

BIOAVAILABILITY OF LEAD AND CADMIUM FROM MINE TAILINGS

TO THE POCKETBOOK MUSSEL (LAMPSILIS VENTRICOSA)

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ABSTRACT

The Big River, a hard-water, alkaline stream that drains an inactive mining district in southeastern Missouri, has been heavily contaminated by mine tailings rich in Pb and Cd. Tailings constitute a significant percentage of the bottom sediments as far as 100 km downstream from the mining district, and Pb and Cd concentrations in the biota are higher there than in upstream areas. We measured concentrations and used a series of progressively stronger reagents to estimate the availability of Pb and Cd in tailings and in streambed sediments at one site upstream (11 km) and two downstream (0.5 and 93 km) from the source of tailings to the river. Sediments were collected with a pump system, maintained under oxygen-free conditions, and extracted in five operationally defined fractions: exchangeable, bound-to-carbonates, bound-to-oxides, bound-to-organic matter, and residual. We also measured Pb and Cd concentrations every 2 wk for 8 wk in pocketbook mussels (Lampsilis ventricosa) that had been transplanted from an uncontaminated stream to the Big River, and in naiades indigenous to various reaches of the river. Both Pb and Cd originally occurred in the ores of the mining district as insoluble sulfide minerals, which should not have been extracted until the residual step; however, relatively little Pb was found in the residual fraction, even in tailings and in streambed sediments 0.5 km downstream from the tailings source. Conversely, >50% of the Cd was in the residual fraction at this site and in tailings; this residual percentage declined with distance downstream. At the downstream sites, the bound-to-carbonate and bound-to-oxide fractions accounted for >50% of both Pb and Cd. Relatively small percentages of Pb and Cd were found in the exchangeable and bound-to-organic fractions at any site. Concentrations of Pb and Cd were higher in the shells and soft tissues of indigenous naiades from the contaminated sites than in naiades from the upstream site, but the concentrations were not correlated with either total concentrations or any of the operationally defined fractions in sediments. Transplanted pocketbook mussels exposed at the contaminated sites rapidly accumulated Pb and Cd in their soft tissues; those placed upstream did not. Concentrations in naiades after 8 wk of exposure were positively correlated with total concentrations in sediment as well as with all operationally defined fractions. Examination of the gut contents of indigenous naiades from the Big River revealed a particle-size distribution much finer than that of surficial stream sediments at the sites of capture. Consequently, the "availability" of particulate

metals to filter-feeding organisms depended upon both the distribution of the metals in the various particle-size fractions and on the strength of the bonds between the metals and the particles.

INTRODUCTION

The term "bioavailability" reflects the premise that for some environmental contaminants, organisms may be exposed to less than the total amount present in their habitat. Early tests to determine the toxicity of Zn and Cu (Sprague 1964) and ammonia (Ball 1967) to fish revealed that water quality factors such as pH and hardness affect toxicity, and suggested that under some environmental conditions even the most hazardous contaminants may be neither toxic nor available. Later studies (e.g., Sunda and Guillard 1976) showed that these effects were due to the differential solubilities of the various chemical forms of toxicants. As a result of these discoveries, bioavailability, in terms of chemical speciation, now forms the basis of the Water Quality Criteria (U.S. Environmental Protection Agency 1980) for many contaminants. Development of the concept has led to the belief that some unavailable, refractory forms of contaminants may be sequestered in sediments for periods of time that can be considered permanent, at least on the ecological time scale, and forms the basis for the use of bioaccumulation tests to evaluate disposal alternatives for contaminated dredged materials (U.S. Army Corps of Engineers 1977).

The bioavailability of an environmental contaminant can be estimated in several ways. For some elemental contaminants, the forms (species) present in water can be determined directly by wet chemistry (e.g., Andreae 1977; Cutter 1978). Alternatively, the total concentration can be apportioned to the various species on the basis of thermodynamic data (e.g., Stumm and Morgan 1981; Ingle et al. 1980). The bioavailability of contaminants in sediments, however, is more difficult to assess because it may not be possible to determine exact chemical forms by direct measurements. Instead, numerous indirect schemes have been devised for quantifying the strengths of the associations between chemical contaminants and sediment particles (e.g., Tessier et al. 1979). These methods generally involve the extraction of contaminants from sediments with a series of progressively stronger chemical reagents, and the measurement of the contaminant concentration in each extract.

As an alternative to chemical methods, a more direct approach to the assessment of bioavailability involves the comparison of contaminant concentrations in sediments and water with concentrations in organisms that are either indigenous to the study area or transported to it and allowed to accumulate contaminants. The methods can also be used together; the concentrations of the various chemical fractions in sediments or water and in the tissues of the organisms can be subjected to statistical analysis (usually correlation or regression) to elucidate the contribution of each fraction to the body burden of the organism (e.g., Luoma and Jenne 1977; Tessier et al. 1984). We report here the results of our efforts using this

latter approach of combined chemical and statistical methods to evaluate the bioavailability of Pb and Cd in southeastern Missouri mine tailings to the pocketbook mussel, Lampsilis ventricosa, a naiaid native to the area.

STUDY AREA

The Big River drains the "Old Lead Belt," an extensively mineralized area of the Ozark Plateau in southeastern Missouri that was mined for Pb and Zn from the time of the first French settlers in the Mississippi Valley until the last mine in the district closed in 1972. Originating as a clear, gravel-bottomed stream in Iron County, the river flows about 50 km eastward to the Old Lead Belt, then essentially northward for about 175 km to its confluence with the Meramec River not far from St. Louis (Fig. 1).

Before 1972, the Old Lead Belt was one of the foremost Pb producing regions in the world. Between 1860 and 1972, more than 7,300 metric tons of Pb were extracted from dolomitic ore containing dispersed galena (PbS), sphalerite (ZnS), and other minerals. This activity resulted in the accumulation of about 227 million metric tons of fine-grained dolomitic tailings divided among five large piles adjacent to the Big River and its tributaries (Kramer 1976); these piles have not been maintained since the mines closed. Because the metal extraction technology used during much of the district's active life was inefficient by modern standards, the tailings contain high concentrations of Pb, Zn, and Cd.

In 1977, attention was focused on the river and its environmental problems when a drainage control structure at an unmaintained pile near Desloge, MO, became clogged during a severe thunderstorm, and an estimated 38,000 m³ of tailings washed into the river when the pile collapsed. This catastrophic event, along with continuing erosion from the Desloge and other tailings piles, has caused the accumulation of substantial quantities of tailings in the river. As a consequence, the naiaid fauna of the most severely affected reaches of the Big River is all but nonexistent (Buchanan 1980), and other components of the benthic community have also been degraded (Whelan 1983).

For our investigations in the Big River watershed, we established study sites near the town of Irondale, upstream from all known sources of tailings to the river; near Leadwood, at the upstream end of the reach affected by tailings; at Desloge, 0.5 km downstream from the tailings break; at Washington State Park, 60 km downstream; and at Brown's Ford, 90 km downstream (Fig. 1).

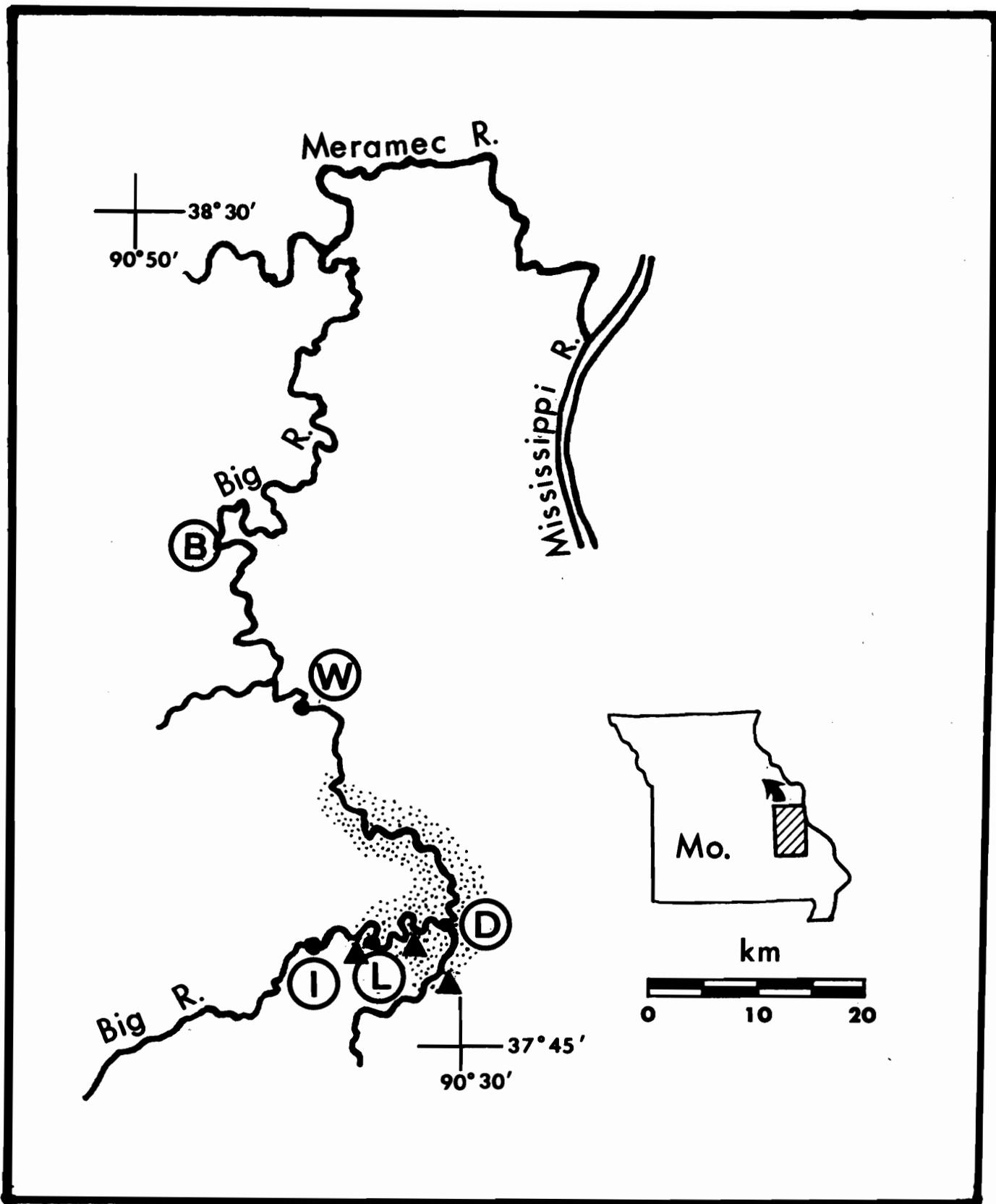


Figure 1. Big River study area, showing three major sources of mine tailings to the river (triangles), river reaches where the sediments are composed primarily of tailings (shaded area), and collection sites at Irontdale (I), Leadwood (L), Desloge (D), Washington State Park (W), and Brown's Ford (B).

METHODS OF STUDY

The general approach used was comparison of the Pb and Cd concentrations in naiades, both indigenous to the Big River and imported from an uncontaminated river, to operationally defined chemical fractions of Pb and Cd and measured size fractions of surficial streambed sediments.

Field Methods

Indigenous naiades, either pocketbook mussels or Britt's shell (Lampsilis reeviana brittsi), were collected in July 1980 from Irondale (n = 5), Leadwood (n = 2), Washington State Park (n = 6), and Brown's Ford (n = 3). Subsequent studies have shown these two species to be functionally equivalent in trace metal content when collected at the same place and time (A. Buchanan, Missouri Department of Conservation, unpublished). The mussels were transported live to the laboratory, held for 48 h in clean, flowing well-water to allow the purging of ingested sediments from their gills and digestive tracts, and then frozen for later processing and metals analyses.

The gut contents of additional indigenous naiades from each site (n = 6) were analyzed without a depuration period to determine the size distribution of ingested particles. These mussels were dissected immediately upon return to the laboratory; all dissection equipment, laboratory-ware, and work surfaces were acid-cleaned as described by Schmitt and Finger (in press). The valves of each mussel were pried apart, the adductor muscles were severed, and the soft tissues were carefully separated from the shells and placed on a surface covered with polyethylene sheeting for further dissection. The digestive tract was separated from the other tissues with forceps, severed with a stainless steel razor blade, and placed on the polyethylene-covered work surface. A longitudinal incision was made with a razor blade to expose the gut contents, which were emptied into a 50-mL borosilicate glass beaker; any particles adhering to the gut wall were rinsed into the beaker with a small volume (~5 mL) of deionized water. Particle size distributions were then determined according to the procedure described later for sediments.

For the study of metals uptake by transplanted, caged naiades, about 200 adult pocketbook mussels were collected in July 1981 from the Bourbeuse River, an uncontaminated tributary of the Meramec, at a site near Owensville, MO. The naiades were transported live to the Big River and placed in cages (1 m x 0.5 m x 0.1 m) constructed of epoxy-coated hardware cloth. Twelve specimens were placed in each of two cages that were staked to the bottom at Irondale, Desloge, Washington State Park, and Brown's Ford. Three specimens were removed from each cage every 2 wk for 8 wk, returned live to the laboratory, and processed as described for indigenous naiades. An additional group of six naiades from the Bourbeuse River was returned directly and similarly processed to obtain an estimate of Pb and Cd concentrations at the beginning of the exposure period.

In August 1981, concurrent with the naiad uptake study, surficial streambed sediments were collected and returned to the laboratory to determine the concentrations and chemical forms of metals they contained. We located "riffle" (scouring) and "pool" (depositional) habitats at the Irondale, Desloge, Washington State Park, and Brown's Ford sites, and collected three sediment samples from each habitat type at each site, using the following technique: A 5.1-cm diameter, gasoline engine-powered diaphragm pump (Homelite^R Model DP-2)^{1/} drew bottom sediments through a 2-mm mesh, 10-cm diameter stainless steel sieve attached to a 5.1-cm diameter, 2-m long section of rigid polyvinyl chloride pipe and a 5.1-cm diameter, 22-m long flexible polyethylene hose. The pump discharged through a similar polyethylene hose into a conical, 200-L settling container (County Plastics, North Babylon, NY) that had been previously acid-washed. The suction side of the apparatus (the pipe-mounted sieve) was pushed by the operator along the bottom at each site, to obtain samples of the upper sediment layers. The conical container was then sealed, purged with N₂ to prevent further oxidation of sediments, and the sediments were allowed to settle for 90 min. The settled sediments were then drawn from the bottom of the conical containers into acid-washed, linear immediately after collection. Samples were packaged in ice and transported to polyethylene 4-L bottles. To avoid oxidation, each sample was purged with N₂ and transported to the laboratory within 24 h after collection. An additional sample of tailings material was collected from the site of the 1977 tailings break at Desloge, placed in a 4-L bottle, and processed as described above.

Laboratory Analyses

After thawing, naiades were prepared for analysis by separating the shell and soft tissues. The soft tissues were homogenized in a blender equipped with stainless steel blades, then lyophilized. The shell was scrubbed with deionized water to remove any attached algae or sediment, air dried, and pulverized in a Spex Mixer Mill^R. Before analysis, the samples were digested by a nitric-perchloric acid procedure; a known weight of sample was digested in an acid-washed Kjeldahl flask with 15-20 mL of 16-N HNO₃ and 2-4 mL of 70%-HC10₄, then heated until the volume was reduced to about 5 mL. Samples were then diluted with distilled water to a volume of 25 or 50 mL and analyzed for Pd and Cd by furnace atomic absorption (AA) spectrophotometry with a Perkin-Elmer model 305B AA spectrophotometer equipped with an HGA 2200 heated graphite atomizer and a model AS-1 autosampling system.

Immediately upon return to the laboratory, sediment samples were placed in a glove box containing N₂. One subsample was retained in the glove box for immediate sequential extraction analysis and two subsamples were set aside for particle size analysis and total trace metal determinations.

The subsample for total trace metal analysis (~1 g dry weight) was digested in a 100-mL Teflon^R beaker with 5 mL distilled water, 2 mL 70%-HC10₄ and 12 mL 40%-HF and heated to near dryness. An additional 8 mL of HF were added and the sample was heated to dryness. Several milliliters of water and 2 mL HC10₄ were then added and the sample was once again heated to

^{1/} Mention of trade names does not imply government endorsement.

dryness. The residue was dissolved in 8 mL of a 1:1 mixture of 6-N HCl and 20 mL of water, diluted to 100 mL with distilled water, and analyzed by AA.

An aliquot (~5 g wet weight) of the subsample retained for particle size analysis was analyzed for total organic carbon (TOC) with a Beckman Model 915 TOC analyzer. The rest of the subsample (~100 g) was dried and sieved (20, 30, 50, 100, 299, and 270 mesh). The fraction that remained (particles less than 60 μ m) was resuspended in deionized water. Grain size distributions of particles in this fraction was determined with a HIAC Model PC-320 particle size analyzer. These procedures were described in detail by Lochmoeller (1982).

The subsample for sequential sediment extraction was handled in the glove box under N_2 throughout the analysis. From this subsample an aliquot (~5 g) was removed for gravimetric determinations of percent moisture. Wet weight aliquots of sediment equivalent to 1 g dry material were weighed into 50-mL polysulfone (PSF) Oak Ridge-type centrifuge tubes with Viton O-ring sealing cap assemblies. The sequential chemical extraction was modified from the procedure of Tessier et al. (1979) as follows: (1) samples were purged with N_2 through the first three steps of the extraction to avoid oxidation; (2) polysulfone centrifuge tubes were substituted for polypropylene (to prevent heat distortion); (3) centrifugation was at 15,000 rpm for 15 min with a Beckman JT-21 centrifuge and JA-17 rotor; (4) a deionized water wash was added to the reagent supernatant; and (5) a shaking water bath was used in the bound-to-oxides and bound-to-organics extraction steps. In addition, care was exercised in the bound-to-organic steps to ensure that all centrifuge tube caps were loose enough to allow the escape of gaseous by-products.

The sequential extraction procedure yielded the following operationally defined fractions:

- (a) "Exchangeable Metals," which include loosely bound ions and complexes associated with the charged surfaces of particles. The sediment was extracted at room temperature for 1 h with 10 mL 1-M $MgCl_2$ (pH 7) with continuous agitation (Burrell wrist-action shakers). After this leaching with $MgCl_2$, liquid-solid separation was achieved by centrifugation at 15,000 rpm for 15 min. Under N_2 atmosphere, the supernatant was removed by pipet and placed in an acid-cleaned borosilicate glass tube. The sediment residue was washed with 10 mL of deionized H_2O and centrifuged for 15 min; this supernatant was added to the first.
- (b) "Metals Bound to Carbonates," which include precipitated metal carbonates as well as any sorbed metals not removed in the previous step. Residue from (a) was extracted at room temperature with 10 mL of 1-M NaOAc and adjusted to pH 5 with HOAc. Because of the fine texture of the sediment samples, extraction time was limited to 5 h. The extraction was followed by the centrifugation and washing steps described in (a).

- (c) "Metals Bound to Fe and Mn Oxides," which may be liberated under the reducing conditions simulated by the procedure. The residue from (b) was extracted with 20 mL of 0.04 M $\text{NH}_2\text{OH}\cdot\text{HCl}$ in 25% HOAc at $96 \pm 3^\circ\text{C}$ for 6 h; centrifugation and a wash with 5 mL distilled H_2O followed.
- (d) "Metals Bound to Organic Matter," which include those associated with the surfaces of organic-rich particles, as well as metals that have been biologically incorporated into living material. To the residue from (c) we added 3 mL of 0.02-M HNO_3 and 5 mL 30% H_2O_2 adjusted to pH 2 with HNO_3 . The mixture was heated to $85 \pm 2^\circ\text{C}$ for 2 h with agitation. A second 3-mL aliquot of 30% H_2O_2 was then added and the sample heated again for an additional 3 h with agitation. After cooling, 5 mL of 3.2-M NH_4OAc in 20% HNO_3 was added and the sample was agitated for 30 min. Centrifugation and a wash with 5 mL H_2O followed.
- (e) "Residual Metals," which include minerals and other refractory forms that are essentially unavailable. The solid from (d) was digested with a 5:1 mixture of HF and HClO_4 . The sample was first digested in a Teflon beaker with 2 mL 70% HClO_4 and 10 mL 40% HF to near dryness. Another 1 mL of HClO_4 was added and the sample was evaporated until white fumes appeared. The residue was dissolved in 12-N HCl and diluted for analysis.

After processing, the extracts and washes from steps (a) through (d) were diluted to a final volume of 25 mL, acidified to pH 2 with HNO_3 , and stored in a cool, dark place until analysis. Metal concentrations in each liquid extract were determined with a Perkin-Elmer Model 5000 AA spectrophotometer equipped with an AS-50 autosampler. We used reagent-matched standards to calibrate the readout of the Model 5000 directly in units of concentration. Low levels of background absorption were observed in the carbonate- and oxide-bound fractions. Background correction for these extracts was made with a deuterium arc lamp continuum. As a check for matrix interferences, a single standard addition was performed for each element on selected individual samples with spiking concentrations approximately equivalent to the concentrations in the samples. Tessier et al. (1979) reported as much as 15% suppression of the analyte signal from matrix effects. In our study, recoveries of spiked Pb and Cd from the first four extraction steps indicated less than 10% signal suppression or enhancement from the sample matrix. This degree of matrix interference was considered negligible, making method-of-additions determinations unnecessary. The procedure is diagrammed in Figure 2.

Detection limits were 0.01 $\mu\text{g/g}$ (ppm) for Pb and Cd in mussels, 0.40 ppm for Cd in sediments, and 4.0 ppm for Pb in sediments. All reported concentrations are per unit of dry weight.

Sequential Extraction of Sediments

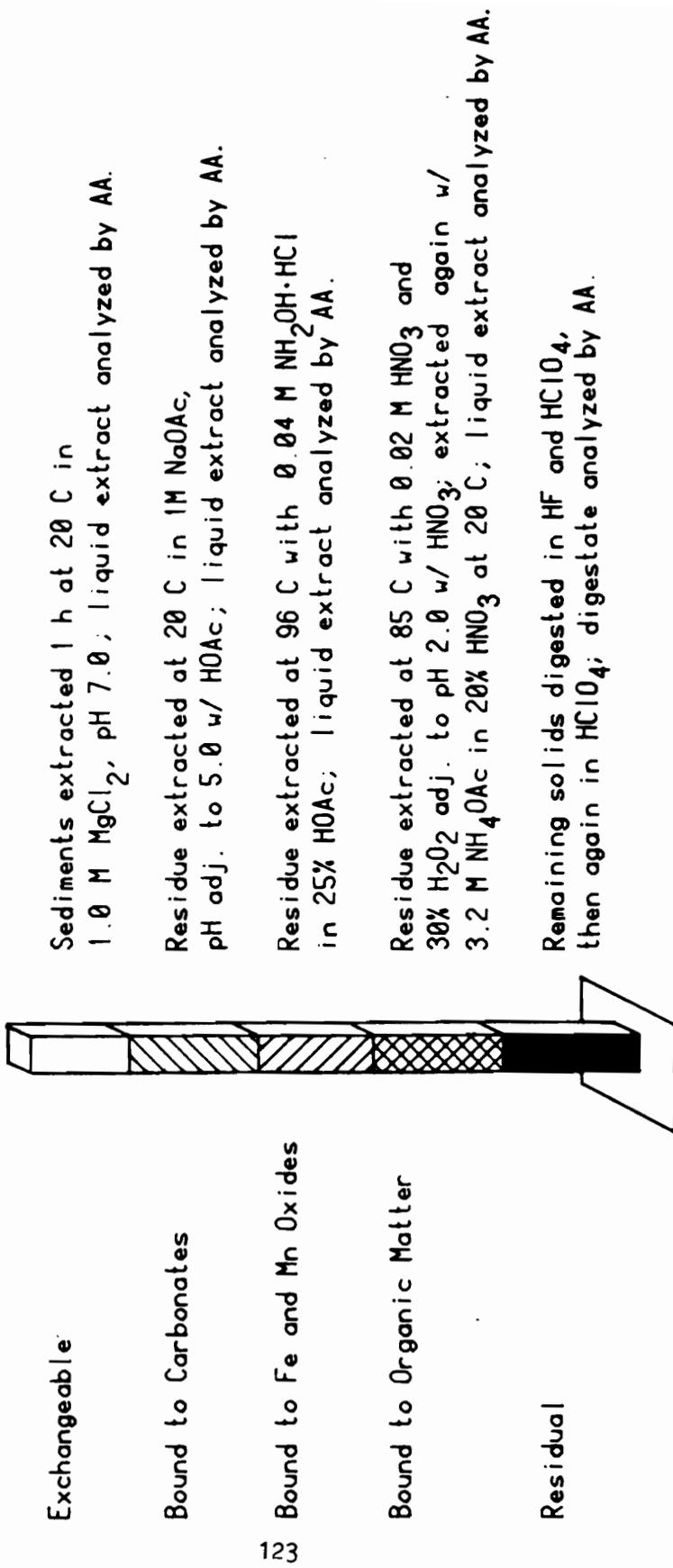


Figure 2. Summary of the sequential extraction procedure used to characterize sediments (modified from Tessier et al. 1979).

The quality control matrices used were NBS bovine liver, tuna, and oyster tissue. Results were deemed acceptable if values generated by laboratory analysis were within 20% or two standard deviations of certified values for the samples. Within each set of samples analyzed, 10% were blanks to monitor for contamination during sample preparation, and an additional 20% were blind replicates and spiked samples.

Statistical Analyses

Simple linear regression was used to model the relation between Pb and Cd concentrations in the soft tissue of mussels with Pb and Cd concentrations in the fine sediments and in various extraction fractions. Separate analyses were performed for indigenous and transplanted mussels at each location. Because these analyses are not independent of one another, the patterns of correlation among sequential sediment fractions and locations are of the greatest interest. Transformations (natural log and angular) were used where necessary to achieve symmetry, linearity, and stable spread of the variables involved. For cases in which sample sizes were small ($n < 5$), formal tests of significance were avoided.

It is important to understand the comparability or equivalency of different methods for estimating total Pb and Cd concentrations in sediment, although this was not a primary objective in our examination of the relationships between metal concentrations, sediment structure, and the bioavailability of Pb and Cd to naiades. In this study, total concentration values were estimated by two methods: (1) independent subsamples for which only the total concentration was determined by AA, and (2) subsamples on which sequential extraction was performed to yield separate values for each fraction. The values determined for each fraction were then summed to calculate total values. A series of sample statistics known as "measures of reliability" (Wilson and Seabaugh 1981) were used to compare sequential totals, Y_i , with independent totals, Y_i . The following five measures were used: (1) bias, defined as the mean difference between sequential totals and

independent totals, $\frac{1}{n} \sum_{i=1}^n (\hat{Y}_i - Y_i)$; (2) root mean square error, defined as

$[\frac{1}{n} \sum_{i=1}^n (\hat{Y}_i - Y_i)^2]^{1/2}$; (3) standard deviation, defined as

$[\frac{1}{n} \sum_{i=1}^n (d_i - \bar{d})^2]^{1/2}$ where $d_i = \hat{Y}_i - Y_i$; (4) percent of

relative differences greater than 10%, where relative difference, $rd_i = (\hat{Y}_i - Y_i)/Y_i$; and (5) correlation (r) between sequential and independent totals.

RESULTS AND DISCUSSION

It was common knowledge that the Old Lead Belt tailings were rich in Pb and Cd, and that these metals were present in Big River sediments. Total Pb levels were about 40 ppm (dry weight) in sediments at Irondale, rose to about 2500 ppm within the Old Lead Belt, and then declined with distance downstream (Table 1). Similarly, Cd levels were <2 ppm at Irondale, rose to about 40 ppm at Desloge, then also declined with distance downstream. The concentrations declined because some tailings have been washed from the streambed, and because the tailings are diluted by clean sediments derived from uncontaminated areas of the watershed. The natural stream sediments are typically dominated by coarse-textured, inorganic material, even in pools. The tailings are dolomite and limestone ground to a texture that resembles beach sand. As a consequence, there is comparatively little fine particulate or organic material in most Big River sediments (Table 1).

Concentrations of Pb, Cd, and TOC differed among habitat types at the three downstream sites. At Desloge and Washington State Park, concentrations of Pb, Cd, and TOC were higher in riffle than in pool sediments, as was the percentage of sediments < 60 μm (Table 1). At Brown's Ford, higher percentages of fine sediments and concentrations of Pb, Cd, and TOC were found in pool than in riffle sediments. At Irondale, Pb and Cd concentrations were uniformly low in both habitats, with higher percentages of fine sediments and TOC concentrations in pool habitat. Collectively, these data suggest a lack of depositional zones in the middle reaches of the river (Desloge-Washington State Park).

It was widely believed that the metals in tailings and the sediments of the Big River were immobile because they originally occurred in the Old Lead Belt ores as sulfide minerals, which are virtually insoluble. Thermodynamic data suggested that dissolved metals would either precipitate or form complexes and thereby remain in biologically inactive forms under the hardwater, alkaline conditions typical of the region. This view was supported by water quality data, which showed that dissolved metals concentrations seldom exceeded drinking water standards. Consequently, the tailings situation was viewed as more of an aesthetic nuisance than an environmental hazard.

The sequential extraction procedure revealed surprisingly low percentages of residual metals and substantial differences between the distribution of Pb and Cd among the fractions at the collection sites. At the upstream site, Cd in the individual extracts was below detection limits; at Desloge, in both tailings and in river sediments, residual Cd accounted for slightly more than half the totals, most of the rest being primarily in the carbonate- and oxide-bound fractions; and further downstream, the sediments contained substantially lower proportions of residual Cd, and much more was in the less tightly bound fractions (Fig. 3). For Pb, however, the residual fractions contained only 24% of the total at the upstream sites and in the tailings; there was little "Exchangeable" Pb at any of the sites (Fig. 4). Downstream, the residual percentages were about the same as for Cd (6%), and the organic

Table 1. Characteristics of sediments finer than 2 mm from riffle and pool habitat at four sites on the Big River.

Characteristic and habitat	Collection Site			
	Irondale	Desloge	Washington State Park	Brown's Ford
Elemental concentrations (ppm, dry weight)				
Total Pb				
Riffle	37.3	2833.0	1590.0	650.0
Pool	42.3	1966.7	1350.0	2633.0
Total Cd				
Riffle	0.1	32.0	5.1	4.6
Pool	0.2	49.0	1.8	7.4
Total organic carbon (ppm, dry weight)				
Riffle	1700	4300	2900	1500
Pool	6000	200	1400	7900
Percentage >60 μ m				
Riffle	96	93	93	92
Pool	90	99	98	55

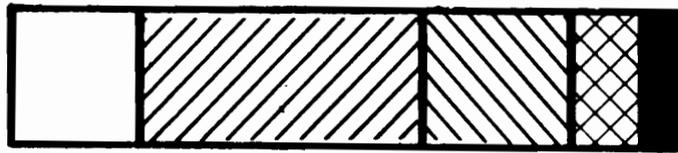
Cd DISTRIBUTION (%), BY LOCATION

DESLOGE

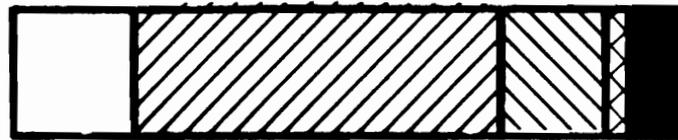
FRACTION

EXCHANGE-
ABLE
CARBON-
ATES
OXIDES
ORGANICS
RESIDUAL

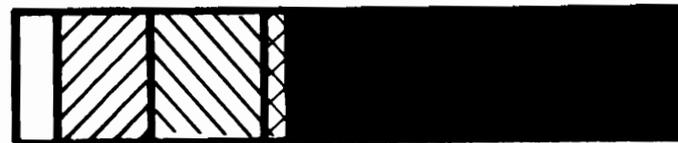
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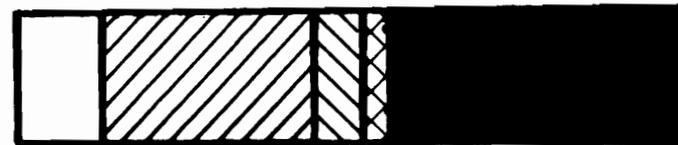
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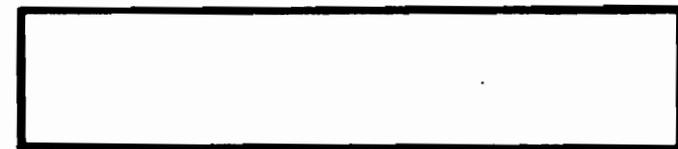
S



T



I



6.4

10.8

32.7

14.8

1.6

TOTAL Cd (PPM)

Figure 3. Mean total concentration and proportional distribution of Cd in sediments from three sites on the Big River and in sediments (S) and tailings (T) from a fourth site at Desloge, Mo. See Figure 1 for locations of sites and Figure 2 for interpretation of chemical fractions.

PB DISTRIBUTION (%), BY LOCATION
DESLOGE

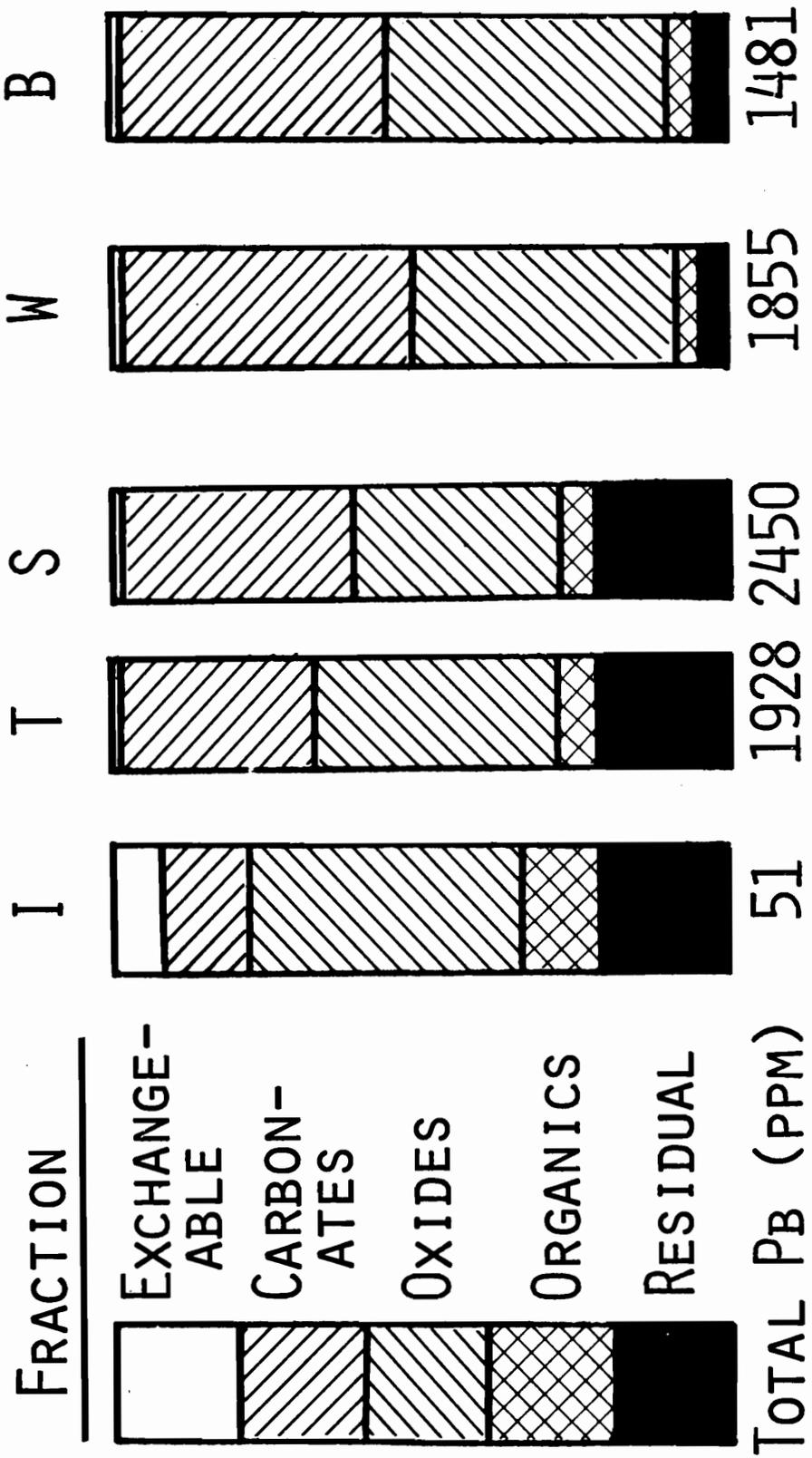


Figure 4. Mean total concentration and proportional distribution of Pb in sediments from three sites on the Big River and in sediments (S) and tailings (T) from a fourth site at Desloge, Mo. See Figure 1 for locations of sites and Figure 2 for interpretation of chemical fractions.

fraction contained <5%. The carbonate- and oxide-bound fractions contained the bulk of the Pb at all sites within and downstream from the tailings area. These results suggested that there were higher concentrations of "available metals" downstream even though the total metal concentrations were lower. One explanation for these observations is that the metal sulfides in the tailings and in the tailings-contaminated sediments are continuously increasing in solubility through biotic and abiotic oxidation of particle surfaces. Once in solution, Pb and Cd are then removed from the water through precipitation as metal carbonates, sorption by Fe and Mn oxides, or binding by living and nonliving organic matter.

Sequential and independent totals for Pb concentrations were not absolutely equivalent (Table 2). The average difference (bias) was 9.4% of the independently derived total, and about 56% of the sequential totals differed from the independent totals by more than 10% of the independent value. Although sequential totals were in closer agreement with the independent totals for Cd than for Pb, with a bias of 3.1%, more than 80% of the values differed by greater than 10% of the independent values. This figure is influenced a great deal by the substantially lower concentrations of Cd than of Pb, which resulted in smaller absolute differences, but a large relative difference, between sequential and independent totals for Cd. Although sequential totals were not absolutely equivalent to independently derived totals for either Pb or Cd, they provided good indices of the independent totals, as indicated by correlations of $r = 0.987$ and $r = 0.990$, respectively (Table 2). A high degree of correlation between sequential and independent totals does not imply equivalence, however, but does indicate that the totals changed in the same manner from sample to sample. Collectively, the measures of reliability suggest that the methods are relative indices of concentration which are consistent with one another, but that comparisons should be made only among totals determined by the same method. Consequently, we used sequential rather than independent totals in all of our analyses of total concentrations except for data from Irondale, where Pb and Cd concentrations in some individual fractions were less than the analytical detection limits.

Concentrations of Pb and Cd in indigenous naiades were much higher at the sites affected by tailings than upstream at Irondale (Fig. 5 and 6), and the log-transformed concentrations in the shells were highly correlated with concentrations in the soft tissues (for Pb, $r = 0.87$, $P < 0.01$; for Cd, $r = 0.74$, $P < 0.01$). The levels in the naiades, however, did not correspond to the levels in the sediments. In the affected zone, Pb in naiades was negatively correlated with total Pb in sediments; concentrations in naiades increased with distance downstream and were highest at Brown's Ford, whereas Pb levels in sediments decreased (Fig. 5).

For Cd, concentrations in the indigenous naiades also did not parallel total concentrations in sediments (Fig. 6). The trend, however, differed from that of Pb; within the contaminated reach, Cd concentrations in both

Table 2. Measures of reliability for comparison of sequentially and independently derived total Pb and Cd concentrations.

Element	Measure of reliability					
	Bias		Root MSE	STD	% Obs > 10%	r
ppm	%					
Pb	128.0	9.4	250.64	215.45	55.6	0.987
Cd	-0.5	3.1	3.86	3.83	83.3	0.990

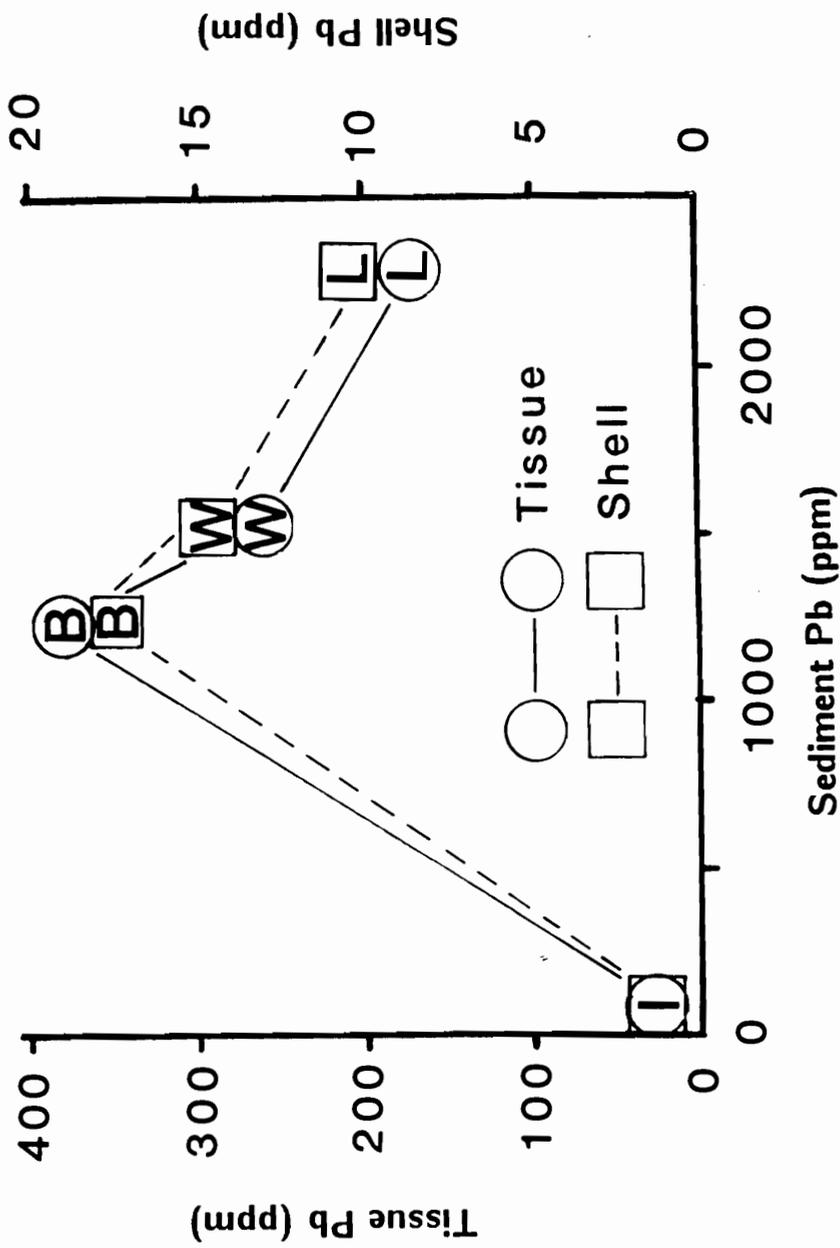


Figure 5. Concentration of Pb in the soft tissues and shells of mussels indigenous to four sites on the Big River vs. total sediment Pb concentration. All values in ppm dry weight; sites as in Figure 1.

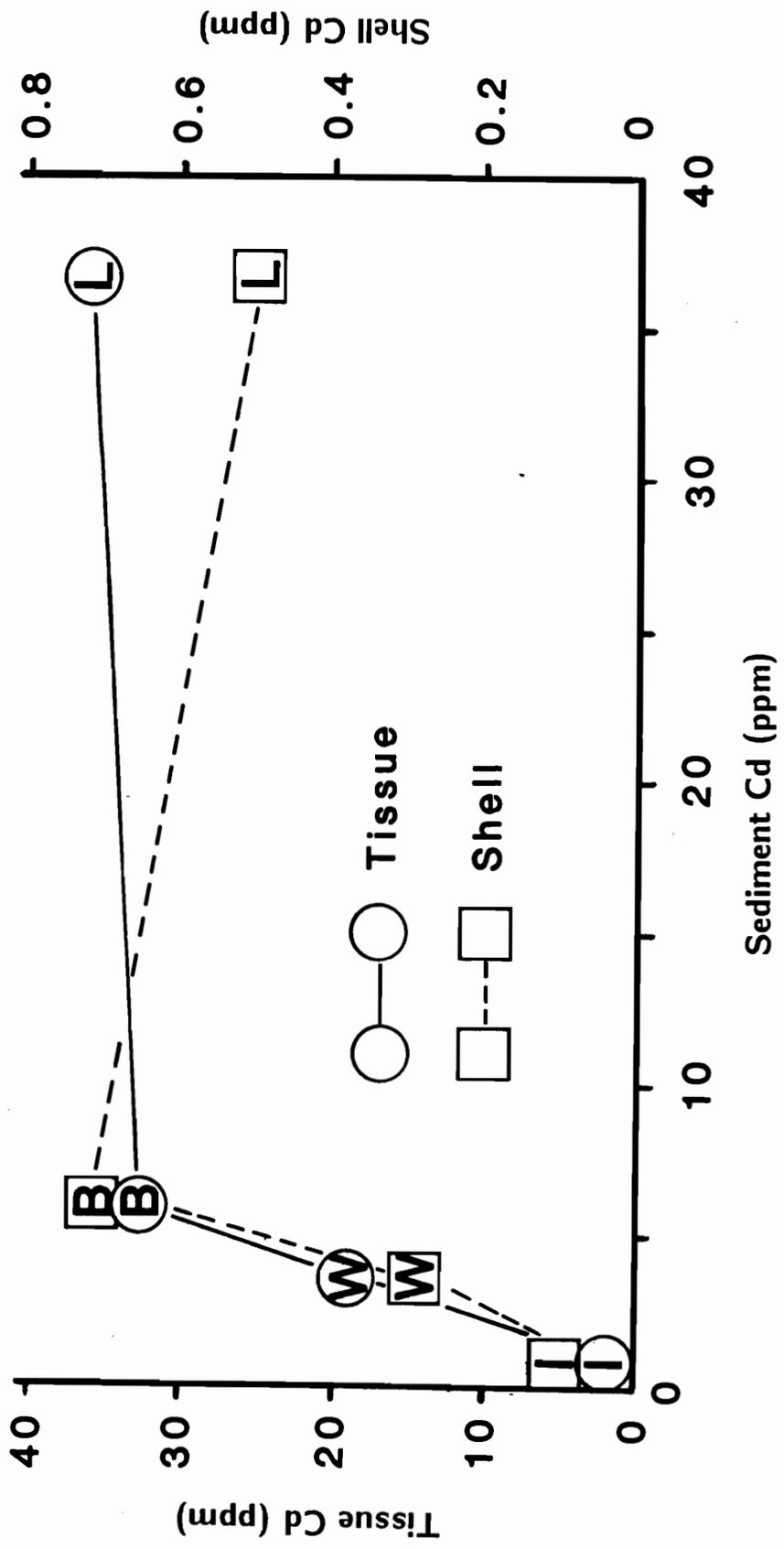


Figure 6. Concentration of Cd in the soft tissues and shells of mussels indigenous to four sites on the Big River vs. total sediment Cd concentration. All values in ppm dry weight; sites as in Figure 1.

sediments and indigenous naiades were lowest at Washington State Park, and higher at both Leadwood and Brown's Ford (Fig. 6); the concentrations of Cd in sediments and naiades were negatively correlated.

The negative correlations between Pb and Cd in sediments and indigenous naiades provided further evidence that the availability of the metals differed among the sites. Using the sequential extraction results, we attempted to determine whether any subset of Pb and Cd concentrations was more closely correlated with concentrations in the naiades than with the total concentration. We created cumulative subtotals by summing concentrations through the steps of the procedure with the assumption that, if a given fraction is available, all metals in preceding, more loosely-bound fractions are also available, and that a given subtotal is equally available at all sites. We also compared concentrations in naiades with concentrations in individual fractions. In all instances, sediment concentrations for Pb and Cd paralleled total concentrations, and all correlations between concentrations in sediments and mussels were negative; no subtotal or fraction was higher at the downstream sites than at the upstream site for either Pb or Cd. It is important to note, however, that naiades from Leadwood are compared with sediments from Desloge in Figures 5 and 6 because no naiades are indigenous to the Big River at Desloge. Although tailings are also present in the river at Leadwood, they come from a different source than at Desloge, and concentrations of Pb and Cd in sediments may be higher at Leadwood than at Desloge (Wixson et al. 1983).

Another aspect of "availability" is particle size. Naiades live at the sediment-water interface and pump through their siphons particles that are a subset of the bed, bedload, and suspended particles present (Churchill and Lewis 1924). We examined the gut contents of pocketbook mussels from several of our sites and found that at only one of these (Washington State Park) had the specimens ingested particles $>60 \mu\text{m}$. Even at this site, particles of smaller size constituted $>90\%$ of the ingested material, despite the obvious domination of the sediments themselves by much coarser particles (Table 1). It was our original intent to statistically adjust the metal concentrations in the sediment samples to the particle-size distribution ingested by the naiades; however, because of the domination of the sediment samples by coarse material, the scarcity of particles $>60 \mu\text{m}$ in the gut contents of the naiades, and the limited number of samples from each site, we restricted our analyses to the relation between the percentage of fines in the sediment samples and their Pb and Cd concentrations.

We fitted a simple linear regression model to the sequential extraction and particle size data from each site:

$$\log_e (\text{conc}) = a + b \left[\arcsine \sqrt{\frac{\% \geq 60 \mu}{100}} \right],$$

where the term on the left represents the log-transformed sequential and total concentrations of Pb and Cd, a and b are estimates of the intercept and regression coefficient (slope), respectively, and the expression in brackets on the right is the proportion of coarse material ($>60 \mu\text{m}$) in each sample

(subjected to angular transformation). The sign of the regression coefficient indicates whether or not the concentration increases or decreases with the proportion of coarse particles in the samples, and can be tested to determine whether the relations differ among the sites. Given a sufficient range of percentages, the back-transformed intercepts should also estimate the concentrations in the particles $<60 \mu\text{m}$ -- concentrations that are "available" to naiades. Unfortunately, the sediment samples contained mostly coarse particles (Table 1), and the confidence limits for the intercept estimates were so large that they were of little value. Many of the slopes were significant, however, and differences among locations were apparent.

For Pb, most of the regression coefficients were negative, as expected, because concentrations of total Pb and concentrations in most fractions were higher in finer than in coarse sediments (Table 3). Several coefficients were positive, however, and the slopes for some fractions differed among the sites. Furthermore, concentrations of Pb at Desloge did not change in the same direction with sediment texture for all fractions. The regression coefficient for residual Pb at Desloge was large (positive), indicating enrichment in the coarser size fractions. The slopes for Cd were similar; most were negative, but a few, including the coefficient for the residual fraction at Desloge, were positive (Table 2). We suspect that the differences noted reflect the fact that residual-rich coarse material derived from tailings has been deposited in the upper reaches of the river, and that more of the metals-enriched fines have been scoured from the bed and deposited downstream, where the coarse material has been derived from the watershed and contains less Pb and Cd. These locational differences were important because they indicated that our original intent to describe the relations between particle size and metals concentrations by grouping data from the four sites and computing a single set of linear or multiple linear regression equations was inappropriate.

Results of the caged mussel study further confirmed the availability of Pb and Cd. Concentrations of both metals in the pocketbook mussels collected from the Bourbeuse River were low (<0.1 to $1.5 \mu\text{g/g}$ for Pb, <0.20 for Cd). Uptake of Pb into the soft tissues was essentially log-linear over the 8-wk accumulation period after an initial period of rapid accumulation, with no sign that the concentrations had reached a plateau at any site; there was no change in concentration at Irondale, the upstream site (Fig. 7). In contrast with the concentrations in the indigenous naiades, the slopes of the uptake curves decreased with distance downstream from the tailings, with both the uptake rates and the 8-wk concentrations essentially paralleling the sediment concentrations (highest at Desloge, intermediate at Washington State Park, and lowest at Brown's Ford). The results for Cd were similar; there was no change at Irondale, uptake rates and 8-wk concentrations at the contaminated sites declined with distance downstream (Fig. 8). Again, the order was different from that of the indigenous naiades. We repeated the correlation analyses with these results (sequential sediment concentrations vs. 8-wk concentrations in pocketbook mussels), and found that in contrast to the results obtained for the concentrations in the indigenous naiades, the uptake rates for both Pb and Cd in transplanted pocketbook mussels were equally well

Table 3. Results of regression analysis^{1/}, as regression coefficients (b) and coefficients of determination (r²), relating concentrations of Pb and Cd in sediments to the percentage of sediments finer than 60 µm (n = 6).

Element, fraction and statistic	Collection Site		
	Irondale ^{2/}	Desloge	Brown's Ford
Pb			
Exchangeable			
b ₂	0	0.24	-1.46
r ²	0	0.04	<u>0.91</u>
Carbonate-bound			
b ₂	-0.96	-1.63	-1.64
r ²	0.49	<u>0.85</u>	<u>0.82</u>
Oxide-bound			
b ₂	-0.58	-1.32	-0.92
r ²	0.21	<u>0.92</u>	0.61
Organic-bound			
b ₂	-1.39	0.01	-0.58
r ²	0.62	0	0.37
Residual			
b ₂	0.79	<u>3.02</u>	-0.84
r ²	0.22	<u>0.62</u>	<u>0.67</u>
Total			
b ₂	-0.28	0.01	-0.23
r ²	0.55	0.01	<u>0.76</u>
Cd			
Exchangeable			
b ₂	0	-2.18	0.55
r ²	0	<u>0.74</u>	0.29
Carbonate-bound			
b ₂	0	1.41	-0.91
r ²	0	0.22	0.42
Oxide-bound			
b ₂	0	-1.96	-1.14
r ²	0	<u>0.98</u>	<u>0.74</u>
Organic-bound			
b ₂	0	0.12	-0.32
r ²	0	0.01	0.12
Residual			
b ₂	0	<u>2.96</u>	-1.10
r ²	0	<u>0.66</u>	0.13
Total			
b ₂	0	1.47	-0.66
r ²	0	0.53	0.37

^{1/} Underscored values differ significantly from 0 (P ≤ 0.05).

^{2/} Values of 0 for Irondale indicate concentrations less than the detection limit for individual fractions.

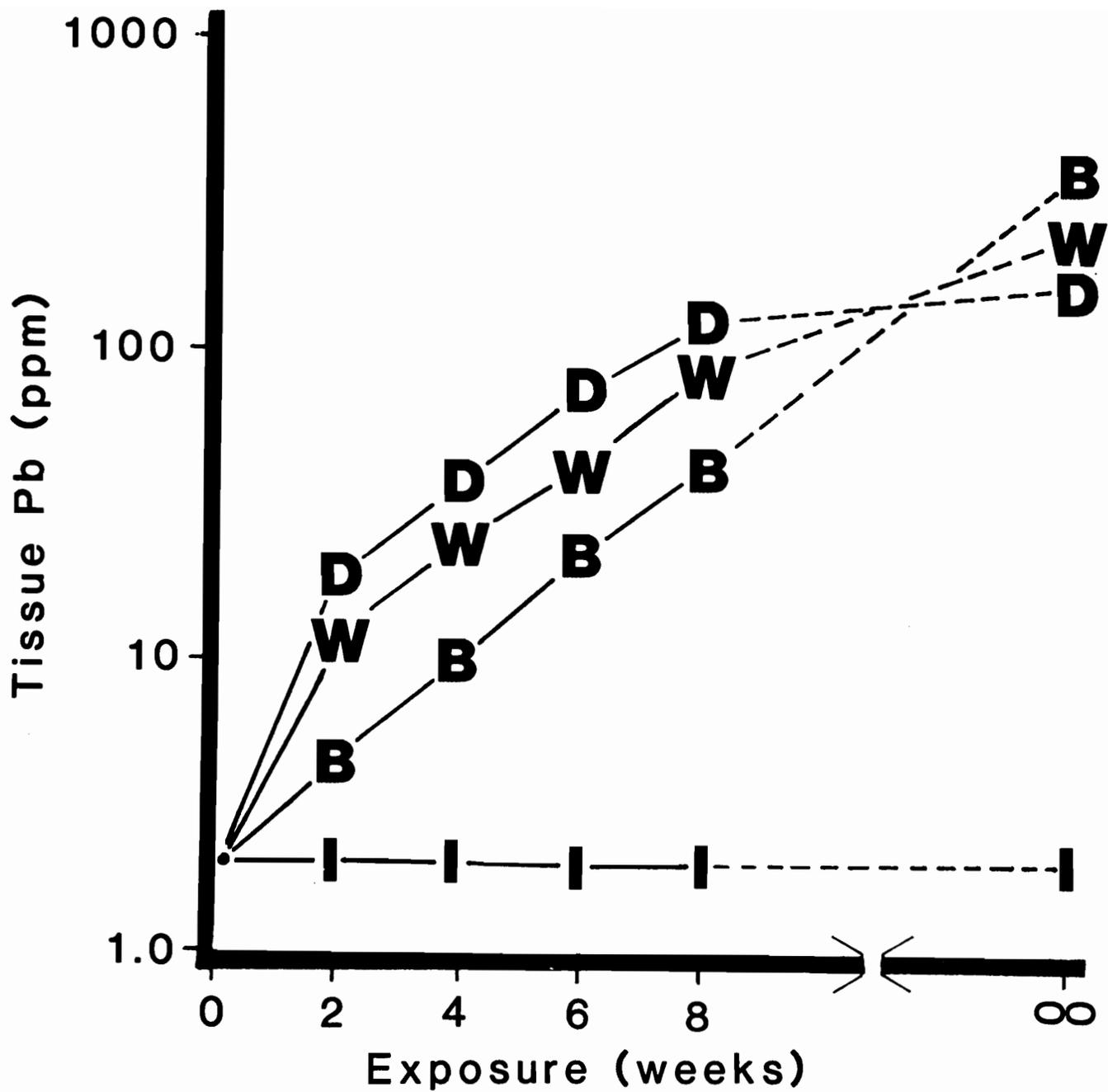


Figure 7. Uptake of Pb by mussels exposed for 8 weeks at four sites on the Big River, and concentration in mussels indigenous to the sites (final value); sites as in Figure 1.

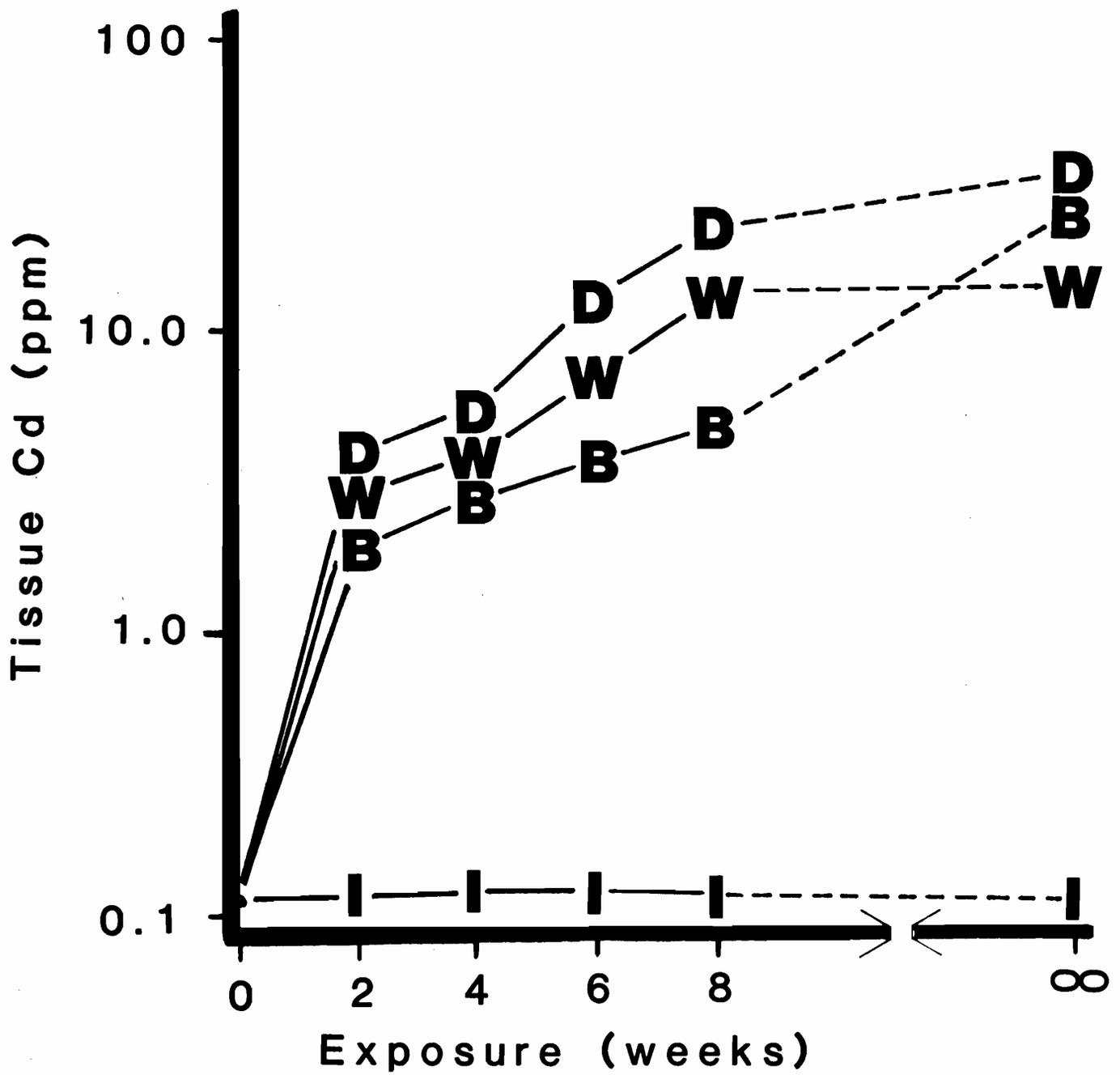


Figure 8. Uptake of Cd by mussels exposed for 8 weeks at four sites on the Big River, and concentration in mussels indigenous to the sites (final value); sites as in Figure 1.

correlated with all of the cumulative sediment concentrations, including total Pb and Cd. A plausible explanation for this anomaly is that the indigenous naiades, some of which were 20 years old, reflected a long history of gross tailings contamination, including the massive influx of tailings that occurred in 1977. Conversely, concentrations in sediment samples and in the transplanted pocketbook mussels secured in cages represented 1981 conditions. We suspect that a considerable volume of Pb- and Cd-rich fine sediments derived from tailings was washed out of the middle reaches of the Big River during the intervening period.

SUMMARY AND CONCLUSIONS

Our indirect approach to the assessment of the availability of sediment-bound metals to Lampsilis can be represented as a matrix of pairs of coefficients (Fig. 9). The first set, the "E's," define the strengths of the associations between metals and particles. The second set, the "P's," represent the size distribution of particles consumed by the organism, which is some subset of the particles present. Both are continuous distributions that can be subdivided into any number of discrete subclasses for measurement. Inasmuch as chemical fractions are dependent variables, the number of these is limited only by analytical resources; however, the size classes are independent variables for which regression coefficients must be determined, and the number of coefficients that can be fitted is constrained by the number of samples. Our results suggest that careful examination is required before large numbers of samples representing heterogeneous points in time or space are grouped for such analyses.

Implicit in the indirect approach we used were some further simplifying assumptions that remain to be verified. First, we assumed that the steps in the sequential extraction scheme bear some relevance to the chemical availability of the metals to the animals. We suggest that they do, but the uptake rates of the caged mussels were correlated with all of the cumulative totals. Closely related to this assumption, we also assumed (when examining the cumulative totals) that all of the metals in preceding fractions were available, and that as a fraction was added to the cumulative total, it was 100% available. In fact, neither of these assumptions may hold; the fractions may be only partly available. And finally, a simple, linear-additive regression approach may not be adequate for examining more than the effect of the size fraction; there may be interactions between particle sizes and chemical fractions, which would occur if a given chemical fraction was more available in one size fraction than in another.

With our limited data, we were able to determine only the coefficients for the border totals of a reduced-rank version of this matrix and gain some insight into the nature of the relations between the two sets of coefficients. We demonstrated that the particulate material in downstream reaches of the river differs qualitatively from tailings and upstream sediments, and the approach provided both chemical and physical evidence that solid-phase Pb and Cd derived from tailings are present and biologically

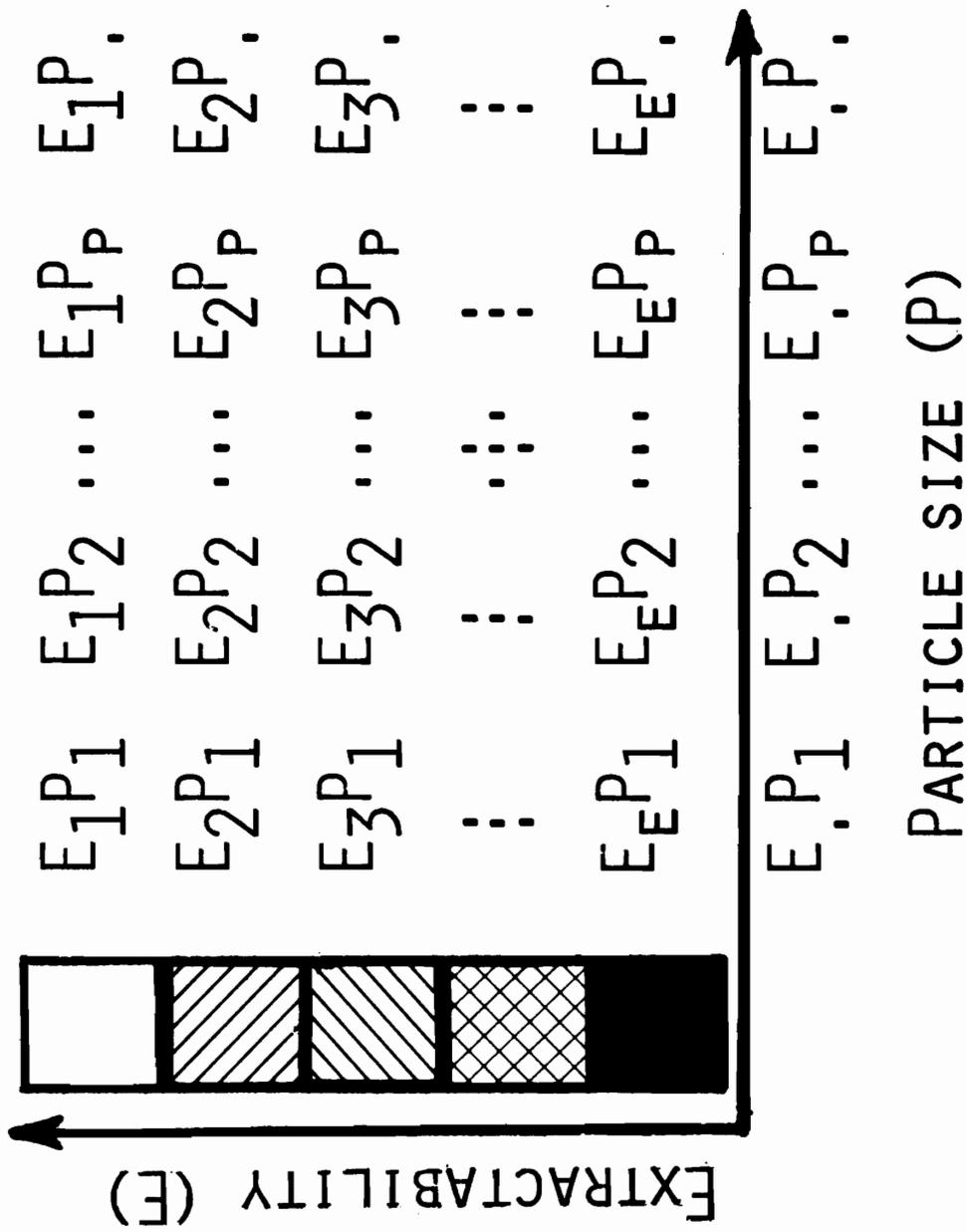


Figure 9 Hypothetical partitioning of an environmental contaminant into availability fractions based on chemical extractability (y-axis) and particle size (x-axis).

available at all sites investigated within and downstream from the Old Lead Belt.

Our data suggest that for Pb and Cd, transplanted caged mussels are better suited than indigenous naiades for use as environmental monitoring organisms in situations requiring information about current conditions. Conversely, indigenous naiades, as long-term integrators, may provide a useful historical perspective.

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LITERATURE CITED

- Andreae, M. O. 1977. Determination of arsenic species in natural waters. *Analytical Chemistry* 49(6):820-823.
- Ball, I. R. 1967. The relative susceptibilities of some species of freshwater fish to poisons -- I. Ammonia. *Water Research* 1:767-775.
- Buchanan, A. C. 1980. Mussels (naiades) of the Meramec River Basin, Missouri. Missouri Department of Conservation, Aquatic Series No. 17. 68 pages.
- Churchill, E. P., Jr., and S. I. Lewis. 1924. Food and feeding in freshwater mussels. *Bulletin of the U.S. Bureau of Fisheries* 39:439-471.
- Cutter, G. A. 1978. Species determination of selenium in natural waters. *Analytica Chimica Acta* 98:59-66.

- Ingle, S. E., J. A. Keniston, and D. W. Schults. 1980. REDEQL.EPAK. Aqueous chemical equilibrium computer program. U.S. Environmental Protection Agency, EPA-600 3-80-049, Corvallis, Oregon.
- Kramer, R. 1976. Effects of a century-old Missouri lead mining operation upon the water quality, sediments and biota of Flat River Creek. Unpublished MS Thesis, University of Missouri, Rolla.
- Luoma, S. N., and E. A. Jenne. 1977. Estimating bioavailability of sediment-bound trace metals with chemical extractants. Pages 343-351 In D. D. Hemphill (ed.). Trace substances in environmental health - X. University of Missouri, Columbia.
- Lochmoeller, T. 1982. Particle size distribution and water quality in natural systems. Unpublished MSc Thesis, University of Missouri, Columbia.
- Schlitt, C. J., S. E. Finger. 1987. The effects of sample preparation on measured concentrations of eight elements in edible tissues of fish from streams contaminated by lead mining. Archives of Environmental Contamination and Toxicology (In press).
- Sprague, J. B. 1964. Lethal concentration of copper and zinc for young Atlantic salmon. Journal of the Fisheries Research Board of Canada 21:17-26.
- Stumm, W., and J. J. Morgan. 1981. Aquatic chemistry. An introduction emphasizing chemical equilibria in natural waters. John Wiley and Sons, New York. 780 pages.
- Sunda, W. and R. R. L. Guillard. 1976. The relationship between cupric ion activity and the toxicity of copper to phytoplankton. Journal of Marine Research 34:511-529.
- Tessier, A., P. G. C. Campbell, J. C. Auclair, and M. Bisson. 1984. Relationships between the partitioning of trace metals in sediments and their accumulation in the tissues of the freshwater mollusc Elliptio complanata in a mining area. Canadian Journal of Fisheries and Aquatic Sciences 41:1463-1472.
- Tessier, A., P. G. C. Campbell, and M. Bisson. 1979. Sequential extraction procedure for the speciation of particulate trace metals. Analytical Chemistry 51(7):884-851.
- U.S. Army Corps of Engineers. 1977. Ecological evaluation of proposed discharge of dredged material into ocean waters. Environmental Protection Agency/Corps of Engineers, Technical Committee on Criteria for Dredged and Fill Material, Vicksburg, Mississippi.

- U.S. Environmental Protection Agency (EPA). 1980. Water quality criteria documents; availability. Appendix B -- Guidelines for deriving water quality criteria for the protection of aquatic life and its uses. Federal Register 45(231):79342-79347.
- Whelan, G. 1983. The distribution of lead and cadmium within a lotic benthic community. Unpublished MS Thesis, University of Missouri, Columbia.
- Wilson, W. W., and J. L. Seabaugh. 1981. Established criteria and selected methods for evaluating crop yield models in the AGRISTARS program. Proceedings of the American Statistical Association 1981:24-31.
- Wixson, B. G., L. E. Elliot, N. L. Gale, and B. E. Davies. 1983. Influences of tailings from the Old Lead Belt of Missouri on sediments of the Big River. Pages 3-11 in D. D. Hemphill (ed.), Trace substances in environmental health - XVI. University of Missouri, Columbia.