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The Effects of Sample Preparation on Measured Concentrations of Eight Elements in Edible Tissues of Fish from Streams Contaminated by Lead Mining

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Abstract. The influence of sample preparation on measured concentrations of eight elements in the edible tissues of two black basses (Centrarchidae), two catfishes (Ictaluridae), and the black redhorse, *Moxostoma duquesnei* (Catostomidae) from two rivers in southeastern Missouri contaminated by mining and related activities was investigated. Concentrations of Pb, Cd, Cu, Zn, Fe, Mn, Ba, and Ca were measured in two skinless, boneless samples of axial muscle from individual fish prepared in a clean room. One sample (normally-processed) was removed from each fish with a knife in a manner typically used by investigators to process fish for elemental analysis and presumedly representative of methods employed by anglers when preparing fish for home consumption. A second sample (clean-processed) was then prepared from each normally-processed sample by cutting away all surface material with acid-cleaned instruments under ultraclean conditions. The samples were analyzed as a single group by atomic absorption spectrophotometry. Of the elements studied, only Pb regularly exceeded current guidelines for elemental contaminants in foods. Concentrations were high in black redhorse from contaminated sites, regardless of preparation method; for the other fishes, whether or not Pb guidelines were exceeded depended on preparation technique. Except for Mn and Ca, concentrations of all elements measured were significantly lower in clean- than in normally-processed tissue samples. Absolute differences in measured concentrations between clean- and normally-processed samples were most evident for Pb and Ba in bass and catfish and for Cd and Zn in redhorse. Regardless of preparation method, concentrations of Pb, Ca, Mn, and Ba in individual fish were closely correlated; samples that were high or low in one of these four elements were correspondingly high or low in the other three. In contrast, correlations between Zn, Fe, and Cd occurred only in normally-processed samples, suggesting that these correlations resulted from high concentrations on the surfaces of some samples. Concentrations of Pb and Ba in edible tissues of fish from contaminated sites were highly correlated with Ca content, which was probably determined largely by the amount of tissue other than muscle in the sample because fish muscle contains relatively little Ca. Accordingly, variation within a group of similar samples can be reduced by normalizing Pb and Ba concentrations to a standard Ca concentration. When sample size (N) is large, this can be accomplished statistically by analysis of covariance; when N is small, molar ratios of [Pb]/[Ca] and [Ba]/[Ca] can be computed. Without such adjustments, unrealistically large Ns are required to yield statistically reliable estimates of Pb concentrations in edible tissues. Investigators should acknowledge that reported concentrations of certain elements are only estimates, and that regardless of the care exercised during the collection, preparation, and analysis of samples, results should be interpreted with the awareness that contamination from external sources may have occurred.

In response to heightened public concern about environmental contaminants, fishery management agencies are frequently asked to determine whether fish in waters under their jurisdiction are safe for human consumption. Suitability for consumption may largely determine the economic value of publicly owned fishery resources. Richkus (1984) outlined problems that can be encountered when

investigators submit fish samples to analytical laboratories; however, the extreme difficulty of measuring Pb at concentrations commonly found in the edible parts of fish was not emphasized. Settle and Patterson (1980) concluded that most analytical laboratories are incapable of accurately measuring Pb concentrations in fish muscle, contending that (1) gross contamination of reagents and laboratory ware by industrial Pb increases detection limits by several orders of magnitude—limits too high to be meaningful; (2) U.S. National Bureau of Standards (NBS) reference materials are contaminated during preparation and their Pb concentrations are also too high for their intended purpose; (3) fish tissue samples are often contaminated during preparation, in either the field or laboratory; and (4) as a consequence of grossly misleading information on the extent of Pb contamination in the human environment, established guidelines for Pb in foods inadequately protect the public.

The influence of certain techniques of sample collection and preparation on Pb concentrations measured by atomic absorption (AA) spectrophotometry in the edible tissues of several species of fish from two streams draining mining districts in southeastern Missouri was investigated. Concentrations of Cd, Zn, Cu, Fe, Mn, Ba, and Ca were also determined. Problems that can be encountered in the study of elemental contaminants are illustrated, and alternative approaches for collecting and analyzing edible tissues of fish for potentially toxic trace elements are suggested.

Study Area

The mineralized areas of southeastern Missouri (Figure 1) have been mined for more than 200 yr. The deposits of the "Old" (inactive) and "New" (active) Lead Belts, as the mining areas are known, are rich in Pb and Zn, and contain lesser amounts of Cd, Cu, Fe, and Ag. Barite ($BaSO_4$) is currently surface-mined in Washington, Jefferson, and St. Francois Counties, and Fe and Mn deposits were previously exploited (U.S. Geological Survey 1967).

The mining districts are drained mostly by the Meramec and Black River systems (Figure 1). The Big River, a tributary of the Meramec, has been heavily contaminated by tailings rich in Pb, Cd, and Zn from mining and ore processing activities in the Old Lead Belt (Whitley 1979; Schmitt and Finger 1982; Schmitt *et al.* 1984; Czarneski 1985). Lead concentrations in the edible tissues of some fishes from the Big River—especially redhorses (*Moxostoma* spp.)—frequently exceed 0.3 μg/g wet weight (Czarneski 1985), a concentration sometimes cited as an upper permissible limit for Pb in foods (Settle and Patterson 1980; Bryce-Smith and Stephens 1983). Although not game species, redhorses are heavily exploited for home consumption in Missouri by gigging (a spearing technique for taking rough fishes). Accordingly, the Missouri Department of Conservation advised anglers to limit their consumption of fish, especially redhorses and other suckers (Catostomidae), from affected reaches of the river (Shirk 1980).

Environmental controls have generally prevented similar problems in the New Lead Belt (Hardie *et al.* 1974), where no elevated concentrations of mining-related contaminants in fish have been reported. Nevertheless, the sediments of Clearwater Lake (660-ha permanent pool; 4,189-ha flood pool), a multi-use impoundment on the Black River (Figure 1), are enriched with Pb, Cd, Cu, Mn, and Zn relative to upstream concentrations (Gale *et al.* 1976; Schmitt and Finger 1982). Clearwater Lake stratifies each summer and the hypolimnion becomes anoxic. Because the discharge is at mid-depth (below the thermocline during summer), concentrations of Fe and Mn in the tailwaters are sometimes relatively high (Schmitt and Finger 1982). Further information on the study area and its environmental problems is available elsewhere (Wixson and Bolter 1972; Gale *et al.* 1973, 1976, 1982; Hardie *et al.* 1974; Kramer 1976; Hocutt *et al.* 1978; Novak and Hasselwander 1980; Schmitt and Finger 1982; Whelan 1983; Schmitt *et al.* 1984; Niethammer *et al.* 1985; Czarneski 1985).

Materials and Methods

Black bass (either smallmouth bass, *Micropterus dolomieui*, or spotted bass, *M. punctatus*), catfish (either channel catfish, *Ictalurus punctatus* or flathead catfish, *Pylodictis olivaris*), and black redhorse (*Moxostoma duquesnei*)—herein termed bass, catfish, and redhorse, respectively—were collected by electrofishing at seven sites on the Big and Black Rivers, in reaches known to be contaminated to various degrees by elemental contaminants from past or present mining operations (Figure 1). Two fish of each of the three taxa were collected at each site except at Brown's Ford, where only one bass was captured, and at Washington State Park, where only one catfish and one redhorse were obtained. Channel catfish were collected at all sites except the Big River at Washington State Park; at this site a section of axial muscle tissue (taken adjacent to the caudal peduncle) was dissected from a large (>20 kg), freshly caught flathead catfish provided by an angler. All other fish were from 30 to 50 cm total length. Smallmouth bass were collected at all sites except at the tailwaters of Clearwater Lake, where spotted bass were substituted. After capture, each fish was weighed, measured, rinsed in river water, and twice-bagged in polyethylene bags. All fish were frozen and returned to the laboratory for processing and analysis.

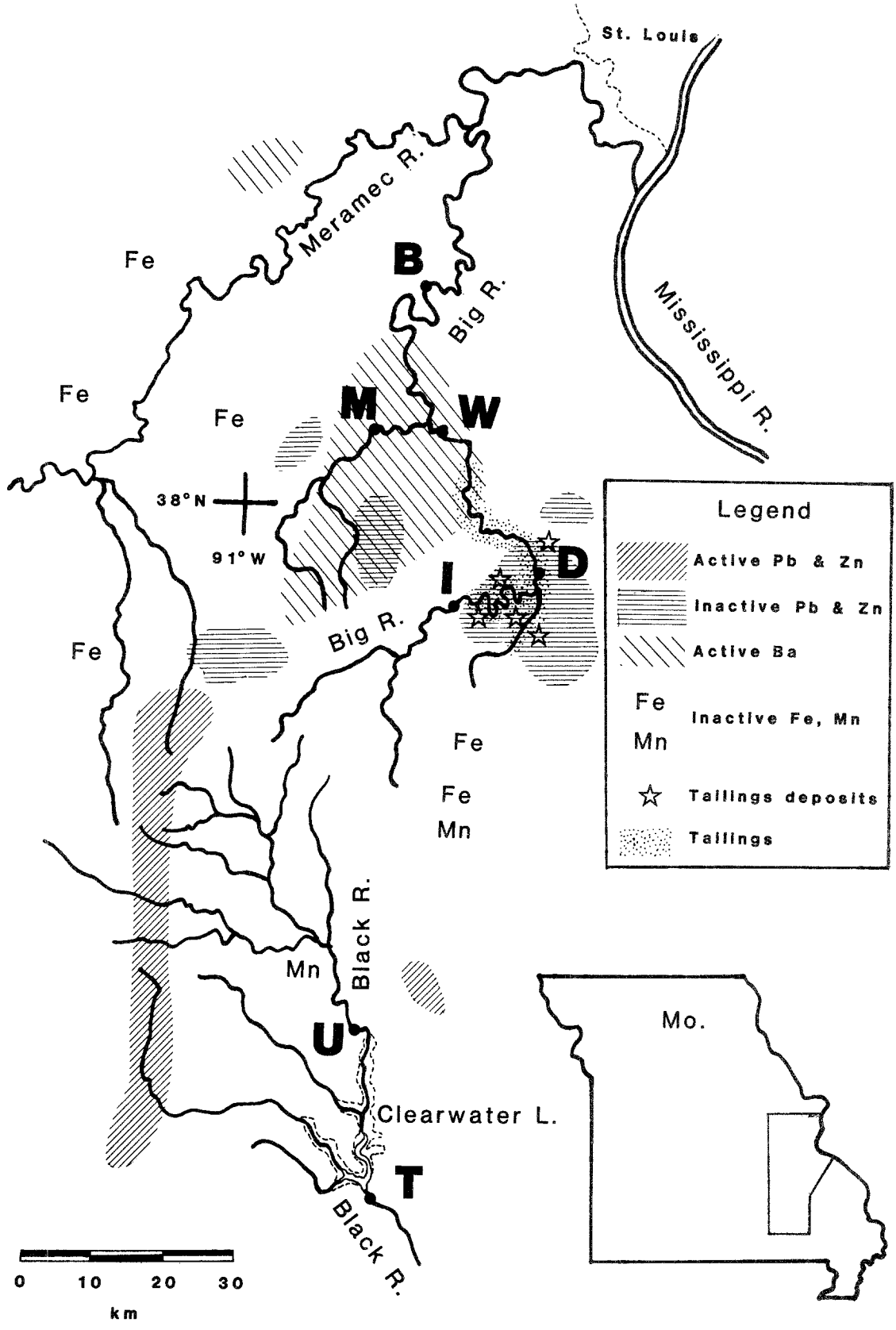

Fig. 1. Active and inactive mining areas of southeastern Missouri, and collection sites on the Big River at Brown's Ford (B), Washington State Park (W), Mineral Fork (M), Desloge (D), and Irondale (I); and on the Black River upstream from (U) and in the tailwaters of (T) Clearwater Lake

In a limited access laboratory clean-room, fish were partly thawed, then scaled with a stainless steel knife that had been washed in laboratory detergent (Alconox®)[1] and rinsed in well water between each fish and each preparation step. The scaled fish were rinsed in ultrapure (15–18 MΩ-cm) water. A skinless, boneless sample of axial muscle (edible tissue) was removed from the right side of each fish with the stainless steel knife. One half of this tissue, the "normally-processed" sample, was immediately refrozen in a polyethylene bag for subsequent digestion and analysis; the remainder was retained for further preparation. Immediately preceeding digestion, the normally-processed samples were thawed and 1-cm^3 subsamples (about 5 g each) of tissue were removed from each with a well water-rinsed, stainless steel razor blade, weighed on a 5-cm × 5-cm piece of polyethylene sheeting, and digested for each analysis. All normally-processed samples were prepared on a Teflon® laboratory counter top that was rinsed with well water between samples. Gloves were not worn during sample preparation, and polyethylene items were used as packaged by the manufacturer.

To prepare a "clean-processed" sample, as defined by Patterson and Settle (1976), the remaining half of the sample was placed on an acid-cleaned sheet of polyethylene, and with acid-cleaned, stainless steel razor blades, all potentially contaminated surface material was removed; this procedure yielded a 1-cm cube (about 5 g) of clean tissue, which was then weighed on a 5-cm × 5-cm sheet of polyethylene. The subsample was placed in a polyethylene bag and frozen for subsequent digestion and analysis. Disposable, talc-free polyethylene gloves, rinsed with 10%-HNO_3, were worn during processing and changed between samples. All tissue was handled throughout the procedure with acid-cleaned forceps, and all polyethylene and stainless steel items were cleaned according to protocol described in detail by Patterson and Settle (1976).

A total of 40 fish were prepared for analysis; of these, both clean- and normally-processed samples were obtained from 39 (there was insufficient material to prepare a clean-processed sample from the Washington State Park catfish). Of the 40 normally-processed samples, four (one redhorse each from Mineral Fork, Irondale, and Desloge, and the catfish from Washington State Park) were replicated (duplicate 5-g subsamples carried through the digestion and analytical procedure) to assess within-fish variability. None of the clean-processed samples were replicated due to the labor intensity inherent in their preparation.

Digestion of both normally- and clean-processed tissue was conducted under identical conditions in the clean room. Quartz Kjeldahl digestion flasks were precleaned by immersion for 10 min in 10%-HF, then placed in a heated (70°C) bath of 16M-HNO_3 for 3 days, removed and rinsed with ultrapure water, immersed in a second heated bath of 0.05%-HNO_3 for 24 hr, rinsed again with ultrapure water, dried at 110°C for 24 hr, and wrapped in polyethylene film until needed. Each subsample was digested with 25 mL of 16-M sub-boiled HNO_3 in a 100-mL Kjeldahl flask over low heat (without boiling) until only 1 mL remained. This digestate was diluted to 50 mL with ultrapure water and analyzed for Pb and Cd with a Perkin Elmer Model 305-B AA spectrophotometer equipped with an HGA-2100 graphite furnace and a deuterium arc background correction. A L'vov platform was used to reduce interferences from sample matrices (Kaiser *et al.* 1981). Analyses for Cu, Zn, Fe, and Mn were performed with a Perkin Elmer Model 603 AA spectrophotometer equipped with a standard air/acetylene burner, a deuterium arc background corrector, and a Model 56 recorder. Concentrations of Ba and Ca, along with a scan of several other elements, were measured with a Jarrel-Ash Model 975 inductively coupled argon plasma (ICAP) spectrophotometer.

The quality control matrices used for this study were NBS bovine liver, albacore tuna, and oyster tissue. All results were within 20% (or 2 SD) of the certified concentrations for the reference materials. Of the samples analyzed, 10% were blanks and 20% were blind replicates and spiked samples. None of the values for blanks exceeded detection limits (0.001 μg/g; 5 ng) for any element. Results are reported as μg/g wet weight.

The Statistical Analysis System (SAS Institute 1982a, 1982b) was used for all data handling and computations. Geometric mean regression (Ricker 1973) and correlation analysis were used to measure the degree of association between the two sets of measurements and overall trends in their accuracy; perfect agreement would yield geometric mean regressions with slopes and correlation coefficients of 1.0 and intercepts of 0. There was insufficient sample size (N) to use the regression technique recommended by Wiener (1982) to detect surface contamination; however, the measured elemental concentrations in the liquid digestates were plotted against the measured mass of tissue digested, and these plots were examined for positive slopes. The molar ratios of Pb and Ba to Ca ([Pb]/[Ca], [Ba]/[Ca])—elements known to accumulate in bony tissue (Settle and Patterson 1980)—were also computed and examined. Three-way analysis-of-variance (ANOVA) in a split-plot design was used to test for differences among individual fish that were attributable to fixed effects of collection location (L), taxon (T), and $L \times T$ interaction, and to fixed within-fish effects of preparation methods (M) and $M \times L$, $M \times T$, and $M \times L \times T$ interactions. In this analysis, differences attributable to individual fish, $F_{(LT)}$, and to $F_{(LT)} \times M$ interaction are random effects corresponding respectively to "Error A" and "Error B" in a traditional split-plot ANOVA. The test of the fixed M effect against the random $M \times F_{(LT)}$ term is equivalent to comparing the two methods with a paired-t test. Examination of the expected mean-squares for this design showed these to be the appropriate sums-of-squares for the required F-tests (Steele and Torrie 1980; Freund and Littel 1981). An F-test was also used to compare the among-fish variances for the two methods, and correlations between measured concentrations of the eight elements and among the deviations between the two methods were computed as indicators of similar sources of elements in the samples. Two-way ANOVA was also performed independently on each set of samples to determine whether effects of L, T, and $L \times T$ were artifacts of sample preparation. A significance level of $P \leq 0.05$ was used in all statistical tests, unless otherwise indicated. All concentration values were transformed [$\log_e (x + 1)$] before analysis.

Results

Concentrations of all the elements measured, except for Mn and Ca, were significantly lower in clean-processed samples, as judged by the M effect in ANOVA (Table 1). The mean difference ($\bar{d}$) between normally- and clean-processed samples, as percentages of the clean-processed means, ranged from 1% for Mn to 90% for Ba (Table 2). Differences between the methods were 95% or greater for

[1] Mention of trade names does not constitute government endorsement.

Table 1. Results of split-plot analysis of variance, as F-values, significance levels[a], and residual-mean-squares, for concentrations of eight elements and two elemental ratios in samples prepared for analysis by two methods

Source of variation	df[b]	Elements								Ratios	
		Pb	Cd	Cu	Zn	Fe	Mn	Ba	Ca	[Pb]/[Ca]	[Ba]/[Ca]
Among fish (F)											
Locations (L)	6, 20	75.71**	2.98*	0.95	2.21	2.09	3.21*	6.36**	2.55	18.45**	2.47
Taxon (T)	2, 20	36.98**	10.24**	10.62**	3.01	2.88	26.59**	1.56	42.56**	7.78**	1.43
$L \times T$	12, 20	62.20**	2.25*	0.96	4.45**	1.02	5.30**	0.96	4.99**	2.95*	0.54
$F_{(LT)}$ (Error A)	20, 19	1.41	2.02	4.46**	1.78	4.81**	2.14	11.17**	1.38	13.94**	6.04**
Within fish											
Method (M)	1, 19	10.84**	6.62*	47.40**	133.15**	68.80**	0.02	29.98**	2.67	223.03**	5.92*
$M \times L$	6, 19	1.52	0.22	1.30	2.03	2.84*	1.53	6.30**	0.79	40.53**	1.67
$M \times T$	2, 19	2.73	0.37	1.84	3.03	0.47	3.14	1.55	5.88*	73.76**	1.80
$M \times L \times T$	11, 19	0.75	0.52	2.66*	1.30	1.85	0.64	1.84	2.47*	26.57**	1.99
$M \times F_{(LT)}$ (Error B)	19, 4	0.02	66.25**	3.25	0.43	1.18	0.09	0.06	0.72	0.01	1.55
Replicates (residual)	4	2.6 $\times 10^{-}$	1.3 $\times 10^{-6}$	8.8 $\times 10^{-4}$	9.6 $\times 10^{-2}$	5.9 $\times 10^{-2}$	2.5 $\times 10^{-1}$	2.0 $\times 10^{-1}$	3.1 $\times 10^{-1}$	9.0 $\times 10^{-7}$	1.9 $\times 10^{-6}$

[a] $*P \leq 0.05$; $**P \leq 0.01$

[b] Numerator, denominator

Pb and Ba in bass and catfish, and for Cd and Zn in redhorse, whereas the differences for Mn in catfish and Ca in redhorse were negligible (Table 2). $M \times T$ interaction was not significant for any element except Ca (Table 1), which implies that for the other seven elements, differences attributable to method of preparation were significant for all three taxa. For Ca, the significance of these $M \times T$ interactions reflected the higher concentrations of this element in the clean-processed redhorse; in bass and catfish, Ca concentrations were higher in normally-processed samples (Table 3). The $M \times L$ interaction was nonsignificant for all elements except Fe and Ba (Table 1), suggesting that the effect of preparation method was independent of collection location for the other six elements, and that cross-contamination of the samples did not occur.

Differences among locations were significant for Pb, Cd, Mn, and Ba (Table 1). As expected, concentrations of these four elements were highest in the sections of the Big River most heavily influenced by mining activities: Desloge, Washington State Park, Mineral Fork, and Brown's Ford (Table 3). Differences among taxa were significant for Pb, Cd, Cu, Mn, and Ca (Table 1). Of these, the overall taxon means for clean-processed samples were highest in redhorse for Pb, Mn, and Ca, and highest in catfish for Cd and Cu (Table 3). The $L \times T$ interaction was significant for Pb, Cd, Zn, Mn, and Ca (Table 1), indicating that the taxon containing the highest and lowest concentrations of these elements differed among the locations studied (Table 3).

Two-way ANOVA, performed separately for the clean- and normally-processed values, revealed that L, T, and $L \times T$ were highly significant ($P < 0.01$) sources of variation for Pb, regardless of preparation method (Table 2). For the other elements, significance of an effect in only one of the ANOVAs indicated that some differences among locations and taxa were attributable to the method of preparation. For example, Cd did not differ significantly among locations or taxa in clean-processed samples, but both main effects and $L \times T$ were significant for Cd in normally-processed samples. For Ba, differences among taxa were significant only in clean-processed samples, with the highest concentrations occurring in redhorse. For Zn, the greater precision afforded by clean-processing the samples apparently increased the resolution sufficiently for L effects to be significant, as it also did for among-taxon differences in Ba concentrations (Table 2).

Variability among fish, $F_{(LT)}$, was significantly greater than variability within fish, $M \times F_{(LT)}$, for Cu, Fe, and Ba (Table 1). For Pb, Cd, Zn, Mn, and Ca, differences between the two digestions and analyses of normally-processed tissues from individual fish were greater than the average fish-to-fish differences; for these elements, $F_{(LT)}$ was not significant. Variability among fish of the same species from a given location was significantly greater for

Table 2. Minimum and maximum concentrations (μg/g), *F*-tests for equality of variances, geometric mean differences ($\bar{d}$) between methods, and regression analyses of elemental concentrations in normally- (N, $N = 40$) and clean-processed (C, $N = 39$) samples

Element and taxon	Minima		Maxima		*F* for unequal variances[a]	Differences		Regression		
	N	C	N	C		$\bar{d}$[b]	%[c]	u[d]	v[d]	r^2[d]
Pb										
Bass	0.008	0.001	0.36	0.099	13.57**	0.073	304.2	0.01	4.35*	0.26
Catfish	0.200	0.002	0.33	0.120	4.92*	0.078	251.6	0.02	2.64*	0.53
Redhorse	0.010	0.006	0.81	1.100	4.10	0.008	36.4	0.03	0.86	0.74
All fish	0.008	0.001	1.10	1.100	1.33	0.053	57.0	0.05**	0.92	0.64
Cd										
Bass	0.002	0.001	0.023	0.020	1.48	0.003	60.0	0.002	1.15	0.85
Catfish	0.009	0.002	0.065	0.085	3.56	0.008	44.0	0.024	1.56*	0.46
Redhorse	0.004	0.002	0.042	0.016	14.72**	0.006	100.0	0.002	2.47**	0.60
All fish	0.002	0.001	0.065	0.085	2.50*	0.005	50.0	0.011	1.01	0.52
Cu										
Bass	0.20	0.12	0.50	0.24	3.75	0.110	59.0	−0.06	1.87	0.09
Catfish	0.16	0.12	0.74	0.99	1.57	0.153	50.8	0.15	0.85	0.21
Redhorse	0.15	0.14	0.38	0.32	1.33	0.064	34.0	0.00	1.31	0.27
All fish	0.15	0.12	0.74	0.99	1.32	0.106	32.5	0.07*	1.06	0.32
Zn										
Bass	4.60	3.30	16.00	6.50	2.51	5.18	113.6	−0.98	1.96	0.03
Catfish	4.00	3.10	24.00	6.10	6.33*	2.31	51.4	−2.03	2.40	0.04
Redhorse	6.10	3.40	20.00	12.00	2.20	5.13	96.2	0.58	1.01	0.54
All fish	4.00	3.10	24.00	12.00	3.77**	4.17	86.9	−0.49*	1.59*	0.20
Fe										
Bass	1.10	1.10	25.00	17.00	1.33	0.68	31.8	0.26	1.23	0.73
Catfish	1.30	0.60	10.00	9.70	2.20	0.73	31.5	0.64	0.92	0.03
Redhorse	1.90	0.30	4.30	5.10	1.64	0.52	61.3	0.86**	0.55**	0.28
All fish	1.10	0.30	35.00	17.00	1.21	0.64	32.0	0.42*	1.06	0.33
Mn										
Bass	0.10	0.08	1.30	0.57	4.82*	0.112	74.7	−0.07	2.13**	0.84
Catfish	0.09	0.16	0.90	1.20	2.68	−0.001	0.3	0.07	0.74	0.65
Redhorse	0.22	0.14	1.70	1.90	1.14	−0.172	18.5	0.09	0.73	0.17
All fish	0.09	0.08	1.70	1.90	1.00	−0.004	0.9	0.08	0.77*	0.54
Ba										
Bass	0.010	0.005	4.40	2.00	2.01	0.20	166.7	0.10	1.58**	0.89
Catfish	0.001	0.009	2.10	0.33	2.51	0.17	212.5	0.06	3.83**	0.57
Redhorse	0.120	0.010	2.30	2.60	2.76	0.16	39.0	0.11	1.00	0.72
All fish	0.001	0.005	4.40	2.60	1.21	0.18	90.0	0.07	1.37**	0.67
Ca										
Bass	75.0	51.0	480.0	166.0	8.23**	78.2	91.5	14.26**	−2.04**	0.12
Catfish	34.0	65.0	270.0	350.0	1.59	42.0	38.2	1.86	1.37	0.10
Redhorse	93.0	74.0	900.0	1090.0	0.36	31.6	7.5	−2.35*	0.60*	0.06
All fish	34.0	51.0	900.0	1090.0	1.35	57.9	35.4	0.84	0.87	0.28

[a] Among fish of the indicated taxon

[b] Geometric mean difference between concentration (μg/g) in normally- and clean-processed samples from the same fish

[c] Geometric mean difference as a percentage of the clean-processed mean

[d] Intercept (u), slope (v), and coefficient of determination (r^2) of geometric mean regression between concentrations in normally- (abscissa) and clean-processed (ordinate) samples from the same fish, and significance of test of the null hypotheses H_0: $u = 0$, $v = 1.0$. $^*P \leq 0.05$; $^{**}P \leq 0.01$

normally- than clean-processed Mn in bass, Zn in catfish, Pb in bass and catfish, and Cd in redhorse (Table 2). Assuming that the residual mean-squares (σ^2) from ANOVA (Table 1) represent within-fish variances for normal processing (and including the Washington State Park catfish) the coefficients of variation [$CV = 100\ (\sqrt{\hat{\sigma}^2}/\bar{X})$] were Pb, 314%; Cd, 9%; Cu, 12%; Zn, 15%; Fe, 18%; Mn, 124%; Ba, 155%; and Ca, 11%.

Regression analysis (normally- vs clean-pro-

cessed values from the same fish) yielded intercepts that differed significantly from zero for Pb, Cu, Zn, and Fe (for all fish combined), for Fe and Ca in redhorse, and for Ca in bass (Table 2). Slopes differed significantly from 1.0 for Pb in bass and catfish, Cd in catfish and redhorse, Zn in all taxa combined, Fe in redhorse, Mn in bass, Ba in bass and catfish, and Ca in bass and redhorse (Table 2). The coefficients of determination for the linear regressions, representing the fraction of the variability in the normally-processed concentration explained by the corresponding clean-processed concentration, ranged from 0.03 for Zn in bass to 0.89 for Ba in bass (Table 2, Figure 2). In two bass (one each from Washington State Park and Mineral Fork), one catfish from Brown's Ford, and one redhorse from Washington State Park, Pb was much higher in normally- than in clean-processed samples; Figure 2 suggests that material on the surface of the normally-processed samples was responsible for their concentrations exceeding 0.3 μg/g. The relation for Cd is similar to that for Pb—three specimens with low clean-processed concentrations (one catfish each from Irondale and Mineral Fork, and one redhorse from Desloge) appear conspicuously far from the overall trend of the data (Figure 2). These points, however, represent different specimens for Cd than for Pb, and all Cd values were less than 0.1 μg/g.

For Mn, there was no apparent trend; as the statistical tests (Tables 1 and 2) suggest, the points appear randomly distributed about a regression line with a slope of ≤ 1.0 and a positive intercept (Figure 2). For Cu, Zn, Fe, and Ba, nearly all of the points lie above the solid lines (Figure 2), indicating that concentrations were consistently lower in clean- than normally-processed samples and that material on the surface of the samples influenced the concentrations. The relation for Ca resembles that of Pb—concentrations in a few fish at the low end were conspicuously higher in normally- than clean-processed samples (Figure 2).

Inspection of individual points in Figure 2 suggests that, of the four normally-processed samples that appear to be most heavily influenced by surface Pb, concentrations of Mn were high in three and Ba and Ca in two. Consequently, the deviations for Pb (difference between the two preparations of each sample) were most strongly correlated with deviations for Mn, less strongly with those for Ba, Ca, Cu, and Fe, and weakly or not at all with Cd and Zn; also, the Ba, Ca, and Mn deviations were themselves correlated (Table 4). Deviations of Cd were correlated with those of Cu and Fe, which in turn were correlated with those of Zn (Table 2). Collectively, these results suggest a common source of Cu, Fe, and Zn, but one different from that of Pb, Ba, Ca, and Mn.

Correlations between the concentrations of most elements resembled those of the deviations. Concentrations of Pb, Ba, Mn, and Ca were closely correlated in both clean- and normally-processed samples (Table 5)—suggesting that regardless of preparation method, samples that were high or low in one of these four elements were correspondingly high or low in the other three. Similarly, Zn was correlated with Pb and Ca in both sets of samples, as were Cu and Fe. Conversely, the correlations of Zn with Mn and Ba and of Mn with Fe that were present in the normally-processed samples were absent in the clean-processed samples, indicating that some of these relations were artifacts of sample preparation.

Figures 3 and 4 illustrate the relation between Ca and the other seven elements. The plot for Pb clearly shows positive correlation with Ca in both sets of samples from tailings-contaminated sites. The relation was most obvious in clean-processed redhorse (Figure 4). Similarly, correlation between Ba and Ca was evident in fish from the barite mining areas; the relation for clean-processed redhorse was especially distinct. Calcium and Mn were closely correlated in all samples, regardless of preparation method, taxon, or collection site; this relation was also especially evident in redhorse. On the basis of their Zn and Ca concentrations, clean-processed samples were clustered in two groups, both of which had positive slopes (Figure 4). The smaller group consisted almost entirely of clean-processed redhorse; the larger contained the rest of the samples, and these groups were separated on the basis of their Ca concentrations. All samples from the most heavily contaminated site (Desloge) occurred in the larger group and these fish alone constituted a group with a positive slope (Figure 4). No correlations were clearly evident between Ca and Fe, Cd, or Cu in either group of samples.

Plotting elemental concentrations in digestates against mass of tissue digested, as recommended by Wiener (1982), yielded graphs that were difficult to interpret for some elements but which clearly showed the effects of material on the sample surface for others. As expected, the plots for normally-processed samples of most elements consisted of randomly distributed points, and if lines were drawn between the points that represent sample pairs (same species, location, and method of preparation) many would have zero or negative slopes (Figure 5). Without large N, one cannot determine with certainty whether individual points deviate from the expected positive relation because

Table 3. Geometric mean concentrations of eight elements in clean- (C) and normally-processed (N) samples of three taxa from seven collection sites in southeastern Missouri, and the results of two-way ANOVA testing the significance of main effects due to locations (L), taxa (T), and $L \times T$ interaction

River, collection site, and taxon	n or (DF)[a]	Element and processing method					
		Pb		Cd		Cu	
		N	C	N	C	N	C
Big River							
Mineral Fork							
Bass	2	0.194	0.018	0.005	0.002	0.418	0.205
Catfish	2	0.126	0.039	0.024	0.007	0.514	0.310
Redhorse	2	0.079	0.087	0.005	0.002	0.225	0.210
Location mean	6	0.132	0.048	0.011	0.004	0.380	0.241
Brown's Ford							
Bass	1	0.130	0.030	0.005	0.003	0.200	0.130
Catfish	2	0.269	0.060	0.016	0.005	0.658	0.175
Redhorse	2	0.626	0.850	0.012	0.008	0.220	0.170
Location mean	5	0.369	0.317	0.012	0.006	0.375	0.164
Washington State Park							
Bass	2	0.275	0.064	0.007	0.003	0.259	0.175
Catfish	1[b]	9.144	—	0.033	—	3.347	—
Redhorse	1	0.433	0.241	0.011	0.009	0.225	0.185
Location mean	5[c]	1.022	0.149	0.014	0.006	0.600	0.180
Desloge							
Bass	2	0.054	0.022	0.014	0.008	0.320	0.145
Catfish	2	0.132	0.060	0.032	0.034	0.484	0.558
Redhorse	2	0.569	0.525	0.034	0.014	0.280	0.160
Location mean	6	0.232	0.182	0.027	0.019	0.358	0.274
Irondale							
Bass	2	0.014	0.006	0.003	0.003	0.269	0.225
Catfish	2	0.058	0.014	0.061	0.045	0.441	0.276
Redhorse	2	0.024	0.025	0.012	0.003	0.334	0.237
Location mean	6	0.032	0.015	0.025	0.017	0.346	0.246
Black River							
Upstream							
Bass	2	0.038	0.008	0.006	0.002	0.325	0.180
Catfish	2	0.049	0.004	0.010	0.012	0.459	0.399
Redhorse	2	0.026	0.025	0.004	0.003	0.264	0.175
Location mean	6	0.038	0.013	0.007	0.006	0.347	0.247
Downstream							
Bass	2	0.015	0.021	0.015	0.013	0.240	0.215
Catfish	2	0.035	0.006	0.011	0.003	0.214	0.135
Redhorse	2	0.015	0.008	0.006	0.004	0.222	0.180
Location mean	6	0.022	0.012	0.011	0.007	0.225	0.176
Taxon means							
Bass	13	0.097	0.024	0.008	0.005	0.295	0.186
Catfish	13[d]	0.314	0.031	0.026	0.018	0.583	0.301
Redhorse	14	0.228	0.220	0.012	0.006	0.252	0.188
Grand mean	40[e]	0.210	0.093	0.015	0.010	0.366	0.326
Two-way ANOVA[f]							
Source							
L	(6, 19[g])	13.83**	5.97**	4.38**	1.04	1.41	0.73
T	(2, 19[g])	8.29**	18.63**	12.28**	3.03	10.93**	3.88*
$L \times T$	(11[h], 19[g])	4.94**	4.23**	3.47**	1.00	1.22	1.33
Error	(19[g])	5.2 $\times 10^{-3}$	6.8 $\times 10^{-3}$	7.6 $\times 10^{-5}$	1.9 $\times 10^{-4}$	6.6 $\times 10^{-3}$	8.7 $\times 10^{-3}$

[a] (Numerator, denominator); [b]For clean-processed, 0; [c]For clean-processed, 4; [d]For clean-processed, 12; [e]For clean processed, 39; [f]F-values, significance levels, and error (among-fish) variances. *$P \leq 0.05$; **$P \leq 0.01$; [g]For clean-processed, 20; [h]For clean-processed, 12.

Table 3. (cont'd)

Element and processing method									
Zn		Fe		Mn		Ba		Ca	
N	C	N	C	N	C	N	C	N	C
13.97	4.33	12.15	6.71	0.63	0.34	1.75	0.82	347.0	90.8
5.67	4.14	6.28	2.70	0.38	0.23	1.61	0.26	194.5	120.0
13.42	6.49	3.00	2.10	0.64	0.63	1.81	1.26	450.0	455.5
10.29	4.90	6.26	3.46	0.55	0.39	1.72	0.73	328.8	170.7
4.60	3.30	4.00	1.50	0.29	0.12	0.10	0.01	106.1	77.0
12.23	4.32	8.44	0.92	0.26	0.28	0.18	0.04	49.8	70.3
11.67	5.09	2.65	1.00	0.97	1.35	1.03	0.90	516.5	734.5
9.95	4.38	4.68	1.06	0.51	0.59	0.44	0.32	140.2	183.6
9.49	4.05	5.14	1.84	0.46	0.18	0.68	0.10	104.5	85.7
23.08	—	10.44	—	4.02	—	2.87	—	2127.5	—
9.38	4.01	2.76	2.83	0.73	0.42	0.60	0.23	384.1	206.0
11.33	4.03	4.72	2.29	1.00	0.29	0.95	0.16	428.2	133.0
17.73	5.76	3.90	1.40	0.13	0.11	0.04	0.01	141.5	73.3
5.12	4.70	2.52	2.13	0.18	0.19	0.03	0.01	55.4	73.2
16.16	10.46	2.81	0.69	0.36	0.38	0.16	0.13	320.0	336.3
10.01	6.62	3.04	1.33	0.22	0.22	0.08	0.05	136.0	122.0
13.28	4.06	3.90	1.89	0.23	0.13	0.15	0.02	398.0	56.2
6.75	3.83	8.26	3.85	0.57	0.60	0.13	0.11	84.1	120.1
9.32	4.37	3.16	1.20	0.52	1.35	0.50	0.37	241.4	526.3
9.45	4.08	4.74	2.14	0.44	0.62	0.25	0.16	206.2	154.0
7.46	4.34	4.35	1.95	0.11	0.08	0.03	0.01	119.4	90.4
7.04	6.05	3.90	3.72	0.24	0.22	0.04	0.02	196.1	107.3
7.50	4.55	3.77	2.02	1.17	1.80	0.21	0.24	614.9	973.5
7.33	4.94	4.00	2.47	0.44	0.55	0.09	0.08	243.5	211.9
7.75	5.70	1.15	1.42	0.11	0.11	0.01	0.01	121.2	141.2
5.73	4.18	2.30	1.65	0.26	0.38	0.01	0.02	97.0	221.5
8.15	4.24	3.02	2.28	1.03	1.01	0.19	0.07	371.6	190.9
7.13	4.67	2.05	1.76	0.42	0.46	0.07	0.03	183.9	181.4
9.73	4.56	4.28	2.14	0.27	0.15	0.32	0.12	163.7	85.5
7.51	4.49	5.06	2.32	0.45	0.31	0.36	0.08	152.0	110.0
10.47	5.33	3.01	1.63	0.76	0.93	0.57	0.41	387.6	419.2
9.19	4.80	4.01	2.00	0.49	0.44	0.42	0.20	221.6	163.7
1.08	4.95**	2.40	1.53	1.04	1.85	10.00**	3.44**	5.48**	0.86
5.27**	4.56*	2.49	0.94	12.23**	28.90**	2.52	5.34**	19.38**	32.57**
1.88	2.80*	1.37	0.81	1.34	1.86	0.36	0.67	5.96**	1.65
9.3 $\times 10^{-2}$	2.5 $\times 10^{-2}$	1.9 $\times 10^{-1}$	2.3 $\times 10^{-1}$	3.4 $\times 10^{-2}$	3.4 $\times 10^{-2}$	6.7 $\times 10^{-2}$	5.5 $\times 10^{-2}$	1.9 $\times 10^{-1}$	3.0 $\times 10^{-1}$

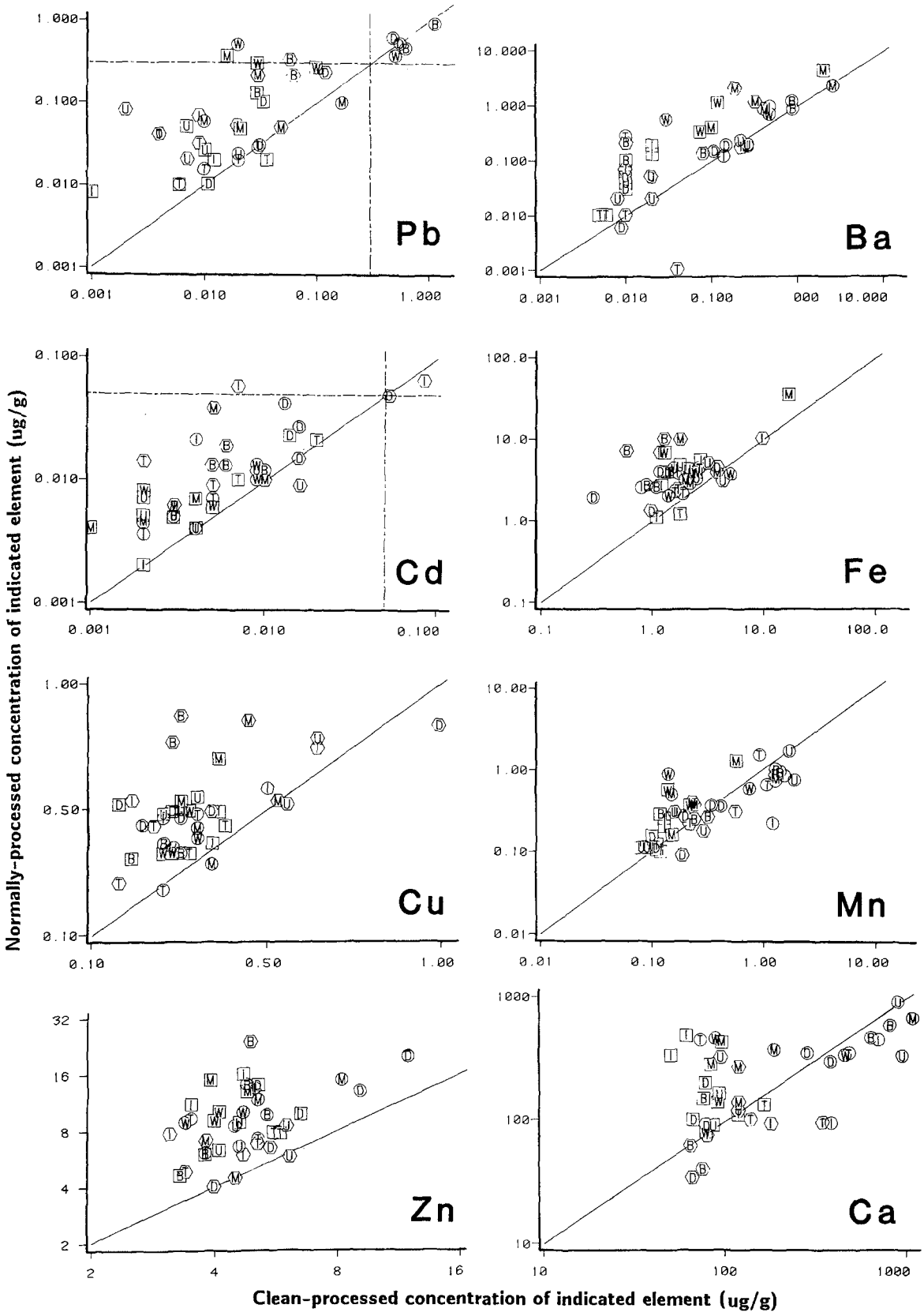

Fig. 2. Concentrations of eight elements (μg/g, wet weight) in normally-processed (*y*-axes) vs clean-processed (*x*-axes) edible tissue samples. Squares represent bass, hexagons catfish, and circles redhorse. Letters correspond to collection sites given in Figure 1. Solid diagonals, $y = x$; broken horizontal and vertical lines for Pb and Cd illustrate derived provisional guidelines for these elements (see text for explanation)

Table 4. Product-moment correlation coefficients illustrating relations among the differences between log-transformed elemental concentrations in normally- and clean-processed samples ($N = 39$)

	Pb	Cd	Cu	Zn	Fe	Mn	Ba	Ca
Pb	—	0.09	0.39*	0.26	0.36*	0.62**	0.50**	0.37*
Cd		—	0.46**	0.17	0.59**	0.04	0.17	−0.05
Cu			—	0.42**	0.79**	0.13	0.27	0.09
Zn				—	0.46**	0.27	0.30	0.44**
Fe					—	0.10	0.21	0.07
Mn						—	0.53**	0.75**
Ba							—	0.42**
Ca								—

H_0: $r = 0.0$. *$P \leq 0.05$; **$P \leq 0.01$

of contamination or because the points represent samples containing different concentrations (Wiener 1982). Nevertheless, several features of Figures 5 and 6 are noteworthy. For Pb, the expected positive relations appear to be present, but poorly defined, in both normally- and clean-processed redhorse from reaches of the Big River contaminated by tailings. For Zn, clean-processing greatly reduced differences among fish; most of the points for clean-processed samples lie along a well described line with a positive slope, irrespective of taxon or location of capture (Figure 6). There was less improvement for Cd, Cu, and Fe; the plots reflect differences among taxa detected by ANOVA (Tables 1 and 2). For Ba, Mn, and Ca, the plots were similar to each other, with the relation poorly defined and with little improvement after clean-processing. For Mn and Ca, differences in the concentrations attributable to taxon were apparent for both methods of preparation (Figures 5 and 6).

The molar ratio [Pb]/[Ca] ranged from 3.2×10^{-6} (in a clean-processed bass from Irondale) to 1.3×10^{-3} (in one subsample of the flathead catfish from Washington State Park). In the split-plot ANOVA, all main effects and interactions were significant, and $F_{(LT)}$ was 14 times greater than $M \times F_{(LT)}$ (Table 1). In normally-processed samples, [Pb]/[Ca] averaged about 5.0×10^{-4} at tailings-contaminated sites and 5.0×10^{-5} at uncontaminated sites (Table 6). The ratio was lower in clean- than in normally-processed samples; however, the interactions were significant (Tables 1 and 6) and tended to obscure the T effects. In clean-processed samples, only L was significant; ratios averaged about 2.3×10^{-5} at sites not affected by tailings, and about 1.6×10^{-4} at affected sites (Table 6).

For [Ba]/[Ca], only M was significant (Tables 1 and 6). Means for [Ba]/[Ca] were lower at sites outside the barite mining district ($\bar{X} \simeq 5.0 \times 10^{-4}$) than at Mineral Fork, Washington State Park, and Brown's Ford ($\bar{X} \simeq 8.0 \times 10^{-3}$), but L was not significant in either ANOVA (Tables 1 and 6).

Discussion

Sources of Error and Variation in Measured Concentrations

The removal of elements from the sample surface by clean-processing in making determinations for certain elements is noteworthy for two reasons. First, concentrations of three essential elements (Zn, Cu, and Fe) varied relatively little among locations and taxa after the effects of material on the surface of the samples were considered (Figures 5 and 6), even though concentrations of these elements in the streams from which the fish were collected varied substantially (Schmitt and Finger 1982). Zinc levels in the clean-processed tissues of most of the fish analyzed were similar (Figure 6). Levels of Cu and Fe varied more than those of Zn, but less than those of Pb and Ba. The point on the plot of Zn in clean-processed tissue (Figure 6) that deviates farthest from the general trend represents a redhorse from Desloge; this fish, in which the clean-processed Zn level was about double the levels in most others, caused the among-location differences for Zn in clean-processed samples to be significant (Table 2). Schmitt *et al.* (1984) showed that blood-Zn concentrations in redhorse from Desloge were also about twice those in fish from other reaches. The second important feature is that differences between the two plots for Zn and Cu (Figures 5 and 6) suggest that clean-processing effectively removed the enriched surface layer from the samples. This enrichment was probably not attributable to the presence of mucosal slimes, which should also have been rich in Pb and Ba (Wiener and Giesy 1979; Wiener 1982; Settle and Patterson 1980); if mucosal slimes caused this enrichment, it is improbable that Zn would have been removed while other elements (notably Pb and Ba) remained on the sample. Although Pb and Ba concentrations were also greater in some normally- than clean-processed samples, the derivations were variable and difficult to interpret. One explanation for these inconsistencies would be that some normally-processed samples were contaminated after preparation; alternatively, the subsamples prepared from each fish (one or more by each method) contained different concentrations of Pb and Ba; or, both events may have occurred. It is improbable that only the normally-processed samples were contaminated because the two sets of samples were simultaneously prepared in the clean-room, stored in identical containers, and digested and analyzed in one batch. Contamination from dust or some other source should have been equally probable for both groups; however, it clearly was not (Figure 2). The alternative explanation therefore seems more plau-

Table 5. Product-moment correlation coefficients illustrating relations between log-transformed elemental concentrations in normally- (above the principal diagonal, $N = 40$) and clean-processed (below diagonal, $N = 39$) samples

Clean-processed	Normally-processed							
	Pb	Cd	Cu	Zn	Fe	Mn	Ba	Ca
Pb	—	0.31*	0.45**	0.50**	0.35*	0.75**	0.58**	0.54**
Cd	0.05	—	0.53**	0.01	0.32*	0.12	0.01	−0.11
Cu	−0.08	0.65**	—	0.22	0.69**	0.24	0.29	−0.02
Zn	0.45**	0.17	0.10	—	0.25	0.46**	0.44**	0.59**
Fe	−0.21	0.41**	0.42**	−0.18	—	0.35*	0.47**	0.12
Mn	0.34*	0.08	−0.06	0.07	0.09	—	0.65**	0.75**
Ba	0.39*	−0.09	−0.05	0.20	0.33	0.54**	—	0.54**
Ca	0.50**	−0.06	−0.16	0.35*	−0.12	0.85**	0.55**	—

H_0: $r = 0.0$. $^*P \leq 0.05$; $^{**}P \leq 0.01$

sible: concentrations of Pb, Ba, Ca, and Mn in the subsamples differed because these elements were distributed heterogeneously among the cubes of tissue.

It is generally known that Pb, Ba, and Mn accumulate in bone, skin, and scales, where concentrations may be orders of magnitude higher than in muscle (Patterson and Settle 1977; Brooks and Rumsey 1974). Although there were no visible fragments of these tissues on the surfaces of the samples, small bones within a cube would not have been detected. Because catostomids possess numerous tiny intermuscular bones, the presence of bone fragments in redhorse samples is highly probable.

The presence of high Pb, Ba, Mn, and Ca concentrations in the flathead catfish from Washington State Park (Table 2) also supports the contention that the apparent overestimation of these elements in some subsamples was caused by their heterogeneous distribution within samples. Duplicate subsamples from this fish yielded the following elemental concentrations (μg/g, wet weight):

Pb	Cd	Cu	Zn	Fe	Mn	Ba	Ca
20.0	0.038	0.71	28.0	9.9	8.0	5.0	71.6
3.9	0.032	0.67	19.0	11.0	1.8	1.5	38.0

Concentrations of elements that accumulate predominantly in the soft tissues—Cd, Cu, Zn, and Fe—were double their respective concentrations in normally-processed bass and redhorse from Washington State Park (Table 2), and the concentrations in duplicate subsamples were similar. Conversely, the concentrations of Pb, Ba, Ca, and Mn—elements that accumulate in the hard tissues—were 5- to 20-fold greater than their respective concentrations in bass and redhorse from the same site (Table 2), and the duplicate values differed substantially (2–5 X). Inasmuch as Pb concentrations in bone increase with age (Barry 1978; Eisler 1984), the inclusion of bone in subsamples from this fish should have had a greater effect on the overall concentration than the inclusion of an equivalent amount of bone in a subsample from a younger fish. Given the sizes of the specimens analyzed relative to published age and growth data (Purkett 1958; Bowman 1970; Pflieger 1975), the flathead catfish was probably 10–15 yr old, or two to three times older than the bass or redhorse. The fact that the sample of edible tissue from the catfish was dissected in the field cannot, however, be completely discounted; the other samples were prepared entirely in the clean-room. Although every attempt was made to clean this sample during preparation for analysis, some handling contamination could have remained.

Reagent blanks carried through the digestion and analytical procedure were never positive for Pb, and some clean-processed samples had Pb concentrations that were at the analytical limit of detection—0.001 μg/g (Table 2). Chow *et al.* (1974), Patterson and Settle (1977), and Settle and Patterson (1980) reported that the Pb concentration in the axial muscle tissue of albacore (*Thunnus alalunga*) from the unpolluted Pacific Ocean was about 0.0003 μg/g, as measured by isotope dilution mass spectrometry, a more sensitive method of analysis than AA. The values of 0.001–0.005 μg/g reported for fish from the least contaminated streams in southern Missouri (Table 2), where several smelters constitute significant atmospheric sources of Pb (Gale *et al.* 1973) and where the bedrock is extensively mineralized, are probably accurate. Moreover, these results show that fish can be prepared and analyzed by AA without significant contamination.

Within-fish variation in normally-processed samples was substantially higher for Pb, Ba, and Mn than for Cd, Cu, Zn, and Fe (Table 1). Settle and Patterson (1980) reported measurement uncer-

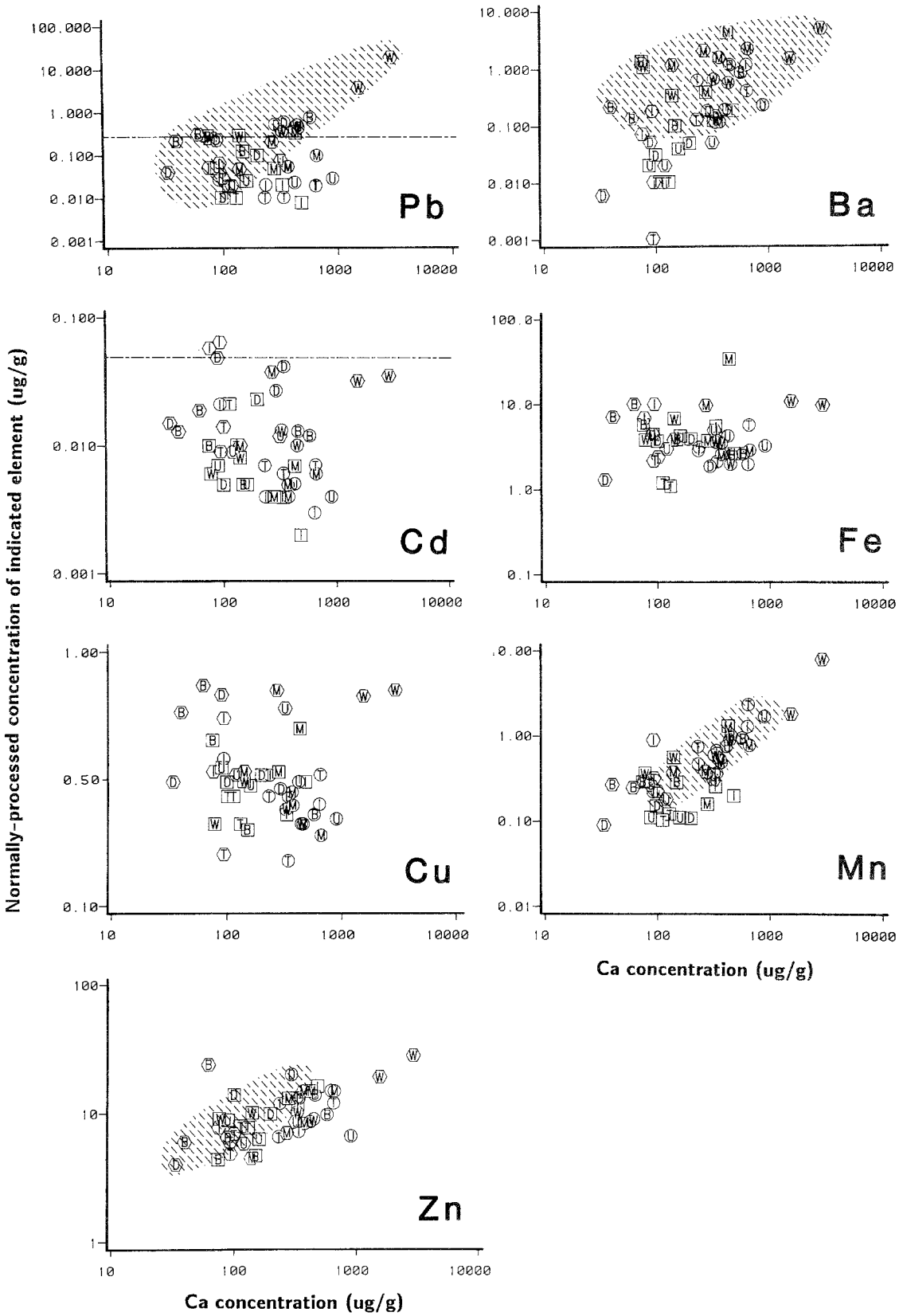

Fig. 3. Concentrations of seven elements (*y*-axes) vs calcium concentration (*x*-axes) in normally-processed edible tissues (all in μg/g wet weight). Shaded areas on panels: Pb—all fish from tailings-contaminated sites; Zn—all fish from Desloge; Ba—all fish from sites within barite mining district; Mn—all redhorse. See Figure 1 for locations and names of collection sites and Figure 2 for explanation of symbols

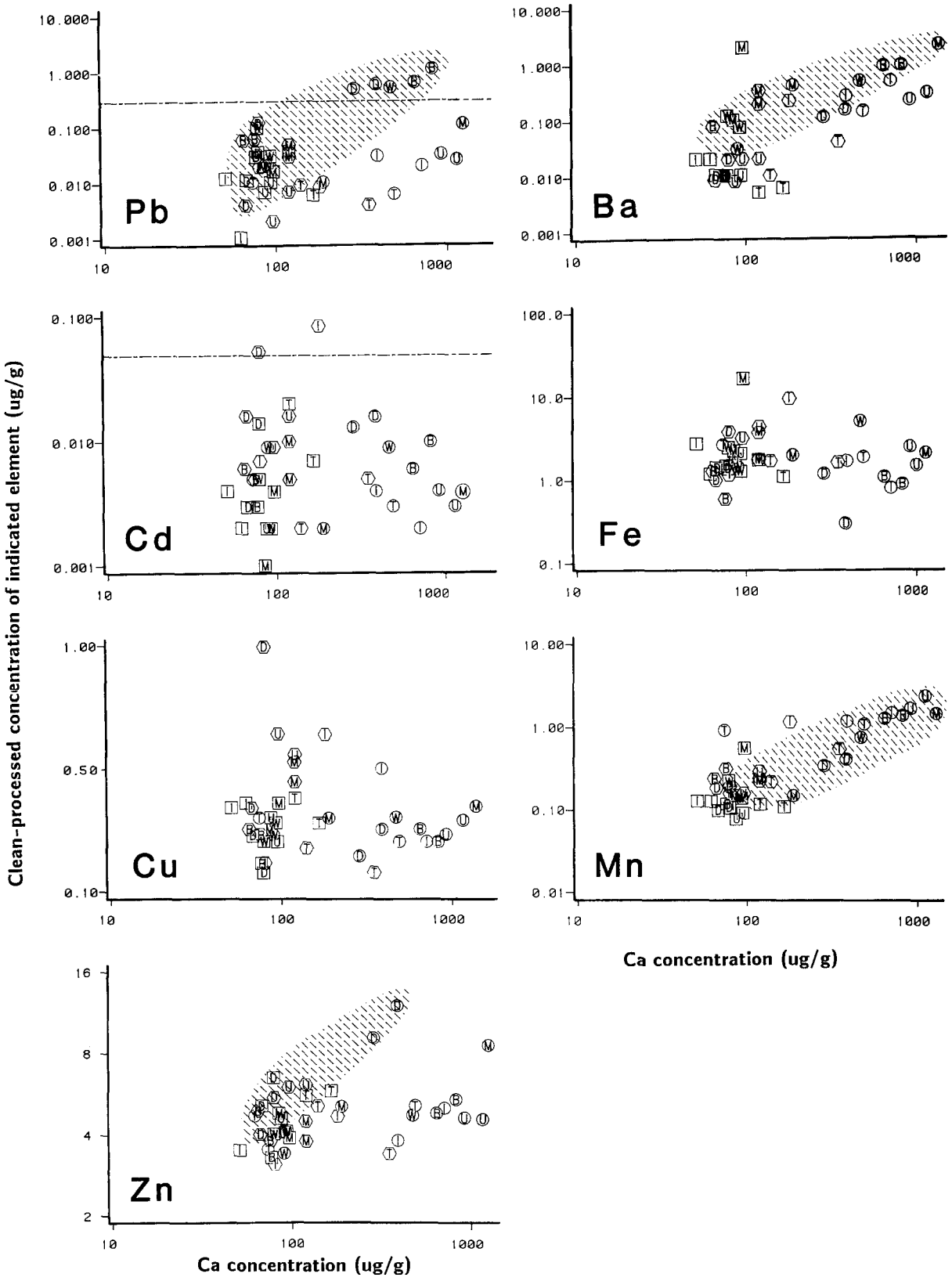

Fig. 4. Concentrations of seven elements (*y*-axes) vs calcium concentration (*x*-axes) in clean-processed edible tissues (all in μg/g wet weight). Shaded areas on panels: Pb—all fish from tailings-contaminated sites; Zn—all fish from Desloge; Ba—13 of 14 fish from sites within barite mining district; Mn—13 of 14 redhorse. See Figure 1 for locations and names of collection sites and Figure 2 for explanation of symbols

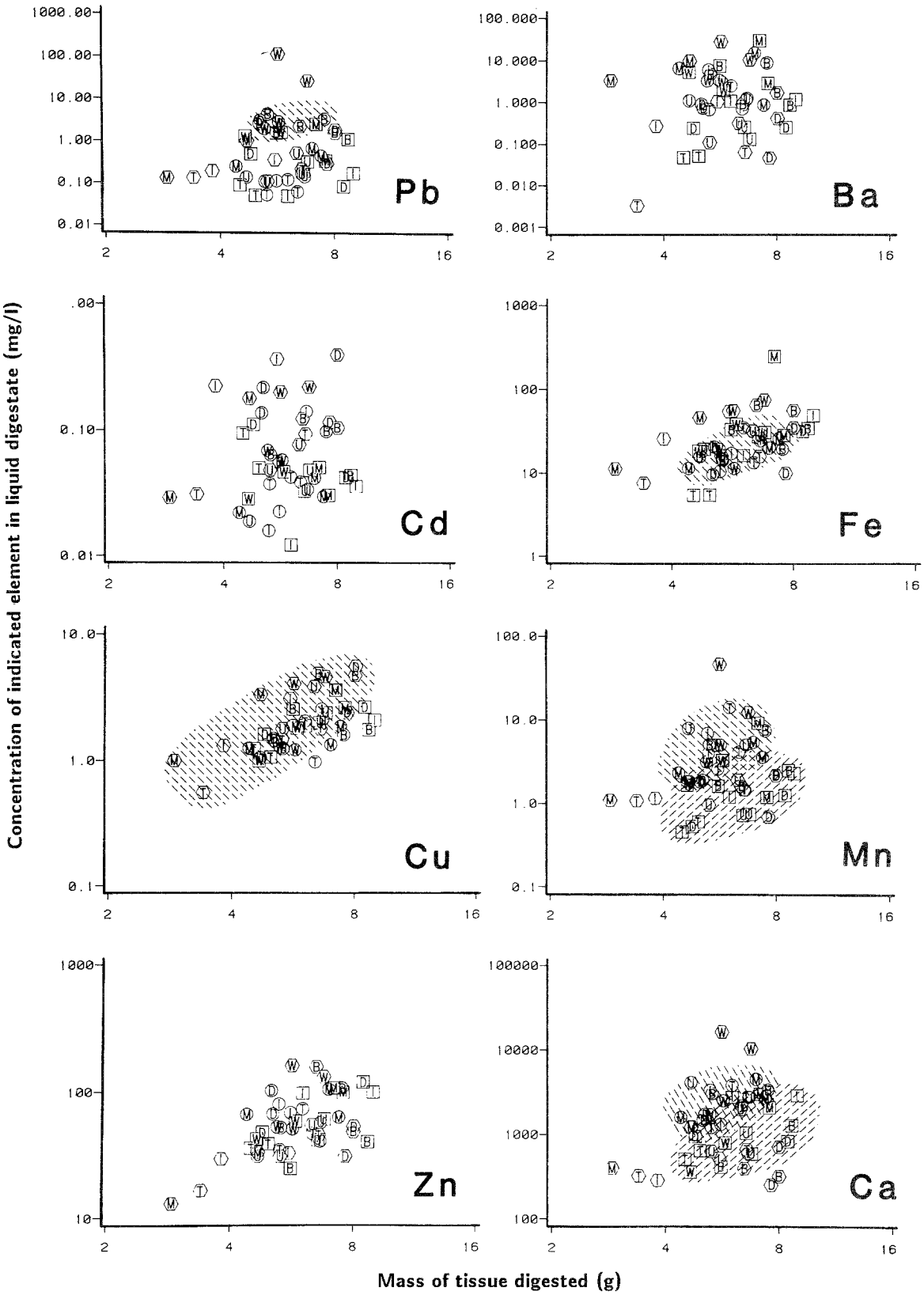

Fig. 5. Concentrations (mg/L) of eight elements (*y*-axes) in liquid digestates vs wet weight (g) of normally-processed edible tissue digested (*x*-axes). Shaded areas on panels: Pb—all redhorse from tailings-contaminated sites; Cu—all catfish; Fe—all redhorse; Mn—13 of 14 redhorse (upper area) and 13 of 14 bass (lower area); Ca—all redhorse (upper area) and all bass (lower area). See Figure 1 for locations and names of collection sites and Figure 2 for explanation of symbols

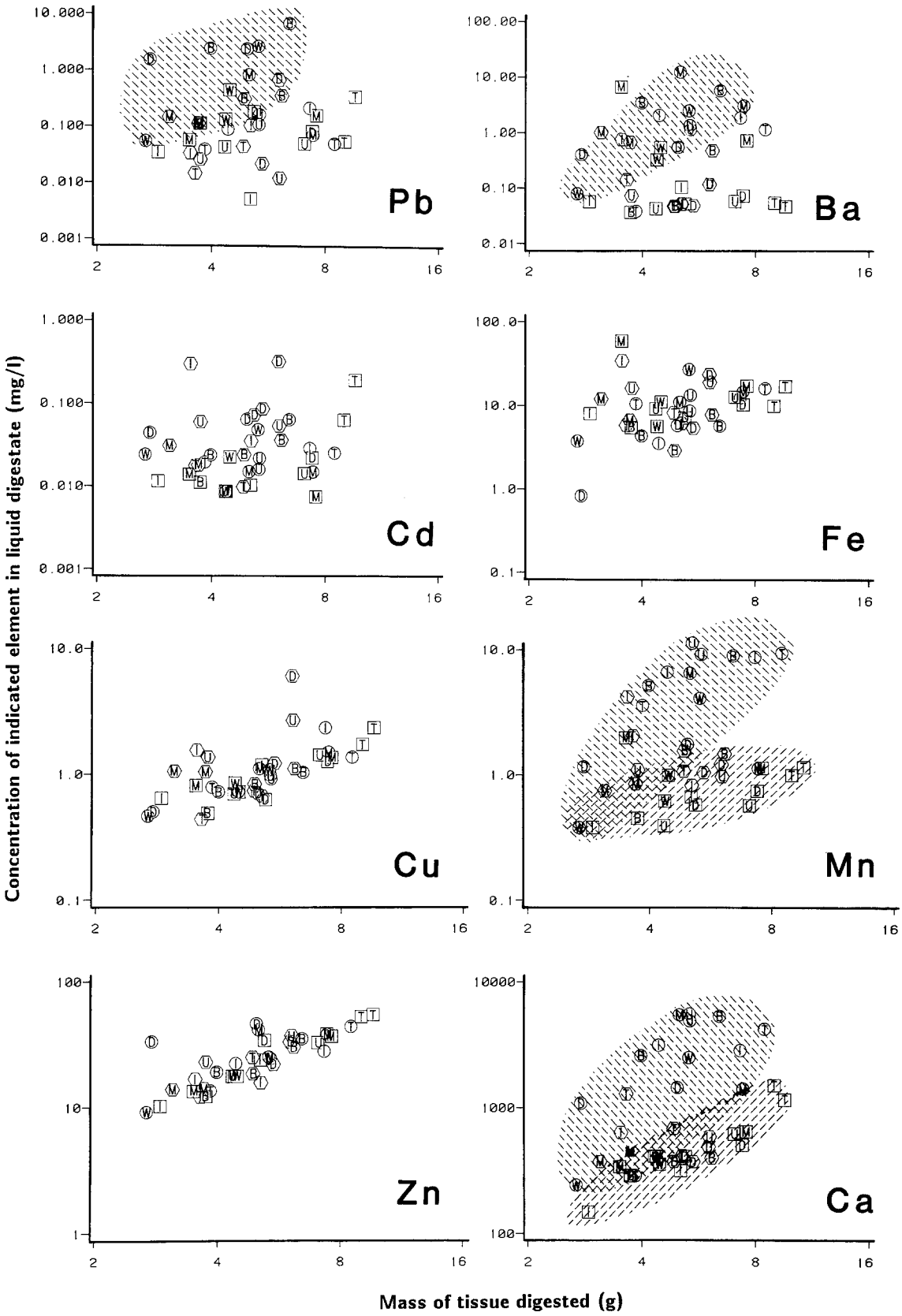

Fig. 6. Concentrations of eight elements (*y*-axes, mg/L) in liquid digestates vs wet weight of clean-processed edible tissue digested (*x*-axes, g). Shaded areas on panels: Pb—all fish from tailings-contaminated sites; Ba—all redhorse from sites within barite mining district; Mn—all redhorse (upper area) and 13 of 14 bass (lower area); Ca—all redhorse (upper area) and all bass (lower area). See Figure 1 for locations and names of collection sites and Figure 2 for explanation of symbols

Table 6. Mean [Pb]/[Ca] and [Ba]/[Ca] molar ratios in normally- (N) and clean-processed (C) samples, and the results of two-way ANOVA testing the significance of main effects due to locations (L), taxa (T), and $L \times T$ interaction

River, collection site and taxon	[Pb/Ca]		[Ba/Ca]	
	N	C	N	C
Big River				
Mineral Fork				
Bass	9.9×10^{-4}	4.1×10^{-5}	1.7×10^{-3}	3.2×10^{-3}
Catfish	1.1×10^{-4}	6.4×10^{-5}	2.4×10^{-3}	6.4×10^{-4}
Redhorse	3.1×10^{-5}	2.1×10^{-5}	8.1×10^{-4}	6.8×10^{-4}
Location mean	7.0×10^{-5}	4.2×10^{-5}	1.5×10^{-3}	1.5×10^{-3}
Brown's Ford				
Bass	4.7×10^{-4}	7.7×10^{-5}	2.9×10^{-3}	3.9×10^{-5}
Catfish	1.0×10^{-3}	1.7×10^{-4}	1.2×10^{-3}	2.0×10^{-4}
Redhorse	2.4×10^{-4}	2.3×10^{-4}	6.1×10^{-4}	3.7×10^{-4}
Location mean	5.0×10^{-4}	1.7×10^{-4}	1.5×10^{-3}	2.4×10^{-4}
Washington State Park				
Bass	5.3×10^{-4}	1.6×10^{-4}	2.5×10^{-3}	3.5×10^{-5}
Catfish	9.2×10^{-4}	——	4.0×10^{-4}	——
Redhorse	2.2×10^{-4}	1.3×10^{-4}	4.8×10^{-4}	2.0×10^{-4}
Location mean	5.6×10^{-4}	1.4×10^{-4}	1.1×10^{-3}	2.7×10^{-4}
Desloge				
Bass	5.9×10^{-5}	5.8×10^{-5}	8.2×10^{-5}	4.1×10^{-5}
Catfish	3.7×10^{-4}	1.5×10^{-4}	1.1×10^{-4}	5.7×10^{-5}
Redhorse	3.5×10^{-4}	3.1×10^{-4}	1.5×10^{-4}	1.1×10^{-4}
Location mean	2.6×10^{-4}	1.7×10^{-4}	1.2×10^{-4}	7.0×10^{-5}
Irondale				
Bass	7.6×10^{-6}	2.5×10^{-5}	1.1×10^{-4}	1.1×10^{-4}
Catfish	1.4×10^{-4}	3.0×10^{-5}	4.3×10^{-4}	2.0×10^{-4}
Redhorse	2.8×10^{-5}	1.0×10^{-5}	6.5×10^{-4}	2.0×10^{-4}
Location mean	5.3×10^{-5}	2.2×10^{-5}	4.3×10^{-4}	1.7×10^{-4}
Black River				
Upstream				
Bass	7.1×10^{-5}	1.8×10^{-5}	7.1×10^{-5}	2.9×10^{-5}
Catfish	4.1×10^{-5}	7.8×10^{-6}	4.8×10^{-5}	5.6×10^{-5}
Redhorse	8.7×10^{-6}	5.2×10^{-6}	1.1×10^{-4}	7.3×10^{-5}
Location mean	4.0×10^{-5}	1.0×10^{-5}	7.5×10^{-5}	5.3×10^{-5}
Downstream				
Bass	2.5×10^{-5}	3.3×10^{-5}	2.5×10^{-5}	1.2×10^{-5}
Catfish	7.2×10^{-5}	7.5×10^{-6}	1.6×10^{-5}	2.8×10^{-5}
Redhorse	6.8×10^{-6}	1.5×10^{-5}	1.5×10^{-4}	6.3×10^{-5}
Location mean	3.1×10^{-5}	1.8×10^{-5}	7.7×10^{-5}	3.4×10^{-5}
Taxon means				
Bass	1.8×10^{-4}	5.8×10^{-5}	1.1×10^{-3}	5.4×10^{-4}
Catfish	3.8×10^{-4}	7.2×10^{-5}	6.6×10^{-4}	2.0×10^{-4}
Redhorse	1.3×10^{-4}	1.2×10^{-4}	4.3×10^{-4}	2.4×10^{-4}
Grand mean	2.2×10^{-4}	7.8×10^{-5}	7.2×10^{-4}	3.4×10^{-4}
Two-way ANOVA[a]				
Source				
L	24.94**	6.84**	2.48	1.91
T	17.98**	1.47	1.14	0.52
$L \times T$	4.81**	1.54	0.71	0.80
Error	1.0×10^{-8}	4.2×10^{-9}	1.2×10^{-6}	8.9×10^{-7}

[a] F-values, significance levels, and error (among-fish) variances

* $P \leq 0.05$; ** $P \leq 0.01$. DF as in Table 3

tainty of ±30% for Pb and Ba in clean-processed albacore muscle, which is one tenth the average within-fish variation for Pb and one fifth that for Ba in normally-processed samples. Within-fish variability for Pb in normally-processed samples was also higher than the 61% CV reported by Heit (1979) for 15 replicate analyses of Pb in the clean-processed edible tissues of a striped bass (*Morone saxatilis*); however, one group of three subsamples analyzed by Heit had a CV of 218%. Repeatability was also relatively poor for Mn and Ca in normally-processed samples. Collectively, these results suggest that these elements were distributed heterogeneously within the samples; however, no clean-processed samples were replicated for comparison.

The epithelial mucus of teleosts in known to impart elemental contaminants to samples of the edible parts of fish (Giesy and Wiener 1977; Settle and Patterson 1980; Wiener 1982). Mucus contains glycoproteins (Fletcher and Grant 1968; Wold and Selset 1977) that can bind many cations through complexation (Coombs *et al.* 1972; Chow *et al.* 1974; Varanasi *et al.* 1975; Varanasi and Markey 1978; Pärt and Lock 1983). Mucus production can be stimulated by certain metal ions (Varanasi and Markey 1978; Lock and Van Overbeck 1981; Eddy and Fraser 1982), indicating a possible protective role (Pärt and Lock 1983); production can also continue after the death of the fish (Wold and Selset 1978), which may be important in the context of determining trace metal concentrations in edible tissues. Varanasi and Markey (1978), who injected living fish with radioisotopes, showed that Pb and Cd can be transferred to the scales and mucus from the blood and suggested that such secretions are an important route for the excretion of toxic cations. Therefore, the external mucus of fish from a metal-contaminated environment, such as the Big River, probably receives Pb both from within the fish and from the surrounding water, and some of the Pb from within may be derived from the diet.

The concentrations of free metal ions, which are controlled by local environmental conditions, probably determine concentrations in mucus. For example, Coombs *et al.* (1972) reported that Cu and Zn were preferentially complexed by mucus; however, Wiener and Giesy (1979), in their study of metal concentrations in bluegill (*Lepomis macrochirus*) from a soft-water, highly organic pond in the Southeast, reported mucus contamination of muscle samples by Pb, but not by Cu, Cd, or Zn. This discrepancy suggests that dissolved organic ligands can bind some metals, rendering them unavailable. Big River water is relatively hard (>180 mg/L as $CaCO_3$) and low in dissolved organic material; accordingly, most dissolved Pb occurs as carbonate and bicarbonate complexes rather than as organic complexes or free hydrated ion (Schmitt *et al.* 1984; Harwood 1984), and complexes may not be available for binding by mucus. Hardness ions (Ca^{+2} and Mg^{+2}) and pH also influence the complexation of divalent trace metals by organic substances (O'Shea and Mancy 1978). Consequently, the concentrations of trace metals in mucus reflect variations in metal abundance and availability for binding; the surface of the fish competes with other ligands and particulate material for metal ions, and concentrations in mucus depend on local environmental conditions.

Mucus is rich in Ca (Van Oosten 1957) and contains calmodulin (Flik *et al.* 1984), a specific Ca-binding protein (Pärt and Lock 1983). Calmodulin may be actively involved in direct uptake of Ca across the gills (Kirschner 1977). Coombs *et al.* (1972) reported that the Ca concentration of mucus in the plaice (*Pleuronectes platessa*) was similar to that in seawater. In hard-water environments, such as the Big River, many potential metal binding sites in mucus may be occupied by Ca. The data clearly show that Ca was present on the surfaces of the normally-processed samples (Figures 5 and 6); however, in redhorse the significantly higher Ca concentrations in clean-processed samples (Figure 4; Tables 2 and 3) probably resulted from the incomplete removal of bone tissue.

According to Patterson (1980), Pb and Ba are processed by organisms incidentally, as trace contaminants of Ca; biological mechanisms that regulate Ca do not operate for Pb and Ba. As a consequence of sequential "biopurification" steps, [Pb]/[Ca] and [Ba]/[Ca] ratios in organisms tend to decline as trophic position rises. For example, Patterson (1980) estimated that [Pb]/[Ca] decreases from about 8.0×10^{-5} in algae to 5.0×10^{-6} in northern anchovy (*Engraulis mordax*) and 1.0×10^{-7} in the skeleton of albacore. The lowest [Pb]/[Ca] values from the Big and Black Rivers were consistent with the mid-trophic values reported by Patterson (1980). The highest ratio occurred in the flathead catfish from Washington State Park. The trophic structure of Ozark streams is less rigid than that of the Pacific Ocean; many stream invertebrates depend on allochthonous organic material, and sharing of food resources among stream fishes is also common (Probst *et al.* 1984). The biopurification mechanism may therefore be poorly defined and not recognizable as among-species differences in [Pb]/[Ca] and [Ba]/[Ca] attributable to trophic position. Direct uptake of Pb or Ba from water, which is more probable in the Big River than in the Pacific Ocean, would also tend to obscure any differences related to diet.

Table 7. Mean concentrations (μg/g wet weight) of Pb in the edible tissue of smallmouth bass, suckers (Catostomidae), and longear sunfish collected from the Big River in independent studies conducted from 1979 to 1981

Species and source	Location				
	Irondale	Leadwood	Desloge	Washington State Park	Brown's Ford
Smallmouth bass					
Gale *et al.* (1982)	0.060	0.200	0.100	—	—
Czarneski (1985)	0.080	0.040	0.170	0.150	0.050
Present study					
Normally-processed	0.014	—	0.054	0.275	0.130
Clean-processed	0.006	–	0.022	0.064	0.030
Black redhorse and other suckers					
Gale *et al.* (1982)	0.080	0.700	0.500	—	—
Schmitt *et al.* (1984)	0.070	0.440	0.790	0.380	—
Czarneski (1985)	0.060	0.310	0.620	0.370	0.430
Present study					
Normally-processed	0.024	—	0.569	0.433	0.626
Clean-processed	0.025	—	0.525	0.241	0.850
Longear sunfish					
Schmitt and Finger (1982)					
Clean-processed	0.020	—	0.310	0.180	0.170
Czarneski (1985)	0.090	0.140	0.390	0.180	0.270
Gale *et al.* (1982)	0.090	0.500	0.500	—	—

Elemental Contaminants in Southeastern Missouri Fishes

Only for Pb did measured concentrations exceed values reported in edible fish tissues from locations outside the Old Lead Belt (Hall *et al.* 1978; Sidwell *et al.* 1978; Wiener and Giesy 1979). Concentrations of Pb in redhorse from tailings-contaminated reaches of the Big River prepared by either method (Tables 2 and 3) were generally 2- to 5-fold the levels reported elsewhere, whereas values for normally-processed samples of bass and redhorse agreed well with those from previous Big River studies (Table 7). The Pb concentration in the flathead catfish from Washington State Park (Table 3) was 10-fold higher than average levels from outside the Old Lead Belt, 17 times higher than the concentration in canned tuna (Settle and Patterson 1980), and far surpassed concentrations reported previously for the Big River. This fish and half of the redhorse from contaminated reaches of the Big River contained at least 0.3 μg/g of Pb, even after clean-processing. In contrast, only three catfish contained more than 0.05 μg/g of Cd, and two of these were from a site not affected by tailings (Figure 2). Neither Cd nor Zn from the Old Lead Belt tailings has accumulated in the edible tissues of fish.

The high levels of Pb in redhorse from tailings-contaminated reaches of the Big River reported by the Missouri Department of Conservation (Shirk 1980; Czarneski 1985) prompted the present study. Perhaps ironically, the data suggest that the edible tissues of these fish contain sufficient Pb to be relatively immune to laboratory contamination; clean- and normally-processed samples were generally in close agreement (Table 2, Figure 2). Evidence from previous investigations also supports the contention that redhorse from the Big River contain unusually high concentrations of Pb and that reported values (here and elsewhere) are not artifact of laboratory contamination. Schmitt *et al.* (1984) found that Pb concentrations in the blood of several species of suckers from the Big River were highly correlated with, but 10-fold higher than, Pb concentrations in the edible tissues. Furthermore, the activity of the erythrocyte enzyme δ-aminolevulinic acid dehydratase (ALA-D) was negatively correlated with Pb in edible tissues and in blood. Schmitt *et al.* (1984) also found that Pb concentrations in blood and in edible tissues were highly correlated. Schmitt and Finger (1982) reported similar findings for longear sunfish (*Lepomis megalotis*). The inactivation of ALA-D is a biochemical response that is specific for Pb, but which is relatively insensitive to Pb added to a blood sample after collection (Hodson *et al.* 1977). The blood samples analyzed by Schmitt *et al.* (1984) and Schmitt and Finger (1982) were collected from living fish with sterile, heparinized needles and syringes. Berman (1976) recommended a similar tech-

nique to minimize the risks of Pb-contamination in the collection of human blood samples.

Allocation of Analytical Resources

The allocation of analytical resources between fish and subsamples that yields the greatest precision is given by the ratio $\sqrt{c\sigma^2/c_F\sigma^2_F}$, where σ^2 and σ^2_F are the estimated within-fish and among-fish variance components from ANOVA, and c_F and c are the total costs of collecting, preparing, digesting, and analyzing each fish (c_F) and subsample (c) (Sokal and Rohlf 1969). It was assumed that $c \simeq c_F$, because laboratory costs outweigh costs of collection on a per sample basis, and the total cost of analyzing a sample (fish) or subsample are essentially equal. Given this assumption, application of the above formula to the variance estimates yielded the following approximate recommended numbers of subsamples per fish: Cd, 1; Cu, 1; Zn, 2; Fe, 1; Mn, 5; Ba, 2; Ca, 3; and Pb, 17. Although σ^2 may have been overestimated by replicating few samples (and even for those, only two subsamples were analyzed), this worst-case illustration clearly shows far greater within-fish variability for Pb than for the other seven elements. Consequently, a sampling plan that calls for the collection of five or fewer small, randomly selected and carefully prepared subsamples from each fish would be adequate for most elements, but not for Pb.

One alternative to intensive subsampling would be to carefully prepare and digest all of the edible tissues from each fish rather than numerous subsamples, either in one large vessel or by cutting up the large pieces, digesting them separately, and compositing the digestates. In their study of bluegill, Wiener and Giesy (1979) found that the digestion of whole fish was necessary to avoid the problems of heterogeneous trace element distributions and surface contamination. Few analytical laboratories, however, can accommodate the preparation and digestion of large fish, and physical homogenization (blending, lyophilization, or grinding) increases the probability of contamination (Mahaffey 1978). Additional preparation steps, the use of numerous containers for digestions, and large reagent volumes will also cause c_F/c to increase with fish size, and if $c_F/c \geq 17$, subsampling would be cost-justified. In any event, the conventional procedure of selecting one or two small subsamples from a large fish will not yield satisfactory estimates of the mean Pb concentration in the edible tissues.

The amount of Pb and Ba in the edible tissues of a fish from contaminated waters appears to be related to the Ca content of the sample, because bone, skin, scales, or (to a lesser extent) mucus inevitably accompanies the muscle tissue. In the present data, almost half of the variability in Pb and Ba could be accounted for by Ca. With large N, one could use analysis of covariance (Huitema 1980) to reduce the variation in such groups of similar samples by statistically adjusting their Pb and Ba concentrations to a common Ca concentration. With smaller N, a similar alternative—examining and statistically testing [Pb]/[Ca] and [Ba]/[Ca]—may be appropriate. ANOVA (Table 1) clearly illustrated the greater precision of the molar ratios over the unadjusted concentrations, and application of the previously described formula to the variance components of the ratios indicated that only four 5-g subsamples per fish would adequately estimate [Pb]/[Ca], and one subsample would be adequate for [Ba]/[Ca]. The data indicate that, even in samples from heavily contaminated sites, the diminution of Ca through replacement by Pb and Ba will not be measurable, and that the ratios should therefore remain unaffected.

Conclusions and Recommendations

Except for Se (Lemly 1985), Hg, and As (Moore and Ramamoorthy 1983), potentially toxic trace elements generally do not accumulate in the axial muscle tissues of fish. Nevertheless, the results of this study and of other investigations cited illustrate how the edible parts of fish from contaminated waters may in fact contain high levels of some elements—Zn, Cu, Pb, and Cd—by inclusion of the skin (with its associated mucus, and, for some fishes, scales), and by contact of the surface of a skinless fillet with mucus. Additional Pb may accrue from the inclusion of bone fragments, either by accident or intention, in the preparation of the fish. Investigators should acknowledge the potential presence of elements from these sources and their effects on measured concentrations and variability.

Of the eight elements measured—Pb, Cd, Cu, Zn, Fe, Mn, Ba, and Ca—only Pb, and to a lesser extent Cd, approached published criteria for maximum allowable concentrations in foods, despite the gross contamination of the Big River by mine tailings and the high concentrations of Fe and Mn in the tailwaters of Clearwater Lake. Consequently, little direct value will be derived from the measurement of Cu, Zn, Fe, and Mn in the edible tissues of fish. The growing concern about environmental Pb suggests that concentrations in fish will be subject to further scrutiny. Because the data indicate that fish from contaminated areas may contain this ele-

ment at concentrations considered by some authorities to be harmful, the following suggestions are offered for potential investigators:

(1) Depending on the method of preparation and the intent of the investigator, mucus on the exterior of the "edible tissues," along with its bound metals, may contstitute part of the sample and not "contamination." In such instances and under current guidelines for dietary Pb levels, NBS reference materials (bovine liver, 0.35 μg/g Pb dry weight; albacore, 0.46 μg/g) are adequate for detecting relevant contamination. This assessment does not, however, rule out the problem of cross-contamination (for example, slime from one sample contaminating another). Overcoming this potential problem requires that all dissecting tools and work surfaces be completely cleaned after each fish has been processed, which is best achieved in the laboratory. Fish should be individually bagged and sealed in the field and frozen whole, then prepared for digestion and analysis under ultraclean conditions—as recommended by Patterson and Settle (1976).

(2) If the intent of the investigator is to differentiate between contaminated and uncontaminated sites on the basis of Pb concentrations in the edible tissues of fish, the amount added by contamination acquires more significance and must be considered. Contamination in the analytical laboratory will be evident at Pb concentrations of 0.5 μg/g or less, and there are no readily available reference materials sufficiently low in Pb to evaluate contamination at this level. The results of this study indicate that clean-processed muscle tissue of bass (also longear sunfish; Schmitt and Finger 1982) from uncontaminated areas contains less than 0.005 μg/g Pb. Although 10-fold higher than the albacore advocated by Settle and Patterson (1980) for use as a reference, this value is sufficiently low to permit detection of contamination in the laboratory. Although the inclusion of such samples in each group to be analyzed would not be foolproof because of the heterogeneity of Pb in the samples, it would nevertheless be preferable to no reference material or to material that contains excessive Pb. Obviously, use of such material implies that (a) fish must be collected from a relatively uncontaminated area, and a pilot survey may be required to locate such an area; and (b) the analytical laboratory must be both able and willing to subject itself to rigorous introspection. Both (a) and (b) will increase the cost of the study—as would the requirement of a 0.001 μg/g detection limit for Pb. Better alternatives include selection of a tissue that accumulates higher concentrations of Pb, such as bone or scales; analysis of the whole fish rather than just the edible tissues; or the selection of a different organism entirely (for example, a mollusc) that accumulates higher concentrations of Pb (Phillips 1977). Any of these measures would yield samples that contain higher concentrations of Pb, would better reflect environmental Pb levels, and would be less affected by laboratory contamination.

(3) For statistical testing, the adjustment of Pb concentrations for the Ca content of the sample may increase relative precision. Furthermore, the measurement of Ca in the sample will provide a margin of quality assurance in that it may explain an otherwise anomalously high or low Pb value caused by the inclusion of a greater than normal proportion of Ca-rich tissue in the sample. The measurement of other elements may prove similarly beneficial.

(4) The frequency distributions of elemental concentrations in fish have been described as "outlier prone" (Giesy and Wiener 1977) because of high variability among individual fish, among tissues within a fish, and even among samples of the same tissue. The results of the present study clearly support this conclusion. Accordingly, statistically reliable estimates for some elements in individual fish, notably Pb, will require unrealistically high numbers of subsamples. Composite samples are frequently analyzed to reduce analytical costs, and Kussmaul and Anderson (1967) described a statistical procedure for allocating resources in such designs; however, the additional preparation required in the compositing step will increase the risk of contamination, as will the digestion and analysis of all the edible tissues because additional digestion vessels and sample preparation are required. Therefore, Pb concentrations determined by any technique should be considered estimates, to be reported and interpreted with full knowledge that the samples may have been contaminated and that the integrity of the results may subsequently be challenged.

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