved and a 10% alkaline earth aliquot removed at this point. The remaining 90% is used for the Pb analysis. This consists of two successive dithizone-chloroform extractions, the second done without the use of complexing agents in order to minimize the blank correction. Extraction yields are 90-95% and typical Pb blanks are about 0.15 ng for the dissolution, 0.3 ng for the first dithizone extraction, and 0.15 ng for the second extraction. The Pb sample is loaded with a slurry of silica gel

and H_3PO_4 onto an outgassed rhenium filament. The Pb concentration is determined after measuring the $^{208}Pb/^{207}Pb$ ratio in a single-focusing, thermal-ionization, solid-source mass spectrometer by use of the following expression:

$$\text{total Pb} = \Big\{\Big[(\text{sample Pb} + \text{tracer Pb} + \text{dissolution blank Pb})\ (\%\ \text{Pb aliquot}) + \text{1st extraction blank Pb}\Big]\ (\text{1st extraction yield}) + \text{2nd extraction blank Pb}\Big\}\ (\text{2nd extraction yield}) + \text{loading blank Pb} + \text{yield tracer Pb}$$

An outgassed oxidized tantalum filament is used for the alkaline earth aliquot. Ba blanks are about 0.4 ng for the complete analysis. The concentration of Ba is determined from the following expression:

$$\text{total Ba} = \Big[(\text{sample Ba} + \text{tracer Ba}) + (\text{dissolution blank Ba}\Big]\ (\%\ \text{Ba aliquot}) + \text{loading blank Ba.}$$

Contrary to common opinion, lead is not necessarily concentrated in higher organisms as it is transported to the higher ends of food chains. In marine ecosystems there are two different processes competing with each other in the transport of lead along food chains: passive adsorption of lead from seawater by chelating agents on organism surfaces to form stable lead complexes (enrichment process); and non-efficient active transport of lead across cell membranes (depletion process). The enrichments or depletions of lead cannot be expressed in such simple terms as concentrations of lead in water or in wet, dry, or ashed tissues. The actual enrichment or depletion of lead is better expressed as a change relative to the more or less fixed bulk of calcium, which is an abundant nutritious metal that flows easily along food chains and is biochemically similar to lead in many respects (Hirao and Patterson, 1974).

Data for the food chain consisting of seawater-kelp (Macrocystis pyrifera) - gastropod (Norrissia Norrissii) are presented in Table 1. Although the results are from single analyses, certain order of magnitude trends are apparent. The data indicate that Sr, Ba, and Pb are enriched relative to Ca in going from seawater to total kelp blade, Sr/Ca increasing by 3, Ba/Ca increasing by 45, and Pb/Ca increasing by 1600. A major fraction of kelp consists of an alginic acid-rich binding matrix, holding the kelp blade cells together (Frei and Preston, 1962). The strengths of alginic acid-metal complexes have been determined (Schweiger, 1964; Haug,1961) and the relative stabilities of those complexes are: Pb >> Ba > Sr > Ca. The binding matrix is in contact with seawater during and after growth (Haug and Smidsrød, 1967), and it is believed that the relative enrichments cited above, which parallel the relative strengths of the metal complexes, result from passive adsorption of Pb, Ba, Sr, and Ca from seawater by the alginic acid-rich matrix.

The gastropod Norrissia feeds on kelp, cutting down into and consuming the blade material (Leighton, 1971). Using the composition of total kelp blade as gastropod food, Sr, Ba and Pb are depleted relative to Ca in going from kelp to total gastropod, Sr/Ca decreasing by 20, Ba/Ca decreasing by 290, and Pb/Ca decreasing by 1800. Analyses of separate organs of the gastropod indicate differences in metal depletions. The biodiminutions cited above refer to shell material but they also apply to total gastropod because the bulk of metals in the gastropod are in its shell. The situation is different for muscle tissue, Sr/Ca decreasing by 5, Ba/Ca decreasing by 9 and Pb/Ca decreasing by 3 , in going from kelp to gastropod muscle.

Table 1 (ng/g fresh wt.)

	Ca	Sr	Ba	Pb
Seawater	410	8.1	.006	.00003
Kelp blade (87.5% H_2O) (M. Pyrifera)	1,250	82.5	.850	.150
Gastropod muscle (66% H_2O) (N. Norrissii)	1,020	14	.080	.040
Gastropod shell (N. Norrissii)	374,000	1210	.875	.025

Distributions of Ca, Sr, Ba, and Pb were determined in the organs of an albacore (Thunnus alalunga), a marine carnivore at the highest level of another marine food chain, seawater-algae-herbivore-carnivore (Patterson and Settle, 1976). These were compared to those in a pine marten (Martes americana), a terrestrial carnivore, to see whether there were differences in distirbution between marine and terrestrial carnivores.

95% of the Ca and Sr, and 70% of the Ba and Pb in tuna are contained in the skeleton (Patterson and Settle, 1976). These distributions, when compared to those in a terrestrial carnivore (Martes americana) (Elias, Hirao, Hinkley and Patterson, 1975), are nearly the same except that the fur of the terrestrial carnivore, free of dermal secretions and aerosol deposits, contains 50% of the total lead body burden. A tuna was observed to contain unusually high concentrations of lead in epidermal mucus, but that is believed to originate from artifact contamination by fishermen.

Although the morphological distributions of the alkaline earths and lead are nearly identical in these terrestrial and marine carnivores, the concentrations are quite different.

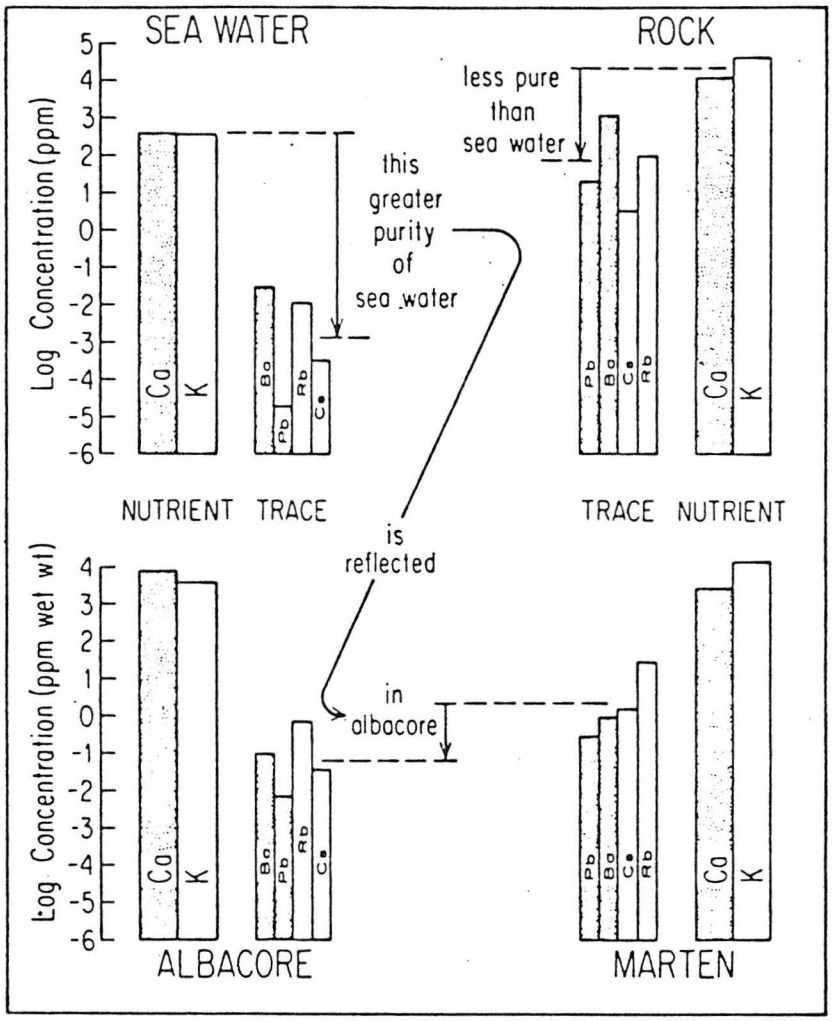

Figure 1. Smaller concentrations of trace metals relative to nutrient metals in seawater compared to rocks, results in smaller concentrations of trace metals in marine animal compared to terrestrial animal. More than 90% of the Pb in the marten is of industrial origin and unnatural. The same may be true for albacore. Seawater concentrations: K, Ca, Rb, and Sr from Goldberg (1963); Ba from Bacon and Edmond (1972); Cs from Folsom (1974); and Pb from Patterson, et al. (1976). Rock concentrations: K, Rb, Cs, Ca, Sr and Ba from Green (1959); Pb from Chow and Patterson (1961). Marten concentrations from Elias, et al. (1975).

The Ba/Ca and Pb/Ca ratios in tuna are about 1/10th of those in marten because much smaller amounts of Ba and Pb are associated with the Ca in seawater than with the Ca in terrestrial soils, which is shown in Figure 1. This does not mean that there

FIGURE 1

is a greater biodiminution of Ba and Pb in albacore than in marten. The total biodiminution is a factor of 1000 for both Ba and Pb relative to Ca in going from rock to carnivore in the marten food chain (Elias, Hirao, Hinkley and Patterson, 1975). In albacore, Ba/Ca is biodiminished only by a factor of 10 in going from seawater to fish, while Pb/Ca is bioamplified by a factor of 10. This marked contrast in the food chain transport of lead between albacore and marten may be due to passive adsorption effects in the marine ecosystem.

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APPENDIX IV

Analysis of Natural and Industrial Lead in Marine Ecosystems

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ANALYSIS OF NATURAL AND INDUSTRIAL LEAD IN MARINE ECOSYSTEMS

In order to illustrate the effect that analytical errors have on interpretations of lead in marine ecosystems, the general relationships of lead in marine food chains and organisms must be described concurrently with a discussion of analytical errors. It has been found that lead is passively sorbed by chelating agents on the surfaces of algae, and this produces high concentrations of lead in bulk algal material [1]. This characterizes the first step in the flow of lead through all marine food chains. In subsequent stages in progressing from primary producer to primary consumer and subsequently to higher carnivorous levels, lead is not readily absorbed into cellular constituents of organisms; instead only a fraction of the ingested lead is retained within organisms. This means that in sequential food chain relationships, higher organisms tend to contain less lead than do their precursors because of the biodepletion of lead. The general aspect of lead in marine food chains is therefore an initial bioenrichment followed by stages of successive biodepletion [1]. When comparing variations in lead concentrations among different organisms to show the effects of biodepletion, data scatter may result from variations in ash or water contents, and in order to eliminate them it is best to consider the concentrations of lead in only the metal fraction of organisms. Furthermore, since lead is such a close relative of calcium that the morphological distributions of both metals in organisms are nearly identical, and since calcium is a nutrient metal which flows easily along food chains, it is best to consider ratios of lead to calcium in organisms at the different trophic levels in food chains. When this is done it can be seen, as shown in Figure 1, that the Pb/Ca ratio increases dramatically at the primary producer level compared to seawater but then declines.

This is the general aspect for most animal food chains, even though specific examples are given here because the main reservoir of both calcium and lead in most animals is the skeleton, and the calcium in this reservoir is highly purified of lead relative to the less pure calcium in food because of selective biochemical processes. Even though proteins in some tissues may tend to concentrate lead relative to calcium the masses of such reservoirs of lead and calcium are insignificant compared to the skeletal reservoir, and the net effect for the entire organism is biodepletion of lead compared to food. Note that the variations cover 3 orders of magnitude. Trustworthy data for phytoplankton are lacking. Valonia is a good model, not only for phytoplankton, but for macro algae as well, because the Pb/Ca bioenrichment ratio is relatively insensitive to the type of complexing agent on algal surfaces. Trustworthy data for zooplankton are lacking and our spiny lobster data provide only an approximate model for these organisms. The anchovy sample analysed was taken from the stomach of an albacore.

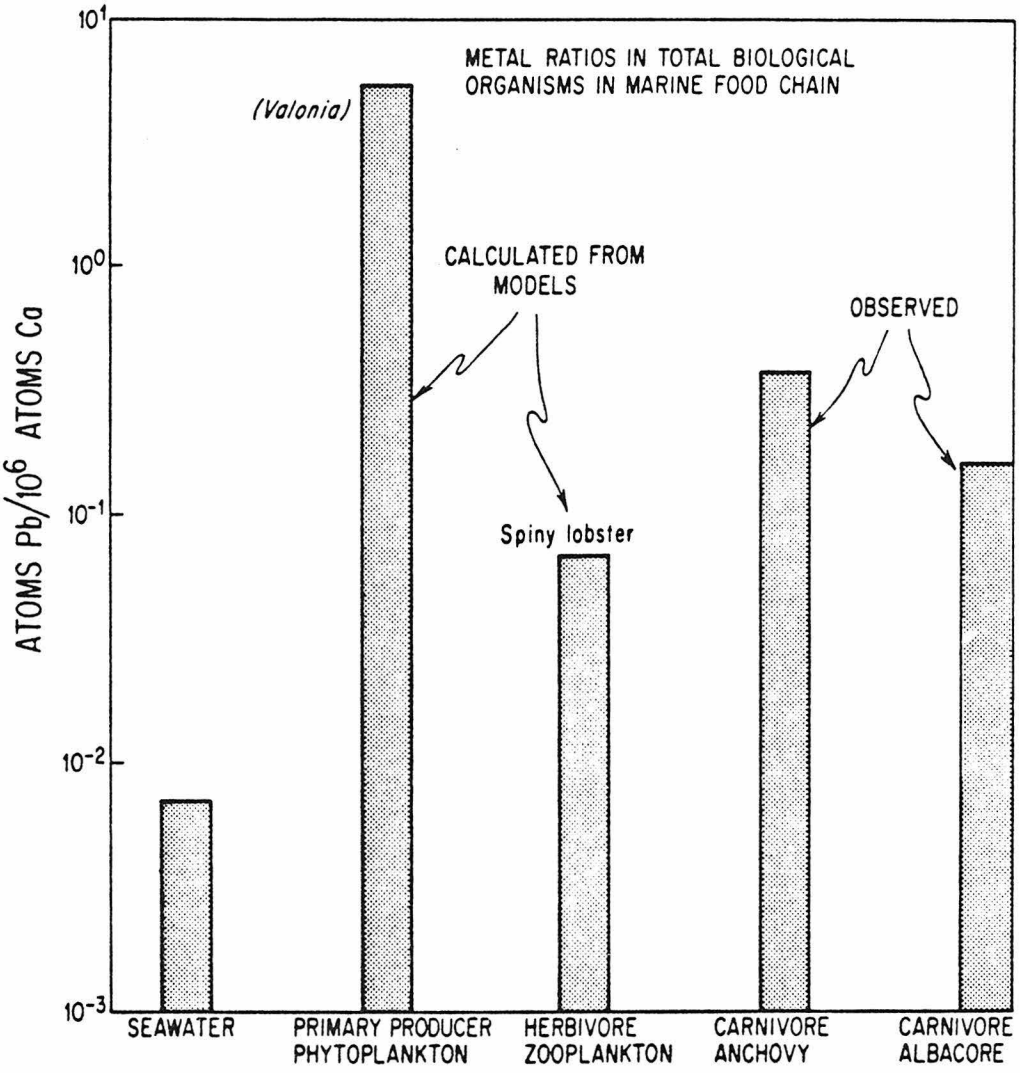

Figure 1. Bioenrichment of lead relative to calcium followed by biodepletion in marine food chains [1] [2].

Figure 1 shows that the biodepletion of lead relative to calcium should be generally applicable to most components of most food chains because calcareous tissues comprise the major reservoir for both calcium and lead in most organisms, and lead is far less efficiently biotransported to that bony reservoir than is calcium. Biodepletion effects continue to operate more or less independently of any particular lead concentration level that may exist because the decimal fraction of lead absorbed into animals from the gut remains relatively fixed, whatever this value may be for a given species, despite large fluctuations of lead concentrations in nutrient mediums. This happens because lead is only inadvertently and inefficiently absorbed as a trace constituent of calcium, and the factors which actually control the decimal fraction of lead absorption, as long as lead remains a trace constituent, are those which control the decimal fraction of calcium absorption [3].

In past work by other investigators, these relationships have been obscured, as shown in Figure 2, by lead contamination during collection, handling, and analysis. The incorrect classical view that all marine organisms concentrate lead from seawater, is based upon erroneous data which suggest uniformly high concentrations of lead at all trophic levels in marine food chains.

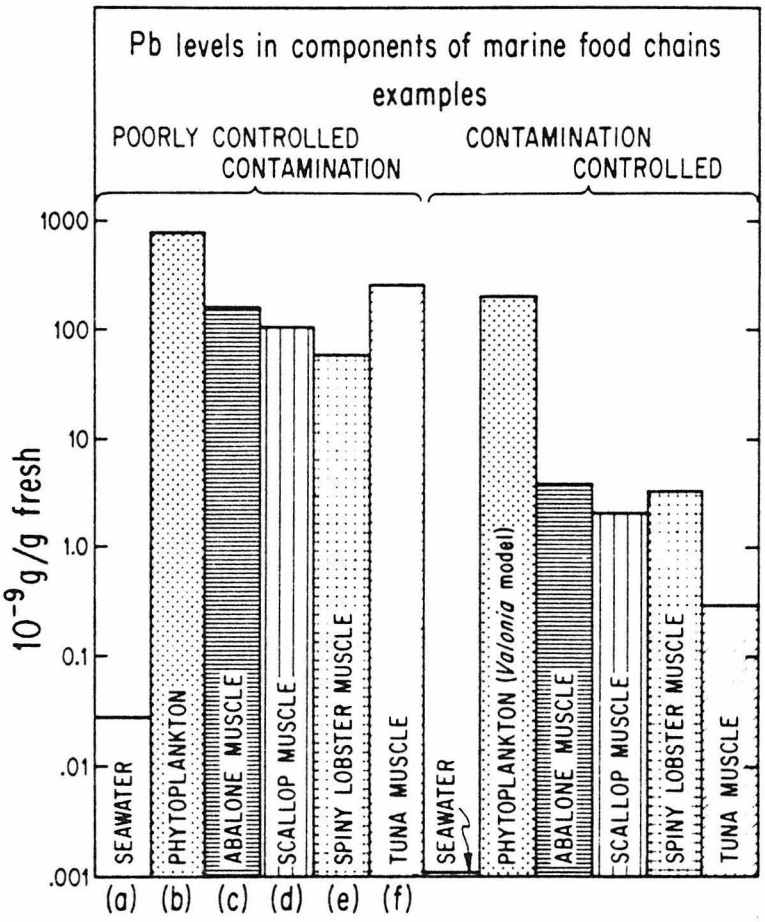

Figure 2. The obscuring of true relations of lead in marine food chains by improper control of lead contamination, which yields erroneous data. Note that the plotted variations of lead in tissues cover 4 orders of magnitude. Values on the left are from recent literature commonly regarded as the best available: (a) M. Tatsumoto and C. Patterson, Common Pb in seawater, Nature, Lond., 199, 350 (1963) T. J. Chow and C. Patterson, Pb and Ba in seawater, Earth and Planetary Science Letters, 1, 397 (1966); (b) J. H. Martin and G. A. Knauer, The Elemental Composition of Plankton, Geochimica et Cosmochimica Acta, 37, 1639-1653 (1973); (c) J. Stewart, M. Schulz-Baldes, Long term Pb accumulation in abalone fed on Pb treated brown algae, Marine Biology, 36, 19-24 (1976); (d & e) T. J. Chow, C. Snyder, H. Snyder, and J. Earl, Pb content in some marine organisms, J. Environmental Science Health - Environmental Science Engineering, A-11, 33-44 (1976); (f) See note (+) in table 6. Values on the right are from data obtained at the CIT biogeochemical laboratory (see ref. [1] [2] [4].

The concentrations of strontium and barium, which are trace metal relatives of calcium, have been studied in marine food chain components as well as lead and calcium [1]. It has been found that the observed concentrations and enrichments of these four metals in total Macrocystis pyrifera relative to seawater are directly proportional to the stabilities of the metal alginates shown in Table 1, and that a brief 0.1 N HCl leach removes these metals in inverse proportion to their respective binding affinities to alginate.

Table 1. Numbers in second column [5] are mequiv. H^+ liberated per ml by addition of metal salt (0.02 mequiv./ml) to solutions containing 0.027 mequiv. half-neutralized polyanions per ml. The alginate titrated is 90% guluronic acid. M. pyrifera contains both guluronic and mannuronic alginic acids. The latter forms stronger Pb complexes but less strong alkaline earth complexes than the former. These second column numbers show that the stabilities of the metal alginates increase in a series Ca<Sr<Ba<<Pb. The equimolar conditions for these data are different from actual field conditions. Numbers in the third column [1] are normalized to calcium because in the field, alginates in M. pyrifera are predominately calcium alginates. The relation among these numbers, which show an increasing enrichment in the series Sr<Ba<<Pb relative to Ca in going from seawater to M. pyrifera, parallels exactly the relation of numbers in the second column.

Metal	mequiv. H^+ liberated from guluronic acid	$\left[\frac{\text{metal}}{\text{Ca}}\right]_{Mp} / \left[\frac{\text{metal}}{\text{Ca}}\right]_{sw}$
Pb	.0024	470
Ba	.0010	56
Sr	.0008	38
Ca	.0004	1

That is, lead contamination of marine algae is related to the amount of lead in the dissolved phase of lead in seawater, and not to the amount of lead in the particle phase. This ionic sorption process on algal surfaces for ionic lead is quite different from that for radionuclides of polonium, plutonium, and americium, where, as has been shown in work pioneered by Folsom and coworkers, particle sorption appears to be the predominant process [6]. The reason for this difference is not yet clear. There may be a similar difference between common lead and Pb^{210} [7], but the actuality of such a difference is still in the process of being established. Near sewage outfalls where the concentration of lead in particles is extremely high, the adherence of relatively few particles on plant surfaces may, in some instances, give anomolously high lead values. In general, however, it is only the dissolved phase that determines the concentrations of lead in marine algae [1] as is shown in Figure 3.

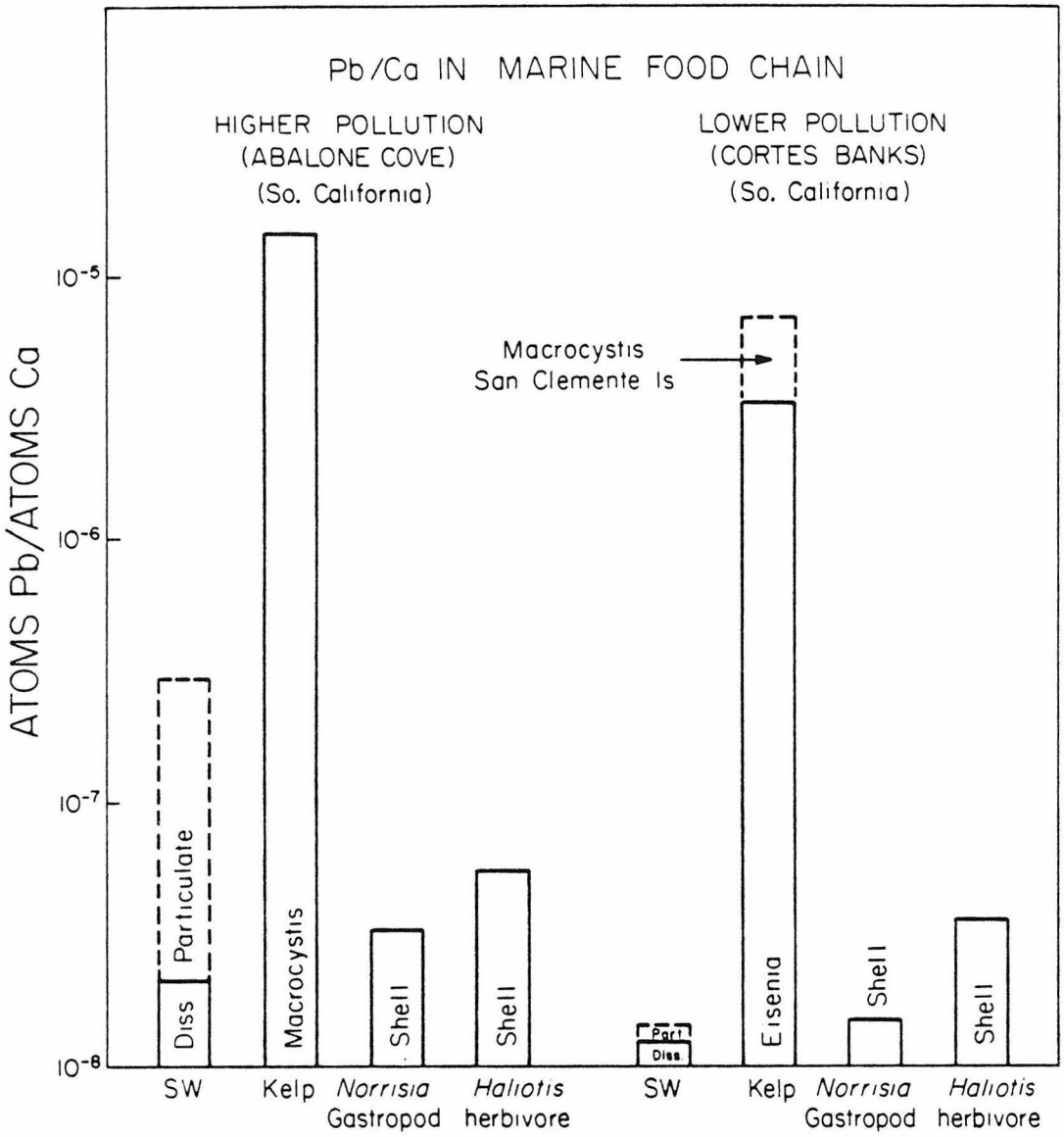

Figure 3. Pollution effects caused by simultaneous small increases of dissolved Pb/Ca and large increases of particle Pb/Ca in seawater. The 2-fold increase of dissolved Pb/Ca that occurs in going from the less polluted Cortes Banks region to the heavily polluted Abalone Cove region is paralleled by a 2-fold increase of Pb/Ca in kelp and in the shells of herbivores. These latter changes are far smaller than the 20-fold difference between particle Pb/Ca at the Cortes Banks region and the Abalone Cove region. As noted in Figure 1, the biodepletion of lead relative to calcium operates independently of lead levels in nutrient mediums, whatever they may be, as long as they are not more than 100-fold above natural levels.

An increase in dissolved lead, caused by pollution, elevates lead concentrations in animals, as the data in Figure 3 show. The concentration of lead in herbivores is related to the concentration of dissolved lead in seawater through their precursor algae.

When other investigators analyse lead in samples of seawater collected and standardized by the CIT laboratory only a few investigators can obtain correct values. This is shown in Table 2.

Table 2. Comparison of actual and observed values of lead in seawaters known by us to have been obtained by different investigators within the last 4 years.

Waters Standardized at CIT and then Analysed by Other Investigators

Method	ng/kg Reported Values	ng/kg True Value	# of Invest.
AA	120 & 150	140	3
AA	100	100	1
DPP	20	40	1
AA	22 & 100	40	2
AA	80 & 1200	25	2
IDMS, AA	30	25	1
ASV	180 to 1300	14	3
AA	50 to 120	14	3
AA	10	10	2
ASV	70 & 500	3	2
DPP	3	3	1
IDMS	2	2	1
AA	1	1	1
Waters Known to have been Contaminated During Collection and Reported by Other Investigators			
AA	100 to 5000	5 - 30 (est)	7
ASV	500 to 5000	5 - 30 "	4
IDMS	700	1 - 2 "	1
IDMS	30	1 - 2 "	(pre 1974 by CIT)

Outside the Caltech laboratory the following investigators are known by us to have correctly analysed for lead at concentrations of 3 ng Pb/kg seawater or in distilled water: H. W. Nürnberg, Director of Institute for Chemistry, KFA Julich, ICH-4, Postfach 1913, D-517 Julich, West Germany; Y. Hirao, College of Science and Engineering, Aoyama Gakuin University, 6-16-1 Chitosedai Setagaya-Ku, Tokyo 157, Japan; and M. Murozumi, Muroran Institute of Technology, 27-1 Mizumoto - cho, Muroran, Japan 050. For correct analyses for lead at 10 ng Pb/kg seawater: J. Martin, Moss Landing Marine Laboratories, Moss Landing, California, USA 95039; and K. Bruland, University of California, Santa Cruz, California, USA 95064. For correct analyses for lead at 25 ng Pb/kg seawater: C. S. Wong, Ocean Chemistry Division, Marine Sciences Directorate, Pacific Region, Department of the Environment, 211 Harbour Road, Victoria, B.C., Canada. For correct analyses for lead at 100 ng Pb/kg seawater: B. J. Presley, Department of Oceanography, Texas A and M University, College Station, Texas, USA 77843. For correct analyses for lead at 150 ng Pb/kg seawater: J. M. Bewers, Chemical Oceanography Division, Marine Sciences Directorate, Atlantic Oceanographic Laboratory, Bedford Institute of Oceanography, Dartmouth, Nova Scotia B2Y 4A2; R. Duce, Graduate School of Oceanography, University of Rhode Island, Kingston, Rhode Island, USA 02881; and W. Fitzgerald, Marine Sciences Institute, University of Connecticut, Avery Point, Groton, Connecticut, USA 06340. Some of these investigators may be able to analyse lead correctly at lower concentrations, but this has not been confirmed by interlaboratory calibration with IDMS in an ultra-clean laboratory. Concentrations of lead in seawater above 15 ng Pb/kg are not significant on a world-wide scale. A number of other investigators,some in prominent oceanographic laboratories, are known by us to have made serious

errors in analyses for lead in standardized samples of seawater in recent years, where concentrations ranged from 1 to 40 ng Pb/kg, and whom we have not been able to recheck in subsequent work. On the basis of a comparison of reasonable estimates of the probable extent of lead contmaination and its probable control in most chemical oceanographic laboratories in the world with those that are required for reliable lead analysis of seawater, it is believed that very few, if any, out of the hundreds of such laboratories in the world, that we have not carried out interlaboratory calibrations with, can obtain correct values for lead in most of the seawaters of the world at the present time. The widely recognized lead-in-open-and-deep-oceans results of Tatsumoto, Chow, and Patterson [8] [9] reported before 1974 are seriously wrong because of contamination during collection, although the analytical methods they developed in their pioneering work were accurate and serve as a foundation for the recent studies that have provided more reliable data.

The discovery that the shallow waters of the open oceans contain only 10 ng Pb/kg and that the deep waters of the oceans contain only one or two ng Pb/kg, instead of many hundreds or thousands of ng Pb/kg, as is still commonly believed, is crucially important, because the correct very small concentrations are an index of ocean reservoirs of lead that are small with respect to the annual input of industrial lead to the oceans, which correctly signifies a large lead pollution effect, while erroneous very large concentrations of lead in seawater incorrectly suggest that ocean reservoirs of lead are hundreds of times larger than annual lead inputs and that lead pollution effects in the oceans are insignificant. In this same sense, true low baseline levels of lead in marine plants and animals correctly show that these substances are much more susceptible to lead pollution effects than when baseline lead concentrations in marine plants and animals are incorrectly reported as being high.

Although there are many thousands of lead analyses reported each year for marine animals, it is doubtful that much of these data are scientifically significant. Most anlayses of invertebrates for example, are reported at levels of 10^{-6} g/g fresh wt. lead in total soft parts. Since the concentrations of lead in the soft tissues of invertebrates are actually on the order of 10^{-9} g/g fresh wt., reports of 10^{-6} g/g fresh wt. levels of lead in whole soft parts can refer only to concentrations of lead in detritus contained in the stomachs of the animals, which can be correct only in those cases of extreme lead pollution where lead is >10 ppm in wet detritus (>100 ppm dry detritus). The true distribution of lead among the various organs of a scallop are listed in Table 3. The region from which this scallop was taken is 200 km from the coast west of San Diego on the seaward side of the channel islands, and it is polluted with industrial lead on an estimated world-scale of about 4-fold above prehistoric natural values. Analyses show about 10 ppm Pb (dry) and 1 ppm Pb (wet) in filtered seawater detritus near the surface in these same waters [10], and about 1000 ppm Pb (dry) and 200 ppm (wet) in sewage solids [11] discharged at the coast of this region. In this outer shelf region, where there is an absence of particles highly polluted with lead from local sources, 80% of the lead in this total animal is in the bone reservoir. Most of the remainder is in food residues in the gut, which lead is not biochemically incorporated into the tissues of the animal. If lead in the "total soft parts" which includes the gut contents but excludes bone, were to be reported in this animal, about the same lead concentration would be observed as for the total animal, as shown in the last column in Table 3, but 90% of the lead in this mixture would not be in tissues at all, but in food residues. Actually, 97% of the total lead biochemically incorporated in tissues of the animal resides in bone, and the concentration of lead in "total soft parts" minus food residues in the gut would

be only 1/10th of that in soft parts which include gut contents, shown in the next to the last column of Table 3.

Table 3. Distribution of lead in organs of scallop (Hinnites multirugosa), 433 g total wt from Cortes Banks, So. California [1].

Organ	% Body	Pb Conc. (µg/g fresh wt)	% Pb Body Burden (incl. gut cont.) (& shell)	% Pb Body Burden (in tissues only)	% Pb Body Burden (in "total soft parts") (excluding gut cont.)	% Pb Body Burden (in "total soft parts") (incl. gut contents)
Shell	75	0.14	77	97	-	-
Muscle	11	0.004	0.3	0.4	11	1.4
Gonad	5.1	0.023	0.9	1.1	31	3.8
Mantle	4.6	0.04*	1.3	1.7	48	5.9
Gut contents	2.3	1.2	20	-	-	88
Gills	1.6	0.01*	0.2	0.2	4	0.2
Gut wall	0.2	0.06	0.1	0.1	3	0.4
Kidney	0.2	0.05**	0.1	0.1	3	0.4
[Total Pb conc. (µg/g fresh wt)]		[0.14]	[0.11]	[0.017]		[0.13]

* est. from bony fish data [2]; mantle epidermal mucus; gills epithelial gill tissue.

** est. from abalone from same location [1].

When this same species grows within several km of sewage outfalls, the concentration of lead in the gut contents is observed to increase 10-fold as a consequence of the addition of sewage solids, shifting the bulk of lead in the total organism away from bone (30%) to the gut contents (70%) [1]. If lead in the "total soft parts" of this animal were to be reported, the observed lead concentration would jump 10-fold to more than 1 ppm, even though the concentration of lead incorporated in total tissues (~95% in bone) had increased only 3-fold, and the concentration of lead in muscle tissue was only 0.01 ppm [1]. Thousands of Pb determinations in Mytilus have been made by investigators monitoring global heavy metal pollution. Although we have only begun studies of Mytilus, our new data, listed in Table 4 and Table 5, make it highly improbable that many, if any, of the analyses previously reported by other investigators are scientifically significant. The 0.2 to 1 ppm Pb concentrations reported earlier in the muscle tissue of this filter-feeder [12] exceed, in many cases, the Pb concentrations determined for digestive organs or shell, erroneously suggesting that Pb metabolism in Mytilus is significantly different than for similar bivalves, such as scallop. However, as may be seen by comparison of Table 3 and Table 5, the morphological distribution of Pb and its significance are essentially identical in both scallop and mussel.

Table 4. Distribution of Ca, Sr, Ba, and Pb in the mussel Mytilus Californianus, 54 g total weight from Punta Banda, Baja California, Mexico.

	Pb	Ba	Sr	Ca
Muscle	.025	.006	2.89	179
Gonad	.068	.007	3.56	228
Gut wall	.309	.081	1.78	132
Periostracum	.949	3.29	115.9	29700
Shell	.320	-	-	360000
Gut Conts.	.635	2.39	9.45	960
Mantle	.042	.078	3.73	240

Note that some tissues are easily cross-contaminated by epidermal mucus containing high concentrations of metals relative to underlying tissues. This is illustrated by the following analyses of Pb and Ba in a sample of muscle tissue from Mytilus from which outer layers were not shaved away and discarded, although it was dissected in a clean room.

Cross-contaminated muscle	.056	.008	2.18	-

Table 5. Distribution of Pb in mussel Mytilus Californianus, 54 g total wt from Punta Banda, Baja California, Mexico.

Organ	% Body	Pb conc. μg/g fw	% Pb Body Burden Incl. Gut Conts. & Shell	% Pb Body Burden In Tissues Only	% Pb Body Burden In "Total Soft Parts" Excluding Gut Conts.	% Pb Body Burden In "Total Soft Parts" Including Gut Conts.
Shell (calcitic)	63	.32	74.6	85.5	-	-
Periostracum	1	.95	3.5	4.0	-	-
Muscle	4	.025	0.4	0.4	3.8	1.8
Mantle	12	.042	1.9	2.0	21.5	8.5
Gills	2	.01*	0.1	0.4	1.3	0.6
Gonad	5.5	.068	1.4	1.6	15.2	6.1
Kidney	3	.05**	0.6	0.8	6.3	2.4
Gut walls	4	.31***	4.6	5.2	51.9	20.7
Gut conts	5.5	.64	12.9	-	-	59.8
[Total Pb conc. (μg/g fw)]			[0.270]	[0.249]	[0.079]	[0.164]

*teleost fish gills ≈ mussel gills (ref. 2)

**abalone kidney ≈ mussel kidney (ref. 1)

***value believed elevated as a result of cross-contamination by gut contents

Common lead is not highly concentrated in gut walls of invertebrates, as has been suggested [13], nor in kidney [14], nor even in fish caecum [13] [15].

The true distributions of lead among the various organs of a tuna fish are listed in Table 6.

Table 6. Distribution of lead in organs of tuna (Thunnus alalunga) [2]. Lead has the same distribution as Ca, Sr, and Ba. Furthermore, this distribution is the same as that in a wild terrestrial carnivore (pine marten - Martes americana) [16], except for lead in the fur of the marten.

Tissue	wt% H_2O	Organ % of body	Pb (μg/g fresh wt)	% of body burden Pb
Bone (vertebra)	29	4.6	0.074	56
Scales	25	1.5	0.059	15
Teeth	3	0.005	0.24	0.2
Total skeleton	28	6.1	0.070	70
Muscle	71	73	0.0003	3.6
Liver	64	1.4	0.009	2.1
Spleen	75	0.06	0.019*	0.2
Kidney	75	0.55	0.006	0.5
Caecum wall	65	1.2	0.004	0.8
Gills(epithelium & lamellae)	73	0.5	0.014	1.1
Dermis	44	1.6	0.013	3.4
Epidermal mucus	80	0.03	0.035	0.2
Stomach contents (anchovy)	58	2.1	0.021	7.2
Remainder	-	13	0.005†	10
Whole fish	-	100	0.006	100

*now believed too high - reinvestigation incomplete

†assumed

We have not yet found any gross differences between the distributions of lead in bony fish and in invertebrates. Table 7 lists the concentrations of lead in tuna muscle, ranging from prehistoric times, to present day ocean environments, to that found in non-soldered, and in lead soldered cans. The 20,000-fold lead pollution factor observed in canned tuna, a marine food commonly eaten by Americans provides stark, indisputable, and chilling proof of the extremity of the lead pollution situation today in the human environment [17]. The average person in the United States and the United Kingdom today contains a body burden of lead which is more than 100 times above natural levels prevailing in prehistoric times [16] [17] [18]. Present lead exposures for the average person in the US and UK are about 1/3 of those that will cause symptoms of classical lead poisoning upon prolonged exposure. The 100-fold lead pollution effect in humans constitutes severe chronic lead insult whose effects probably lead to significant reductions in mental acuity, increases in mental irrationality, reduction in resistance to malignancies and infectious diseases, and damages to germ plasm [17]. This situation which applies to entire populations has been ignored by responsible

authorities in the US and the UK, while they have devoted their attention instead to deleterious effects on small segments of populations caused by lead pollution exposures in excess of typical ones. This statement is so extreme it is difficult to take seriously. Unfortunately, the cold facts are that the entire earth's biosphere is polluted by an annual production of industrial lead that is 100-fold larger than the total amount of lead circulating each year through the earth's biomass, that cities and towns throughout the world are vast sewers of industrial lead, that the average person in the industrialized regions of the earth does indeed contain body burdens of lead that are 100-fold larger than natural ones existing in prehistoric times, that such persons are hovering on the brink of classical lead poisoning, and that there are no thresholds of exposure for deleterious effects from lead [17]. One of the main reasons for the existence of this situation is that for decades a vast profusion of erroneous lead analyses engendered by engineering technology have obscured the true magnitudes of lead pollution in foods.

As shown in Table 7, the shocking and certainly poisonous contamination of fish muscle by lead in lead-soldered cans was overlooked through analytical error on the part of a prominent US Federal surveillance laboratory that was carrying out a study program on behalf of the US Food and Drug Administration. The report issued by that laboratory in effect reinforced the erroneous belief by the US FDA that Pb-soldered cans elevated lead levels in foods only a few-fold and that natural levels of lead in one of the purest of foods available with respect to lead were an erroneous 10^{-7} g/g instead of the true 10^{-11} g/g fresh wt.

Table 7. Failure of US Federal surveillance laboratory (Fsl) to discover gross Pb contamination in tuna muscle (albacore - Thunnus alalunga) in its report to the US Food and Drug Administration.

Type of Muscle		Reported Pb (μg/g fresh wt)
Prehistoric in sea (natural)		0.00007 (est. from 4 x Pb poll. of oceans) [4]
Present day in sea (contaminated)		0.00030 (dissect. & anal. CIT) [2]
Non Pb-soldered can*	"	0.00700 (indus. cut, canned; anal. CIT) [17]
Dried powder in plastic bag	"	0.40000 (indus. cut, dried, ground; anal. CIT) [17]
Pb-soldered can	"	1.40000 (indus. cut, canned; anal. CIT) [17]
Present day in sea	"	0.40000 (dissect. & anal. Fsl) (error)+
Pb-soldered can	"	0.70000 (indus. cut, canned; anal. Fsl)+
Present day in sea**	"	0.02000 (dissect. CIT; anal. Fsl) (error)+

*"Chicken of the Sea" chunk albacore in small die-punched cans which lack both a soldered side seam and a non-soldered bottom crimp seal. "Chicken of the Sea", prepared by Van Camp Co., is also packed in small Pb-soldered cans which show the above high lead content, therefore examine can for this soldered side seam and non-soldered bottom crimp seal.

**CIT standardized Pb conc = 0.00040

+In our cooperative laboratory intercalibration program, laboratories reporting erroneous results are not identified. This is done to ensure continued participation by other laboratories in the intercalibration program and the improvement of their analytical techniques for lead.

Tuna muscle that had been dissected from a whole fresh fish at CIT was sent to the Federal surveillance laboratory. The lead concentration reported for this sample was 20-fold less than their previously reported average for lead in muscle of 27 fresh fish of the same species from the same region. This value is listed in Table 7. Part of this reduction for the later sample may be attributed to a different and more careful analytical technique (flameless AA earlier, ASV later), but part is probably also due to reduction of lead contamination during dissection, because this process was carried out in the CIT laboratory. The later analysis was still 50-fold (5000% if such a term exists) too high. Variations of lead concentrations in muscle among tuna are small [2].

This is not an isolated incident. It is rather certain that only a handful of ultra-clean, IDMS laboratories in the world have the potential ability to correctly analyze lead in tuna muscle at this time, and each of these, now devoted to studies of lead in igneous minerals, would require a year or more of preparatory work before they could report publishable data on fish muscle. This means that the US Federal surveillance laboratory mentioned here does not stand alone, but is one in a population of thousands at this time in the world. This is clear evidence that man does not now have the ability to control the environment that he, through engineering technology, has created. In nearly every case where we have been able to check analyses of plant and animal materials for lead by other laboratories that use conventional, rapid methods of analyses, serious errors have been revealed. One instance involves the participation of the CIT laboratory in the US Bureau of Land Management Baseline Study of Outer Continental Shelf Regions [10]. In another instance it has been found that all of the many reported analyses of lead in old tree stemwood are, without exception, erroneously high by about 1000-fold [17]. Entire human populations have been unnecessarily exposed to harmful concentrations of lead in their foods for decades as a direct consequence of the mistaken belief that sophistication of instrumentation by itself is the better modern successor of plain human integrity and perserverance.

Lead analyses in the CIT laboratories are carried out under ultra clean conditions using thermal ionization mass spectrometric isotope dilution. This is an absolute, yield independent method, which does not use standards or working curves, and is the method used to prepare standardized references for DPP, AA, SRF, AE, NA, etc. analytical methods [2] [10] [19].

A weighed amount of one lead isotope tracer is added to the sample and mixed with the unknown weights of different sample lead isotopes of the lead in that sample by chemical homogenization. The purified lead isotope mixture is chemically isolated by solvent extraction, or by ion column chelation, or by electrolysis, and then analysed in a mass spectrometer to find the ratio of the tracer lead isotope to the sample lead isotope. The measurements can easily be made with less than 1% error using nanogram amounts of isotopes. Lead concentrations determined in the CIT laboratory are obtained using contamination control procedures that are exceedingly thorough and they are not low because solvent extraction steps fail to extract all the lead, or because some of the lead adsorbs on the walls of collectors, or because the analytical method overlooks lead in particles. Total leads in seawater are measured in samples boiled to dryness with isotope tracer in aqua regia in ultra-pure quartz, for example. The amounts of lead adsorbed on various surfaces under different conditions have been studied carefully in the CIT laboratory.

Most of the reports of lead adsorptions and of lead contamination from containers [20] are of little significance in marine lead chemistry because the actual amounts of lead involved in these two processes under the optimum conditions required for correct lead analysis are much smaller than typical amounts of lead

contamination encountered in most laboratories, and are far below the trustworthy analytical working limits for lead in most laboratories. Most of the lead in seawater that becomes adsorbed on conventional polyethylene or teflon surfaces at pH 8 is associated with particles, and is not of the readily exchangeable, or active type of lead. That is, it is charged particles containing lead that migrate to and adhere to the plastic surfaces. This process occurs within hours, and it is enormously modified by the pretreatment history of the plastic surface, being less effective for recently acidified surfaces, and most effective after >6 weeks exposure to water at pH 8. It generally involves only a fraction of the particle form of lead, and amounts, in most cases of open seawater, to <0.1 ng Pb/kg unacidified seawater. This adsorbed lead can be released or its formation prevented with 0.01 N acid. Contamination by container walls is far more serious. After washing conventional polyethylene with acetone briefly, followed by pure 4 N HNO_3 at 55°C for 3 days, followed by ultra-pure 0.01 N HNO_3 at 55°C for 1 day, followed by ultra-pure 0.01 N HNO_3 at 55°C for 5 days, followed by storage with cold ultra-pure 0.01 N HNO_3 until just before use, that plastic, if virgin and not made of recycled plastic, will generally yield 1 picogram Pb/cm^2/day at 55°C and 0.1 picogram Pb/cm^2/day at 20°C to 0.01 N acid solutions every day for many months. Pb contamination from FEP teflon cleaned in a similar manner is generally about twice as high. It must be emphasized that floods of lead contamination, amounting to tens and sometimes hundreds of nanograms will be released from plastic containers upon exposure to dilute acids if they are previously cleaned only by heating with acid and then rinsed and allowed to dry before use. Proper measurement of lead in reagents and in contamination from containers is an absolute requirement for proper control of lead contamination in the laboratory during analyses. These measurements cannot be made by simply omitting the sample from the total procedure. Each separate phase of the procedure must be broken down into its many separate parts and each part then analysed for lead as though it were a sample. This is extremely difficult, but analysts must perservere and devote far more time and effort to this work than to the actual analysis of samples, because it is the degree of success at this level which determines their integrity and the degree of reliability of their lead analyses in samples at higher levels of lead concentrations. For relative methods such as AA, ASV, XRF, etc., this success cannot be achieved with absolute assurance without cross checking by the absolute method of IDMS. The single most crucial indicator of laboratory success is the preparation of distilled water containing less than 1 ng Pb/kg together with proof of that value. This, of course, is an absolute prerequisite for reliable monitoring of lead contamination of natural water samples during collection, storage, and analysis.

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APPENDIX V

Impact of Man on Coastal Marine Ecosystems

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IMPACT OF MAN ON COASTAL MARINE ECOSYSTEMS

It is now believed by some of us that lead concentrations in coastal surface waters that are involved in natural circulation processes do not increase to levels above 50 ng/kg unless the waters are within sewage plumes or within heavily contaminated plumes from harbors and industrial bays [1] [2] [3]. Contrary to widely accepted beliefs, we and our co-workers do not believe that lead concentrations in most coastal surface waters commonly approximate 1000 - 2000 ng/kg. Among the largest sewage outfalls in the world, those emanating from the Southern California metropolitan area do not elevate lead concentrations in surface seawaters above 50 ng/kg, when mixed over a relatively small area of 10000 km^2 to an average depth of 300 m [1]. Total lead concentrations within the direct plumes from these sewage outfalls do range from 100 - 1500 ng/kg in coastal regions, however. Where industrial outputs are smaller, lead concentrations in surface waters range from 5 - 20 ng/kg off the coast of western United States. It has been found that surface concentrations of lead in the oceans parallel the concentrations of Pb^{210} whereby both common lead and Pb^{210} surface concentrations are low in biologically productive regions near the continental shelves, and are high in biologically barren regions of the open oceans [2] [4]. In the north Pacific this means that the concentrations of common lead in surface waters increase from levels of about 5 ng Pb/kg seawater over the shelf to 15 ng Pb/kg seawater in the open waters of the central northeast Pacific [2]. The relationship of concentrations of Pb^{210} in these same surface waters have been found to be parallel [2] [4]. Sparse measurements indicate that the vertical distribution of common lead does not change significantly in going from deep waters near the continental shelf of western North America to deep waters in the open NE Pacific [2]. Surface concentrations of common lead near the coast of western North America vary from 5 - 15 ng Pb/kg seawater mainly as a result of either temporal variations in biological productivity or temporal variations in the amount of industrial lead introduced to the coastal waters. These two processes have been distinguished by parallel variations, or lack of them, in Pb^{210} concentrations [2] [4].

The above data for lead in surface waters refer to total lead which is the sum of both highly reactive, or dissolved lead, presumably existing in the form of molecular $Pb(CO_3)$ [5] [6], and chemically inactive, or particle lead. In surface waters containing 15 ng of total Pb/kg seawater or less, the fraction of particle phase lead is found to be about 10%, or less. This fraction is maintained at depth, except for the possible existence of a layer extremely rich in lead particles at 3000 to 3500 m in the NE Pacific (5 ng particle Pb/kg coexisting with 2 ng dissolved Pb/kg) [2]. It is not yet known if this layer is an artifact related to collection procedures, although it has been confirmed in the samples that were collected by replicate analyses of them. In shallow waters more highly polluted with industrial lead

which are very near the coasts, the fraction of particle lead is generally higher, increasing to as much as 90% of the total [1]. Within a few km of the coasts, lead concentrations decrease in progressing seaward from the coast, and most of this decrease is associated with a decrease in the concentration of particle lead, rather than dissolved lead [1].

The concentration of industrial lead in seawater particles is usually extremely high, averaging about 200 ppm Pb in wet sewage particles and 1 ppm Pb in wet coastal seawater particles for example [1] [2] [7]. When anthropogenic particles are introduced to seawater, a fraction of their original lead is released in soluble form. For sewage particles this immediately released soluble fraction is only about 10% [1], while for atmospheric particles enriched with lead from auto exhausts and introduced as wet and dry fallout the immediately released soluble fraction is about 50% [8]. The soluble fraction from sewage does not increase with time in seawater, but that from aerosols containing auto exhaust lead does. The soluble form of industrial lead pollution in coastal waters enters marine ecosystems by sorption on algal surfaces, while industrial lead pollution in the form of particles does not, for the most part, enter marine food chains by surface adhesion to algal surfaces. Instead, particle lead pollution suspended in the water column enters marine food chains through filter feeders. Even though soluble lead is enriched with respect to calcium by sorption on algal surfaces in going from seawater to algae, herbivore grazers feed on material which contains far smaller polluted Pb/Ca ratios than do filter feeders, because lead concentrations in detritus particles are so extremely high. That is, concentrations of pollutant lead in the detritus particle food of filter feeders are in the 10^{-6} g/g range, but only in the 10^{-8} g/g range for the algae

Table 1. Effects of different levels of lead pollution on lead concentrations in tissues of a filter feeder and a grazer [9]. Seawater values are estimated from only a few measurements in the face of large temporal variations of these values [1] [2].

Animal	Substance	Cortes Banks (diss. Pb poll. ~ 4-fold above natural levels) Pb/Ca (wt)	Abalone Cove (diss. Pb poll. ~ 20-fold above natural levels) Pb/Ca (wt)
-	seawater detritus	10000 x 10^{-7}	200000 x 10^{-7}
Scallop (Hm)	gut contents	17000 "	83000 "
"	muscle	250 "	360 "
"	shell	4 "	12 "
-	seawater diss. metals	0.5 "	1 "
Gastropod (Nn)	food	170 "	680 "
"	muscle	290 "	1200 "
"	shell	1 "	2 "

food of grazers, while concentrations of calcium in both detritus and algae are similar [9]. Surprisingly, the tissues of both herbivore grazers and filter feeders show about the same Pb/Ca ratios [9]. This is illustrated in Table 1. It is believed that high pHs in the stomachs of the filter feeders, which depresses the availability of lead relative to calcium, may account for this. It must be emphasized, however, that for a given species of animal the effects of increasing the concentration of pollutant lead in the environment are reflected by correspondingly elevated Pb/Ca ratios in both soft tissues and shell of that species. This is shown in Table 1 where increases of lead in the food of the animals produces increases in Pb/Ca ratios in muscle and shell [9]. Despite enormous (200-fold to 4000-fold) biodepletions of lead relative to calcium in going from food to the main calcium reservoirs in bone tissues of these animals, the amounts of lead in these reservoirs are still delicately regulated by quite small (4-fold) changes in the Pb/Ca ratio in food caused in turn by small variations in levels of lead pollution. We use the term "small" for 400% increases in lead pollution because lead pollution increases in city and town environments are 10000-fold to 100000-fold above natural levels. Lead is bioenriched relative to calcium in going from food to the small and highly dynamic calcium reservoir in the muscle tissue of N. norrisii. This contrast between bioenrichment and biodepletion of lead relative to calcium in different tissues of the same animal is not unusual in marine animals, because it has also been observed in abalone and in tuna [9] [10]. This contrast between muscle and bone has not been observed within 7 different kinds of terrestrial animals [11] although reports in the literature (which may be erroneous because muscle is easily contaminated with lead) indicate that the same contrasting phenomenon does exist in humans and other terrestrial animals. If, as is probable, the Pb/Ca ratio in plasma supplying nutrients to the muscle cells is less than that within the cells, then the residence time of lead within those cells must be longer than that for calcium. Despite the close similarity of Pb/Ca ratios in shell and dissolved in seawater, studies with radioactive Ca^{45}, show that most of the Ca in the shell enters these animals through the stomach [12]. It should also be explained that the 5-fold lead pollution effect on animal food illustrated in Table 1 is super-imposed on an overall lead pollution of the ambient environment, which on a world-wide scale, seems to amount to about a 4-fold increase above natural prehistoric levels, producing an overall lead pollution factor of about 20-fold in the Abalone Cove region cited in Table 1. This is justified in the southern California region by the data in Table 2, where it can be seen that the industrial lead input to coastal waters near Los Angeles is an order of magnitude greater than the prehistoric natural input of lead to the same region. These same data show that the advection of ambient seawater lead (which is about 75% industrial) in and out of the region is the same order of magnitude as the industrial input at this locality.

The increase of industrial lead in coastal marine ecosystems is recorded in basin sediments. Goldberg and Chow pioneered these observations in the southern California region by a combination of reliable Pb^{210} dating of the sediments with identification through stable lead isotopic tracers of the industrial source of the increased amount of lead in the more recent sediments [13]. We have confirmed these lead isotopic findings by more detailed studies of the Santa Barbara Basin sediments that are shown in Figures 1 and 2 [14]. Increasing concentrations of lead are associated with changes in the isotopic composition of lead which match changes in both the amounts and isotopic composition of atmospheric industrial lead aerosols in

Table 2. Mass balance of lead in the Southern California Bight. This is a 40 km wide coastal water strip between San Diego and Pt. Conception bounded by chain of channel islands on seaward side.

(tons Pb/yr/12000 km²)

Type of Input	
Ambient seawater lead entering Bight by advection	280
Dry aerosol deposition lead	280
Sewage lead	200
Storm runoff lead plus dry season flow from storm drains	170
Prehistoric natural river clay lead	40
Rain lead	20
Industrial river dissolved lead	20
Prehistoric natural river dissolved lead	1
Type of Output	
Ambient seawater lead leaving Bight by advection	280
Addition of lead pollution to ambient seawater leaving Bight	530
Industrial lead accumulated in sediments within Bight	160
Natural clay lead accumulated in sediments within Bight	30
Natural clay lead leaving Bight by advection	10

Data revised from: C. Patterson and D. Settle "Contribution of lead via aerosol impact to the So. California bight", Journal de Recherches Atmospheriques, Numero Special 8, 957-960, (1974); and Huntzicker, J. J. S. K. Friedlander and C. I. Davidson, "Material balance for automobile emitted lead in the Los Angeles basin", Environmental Science and Technology 9, 448 (1975).

Southern California. Atmospheric industrial lead aerosols generated mainly from auto exhausts in California possess Pb^{206}/Pb^{207} ratios which changed from 1.145 in 1964 to 1.20 in 1972 to 1.25 in 1977. The calculated lead isotopic compositions of mixtures of industrial lead coming from such aerosols with lead in the sediments at 1940 agree within experimental error of measured isotopic values. This is conclusive proof that the lead is anthropogenic, that it originates mainly from gasoline lead, and that the lead concentration changes are not derived from natural climatic, biologic, or diagenetic processes. It must be emphasized that the changes in the concentrations of lead in the sediments do not necessarily reflect changes on a 1 to 1 basis of lead concentrations in overlying waters.

The anthropogenic lead input flux we observed in this inner coastal basin is the same as that reported earlier by Goldberg and coworkers, that is, about 2 μg $Pb/cm^2/yr$ in excess of a prehistoric flux of about 1 μg $Pb/cm^2/yr$. As might be expected, coastal basins further out on the continental shelves show smaller but still observable anthropogenic lead additions, about 1/20th in excess of natural fluxes in some cases [15]. Other measurements have been made of increased lead deposition fluxes in recent decades in sediments at other coastal locations in the world, but these measurements have not used lead isotopic tracers to identify the sources of the extra lead. It is hoped that the isotopic tracer experiment for leaded gasoline now being carried out in Italy will provide additional helpful information on this subject within a few years. Most studies relate to bays that have been heavily industrialized and highly perturbed by lead effects that cannot be generalized on a world scale. On the east coast of North America, for example, Narragansett Bay shows

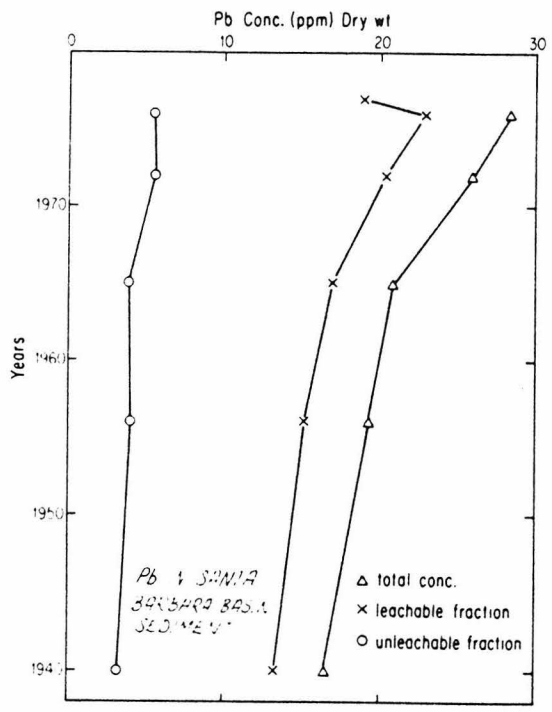

Figure ___ The occurrence of lead in sediments from the anoxic region of the Santa Barbara Basin, southern California. Samples collected and dated by A. Soutar and co-workers.

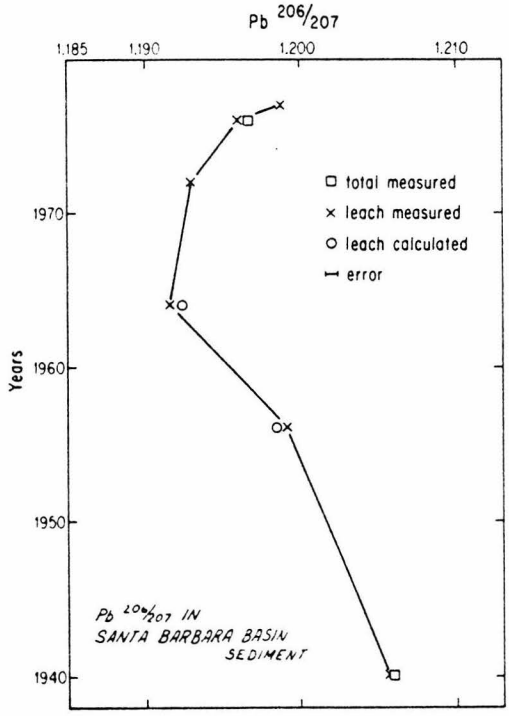

Figure 2. Secular changes in the isotopic compositions of lead in Santa Barbara Basin sediments.

an anthropogenic lead input of 170 μg Pb/cm^2/yr, compared to a prehistoric flux of 3 μg Pb/cm^2/yr [16]. However, much more modest lead pollution effects are also observed in bays near heavily industrialized regions. West of Kiel, in the Baltic Sea, for example, Eckernförder Bucht shows an anthropogenic lead input of 2 μg Pb/cm^2/yr in excess of natural background [17]. These two examples represent the range of local anthropogenic effects reported in coastal regions of the world. It has been claimed, on the basis of the observed presence of coal residues in the two examples given above, that the source of the anthropogenic lead is coal, but this is not necessarily true, because the isotopic compositions of the leads have not been determined. It has been estimated on the basis of temporal changes in coal residue concentrations that at least 1/3rd of the excess anthropogenic lead flux in each of the above examples probably originates from auto exhaust lead. The latter source may account for much of the recent industrial lead, while losses from lead smelters and widespread use of lead account for most of the rest in earlier times.

This work was supported by the IDOE Office of the National Science Foundation through grant OCE74-24362 A02 and by the Bureau of Land Management, US Department of Interior contract 696-16. This article is a summary of an oral presentation which includes discussion of some unpublished work by Schaule and Patterson [2], Hirao, Elias, and Patterson [11], as well as by the listed authors. Manuscripts of these studies are in preparation and will be published in full technical detail. Contribution No. 3050: Publications of the Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, California 91125, USA.

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