Metal Body Burden in Stoneroller Minnows (*Campostoma anomalum*) from the Bayou Creek System

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INTRODUCTION

This report presents the results for eight metals of concern (MOC, *i.e*. Ag, Be, Cd, Cr, Cu, Ni, Pb and Zn) analyzed in thirty-two stoneroller minnows (*Campostoma anomalum*) collected from Big and Little Bayou Creeks on September 29-30, 1997. Sampling was conducted jointly by personnel from UK, FFOU, and U.S. Fish and Wildlife. The objectives were to: **1) use whole-body metal residues to identify sources, magnitude, and spatial distribution of bioavailable metals within the Bayou Creek system; 2) estimate downstream movement of metal contamination in Big Bayou Creek from upstream point-source discharges; and 3) identify stream sectors where metal stressors potentially may produce biological and/or ecological effects.**

MATERIALS AND METHODS

Fish collection

Fish were collected by use of back-pack shockers, and those fish that did not meet size requirements were returned to the stream. Stoneroller minnows were placed in plastic bags, tagged, and placed on ice (4 ^{o}C) for transport to the laboratory. Fish were stored in the freezer (-15 ^{o}C) until analyzed.

Fish Digestion

Stoneroller minnows selected for analysis were prepared according to modified procedures described by Shaw *et al.* (1998), Hogstrand *et al*. (1996), and U.S. EPA (1996). Whole-body samples were wet-weighed and placed in 125-mL Erlenmeyer flasks. The samples were digested with TraceMetal grade concentrated HNO_3 (3.0 mL/g wet weight) and heated until approximately 1.0 mL remained. The samples were allowed to digest at room temperature for 24 h. Once complete digestion was achieved, 0.75 mL of 30% H_2O_2 per gram wet weight was added to each sample followed by heat-instilling until dry. The samples were then reconstituted to 10.0 mL with 0.5 % HNO_3 in deionized water. Samples were filtered through a Gelman Sciences Type A/E glass fiber filter to remove suspended particulates. The filters were rinsed with 0.50 % HNO_3 prior to use. Filtrates were taken to a final volume of 10.0 mL. Most assays were based on whole-body metal, but selected stonerollers were digested and analyzed with the gastrointestinal (g.i.) tract removed. The g.i. tracts removed from these specimens also were analyzed.

Metal Analyses

Analyses of stoneroller tissue samples were performed using a Varian AAS (Model Spectra AA-20) with flame or a graphite furnace equipped with a deuterium lamp for background corrections (U.S. EPA, 1997). All gases used were ultra pure carrier grade. Calibration curves were based on five standards. The instrument was programmed to take three readings per sample and average the absorbance. Instrument blanks (0.5 % HNO_3) and check standards were processed with all samples. Sample concentrations were then corrected for deviations from the standards, and fish wet weight was factored into the calculation of final values.

Estimating Bioavailable Metal

The following background information is presented to provide a **basis for data analyses** given in the FFOU study. U.S. EPA has moved away from basing metal criteria for the protection of aquatic life on total recoverable (TR) to dissolved concentrations (U.S. EPA, 1996). Unfortunately, dissolved concentrations of such metals as Ag and Cd down to low or sub-part-per-billion concentrations that may affect aquatic life are difficult or impossible to quantify accurately with present technology (Bergman and Dorward-King, 1997). It appears that most states will have difficulty implementing these new metal regulations. Even if accurately quantifiable, dissolved concentrations of metals do not reflect all potential **"bioavailable metal"** or metals included in **"dietary uptake"** (Timmermans *et al*., 1992; Young and Harvey, 1991; Sanders *et al*., 1990). In a recent study by Schmittschmitt (1996), trace amounts of Ag that bioaccumulated in algae **blocked reproduction in a primary consumer**, clearly indicating food web transfer.

Whole-Body Burden

Body burden (BB) data (*i.e.,* whole-body chemical residues) have proven useful in ***1***) identifying source/magnitude and calculating concentrations of "bioavailable" metals, ***2***) assessing comparative levels of stress affecting biota of different habitat, trophic, or behavioral characteristics and ***3)*** quantifying ecological impact traceable to pollution. Fish used as **sentinel monitors** integrate exposure from all avenues over time and spatial distribution (McCarthy and Shugart, 1990). When uptake exceeds depuration, the accumulated BB **provides an *in vivo* index to chronic exposure and, we believe, to survivorship**.

For example, in Big Bayou Creek that was moderately impacted by metal-containing industrial effluents in 1987-88 (Birge *et al*., 1988, 1992), the mayfly (*Stenonema sp.*) had substantially higher residues of cadmium (Cd), chromium (Cr), copper (Cu), and zinc (Zn) than the caddisfly (*Cheumatopsyche sp.*). These results are presented in Figure 1, where order and ratio of metal uptake are given for three sentinel organisms: mayfly, caddisfly, and stoneroller minnow. Overall density (no./m^2) of mayflies, which are considered metal-sensitive, dropped precipitously from 9,057 at the upstream reference station to 336 in the most impacted sectors; whereas the abundance of caddisflies quadrupled. The latter are known to be metal-tolerant and opportunistic (Plafkin *et al.*, 1989; Klemm *et al.*, 1990; Barbour *et al.*, 1997). The stoneroller minnow, classified as having intermediate metal tolerance (Barbour *et al.*, 1997; Plafkin *et al.*, 1989), exhibited intermediate BB. It was concluded, therefore, that metal body burden increased in the order from the least metal-sensitive to the most metal-sensitive taxa. Since these initial studies, patterns of BB given for Cu, Cd, Cr, and Zn have been observed for nickel (Ni) and silver (Ag). Results given in Figure 1 further indicate that the mayfly either assimilates more metal than the caddisfly or depurates metals less effectively. Sediment in g.i. tract contents of mayflies did not account for the 40:1 or 50:1 ratios observed for Cd and Cr. For example, sediment Cd (μg/g) was 1.16 at the reference station and only 1.19 at the point of mayfly impact (Birge *et al*., 1998). Various studies indicate that there may be metal, species, or site-specific differences in metal uptake (Cain *et al*., 1992; Timmermans *et al*., 1992; Martin, 1979). However, Borgman *et al.* (1991) found bioaccumulated Cd in *Hyalella azteca* to be more reflective of chronic toxicity than water concentrations.

Figure 1. Order and ratio of metal uptake in sentinel organisms.

Cu: *Stenonema* > *Cheumatopsyche* > *C. anomalum*
(2:1.5:1)

Cd: *Stenonema* > *C. anomalum* > *Cheumatopsyche*
(40:10:1)

Cr: *C. anomalum* > *Stenonema* > *Cheumatopsyche*
(50:35:1)

Zn: *Stenonema* > *C. anomalum* >*Cheumatopsyche*
(5:4:1)

The stoneroller minnow was selected as the sentinel monitor for the Bayou Creek system, and its BB values for representative metals (*e.g.,* Ag, Cd, Cu) were compared with ecological parameters. When 1987-88 data were pooled for unimpacted upstream stations, the effluent receiving zone, and downstream stations, high inverse correlations (r = -0.95) were obtained **with number of macroinvertebrate taxa** (*i.e*., species richness) and **Protocol III bioassessment scores** (Plafkin *et al.*, 1989; Barbour *et al.*, 1997). Correlation coefficients (r) for BB versus the EPT index (*i.e.* mayflies, stoneflies, caddisflies) ranged from -0.82 for Ag to -0.94 for Cd.

Bioavailable Metals and Ecological Metrics

BB determinations (μg/g) obtained with the stoneroller minnow also were used to develop **multipliers** that were applied to total recoverable (TR) water column metal to calculate "bioavailable metal". The formula for this conversion was as follows:

$$M_{BF} = M_{BB} / M_{RBB} * M_{TR}$$

Where

M_{BB} = Body Burden of Sentinel Organisms; M_{RBB} = Reference Body Burden; M_{TR} = Instream Total Recoverable Metal, and M_{BF} = Bioavailable Metal Fraction.

Concentrations of TR and bioavailable Cu based on earlier studies (Birge *et al*., 1988; 1992) are given in Figure 2, together with BB values for the stoneroller minnow. As indicated below, calculated **bioavailable metal fractions correlated strongly with whole-body burden** in the stoneroller minnow and with ecological metrics. Only 13-20% of metal remained bioavailable 7.6 Km downstream of the PGDP effluent receiving zone.

The TR concentrations (mean ± SD) for copper were 0.75 ± 0.54, 3.7 ± 1.5, 3.8 ± 1.55, and 2.4 ± 1.6 μg/L for stations BB2, BB4, BB6 and BB9, respectively (Birge *et al*., 1992). Although TR metal persisted downstream, **BB in stoneroller minnows decreased commensurately with improving ecological conditions, decreased metal bioavailability, and reduced impact**. Significant inverse correlations (r) were obtained for calculated bioavailable metal versus the number of macroinvertebrate taxa (-0.95 Ag, Cu); bioassessment scores (-0.97 Ag; -0.99 Cu); and the EPT index (-0.82 Ag; -0 87 Cu). BB values were more reliable in identifying sources and magnitude of impact on resident biota than were data for effluent or stream water toxicity. For example, correlations (r) between chronic toxicity data determined with the fathead minnow and *Ceriodaphnia dubia* (Weber *et al*., 1989) versus selected ecological parameters (*e.g*., no. taxa, BA scores) only ranged from 0.15 to 0.56.

RESULTS

FFOU Stoneroller Minnow Study

Metal body burden (BB) values for 8 metals analyzed in 32 stoneroller minnows collected in September 1997 are given in Table 1, together with calculated mean values and standard deviations. Except for Ag, BB concentrations observed in Big Bayou Creek were lower at the upstream reference station and increased at stream stations (*e.g.* BB4, BB5, BB6) within the effluent-receiving zone. However, unlike results obtained in 1988 (Birge *et al*., 1998), BB metal values often were as high or higher downstream as compared with the effluent zone. For example, values for Cu (Fig. 3) were approximately four to eight times higher at BB4 through BB9, as compared with BB1 (*i.e.* upstream reference).

An unexpectedly high value for Ag was observed at BB1 and there were other indications that this sector of Big Bayou Creek has received some "localized" impact. Ag was reanalyzed in three additional minnows and the results were in the same range (Table 2). In this follow-up study, assays also were conducted to determine if g.i. tract contents appreciably affected BB results. However, taking into account standard deviations, the mean values of 488 and 627 did not differ significantly. Based on results for Ag and earlier findings given for certain sediment metals (Birge *et al.,* February 1998), BB3 temporarily may provide a better reference point than BB1. However, it is recommended that the upstream reference station be relocated. **The high BB values for lead (Pb)** were another notable difference from 1988 results, in which Pb was not detected in minnows from any of the Big Bayou Creek stations. The 1997 Pb values were estimated to be about 50 to 100 times greater than in 1988 after converting BB to wet weight and using 0.5 of the 1988 detection limit as a point of reference (*i.e.* 6.5 µg Pb/g). Mean BB metal values are presented graphically in the Appendix (Figures A1-A8). Minnows were less abundant in 1997 compared with 1988, and repeated collections at BB9 yielded only one specimen.

Relationship of metal BB, total recoverable metal and calculated bioavailable metal concentrations for the water column are shown in Figures 3 through 7. The results for Cu (Fig. 3) differed dramatically from those given in 1988 (Fig. 2). Tissue Cu and bioavailable metal in 1997, compared with values for the reference point (BB1), were substantially higher at all downstream stations. This supports the premise that metal pollution has extended much further downstream during the intervening years. Effluent 006 is the most downstream continuous outfall into Big Bayou Creek. Based on mile points (MP), stations BB7 (MP 9.5), BB8 (MP 7.4), and BB9 (MP 2.8) are downstream of the 006 effluent (MP 10.4) by 0.9, 3.0, and 7.6 Km, respectively. It appears, therefore, that metal pollution has extended more than 7 Km downstream of 006. Generally similar results were obtained for the other metals, as illustrated in Figures 4 through 7 for Pb, Ni, Ag, and Be. BB Zn showed the least variation among stream stations both in 1988 and 1997. This likely was due to the fact

Figure 2. Instream Bioavailability of Copper in stoneroller minnows from Big Bayou Creek collected 1987-89.

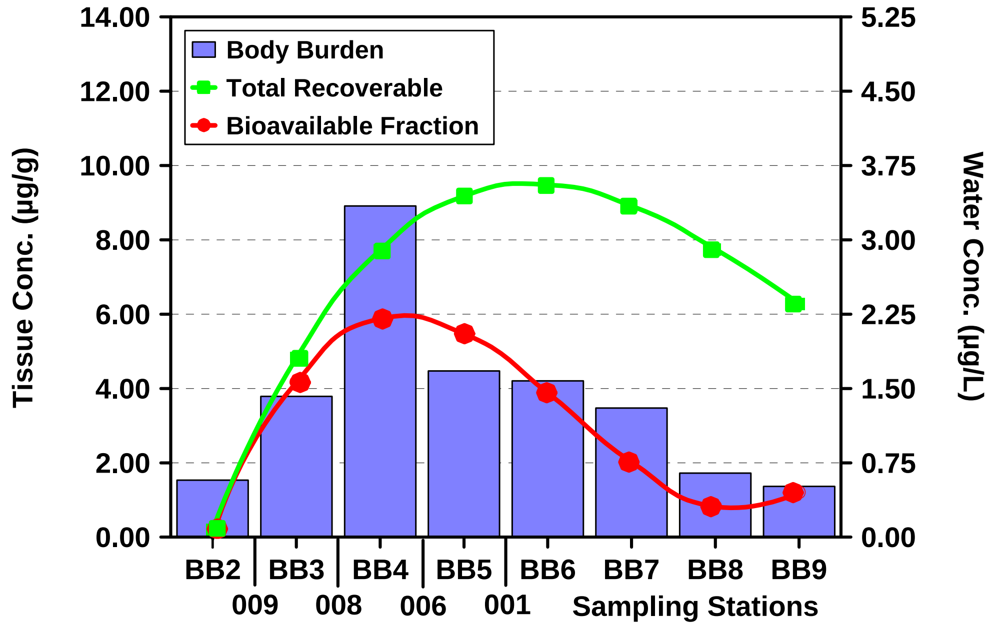
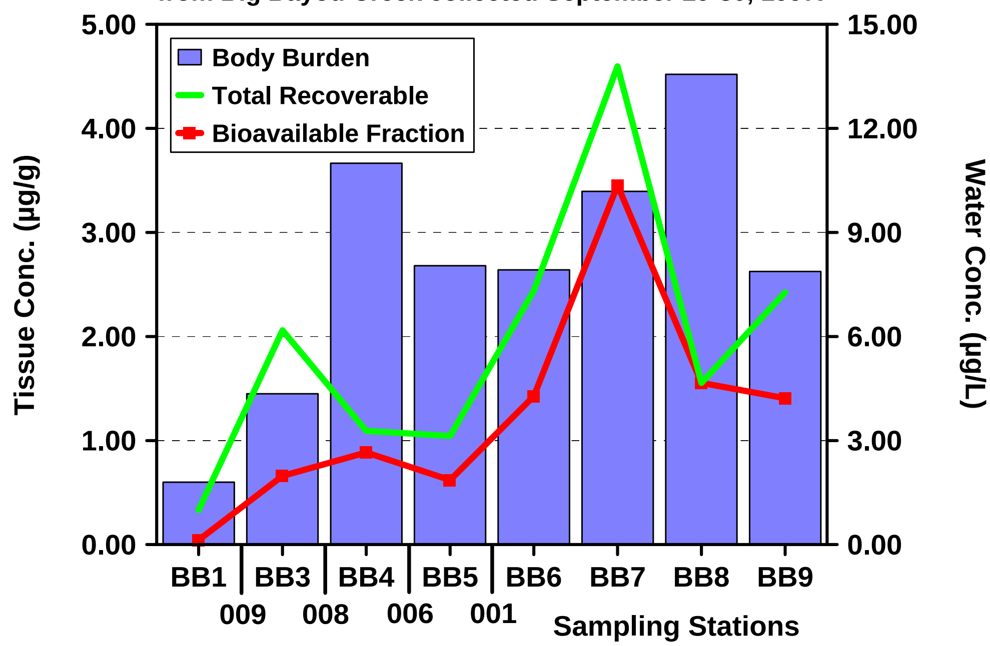


Figure 3. Copper concentrations in stoneroller minnows from Big Bayou Creek collected September 29-30, 1997.



that fish effectively down-regulate Zn (Hogstrand, 1996). **It should be noted that this initial data set is subject to some sampling error and other variables, and more in-depth study will be required to assess fully the extent of metal pollution within the Bayou Creek system.**

Differences in metal pollution that have occurred since 1988 are treated further in Tables 3 and 4. In these calculations, BB metal at station BB4, where highest BB values generally occurred in 1988, was used as a point of reference for determining ratios of metal BB observed at other stations. Setting the value at one for BB4, ratios of Cu and Ag decreased proportionately upstream and downstream in 1988. For example, Cu BB in minnows from stations BB1 and BB9 was 17% of that recorded for BB4. By comparison, 1997 ratios for downstream stations ranged from 72% to 123% of the BB4 value, indicating a downstream progression and higher relative magnitude of metal pollution. Even more dramatic results were obtained for Ag and Pb (Tables 3, 4).

Stoneroller minnows were much less abundant in Little Bayou Creek and the sample was not sufficient to permit an initial assessment. However, there were indications that metal residues were as high or higher at the most downstream station, LB4. This was particularly evident for Cr. As given in an earlier study (Birge *et al.,* February 1988; Fig. 4) sediment Cr also was elevated at LB3 and LB4.

CONCLUSIONS

Metal BB residues in minnows and other information that we have submitted to FFOU over the past year support the initial premise that **1)** metal pollution in the Bayou Creek system, especially Big Bayou Creek, now exceeds by a considerable margin that reported in 1988 (Birge *et al*., 1988; 1992); **2**) metal pollution extends much further downstream of the PGDP effluents than previously reported; and **3)** bioavailable fractions of such metals as Ag, Be, Cd and Pb are higher and more widespread. Metal pollution may pose a threat to environmental health as far downstream as the Bayou Creek confluence with the Ohio River. Findings that support this premise are as follows.

- **Metal residues** in stoneroller minnows collected in 1997 generally were higher, especially downstream, than reported in 1988. For example, whole body residues of Pb, an important contaminant affecting aquatic life and human health, were about **50** to **100** times higher than found in 1988. This is particularly important due to the fact that Pb has a rather low potential for bioconcentration in fish.

- Total recoverable **water column metal** concentrations at downstream stations often approached those reported for the effluent receiving zone.

- **Bioavailable metal fractions** were as high or higher at one or more downstream stations (*e.g.* BB7-BB9) than in the effluent receiving zone and they most always were higher than recorded for the upstream reference station.

- Based on 1988 data, **bioavailable Cu** decreased progressively from about 80% of total recoverable metal at BB4 to 13-20% at the lower stream stations (*i.e*. BB8, BB9). In 1997 the calculated **bioavailable Cu** at downstream stations (BB7-BB9) was as high or higher than observed in the effluent-receiving zone. This trend also was observed for other metals and is a further indication of the downstream movement of metal pollution.

- **Sediment metal** concentrations often were as high or higher at downstream stations (BB7-BB9) as for effluent receiving stations (BB3-BB6).

- **Periphyton Autotrophic Indices** for downstream stations (BB8, BB9), as determined in 1996-97 (Birge *et al.*, March, 1998), exceeded acceptable limits (50-200). For example, the 1996 value for BB8 was 551. The stoneroller minnow is a benthic herbivore that grazes on periphyton.

- Values for **conductivity** reported to FFOU in January 1998 were two to three times higher than previously noted (Birge *et al*., 1992). For example, the value for BB6 was 1048 μS, compared with a previous range of 356-513 μS. Conductivity is a measurement of ionic constituents, including metal ions.

- Values determined in 1997 for dissolved oxygen and temperature at certain stations also were outside guidelines given by the Commonwealth of Kentucky (KDEP, 1990).

- In our January report (Price and Kercher, 1998), we listed five **areas of concern** regarding the internal **PGDP monitoring** of their KPDES effluents. The question was raised as to the efficiency and dependability in screening important priority pollutants. Data gaps, variations in analytical procedures and changes in reporting were among our concerns. There were no clear indications that 1) toxicity reductions have occurred over the past 10 years for most of the KPDES chemicals we reviewed or 2) that a comprehensive in-house monitoring program has been maintained over the years.

The results for 1997 are preliminary and more sampling will be required to validate this premise. Nevertheless, the **considerable agreement of findings for metals in water, sediments and the stoneroller minnow**, together with other documentation cited above, raises important questions and more comprehensive study is indicated. It is our recommendation that this spread of pollution within the Bayou Creek system, possibly affecting the Ohio River, be given **high priority**. A suggested sampling schedule is being forwarded to FFOU under separate cover.

Metals are among the most important priority pollutants identified under the **Clean Water Act**. They receive considerable attention by U.S. EPA, other federal agencies and most all States in regard to monitoring programs for watershed systems and other surface waters. They often dominate the list of chemicals included in State permits issued under the National Pollutant Discharge Elimination System. A substantial list of metals are included among the environmental enforcement statues of most States, including Kentucky. Metals also comprise a big portion of those parameters given in KPDES permits issued for the 16 or more PGDP effluents. In addition to their relevance to water quality and ecological integrity, some metals (*e.g.* Pb, Hg) are important to human health.

Table 1. Metal body burden in the stoneroller minnow (*Campostoma anom*alum) from the Bayou Creek system.

Sample Name	Whole Body Wt. (g)	µg/Kg Ag	Be	Cd	Cr	Cu	Ni	Pb	µg/g Zn
BB1093097MFSR1A	4.66	1163.5	16.27	147.7	58.7	809.8	376.4	48.8	9.36
BB1093097MFSR2A	4.32	483.2	16.20	171.5	243.8	711.9	691.4	78.7	24.35
BB1093097MFSR3A	4.49	2142.2	14.43	193.6	138.2	807.9	1062.4	49.1	23.16
BB1093097MFSR4A	3.89	46.0	20.62	259.9	447.4	72.8	1215.9	54.9	30.49
Mean	4.34	958.7	16.88	193.2	222.0	600.6	836.5	57.9	21.84
Std. Dev.	0.33	913.2	2.63	48.2	168.3	354.8	377.6	14.2	8.92
BB3093097MFSR1A	1.88	51.9	84.89	198.4	547.8	2349.9	1197.9	117.0	32.50
BB3093097MFSR2A	0.93	191.9	110.00	248.9	332.8	1527.5	1331.2	68.7	29.14
BB3093097MFSR3A	1.48	30.5	59.80	185.0	236.4	650.5	664.2	67.2	32.43
BB3093097MFSR4A	1.70	38.9	60.18	342.4	366.3	1271.2	792.4	98.3	31.59
Mean	1.50	78.3	78.72	243.7	370.8	1449.8	996.4	87.8	31.42
Std. Dev.	0.41	76.2	23.93	71.3	130.2	704.1	318.7	24.1	1.57
BB4093097MFSR1A	2.62	62.6	51.83	234.3	345.2	6173.2	1386.6	143.1	38.97
BB4093097MFSR2A	2.99	30.9	45.15	324.3	150.2	3412.3	1244.1	53.0	31.20
BB4093097MFSR3A	2.40	30.4	35.75	155.4	164.1	1292.7	1160.4	97.8	38.17
BB4093097MFSR4A	3.01	31.2	37.18	211.4	426.5	3784.5	1883.4	99.1	38.24
Mean	2.76	38.8	42.48	231.3	271.5	3665.7	1418.7	98.3	36.64
Std. Dev.	0.30	15.9	7.48	70.3	136.3	1999.8	323.6	36.8	3.65

Table 1, continued. Metal body burden in the stoneroller minnow (*Campostoma anom*alum) from the Bayou Creek system.

Sample Name	Whole Body Wt. (g)	µg/Kg							µg/g
		Ag	Be	Cd	Cr	Cu	Ni	Pb	Zn
BB5093097MFSR1A	1.96	535.6	60.82	275.4	293.6	4155.6	1139.3	120.4	45.7
BB5093097MFSR2A	2.42	29.9	30.83	213.2	58.0	1131.9	512.8	74.1	20.6
BB5093097MFSR3A	1.49	469.6	54.98	344.4	178.6	3579.4	1351.7	70.9	46.4
BB5093097MFSR4A	7.79	248.1	29.76	145.0	56.6	1853.7	691.1	62.6	24.8
Mean	3.42	320.8	44.09	244.5	146.7	2680.1	923.7	82.0	34.4
Std. Dev.	2.94	229.6	16.12	85.3	113.4	1421.9	388.4	26.0	13.6
BB6093097MFSR1A	2.95	37.2	54.34	187.8	512.8	1320.1	673.6	64.2	31.4
BB6093097MFSR2A	4.65	26.3	23.63	131.5	208.0	717.1	615.7	46.4	14.8
BB6093097MFSR3A	5.02	192.0	38.86	151.5	348.1	1643.7	793.2	105.2	28.7
BB6093097MFSR4A	1.87	213.9	86.26	204.6	1579.9	6881.5	1967.9	1052.9	55.6
Mean	3.62	117.3	50.77	168.9	662.2	2640.6	1012.6	317.2	32.6
Std. Dev.	1.48	99.3	26.77	33.3	624.3	2853.2	641.2	491.1	17.0
BB7093097MFSR1A	2.35	148.1	147.70	193.3	841.2	3536.3	1039.1	112.7	39.7
BB7093097MFSR2A	2.46	748.6	137.32	195.1	986.7	4113.0	1489.4	123.3	39.1
BB7093097MFSR3A	2.23	421.1	108.74	302.7	827.3	3453.5	1012.6	83.2	43.3
BB7093097MFSR4A	2.35	208.9	210.04	268.2	1818.0	2474.6	1751.1	141.7	<4.3
Mean	2.35	381.7	150.95	239.8	1118.3	3394.4	1323.1	115.3	40.7
Std. Dev.	0.09	271.2	42.70	54.5	472.0	679.7	359.6	24.5	2.3

Table 1, continued. Metal body burden in the stoneroller minnow (*Campostoma anom*alum) from the Bayou Creek system.

Sample Name	Whole Body Wt. (g)	µg/Kg							µg/g
		Ag	Be	Cd	Cr	Cu	Ni	Pb	Zn
BB8093097MFSR1A	1.24	95.6	109.84	281.6	1026.7	3827.6	1512.1	115.9	54.6
BB8093097MFSR2A	2.42	69.4	82.64	230.7	1576.2	4419.7	2488.0	109.1	33.4
BB8093097MFSR3A	2.51	148.9	74.30	184.5	1142.4	7425.4	1868.9	101.9	40.4
BB8093097MFSR4A	1.10	83.1	162.91	306.6	1153.2	2404.3	1595.5	116.2	37.6
Mean	1.82	99.2	107.42	250.8	1224.6	4519.2	1866.1	110.8	41.5
Std. Dev.	0.75	34.8	39.98	54.4	241.3	2114.0	441.7	6.8	9.2
BB9093097MFSR1A	5.73	1156.1	34.64	136.2	357.8	2625.4	1326.7	75.5	25.4
LB2A093097MFSR1A	13.18	33.7	28.55	153.0	144.4	784.1	647.8	78.3	13.7
LB4093097MFSR1A	4.04	33.0	29.13	165.9	179.1	744.0	672.3	66.0	23.9
LB4093097MFSR2A	7.91	206.4	64.34	146.6	1231.1	2024.3	861.1	90.8	18.8
Mean	5.98	119.7	46.73	156.3	705.1	1384.1	766.7	78.4	21.3
Std. Dev.	2.74	122.6	24.89	13.6	743.8	905.3	133.5	17.5	3.6
Fathead020897MFH1A	3.97	25.3	58.84	179.9	286.9	496.4	549.8	<32.8	37.6

Table 2. Reanalysis of Silver in Stoneroller Minnows (*Capostoma anomalum*) from Whole Body Minus Gut and Whole Body With Gut.

	Concentration (µg/Kg)		
Sample Number	Whole Body Less Gut	Gut	Whole Body Plus Gut
BB1093097MFSR5A	460.9	475.6	936.5
BB1093097MFSR6A	326.9	150.2	477.1
BB1093097MFSR7A	675.2	1256.2	1931.4
Mean	487.7	627.3	1115.0
Std. Dev.	143.4	464.1	607.0
BB1093097MFSR1A			1163.5
BB1093097MFSR2A			483.2
BB1093097MFSR3A			2142.2
BB1093097MFSR4A			46.0
Mean			958.7
Std. Dev.			790.9

Table 3. Ratios of Stoneroller Mean Copper and Silver Body Burden in 1988 *vs* 1997[a].

Sampling Station	1988-89 **Mean Copper** (µg/g)	1988-89 Ratio of Means Station/BB4	1997 **Mean Copper** (µg/g)	1997 Ratio of Means Station/BB4	1988-89 **Mean Silver** (µg/g)	1988-89 Ratio of Means Station/BB4	1997 **Mean Silver** (µg/g)	1997 Ratio of Means Station/BB4
BB1	---	---	0.60	0.16	---	---	0.96	24.74
BB2	1.53	0.17	---	---	0.10	0.15	---	---
BB3	3.79	0.43	1.45	0.40	0.36	0.52	0.08	2.02
BB4	8.91	1.00	3.67	1.00	0.68	1.00	0.04	1.00
BB5	4.48	0.50	2.68	0.73	0.56	0.82	0.32	8.28
BB6	4.21	0.47	2.64	0.72	0.33	0.48	0.12	3.03
BB7	3.48	0.39	3.39	0.93	0.20	0.29	0.38	9.85
BB8	1.73	0.19	4.52	1.23	0.12	0.18	0.10	2.56
BB9	1.49	0.17	2.63	0.72	0.07	0.10	1.16	29.83

[a] Metal residue values for stream station were divided by those from BB4, the station where maximum metal body burden (*i.e.* bioavailable metal) concentrations was observed in 1988.

Table 4. Ratios of Stoneroller Mean Cadmium and Lead Body Burden in 1988 *vs* 1997[a].

Sampling Station	1988-89 **Mean Cadmium** (µg/g)	1988-89 Ratio of Means Station/BB4	1997 **Mean Cadmium** (µg/g)	1997 Ratio of Means Station/BB4	1988-89 **Mean Lead** (µg/g)	1988-89 Ratio of Means Station/BB4	1997 **Mean Lead** (µg/g)	1997 Ratio of Means Station/BB4
BB1	---	---	0.19	8.29	---	---	0.58	0.59
BB2	0.34	0.46	---	---	<0.05	1.00	---	---
BB3	0.09	0.12	0.24	1.05	<0.05	1.00	0.88	0.89
BB4	0.74	1.00	0.23	1.00	<0.05	1.00	0.98	1.00
BB5	0.37	0.50	0.24	1.06	<0.05	1.00	0.82	0.83
BB6	0.22	0.30	0.17	0.73	<0.05	1.00	0.32	3.23
BB7	0.27	0.37	0.24	1.04	<0.05	1.00	0.12	1.17
BB8	0.13	0.18	0.25	1.08	<0.05	1.00	0.11	1.13
BB9	0.22	0.30	0.14	1.70	<0.05	1.00	0.76	0.77

[a] Metal residue values for stream station were divided by those from BB4, the station where maximum metal body burden (*i.e.* bioavailable metal) concentrations was observed in 1988.

Figure 4. Lead concentrations in stoneroller minnows from Big Bayou Creek collected September 29-30, 1997.

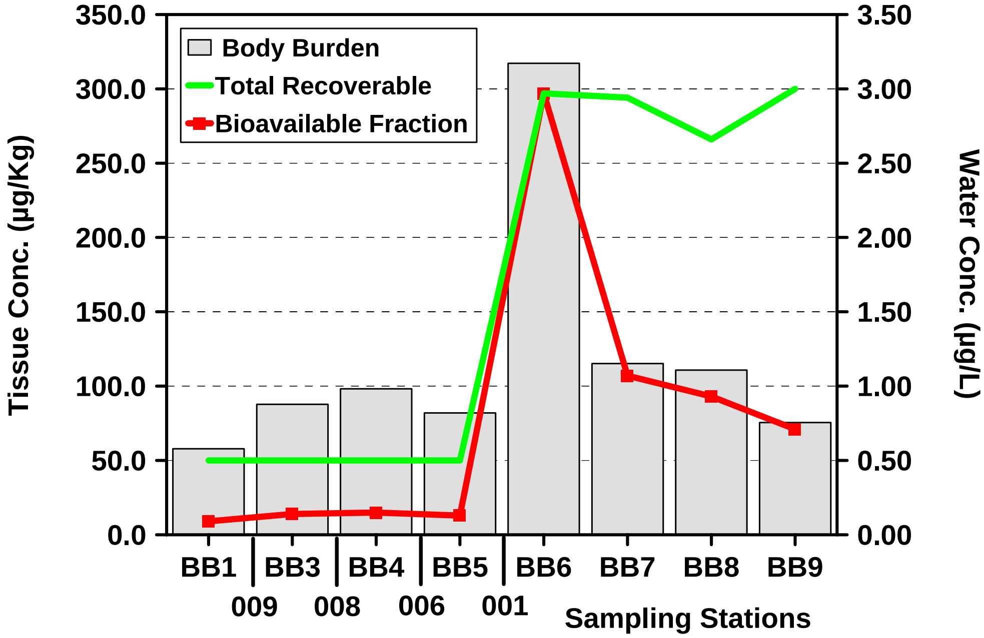


Figure 5. Nickel concentrations in stoneroller minnows from Big Bayou Creek collected September 29-30, 1997.

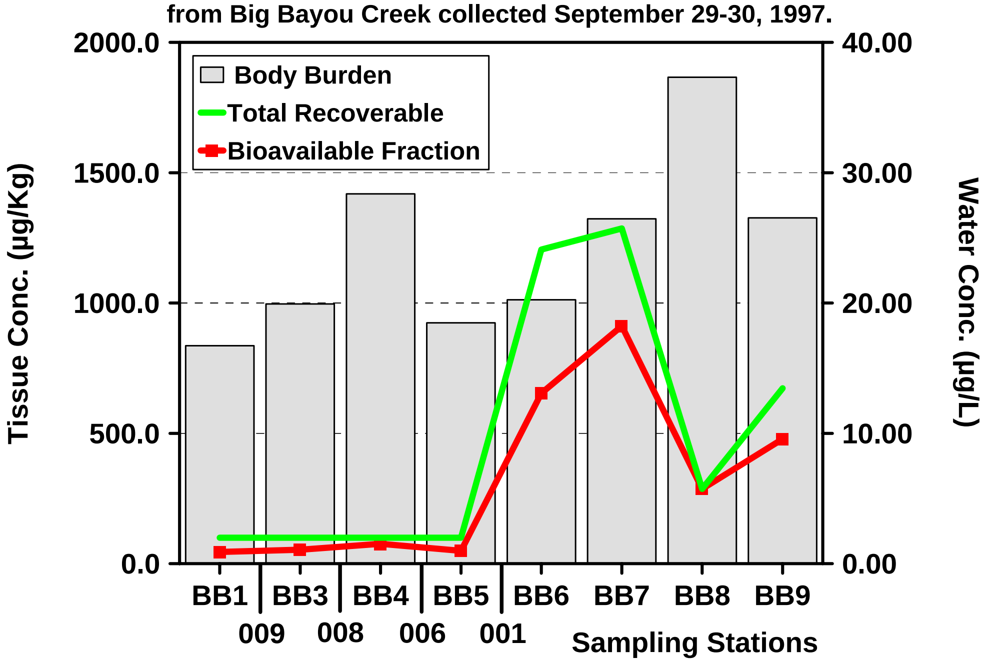


Figure 6. Silver concentrations in stoneroller minnows from Big Bayou Creek collected September 29-30, 1997.

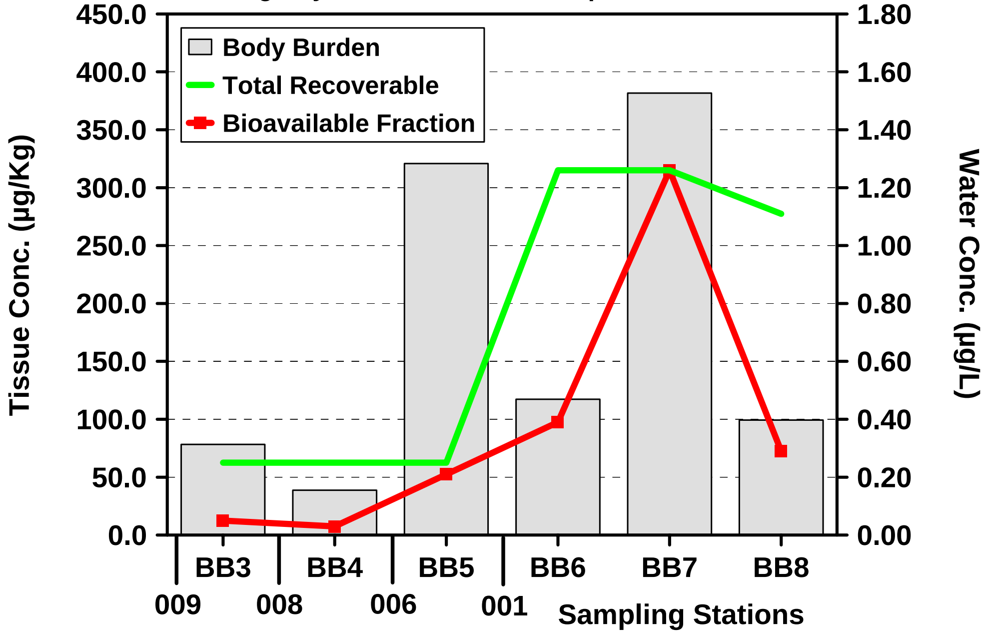
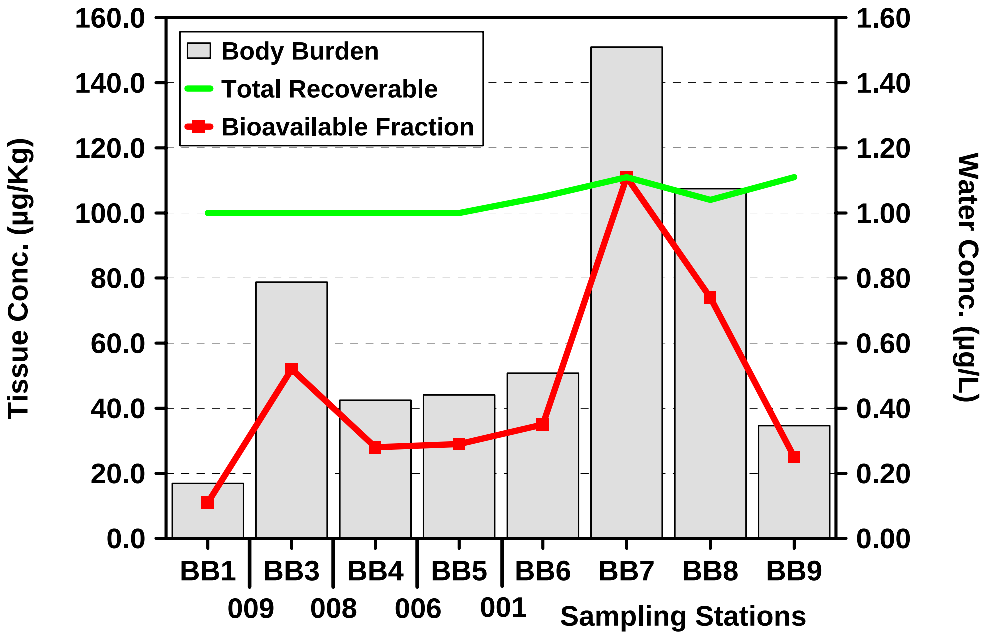


Figure 7. Beryllium concentrations in stoneroller minnows from Big Bayou Creek collected September 29-30, 1997.



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APPENDIX

Stoneroller Minnow Body Burden for Eight Metals, Bayou System 1997

Relationships Among Body Burden Metals, Water Column Metals and Sediment Metals

Table A-1

Figures A-1 through A-12

APPENDIX

Stoneroller Minnow Body Burden for Eight Metals, Bayou System 1997

The Bayou system was surveyed for stoneroller minnows on September 30, 1997. Sampling stations included the upstream reference station BB1 and stations BB3-BB9 on Big Bayou Creek; and LB2/2A and LB4 on Little Bayou Creek. Stonerollers were analyzed (*i.e*. whole body) for eight metals of concern (MOC), including Ag, Be, Cd, Cr, Cu, Pb, Ni, and Zn. Detailed results are given above in Table 1 (see text), and mean values for each MOC are illustrated below in Figures A-1 through A-8.

Little Bayou Creek

Only a few stonerollers were found in Little Bayou Creek. These were analyzed for the eight MOC. Except for cadmium and lead, mean metal body burdens were consistently higher at the downstream station LB4 than at LB2/2A. Values at LB4 were 0.05, 1.50, and 22 ppm for Be, Cu and Zn, respectively. More study is indicated.

Big Bayou Creek

Beryllium (Be) mean body burden was 16.9 ppb at the reference site BB1 (Figure A-1). By comparison, the values at stations BB7 (151 ppb) and BB8 (107.4 ppb) were an order of magnitude higher. The lowest value at BB9 (34.6 ppb) was approximately twice that for the reference site.

Cadmium (Cd) mean values were relatively uniform at all sampling stations (Figure A-2), including the reference BB1 (193.2 ppb). Mean body burdens for this metal ranged from 136 ppb at BB9 to 251 ppb at BB8.

Chromium (Cr) mean body burden at BB1 was 222 ppb (Figure A-3). Mean values ranged from 147 ppb at BB5 to 1225 ppb at BB8. The latter was approximately six times greater than the reference value.

Copper (Cu) mean body burden at BB1 was 601 ppb (Figure A-4). Downstream, mean values ranged from 1450 ppb at BB3 to 4519 ppb at BB8. These value were approximately two to seven times greater than that for the reference site.

Lead (Pb) mean body burden at BB1 was 58 ppb (Figure A-5), and this value was moderately to greatly exceeded at most downstream stations. At stations BB6, BB7 and BB8, mean body burden was 317, 115, and 111 ppb, respectively. These values were about two to five times greater than the upstream reference.

Nickel (Ni) was elevated at all stations below BB1 (Figure A-6). The Ni BB at BB1 was 837 ppb, whereas mean values at the other stations ranged from 924 ppb at BB5 to 1866 ppb at BB8.

Silver (Ag) mean body burden from stations BB3 through BB8 (Figure A-7) ranged from 39 ppb (BB4) to 382 ppb (BB7), with a sharp increase at BB9 (1156 ppb). The value for BB1, the upstream reference site, was verified by reanalysis and there are some indications of localized contamination at this site. In this instance, station BB3 was considered a more appropriate reference.

Mean zinc (Zn) body burden was 22 ppm at BB1 (Figure A-8). With the exception of BB9 (25 ppm), mean values at other stations were somewhat uniformly elevated, ranging from 31 ppm at BB3 to 42 ppm at BB8.

From the preceding results, it is apparent that the spatial pattern of metal bioaccumulation by the stoneroller minnow varied somewhat for each metal. Generally, maximum bioaccumulation was observed at a site within the effluent-receiving zone (*i.e*. BB3-BB6) or downstream thereof, *i.e*. BB7, BB8, BB9.

Relationships Among Body Burden Metals, Water Column Metals and Sediment Metals

Results of water column and body burden metal analyses are summarized in Table A-1. Water column metals were determined as total recoverable, and bioavailable fractions were calculated as given above. These results clearly indicate increased metal pollution in lower Big Bayou Creek. Metal BB in the stoneroller minnow and bioavailable metal consistently were higher at BB8 and/or BB9 than at the upstream reference (BB1). The relationships among total recoverable metal, calculated bioavailable metal, stoneroller body burden, and sediment metal concentrations for Cu, Ag, Zn and Be are further integrated in Figures A-9 through A-12. Generally, sediment metal concentrations were elevated in stream sectors where stoneroller body burden values also were elevated. Often, this occurred within the effluent receiving zone and downstream as far as stations BB8 and BB9. Depending on the metal, water column concentrations, including bioavailable fractions, tended to peak at BB6 to BB8, where metals in sediments and stoneroller minnows also were elevated. Results support the view that metal pollution in Big Bayou Creek has spread downstream as compared with findings in earlier studies (Birge *et al.*, 1988, 1992).

Table A-1. Determinations of bioavailable metal fractions in stoneroller minnows collected September 29-30, 1997 from Big Bayou Creek.

	Sampling Station							
Metal / Matrix (µg/Kg)	BB1	BB3	BB4	BB5	BB6	BB7	BB8	BB9
Beryllium								
Body Burden	16.9	78.7	42.5	44.1	50.8	151.0	107.4	34.6
Total Recoverable	1.00	1.00	1.00	1.00	1.05	1.11	1.04	1.11
Bioavailable Fraction	0.11	0.52	0.28	0.29	0.35	1.11	0.74	0.25
Cadmium								
Body Burden	193.2	243.7	231.3	244.5	168.9	239.8	250.8	136.2
Total Recoverable	0.25	0.25	0.25	0.25	0.84	0.76	0.84	0.83
Bioavailable Fraction	0.19	0.24	0.23	0.24	0.57	0.73	0.84	0.45
Chromium								
Body Burden	222.0	370.8	271.5	146.7	662.2	1118.3	1224.6	357.8
Total Recoverable	0.64	0.95	1.16	0.97	1.94	1.31	1.21	2.42
Bioavailable Fraction	0.12	0.29	0.26	0.12	1.05	1.20	1.21	0.71
Copper								
Body Burden	600.6	1449.8	3665.7	2680.1	26406	3394.4	4519.2	2625.4
Total Recoverable	1.00	6.18	3.28	3.13	7.32	13.79	4.66	7.27
Bioavailable Fraction	0.13	1.98	2.66	1.86	4.28	10.36	4.66	4.22

[a] Bioavailable fractions were based on stoneroller body burden and water column total recoverable metals.

Table A-1, continued. Determinations of bioavailable metal fractions in stoneroller minnows collected September 29-30, 1997 from Big Bayou creek.

	Sampling Station							
Metal / Matrix	BB1	BB3	BB4	BB5	BB6	BB7	BB8	BB9
Lead								
Body Burden	57.9	87.8	98.3	82.0	317.2	115.3	110.8	75.5
Total Recoverable	0.50	0.50	0.50	0.50	2.97	2.94	2.66	3.00
Bioavailable Fraction	0.09	0.14	0.15	0.13	2.97	1.07	0.93	0.71
Nickel								
Body Burden	836.5	996.4	1418.7	923.7	1012.6	1323.1	1866.1	1326.7
Total Recoverable	2.00	2.00	2.00	2.00	24.11	25.72	5.74	13.46
Bioavailable Fraction	0.90	1.07	1.52	0.99	13.08	18.24	5.74	9.57
Silver								
Body Burden	958.7	78.3	38.8	320.8	117.3	381.7	99.2	1156.1
Total Recoverable		0.25	0.25	0.25	1.26	1.26	1.11	
Bioavailable Fraction	---	0.05	0.03	0.21	0.39	1.26	0.29	0
Zinc[b]								
Body Burden	21.8	31.4	36.6	34.4	32.6	40.7	41.5	25.4
Total Recoverable	2.08	3.13	8.48	6.33	15.12	11.46	14.53	7.67
Bioavailable Fraction	1.09	2.37	7.48	5.24	11.88	11.24	14.53	4.69

[a] Bioavailable fractions were based on stoneroller body burden and water column total recoverable metals.
[b] Zinc values are in (µg/g).

Figure A-1. Mean Beryllium Values in Stoneroller Minnows from Bayou Creek System Collected September 30, 1997.

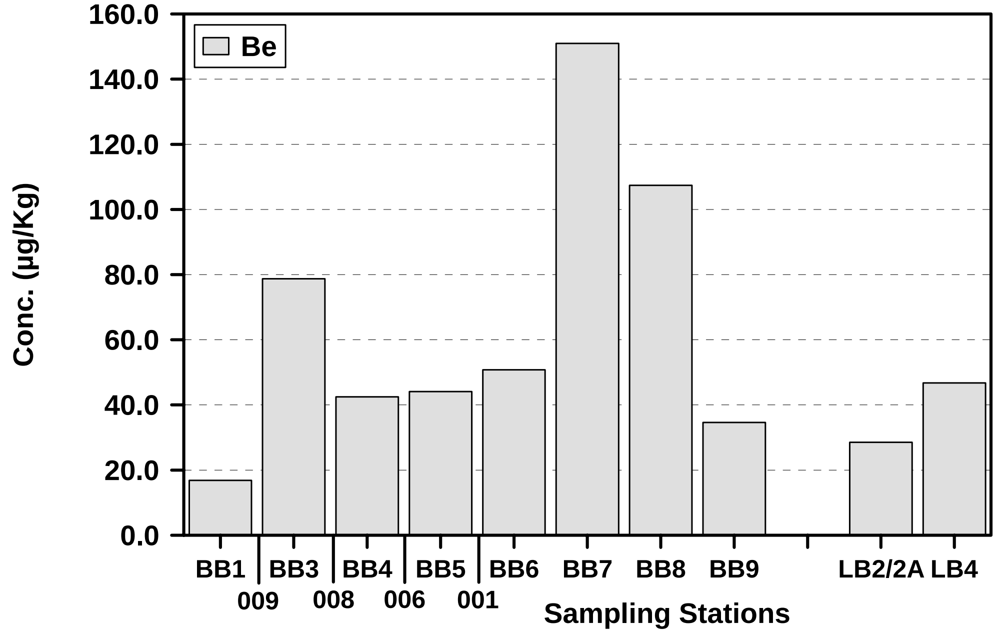

Figure A-2. Mean Cadmium Values in Stoneroller Minnows from Bayou Creek System Collected September 30, 1997.

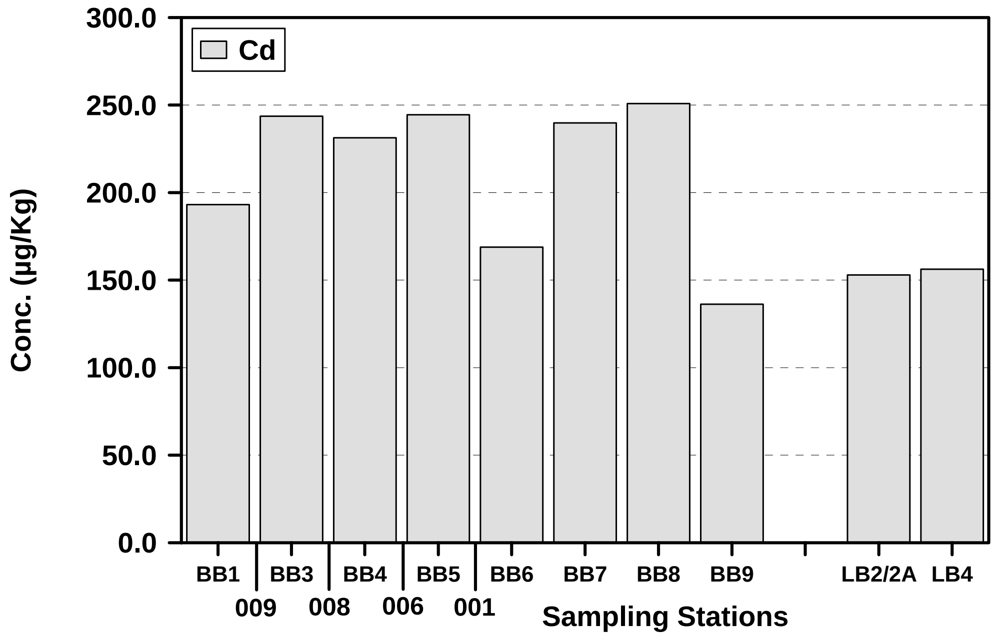

Figure A-3. Mean Chromium Values in Stoneroller Minnows from Bayou Creek System Collected September 30, 1997.

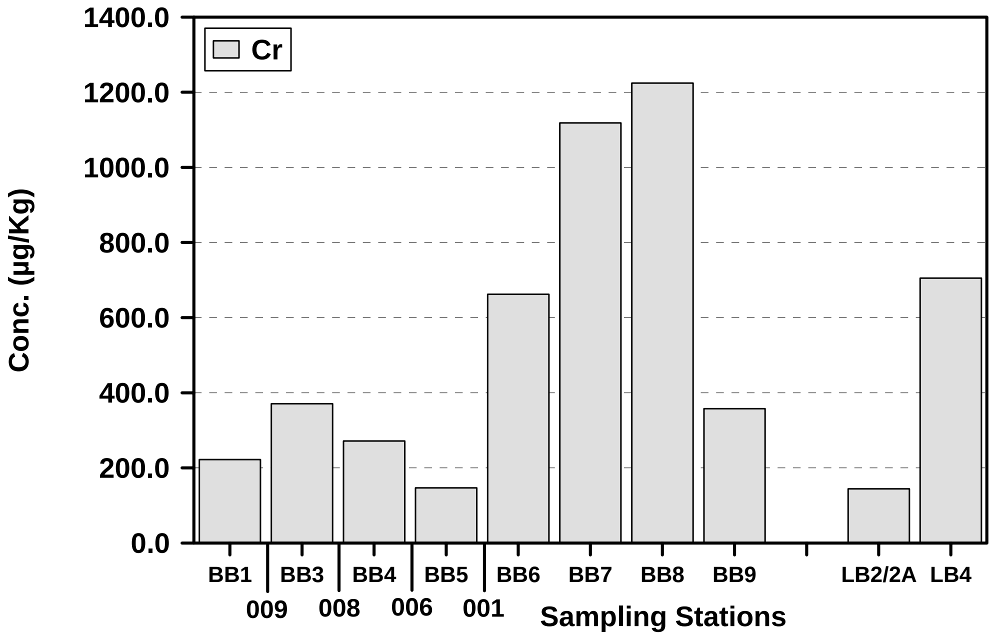

Figure A-4. Mean Copper Values in Stoneroller Minnows from Bayou Creek System Collected September 30, 1997.

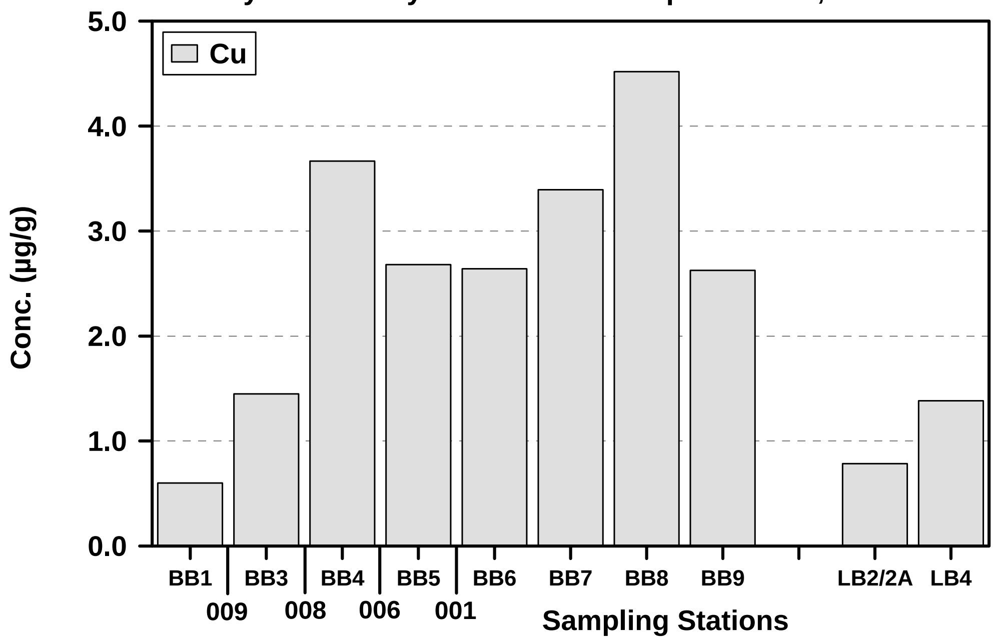

Figure A-5. Mean Lead Values in Stoneroller Minnows from Bayou Creek System Collected September 30, 1997.

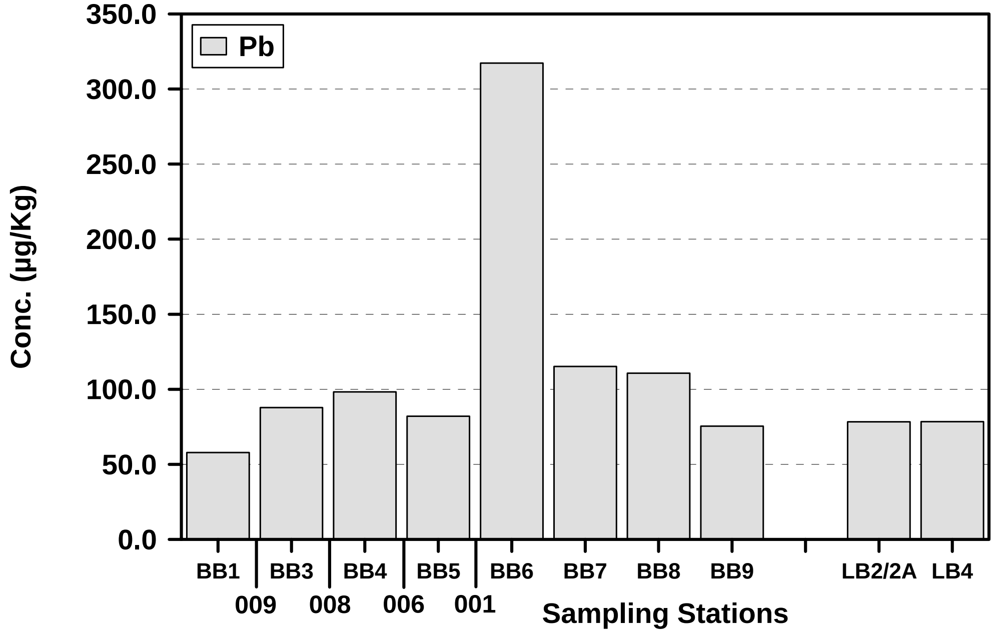

Figure A-6. Mean Nickel Values in Stoneroller Minnows from Bayou Creek System Collected September 30, 1997.

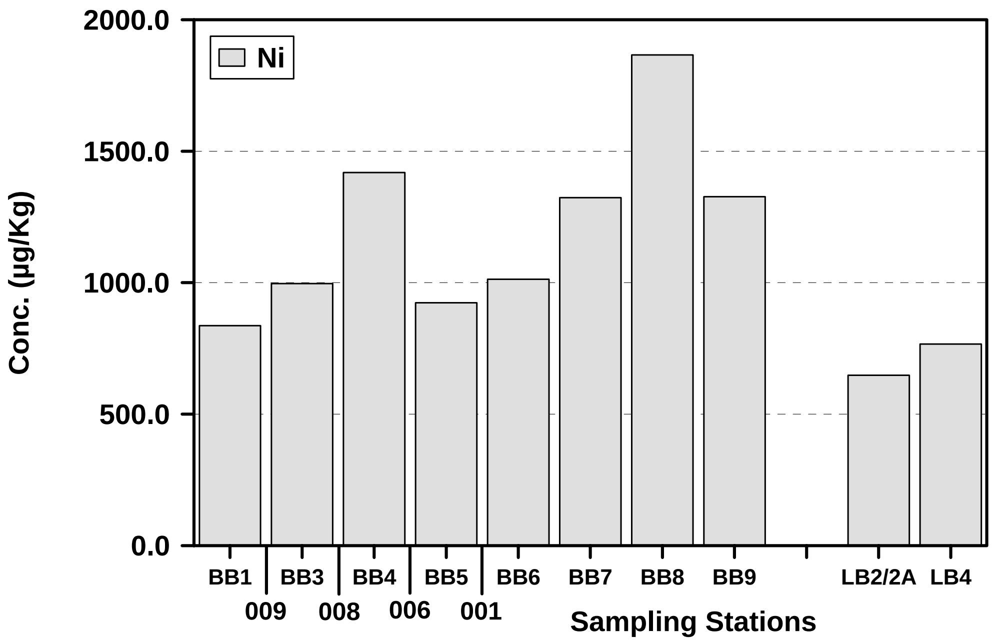

Figure A-7. Mean Silver Values in Stoneroller Minnows from Bayou Creek System Collected September 30, 1997.

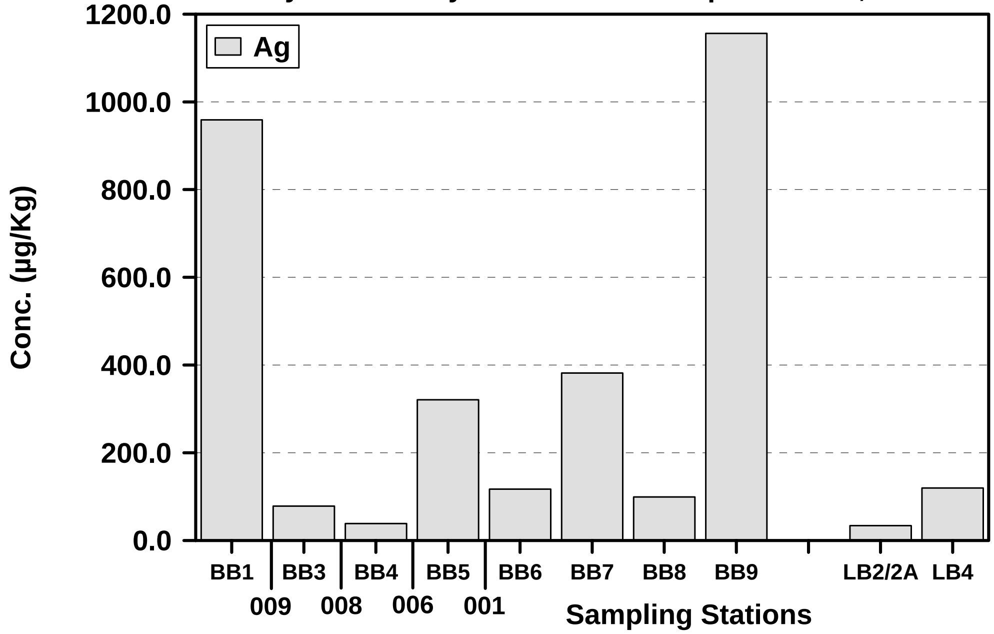

Figure A-8. Mean Zinc Values in Stoneroller Minnows from Bayou Creek System Collected September 30, 1997.

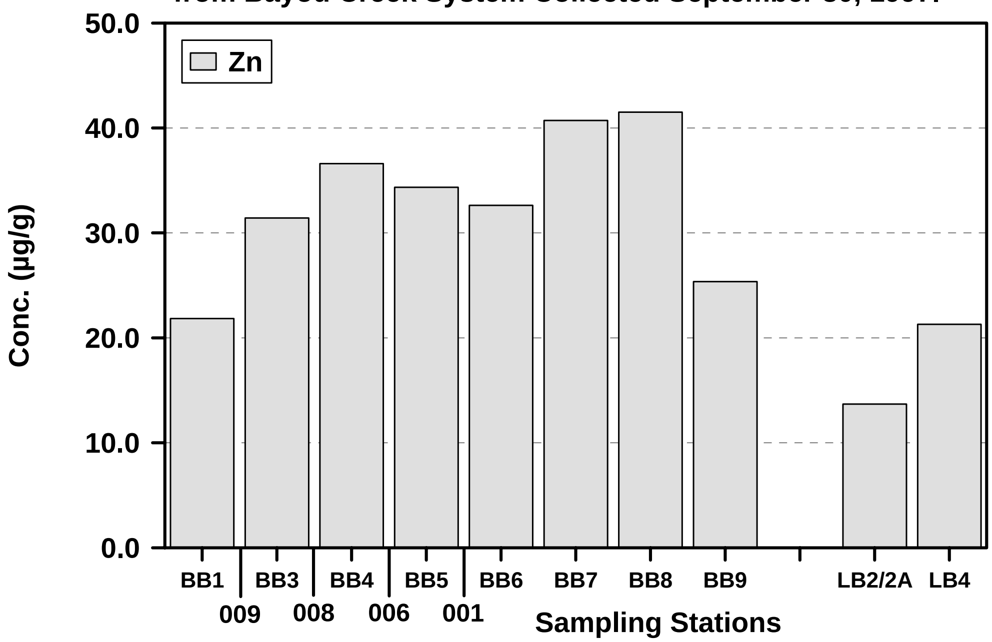

Figure A-9. Copper concentrations in stoneroller minnows from Big Bayou Creek collected September 29-30, 1997.

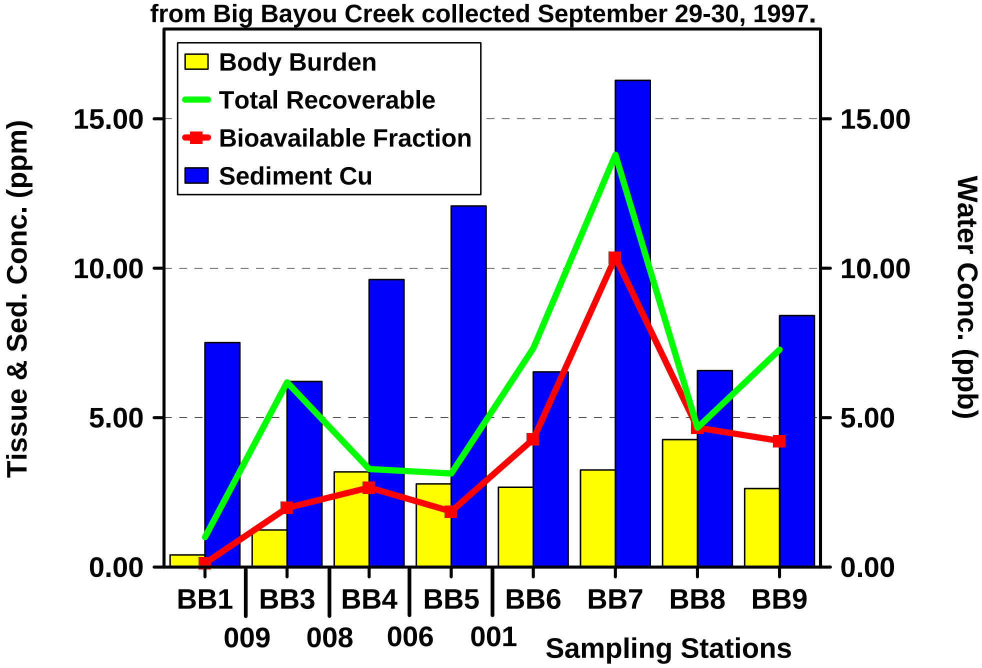

Figure A-10. Silver concentrations in stoneroller minnows from Big Bayou Creek collected September 29-30, 1997.

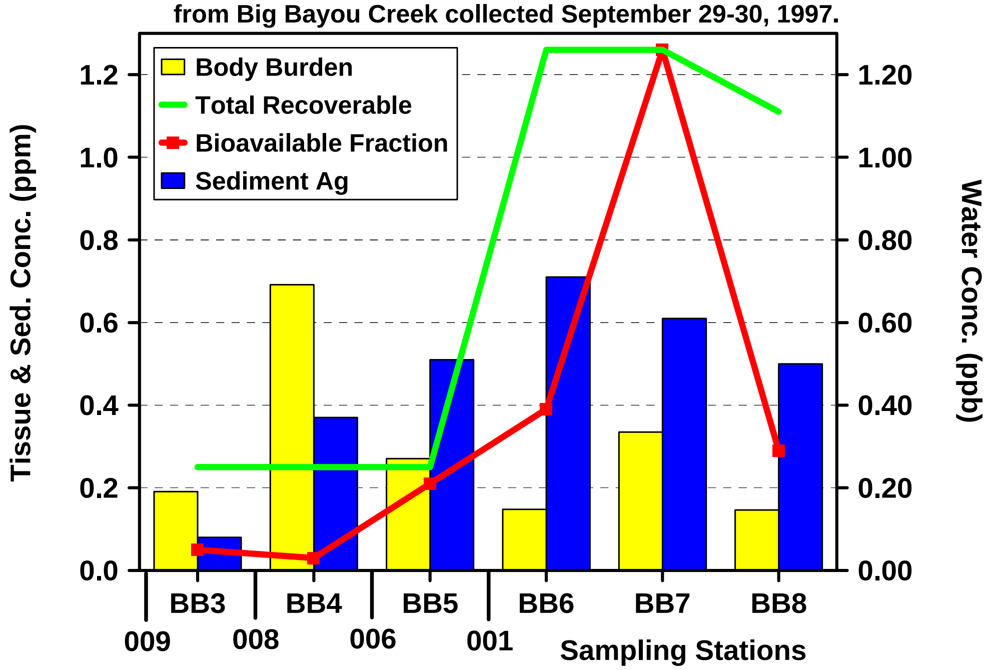

Figure A-11. Zinc concentrations in stoneroller minnows from Big Bayou Creek collected September 29-30, 1997.

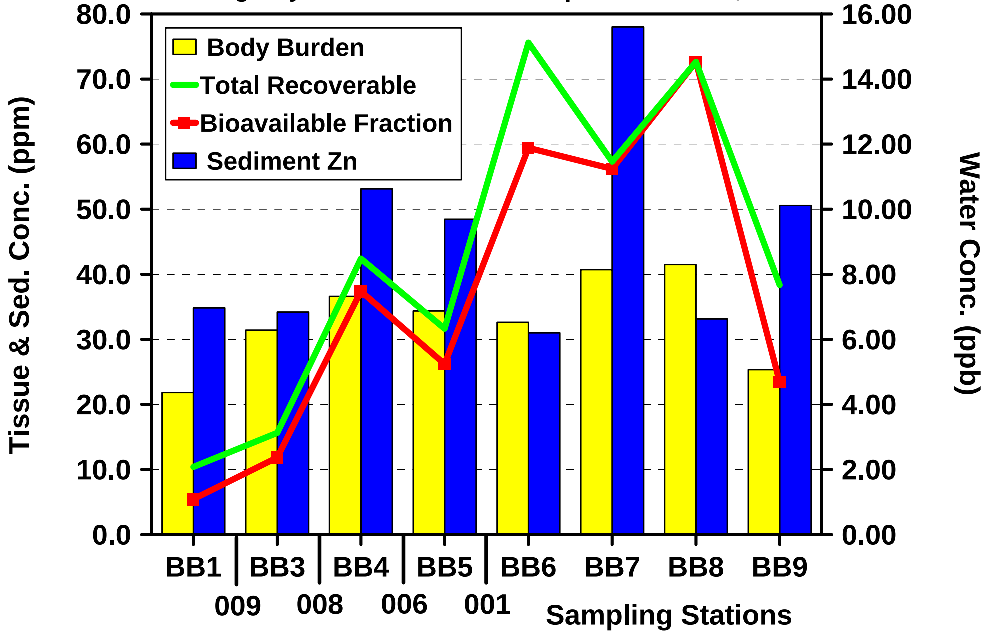

Figure A-12. Beryllium concentrations in stoneroller minnows from Big Bayou Creek collected September 29-30, 1997.