Bioavailability of Trace Metals to Green Sunfish (*Lepomis cyanellus*) Exposed to Suspended Sediments from Sites on the Upper Mississippi River

Report Submitted by

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to

U.S. Fish and Wildlife Service
Rock Island Field Office
Rock Island, Illinois

April 1995

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ACKNOWLEDGEMENTS

The completion of the project would not have been possible without the technical assistance of Todd Johnson, Ken Phillips, and Dr. Eric L. Morgan. Additional thanks goes to Dr. Jim Weiner, Dr. William Gingerich, Verdel Dawson of the Upper Mississippi Science Center, National Biological Service, and Rick Nelson of the La Crosse Fish Health Center, U.S. Fish and Wildlife Service. This work was conducted by Dr. Colleen Caldwell and Mark Steingraeber of the Section of Fisheries Contaminants, Upper Mississippi Science Center (National Biological Service), La Crosse, WI, (formerly the La Crosse Field Research Station, Midwest Science Center, National Biological Service). Financial support for this study was provided by the U.S. Fish and Wildlife Service, Division of Ecological Services, Rock Island, IL.

EXECUTIVE SUMMARY

A unique laboratory exposure system was used in applying environmentally relevant conditions (river traffic simulations) and environmentally relevant sediment concentrations (total suspended solids 300 mg/L) to determine the bioaccumulation and biological response of sediment-associated contaminants in fish. We used sediments collected from areas of the upper Mississippi River reported high in metal concentrations and assessed the bioaccumulation of sediment-associated arsenic, cadmium, copper, lead, and zinc to green sunfish following 28 days of intermittent pulses of contaminated suspended sediments and sediments not in suspension (bedded). Blood enzyme activity (δ-aminolevulinic acid dehydratase, ALA-D) was assessed in the fish as an indicator or biomarker of exposure to lead present in the contaminated suspended and bedded sediments.

Results of the metal analysis of filtered and unfiltered water from all treatments indicated that the majority of the metals were associated with particulate matter in suspension. The bioconcentration of copper, lead, and zinc were greater in fish exposed to suspended sediments than bedded sediments as evidence of greater body burden. Arsenic and cadmium concentrations in whole fish were not significantly different in suspended or bedded tests due to low ambient concentrations. The suspended test resulted in greater blood lead (0.02 - 0.2 ug/mL) and blood zinc (8.80 - 14.5 ug/mL) concentrations than were observed in the bedded tests. And although neither blood lead nor zinc significantly correlated with ALA-D activity, the enzyme was significantly reduced in fish subjected to suspended contaminated sediments compared to fish exposed to bedded contaminated sediments.

INTRODUCTION

Trace metals that are discharged into rivers tend to associate with suspended sediments that eventually accumulate in areas of reduced current velocity. In portions of the Upper Mississippi River (UMR) for example, fine-grained sediments (<4 m) and particulate organic matter strongly adsorb several metals, including cadmium, chromium, copper, lead, and zinc (Bailey and Rada 1984). This association can facilitate contaminant transport for considerable distances downstream of source areas (Rada et al. 1990).

Wind-generated waves, channel maintenance practices, and navigation activities (commercial and recreational) can cause the resuspension of surficial layers of bed sediments in a variety of riverine habitats (Sparks 1984, Smart et al. 1985, Johnson 1992, Adams 1993, Sullivan 1993). Bed sediment disturbances that mobilize fine-grained sediments may influence the bioavailability of some sediment-associated metals by altering metal ion speciation among various environmental compartments (e.g., complexed to organic colloids, dissolved in water, adsorbed to suspended solids). For instance, metal bioavailability to fish may increase via enhanced respiratory uptake if prevailing conditions favor metal ion desorption from sediments to water. However, little is known or understood of the environmental partitioning of metals between the sediment-sorbed and aqueous phases because of the complex relationship of site-specific sediment and water quality factors (e.g., grain-size, organic carbon content, pH, temperature, hardness) that influence these interactions (Wiener et al. 1984; Luoma 1989). Therefore it is difficult to predict to what extent, if any, sediment resuspension will alter metal bioavailability.

Metal bioavailability to fish is often assessed by whole-body or tissue residue analysis. However, the detection of altered biochemical pathways may offer a more sensitive means of determining exposure to certain metals. For example, the erythrocyte enzyme δ-aminolevulinic acid dehydratase (ALA-D) catalyzes the formation of porphobilinogen (PBG), a hemoglobin precursor, from aminolevulinic acid. Lead exposure inhibits ALA-D activity thereby decreasing PBG levels in blood and urine (Finelli 1977). Hodson (1976) first proposed the utility of ALA-D as a biomarker of chronic lead exposure for fish. Several investigators have since observed reduced ALA-D activity in fish following waterborne exposure to lead at sublethal concentrations (Johansson-Sjobech and Larsson 1979; Schmitt et al. 1984). Lead exposure can cause subtle behavioral changes in higher organisms due to degenerative effects on the nervous system. The periodic evaluation of biomarkers such as ALA-D activity may offer an early means of detecting potentially hazardous environmental conditions at the cellular or tissue level rather than waiting for adverse responses to become manifested at the population or community level.

A recent survey of contaminants in surficial UMR bed sediments (Young 1991) revealed several sites where concentrations of arsenic, cadmium, copper, lead, or zinc were far in excess of that recommended for the protection of aquatic life (U.S. EPA 1977). Some of these sites are located within (or near) the Mark Twain National Wildlife Refuge and the Upper Mississippi River National Wildlife and Fish Refuge. These sanctuaries are managed by the U.S. Fish and Wildlife Service (USFWS) to maintain and enhance habitats for a variety of species of fish and wildlife. Possible sources of metals that enrich these sediments include industries and municipalities that have permits to discharge treated wastes into the UMR within or near the refuge boundaries.

The resuspension and transport of sediments that may be metal-enriched is difficult to prevent in the UMR. For instance, the passage of towboats with barges increases main channel and side channel total non-filterable residue (TNFR) concentrations (Smart et al. 1985; Adams 1993; Adams et al. 1993; Adams and Delisio 1993) to levels that are occasionally 10-fold greater than ambient background concentrations and can exceed 300 mg/L for extended periods of time (Adams 1993; Adams and Delisio 1993). Recreational boating activity also increases TNFR concentrations, particularly in side channel and backwater habitats (Smart et al. 1985; Johnson 1992). Peak activity by all vessels on the UMR occurs during summer months when a daily average of 12 commercial and 24 recreational vessels travel between adjacent pools using the navigation locks (Edlund 1992). Moreover, confined recreational boating activity (i.e., restricted to day trips within a single pool) causes an additional increase in river traffic, particularly on weekend and holiday afternoons. These periods of peak boating activity can increase TNFR and turbidity levels near the sediment-water interface across the width of the channel (Johnson 1992). Biological effects of such navigation activities that disturb contaminated sediments are difficult to predict. Potential adverse effects of contaminated sediment resuspension on resident biota should be evaluated on a site-specific basis under environmentally relevant conditions to assist UMR resource managers in making well-informed policy decisions.

We assessed (1) the bioavailability of a suite of sediment-associated metals to green sunfish (*Lepomis cyanellus*), a common UMR panfish species, following chronic exposure to intermittent pulses of suspended UMR sediments and (2) the blood ALA-D activity of these fish as a potential biomarker of chronic exposure to lead-contaminated suspended sediments. Responses were

evaluated relative to those of fish exposed to an equal mass of undisturbed (i.e., bedded) sediment from the same UMR site. These objectives were formulated into the following test hypotheses:

I. H_o: [metal in fish]$_{\text{bedded sediment}^*}$ = [metal in fish]$_{\text{suspended sediment}^*}$

H_a: [metal in fish]$_{\text{bedded sediment}^*}$ > or < [metal in fish]$_{\text{suspended sediment}^*}$

II. H_o: fish ALA-D activity$_{\text{bedded sediment}^*}$ = fish ALA-D activity$_{\text{suspended sediment}^*}$

H_a: fish ALA-D activity$_{\text{bedded sediment}^*}$ > or < fish ALA-D activity$_{\text{suspended sediment}^*}$

where "*" represents sediment from a single UMR location.

MATERIALS AND METHODS

Study Design

We used the sediment exposure system described by Cope et al. (*in review*) to conduct both suspended and bedded sediment tests (Fig 1). A randomized block experimental design of four UMR sediment test treatments (Pool 12, Pool 14, Pool 25, Open River), a sediment control treatment (Pool 10), and a water control treatment (containing no sediment) was used for both tests. Each treatment consisted of three exposure chambers (i.e., experimental units) in which test conditions were replicated.

The exposure system was adapted for intermittent suspended sediment testing by use of a programmable electronic timer (ChronTrol® XT, San Diego, CA) that controlled the daily frequency and duration of chamber rotation to simulate sediment resuspension events associated with peak UMR commercial and recreational navigation activity. Twelve commercial and twenty-four recreational resuspension events were scheduled daily, Monday through Friday (Table 1). The duration of each simulated commercial and recreational events was 30 and 15 min, respectively. A similar schedule was followed on Saturdays and Sundays except between 1200-1830 hours, when sediments were continuously suspended to simulate the combined effects of normal commercial and maximum recreational boating activities. Thus, sediments were in suspension for 50% of the time on weekdays and 60% of the time on weekends.

A portion of the sediment and water in each chamber was replaced every fourth day (through day 24) of the 28-d suspended sediment test. This was done to replenish concentrations of sediment-associated metals that could have decreased over time due to biouptake by test fish or binding to the inner surfaces of the glass exposure chambers. An estimated two-thirds of the sediment mass and water volume in each chamber was removed on renewal days and replaced with near equivalent portions of fresh sediment and test water. The cumulative mass of wet sediment to which fish in each treatment were exposed over the 28-d suspended sediment test is listed in Table 2.

Metal bioavailability to fish exposed for 28 days to intermittent pulses of suspended sediment was evaluated relative to that of fish exposed for 28 days to an equivalent cumulative mass of bedded sediment from the same site. Bedded sediment testing was conducted with the same exposure apparatus used for the suspended sediment test with the following modifications. First,

chambers were stationary throughout the 28-d test so that sediments remained bedded unless disturbed by fish bioturbation. Secondly, the entire mass of sediment used over the 28-d bedded test was placed in exposure chambers six days prior to the start of the test. By adding all of the test sediment to the exposure chambers in one "dose" and allowing it to settle for several days before fish were introduced, the fish were never exposed to suspended sediments. Moreover, although the sediment used for the bedded test was introduced as one large "dose" rather than portioned into several smaller "doses", fish in both tests were exposed to the same cumulative mass of sediment and sediment-associated metals over the 28-d period (Table 2). Finally, fresh test water was continuously pumped into the exposure chambers near the sediment-water interface at a rate of 5.5 ($\pm$1.1) mL/min. Maintenance of this flow rate insured that the volume of water exchanged in exposure chambers during the 28-d bedded sediment test (~222 L) was similar to that exchanged during the 28-d suspended sediment test (225 L).

Sediment Collection and Characterization

Test sediments were collected near four UMR sites reported to have elevated concentrations of certain metals including arsenic, cadmium, copper, lead, and zinc (Young 1991). Included were locations in navigation Pools 12 (Wise Lake), 14 (Swan Slough), 25 (Batchtown Middle Pool), and the Open River near Herculeneum, MO (Table 3). These areas encompass diverse riverine habitats that range from isolated backwater lakes and sloughs to the main channel border. A control sediment (i.e., thought not to be metal enriched) was collected from a backwater area in Pool 10

(Methodist Lake). Sample site coordinates (latitude and longitude) were obtained with a Loran-C receiver calibrated at a nearby benchmark location.

Sediment collection, handling, and storage procedures were consistent with quality assurance and quality control guidelines of the National Biological Service-Midwest Science Center (Columbia, MO). Several grab samples of sediment were collected at each site with a stainless steel van Veen dredge. Overlying water was siphoned off and a stainless steel spatula was used to remove and place the surficial sediments (i.e., from the uppermost 5 cm) into acid-washed (10% HNO_3) glass containers. Containers were filled to the rim with sediment, sealed with Parafilm®, and shipped to the laboratory on ice. Large debris was removed from each sample in the laboratory by successively sieving the wet sediment through 2.0 and 1.0 mm stainless steel mesh sieves into a stainless steel bowl. Sieved sediments were homogenized in the bowl with a stainless steel spoon, returned to their original containers, sealed with Parafilm®, and refrigerated (4°C).

Consolidated sediments and overlying water in each container were later re-homogenized and subsamples taken to characterize the physical and chemical composition of the substrate collected at each site (Appendix A). Results of only the physical analyses were available prior to the start of the suspended and bedded sediment toxicity tests (Table 4). The volatile residue content of sediments was consistently low ($\leq$ 10%) at all treatment sites. However, the total solids content varied widely (29-60%) among sites and bulk density followed a similar pattern (Pool 14 > Open River > Pool 12 > Pool 25 > Pool 10). Textural analysis of sediments indicated particle distributions dominated by silt- and clay-sized grains at all sites. The combined proportion of silt

and clay was uniform (95-99%) at all but the Open River site, where there was a substantially greater sand content (18%).

The overwhelmingly fine-grained textural composition of the surface sediments we collected at most of the sites should have predisposed them to be metal-enriched if (1) recent inputs of significant quantities of metals occurred upstream of or adjacent to the sample sites or (2) recent sediment disturbance activities exposed contaminated layers of sediment previously buried at these sites. Results of chemical analyses (Table 5) indicated substantial metals enrichment at only the Wise Lake site in Pool 12. Sediment from this site, located adjacent to several abandoned lead mines near Galena, Illinois, had concentrations of lead and zinc in excess of reported sediment toxicity threshold values (Long and Morgan 1991). Metal concentrations in sediments we collected from Pools 14, 25, and the Open River were much less than previously reported values for corresponding locations (Young 1991). However these sediment chemical data were not available until after laboratory toxicity testing was completed. Therefore, all of the test sediments collected were used under the assumption that each was metal-enriched to some unknown degree.

Water Quality

Unfiltered UMR water from Lake Onalaska (Pool 7) was collected weekly and used as the test water throughout the study to further simulate realistic environmental conditions. River water was pumped from ~0.3 m below the surface into a covered 950 L fiberglass tank and transported 5 km to the laboratory where it was transferred to a similar reservoir, vigorously aerated, and tempered (22±1°C). Water for the bedded sediment test was collected from under 0.4-0.6 m of ice.

Water for the suspended sediment test was collected during the open water season and had substantially reduced levels of hardness, alkalinity, and conductivity because of snowmelt dilution. This water was ameliorated to a quality similar to that used in the bedded test by adding a mixture of several salts ($NaHCO_3$, $CaSO_4 \cdot 2H_2O$, $MgSO_4$, and $KHCO_3$) to the reservoir.

A nominal TNFR test concentration of 300 mg/L during chamber rotation was chosen to represent the maximum anticipated suspended sediment level associated with UMR navigation activity (Adams 1993). The mass of wet sediment from each site needed to achieve this TNFR concentration in exposure chambers containing 45 L of test water was initially estimated based on the total solids content. A preliminary test was conducted to assess the accuracy of these estimates in achieving the desired TNFR test concentration at a fixed location (mid-depth) inside replicate chambers (n=3) under actual test conditions (i.e., chamber rotational speed of 3.3 revolutions/min, bubbling airstone and fish present, periodic sediment-water renewals). Water samples were collected near the midpoint of the 15 and 30 min rotational periods. The duration of chamber rotation before water samples were collected did not effect the observed TNFR concentrations. Mean TNFR concentrations for all but one treatment were greater (338-382 mg/L) than the desired nominal value. Based on these preliminary results, we assumed a linear relationship between the mass of wet sediment added to exposure chambers and the observed TNFR concentrations to derive the mass of wet sediment that was placed in each treatment exposure chamber with 45 L of water at the start of the suspended sediment test (Table 6).

Standard water quality variables were determined in each exposure chamber at different intervals during the tests: pH, temperature, and dissolved oxygen were measured *in situ* daily;

hardness, alkalinity, and conductivity were measured in samples collected near the start, middle, and end of the 28-d tests; ammonia, TNFR, and turbidity were measured in samples collected weekly (Table 7). All samples were collected from a standard (mid-depth) location in each chamber. Suspended sediment test water samples were collected from revolving chambers after the mid-point of a rotational period. Moreover, two additional water samples were collected during the suspended sediment test to document TNFR concentrations after the chambers were stationary for periods of nearly 11.25 and 90 min, respectively.

All standard water quality variables, except turbidity and TNFR, were similar and ammonia levels were non-toxic in each treatment during the bedded and suspended sediment tests (Table 7). Mean treatment turbidity in rotating chambers during the suspended test was greater (7- to 24-fold) than that in corresponding stationary treatment chambers during the bedded test. Mean treatment TNFR concentrations likewise varied (10- to 45-fold) between the tests, ranging 3-12 mg/L during the bedded test and 121-152 mg/L (time-weighted values) during the suspended test. Moreover, mean treatment TNFR concentrations during the suspended sediment test ranged from 28-55 mg/L when exposure chambers were stationary and from 204 to 250 mg/L (68 to 83% of the nominal test value) when chambers were rotating. These findings suggest that our laboratory exposure system adequately simulated environmentally relevant changes in suspended sediment intensity comparable to that associated with UMR navigation activities.

The concentration of metals (As, Cd, Cu, Pb, Zn) in treatment test waters was measured in samples collected just before fish were placed into exposure chambers on day 0 of the 28-d tests. Bedded sediment test water samples were collected 6 days after sediment was introduced (i.e., just

before water began to overflow from the chamber) and suspended sediment test water samples were collected during a rotational period 1 d after sediment was introduced (i.e., following 24 h of scheduled intermittent rotation). Both an unfiltered and a filtered (0.4 m polycarbonate) sample aliquot were preserved (1 mL Ultrex® HNO_3/125 mL) and later analyzed (Appendix A) to assess metal partitioning between the dissolved and particulate phases.

The total concentration of metals in unfiltered UMR water used for both tests (i.e., the water control treatments) was low (Tables 8, 9), especially during the suspended sediment test when concentrations of copper, lead, and zinc were presumably diluted by snowmelt runoff. The concentration of metals in unfiltered water was greater for all sediment treatments during the suspended test. This difference was most apparent in the Pool 12 treatment where total lead and zinc concentrations increased 27- and 19-fold, respectively. However, sediment resuspension did not substantially alter treatment metal concentrations in filtered water samples from the low levels observed during the bedded sediment test. Therefore despite intermittent sediment resuspension, most water column metals were apparently particulate-bound.

Fish Care and Sampling

Green sunfish were obtained from the USFWS fish hatchery in Genoa, WI, and acclimated to the 22°C test temperature over a 1 week period with flowing, tempered well water (pH 8.1; alkalinity 106 mg/L as $CaCO_3$; hardness 141 mg/L as $CaCO_3$). Fish were maintained in this water for 4-6 months under a 16 h L: 8 h D photoperiod and were fed daily a 2% body weight ration of a commercially prepared trout starter diet (No. 3, Nelson and Sons, Murray, UT). Metal

concentrations (μg/g dry weight) in this diet were 3.5 for arsenic, 0.21 for cadmium, 7.2 for copper, 1.9 for lead, and 164 for zinc.

Fish were separated into two size groups with a standard grader (12.7 mm wide openings) two weeks before the start of testing. The larger fish (i.e., those retained in the grader) had a mean total length of 108 mm (range 98-115 mm) and a mean wet weight of 23.2 g (range 15.6-32.6 g). Most of these fish were used for the first test (bedded sediment exposure), while the smaller fish (i.e., those not retained by the grader) were maintained for 8 additional weeks until used for the second test (suspended sediment exposure). It was anticipated that the smaller fish would reach similar size of the first group of fish in time for the second test. As a result of time constraints, the suspended test was conducted despite the smaller size of the second group of fish having a mean total length of 95 mm (range 81-135 mm) and a mean wet weight of 11.4 g (range 6.3-22.0 g). It appeared that the smaller fish had somewhat greater metal bioaccumulation following exposure to contaminated sediments compared to a "larger" counterpart within the same test. However, overlap between the two tests and between the two size ranges contained only a few fish. Thus, evidence for a disproportionate bioaccumulation of metals in smaller fish compared to larger fish is inconclusive.

One day before the start of testing, blood and tissue samples were collected from 3 groups of 10 green sunfish taken directly from the laboratory holding tank to evaluate background levels of the test endpoints. Seven fish from each chamber were bled from the caudal vessel with a heparinized (100 units/mg) syringe (3 cc, 1 inch-21 gauge needle). Blood from each of the fish was dispensed into individually labelled 2 mL centrifuge tubes. A portion of this blood (250μL) was removed from each of the tubes, placed in a cryovial, and briefly vortexed. A 500μL aliquot of this

pooled blood was transferred to a second cryovial for ALA-D and hemoglobin (Hb) analysis while blood remaining in the original cryovial was reserved for lead and zinc analysis (Appendix A). Cryovials containing blood were stored in an ultracold (-80°C) freezer until these analyses. Blood samples of individual fish that remained in centrifuge tubes were centrifuged (gravity x 2000, 4°C) for 10 min. Plasma was collected in labelled hematocrit tubes and frozen (-80°C) for subsequent glucose and chloride analysis (Appendix A). The seven bled fish from each chamber were placed in a Ziploc® bag and frozen (-80°C) until homogenized into a composite sample (3 per treatment) for lead and zinc analysis (Appendix A). The three remaining fish in each chamber were placed in separate Ziploc® bags and stored similarly until each was homogenized and a portion analyzed (9 samples per treatment) for cadmium, copper, lead, and zinc content (Appendix A).

At the start of testing (day 0), ten fish were randomly selected, weighed (as a group), and placed in each exposure chamber. Fish in all chambers were fed the trout starter diet, every other day (from day 1 to 27), at about 1% of body weight. At the end of testing (day 28), individual fish were netted and removed from chambers, anaesthetized in tricaine methanesulfonate (200 mg Finquel®/L), rinsed in deionized water, blotted, weighed, and measured (total length).

Metal Analyses and Quality Assurance

Test matrices were analyzed for metals content at the Environmental Trace Substances Research Center (Columbia, MO) following standard analytical procedures (Appendix A). Samples of sediment, fish tissue, and fish feed were initially prepared by homogenizing and freeze drying to determine moisture content. An aliquot of each sample for cadmium, copper, and lead analysis was

digested with sub-boiling HNO_3, reconstituted with deionized water, and quantified by graphite furnace atomic absorption spectroscopy (GFAA). The method of standard additions was used to assess and correct for sample matrix interferences. A second aliquot of each sample was digested in a mixture of sub-boiling acids (HNO_3, $HClO_4$, HCl) and reconstituted to quantify zinc (and aluminum in sediments) by inductively coupled plasma (ICP) analysis. Quality control samples were analyzed after every 10-15 sample analyses and the instrument recalibrated if values drifted > 5%.

Analytical accuracy was evaluated by the recovery of metals from certified reference materials (CRMs) and from spiked samples. The mean recovery of metals from CRMs was nearly complete (Table 10), suggesting a high degree of analytical accuracy. Mean metal recoveries from spiked samples were likewise satisfactory (Table 11). Analytical precision of metal determinations in each matrix was evaluated by duplicate analysis of about 10% of the samples. The mean percent difference for these analyses was generally low (Table 12), suggesting good precision. Notable exceptions to this were copper and zinc in water, and lead in tissue. Moreover, precision could not be evaluated for several samples because of metal concentrations below detection limits.

Statistical Analyses

Statistical analyses were performed with PC-SAS software (SAS Institute 1987). A one-tail Student's *t*-test was used to determine significant differences in whole-body metal bioaccumulation ($P < 0.05$) between the bedded and suspended sediment tests for each treatment. The Pearson's Correlation Coefficient was used to describe the relationship between blood lead concentrations and ALA-D activity.

RESULTS

Contribution of Metals from Sediment, Feed, and Test Water

The total mass of metal and the form by which it was introduced into exposure chambers of each treatment was consistent for the bedded and suspended sediment tests (Appendix B). The estimated cumulative mass of arsenic, copper, lead, and zinc from all sources (i.e., food, sediment, and water) placed in sediment treatment exposure chambers over the 28-d exposure typically varied < 10% between the two tests. The relative contribution of these metals from all sources was also consistent for each sediment treatment between the two tests (maximum variation < 10% for arsenic, copper and zinc; < 15% for lead). Thus the relative contribution of copper, lead, and zinc from food and water may explain the greater body burdens of in the Water Control treatments for both suspended and bedded tests (see *Metal Bioaccumulation by Fish*).

The estimated cumulative mass of cadmium placed in exposure chambers of most treatments was substantially greater however during the suspended sediment test due to an increased

concentration (5-fold) of cadmium present in the Mississippi River water used for this test. This change substantially altered the relative contribution of cadmium from sediment and water between the two tests for each treatment.

Metal Bioaccumulation by Fish

All fish analyzed for arsenic in the bedded (n=54) and suspended tests (n=54) had concentrations above the detection limit (0.5 ug/g) (Fig 2). The concentrations ranged from 1.4 to 2.3 ug/g with no significant difference between the two tests for all treatments ($P > 0.05$).

Similarly, cadmium in fish did not significantly differ between the two tests for all treatments ($P > 0.05$) (Fig 3). Of the 54 fish analyzed for cadmium in the bedded test, 40 had concentrations below the detection limit (0.015 ug/g). Cadmium concentrations ranged from below the detection limit to 0.023 ug/g. Of the 54 fish analyzed for cadmium in the suspended sediment test, 35 had concentrations below the detection limit. The concentrations ranged from 0.002 to 0.1 ug/g. Although not significant, cadmium concentrations were greater in fish subjected to suspended sediments from Pool 12, 25, and Open River than in bedded sediment tests for the same sites.

Copper concentrations in whole fish significantly differed between the two tests for Water Control, Pool 25, and Open River ($P < 0.05$) (Fig 4). All fish analyzed in both bedded (range from 1.0 to 2.5 ug/g) and suspended sediment tests (range from 1.2 to 21.9 ug/g) had copper levels above the detection limit (0.2 ug/g). Although not significant ($P = 0.17$), copper concentrations in fish exhibited a greater trend in Pool 12 suspended tests (4.6 ug/g, $\pm$ 2.00 standard error (SE), n=9) compared to the bedded tests (1.5 ug/g, $\pm$ 0.09 SE, n=9).

Lead in whole fish significantly differed between the two tests for Water Control, Pools 10 and 14 ($P < 0.05$) (Fig 5). Of the 54 fish analyzed in the bedded test, seven fish had concentrations below the detection limit (0.05 ug/g). Lead concentrations ranged from 0.05 to 0.2 ug/g. Fourteen of the 54 fish in the suspended test had concentrations below the detection limit with a range from 0.1 to 5.2 ug/g. Lead concentrations in Pool 12 did not significantly differ between the suspended and bedded tests ($P > 0.18$). Two fish had elevated lead concentrations (5.2 and 2.1 ug/g) in the suspended test resulting in a mean of 0.95 ug/g ($\pm$ 0.57 SE, n=9). Removing the two outliers results in a mean lead concentration of 0.18 ug/g ($\pm$ 0.04 SE) which is greater (but not significant) than 0.11 ug/g ($\pm$ 0.04 SE) obtained in fish exposed to bedded sediments from Pool 12.

Zinc concentrations were well above the detection limit (0.1 ug/g) in all fish for both tests, and were significantly greater ($P < 0.05$) in the suspended sediments tests for Water Control, Pools 10 and 12 (Fig 6). Although not significant, zinc concentrations were greater in Pools 14, 25 and Open River in the suspended test comparted to the bedded test. Zinc ranged from 82 to 138 ug/g in the suspended tests and from 83 to 129 ug/g in the bedded tests.

Blood Chemistry

Blood lead levels were below the detection limit (0.001 ug/mL) in 16 of 17 samples collected from green sunfish in the bedded sediment test (Fig 7). In contrast, two of 17 samples were below the detection limit for lead in the suspended test (Fig 7). The bedded test resulted in blood lead ranging from below detection to 0.03 ug/mL, and the suspended test resulted in blood lead levels ranging from 0.02 to 0.2 ug/mL. In contrast to blood lead, blood zinc concentrations were above

the detection limit in both suspended and bedded tests (Fig 8). Blood zinc in the bedded test ranged from 7.37 to 8.86 ug/mL in contrast to blood zinc in the suspended test habing a range of 8.8 to 14.5 ug/mL. Changes in blood lead and blood zinc concentrations with respect to treatments and tests were not significantly correlated to -aminolevulinic acid dehydratase (ALA-D) activity ($P > 0.05$).

ALA-D in green sunfish was significantly reduced ($P < 0.05$) after 28 days in all treatments of the suspended test compared to green sunfish maintained in well water (Fig 9). In contrast ALA-D activity was not significantly different among the treatments in the bedded test when compared to fish from well water (Fig 10). However, no significant differences were observed for ALA-D activity between the bedded and suspended tests for each treatment ($P > 0.05$). No significant differences were observed in fish maintained in well water (not shown in Fig) and sampled at the end of each 28-d test ($P > 05$). In the bedded test, ALA-D activity ranged from 356.68 to 540.6 nmoles PBG/g/hr in fish exposed to Water Control and Pool 14 bedded sediment, respectively. Similarly, ALA-D activity ranged from 280.1 nmoles PBG/g/hr in fish exposed to suspended sediments from Pool 10 to 521.1 nmoles PBG/g/hr in fish maintained in well water (not shown in Fig).

The suspended test resulted in significantly lower hemoglobin concentration (Hb) for all treatments compared to Hb in the same treatments for the bedded test ($P < 0.05$) (Fig 11). Hemoglobin did not significantly differ in fish between treatments for the bedded test and fish maintained in the well water. Changes in Hb with respect to treatments and tests were not significantly correlated to ALA-D activity ($P > 0.05$).

DISCUSSION

Sediments are a major repository for contaminants in surface waters. In aquatic environments, chemicals including organic compounds and heavy metals can accumulate several orders of magnitude greater than the overlying water. However, since contaminants rarely occur singly and under conditions that are easily controlled for, it is difficult to characterize bioavailability and thus impact to the aquatic biota. Analysis of bulk sediment concentrations is often limited to chemical characterizations and simply do not reflect bioavailability and long term adverse effects (Ingersoll and Nelson 1990).

Metals in sediments strongly adsorb to fine particles such as clay or silt which eventually settle and are buried. It is likely that metals in the surficial layers of sediment become resuspended when disturbed by wind-induced wave action, or navigation (commercial and recreational) and channel maintenance practices (Sparks 1984; Smart et al. 1985). Where once the contaminant-associated sediments are in suspension, metal ion speciation will be more likely to occur among the various environmental compartments (e.g., complexed to organic colloids, dissolved in water, adsorbed to suspended solids). Partitioning of the metals between water and sediment will depend upon factors including temperature, pH, salinity, redox potential, total organic carbon, grain size of sediment, and sediment mineral constituents (oxides of iron, manganese and aluminum) (Luoma and Bryan 1978; U.S. EPA 1989; Bryan and Langston 1992). Not only are the physical and chemical processes that regulate metal partitioning among sediments and the aqueous phase complex, but their cumulative effects on metal speciation are difficult to identify and control for.

The greatest proportion of the metals introduced into the test chambers was contributed with sediments. Results of metals analysis of filtered and unfiltered water samples indicated the majority of water column metals were associated with particulate matter in suspension. Therefore very little metal was truly dissolved and readily bioavailable for branchial uptake. Ingestion of metal-enriched sediments and subsequent metal uptake via digestive processes could contribute to increased metal bioavailability (see reviews by Luoma 1983 and 1989). However this mechanism is not suspected to have contributed to significant metal bioaccumulation in the present investigation because most of the sediments we tested were considered unenriched with toxic metals.

Bioavailability of Lead as Indicated by ALA-D Activity and Hemoglobin

The processes and products of industrialization have resulted in an increased occurrence and redistribution of lead throughout the air, soil, sediment, and water systems. Although lead is ubiquitous in the earth's crust, it does not biomagnify in aquatic foodchains. It is neither essential nor beneficial, and is toxic in the inorganic as well as the organic state. The status of knowledge of the toxicological effects on human health and wildlife is almost a complete picture. However, not as much information is available on the effects of lead in aquatic organisms. Generally, lead affects the peripheral nerves and central nervous system as well as the kidney and hematopoietic system. Overt signs of acute lead poisoning are muscle tremors, convulsions and renal failure usually ending in death (Goldberg 1977). In contrast to lead's immediate effects at high doses, chronic sublethal levels result in cumulative toxic effects that are subtly manifested over time by changes in behavior, lethargy/hyperactivity, locomotor malfunction, anemia, and skeletal deformities. In brook trout

(Salvelinus fontinalis) exposed to low concentrations of lead (0.12 mg/L), skeletal deformities as well as abnormal behavior were observed after three generations (Holcombe et al. 1976). Exposure to sublethal levels of waterborne lead for four weeks (0.5 mg/L) altered the reproductive behavior in fathead minnows (Pimephales promelas) (Weber 1993). Chronic sublethal levels of lead potentially pose a serious contaminant threat to aquatic ecosystems if reproductive potential is substantially impaired.

Measuring lead concentrations in water alone may not accurately reflect biological effects in fish (Hodson et al. 1984). Chemical factors such as the presence of reduced iron strongly influence lead's potential to bioaccumulate (Luoma and Bryan 1978). Hodson (1976) and Hodson et al. (1977) observed lead in water correlates well with lead in blood. Thus the added analysis of lead concentrations in blood is essential to begin linking lead exposure with biological effects.

The expected dose-response of lead exposure and ALA-D was not observed in this study, presumably, due to the variability of blood lead among individual fish that were pooled. Environmental lead exposure resulted in a dose-dependent decrease in erythrocyte ALA-D activity in fish (Johansson-Sjobeck and Larsson 1979). The authors observed a 21% and 74% decrease in ALA-D activity following 30 days of exposure to 0.01 and 0.08 mg/L of lead in water, respectively. A similar decrease in ALA-D activity (range from 33 to 46%) was observed in this study with total lead concentrations in water in the suspended tests for all treatments although aqueous lead concentrations was well below the sublethal limit (0.27 ppb) (Spry and Wiener 1993).

Although changes in ALA-D activity and corresponding hematological effects have been used to determine the degree of lead exposure in humans (Marcus and Schwartz 1987), birds

(Gonzales and Tejedor 1992), and mammals (Wigfield et al. 1985), reduced ALA-D activity and the resulting hematological effects in fish remains poorly defined and inconsistent. Haux and Larsson (1982) demonstrated that 0.3 mg/L of lead decreased hemoglobin levels in rainbow trout. In contrast, Santos and Hall (1990) did not observe hematological changes in the eel (Anquilla anquilla) exposed to similar lead concentrations. Similarly, Schmitt et al. (1984) did not observed significant hematological changes in fish having a 67% reduction in ALA-D activity with blood lead concentrations of 0.02 mg/L. Although significant reductions in Hb concentrations were observed in this study for green sunfish subjected to suspended sediments, Hb concentrations did not correlate with observed ALA-D activity.

Hematological effects of lead in fish needs further clarification (beyond the scope of this study). However, there is no doubt that ALA-D inhibition is highly specific for blood lead in fish. Prolonged exposures to cadmium did not decrease blood ALA-D activity in rainbow trout (Johansson-Sjobeck and Larsson 1977). And, it is believed that other heavy metals (copper, mercury, zinc), and polychlorinated biphenyls do not reduce blood ALA-D activity (see review by Hodson et al. 1984). Although zinc has been shown to have an ameliorative effect on blood ALA-D in the presence of lead in fish (Schmitt et al. 1984, 1993) and mammals (Finelli 1977), we did not observe any interactive effect with lead.

SUMMARY AND RECOMMENDATIONS

Various test systems have been developed to assess the effects of suspended contaminants on aquatic biota (Cope et al. *in review*). The greatest disadvantage these systems had was the inability to quantify and analyze the distribution and partitioning of the contaminants bound to the sediment, dissolved in the water, and accumulated by aquatic organisms. We used a test system similar to that used and described by Cope et al. (*in review*; 1994) and successfully quantified the distribution of metals among compartments of sediment, water, and fish. Future recommendations using the same test system would be to conduct controlled tests with contaminated sediments from one location under a range of environmental test conditions (i.e., temperature, and nominal suspended solids concentrations) and a range of sizes of fish (but maintaining fish of similar size within tests).

LITERATURE CITED

Adams, J.R. 1993. Sediment concentration changes caused by barge tows. Illinois State Water Survey, Champaign, Il.

Adams, J.R., and E. Delisio. 1993. Temporal and lateral distributions of resuspended sediment following barge tow passage on the Illinois River. Illinois State Water Survey, Champaign, Il.

Adams, J.R., N.G. Bhowmik, and E. Delisio. 1993. Measuring resuspension of sediment by barge tows. Illinois State Water Survey, Champaign, Il.

Bailey, P.A. and R.G. Rada. 1984. Distribution and enrichment of trace metals (Cd, Cr, Ni, Pb, Zn) in bottom sediments of Navigation Pools 4 (Lake Pepin), 5 and 9 of the Upper Mississippi River. In: Wiener, J.G., Anderson R.V., McConville, D.R. (eds). Contaminants in the Upper Mississippi River. Butterworth Publ. Boston, MA, pp 119-138.

Bryan, G.W. and W.J. Langston. 1992. Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: a reivew. Enivron. Pollut. 76:89-131.

Cope, W.G., J.G. Wiener, and M.T. Steingraeber. *In review.* Test system for exposing fish to suspended, contaminated sediments. Submitted to *Environmental Pollution.*

Cope, W.G., J.G. Wiener, M.T. Steingraeber, and G.J. Atchison. 1994. Cadmium, metal-binding proteins, and growth in bluegill (*Lepomis macrochirus*) exposed to contaminated sediments from the Upper Mississippi River Basin. Can. J. Fish. Aquat. Sci. 51:1356-1367.

Edlund, M. 1992. U.S. Army Corps of Engineers, St. Paul, MN, unpublished data.

Finelli, V. 1977. Lead, zinc, and δ-aminolevulinic dehydratase. In: S. Lee and B. Peirano (eds.). Biochemical Effects of Environmental Pollutants. Ann Arbor Science Publishers, Ann Arbor, MI, p.351.

Goldberg, A.M. 1977. Neurotransmitter mechanisms in inorganic lead poisoning. In: Biochemical Effects of Environmental Problems. S.D. Lee (editor). Ann Arbor Science Publishers, Inc. pp. 413-423.

Gonzales, M. and M.C. Tejedor. 1992. δ-ALAD activity variations in red blood cells in response to lead accumulation in rock doves (Columba livia). Bull. Environ. Contam. Toxicol. 49:527-534.

Haux, C. and A. Larsson. 1982. Influence of inorganic lead on the biochemical blood composition in the rainbow trout, Salmo gairdneri. Ecotoxicology and Environmental Safety 6:28-34.

Hodson, P.V. 1976. δ-Aminolevulinic acid dehydratase activity of fish blood as an indicator of harmful exposure to lead. J. Fish. Res. Board of Can., 33:268-271.

Hodson, P.V., B.R. Blunt, D.J. Spry, and K. Austen. 1977. Evaluation of erythrocyte - aminolevulinic acid dehydratase activity as a short-term indicator in fish of a harmful exposure to lead. J. Fish Res. Board Can. 34:501-508.

Hodson, P.V., B.R. Blunt, and D.M. Whittle. 1984. Monitoring lead exposure of fish. In: V. Cairns, P.V. Hodson, and J.O. Nriagu (eds). Contaminant effects on fisheries. J. Wiley, New York, p.87.

Holcombe, G.W., D.A. Benoit, E.N. Leonard, and J.M. McKim. 1976. Long-term effects of lead exposure on three generations of brook trout (Salvelinus fontinalis). J. Fish. Res. Board of Canada 33:1731-1741.

Ingersoll, C.G. and M.K. Nelson. 1990. Testing sediment toxicity with *Hyallela azteca* (Amphipoda) and *Chironomus riparius* (Diptera). American Society for Testing and Materials. Standard Technical Publication 1096. p. 93-109.

Johansson-Sjobeck, M-L. and A. Larsson. 1977. The effect of cadmium on the hematology and on the activity of delta-aminolevulinic acid dehydratase (ALA-D) in blood and hematopoietic tissues of the flounder, Pleuronectes flesus L. Statens Naturvardsverk. NBL Rapport 60. pp.22.

Johansson-Sjobeck, M-L. and A. Larsson. 1979. Effects of inorganic lead on delta-aminolevulinic acid dehydratase activity and hematological variables in the rainbow trout, *Salmo gairdneri*. Arch. Environm. Contam. Toxicol. 8:419-431.

Johnson, S. 1992. Recreational boating impacts investigations interim report. Minnesota Department of Natural Resources, Lake City, MN.

Long, E.R., and L.G. Morgan. 1991. The potential for biological effects of sediment-sorbed contaminants tested in the national status and trends program. Office of Coastal and Estuarine Assessment, National Oceanic and Atmospheric Administration, Seattle, WA.

Luoma, S.N. and G.W. Bryan. 1978. Factors controlling the availability of sediment-bound lead to the estuarine bivalve *Scrobicularia plana*. J. Mar. Biol. Ass. U.K. 58:793-802.

Luoma, S.N. 1983. Bioavailability of trace metals to aquatic organisms: A Review. Sci. Tot. Environ. 28:1-22.

Luoma, S.N. 1989. Can we determine the biological availability of sediment-bound trace elements? Hydrobiologia 176/177:379-396.

Rada, R.G., J.G. Wiener, P.A. Bailey, and D.E. Powell. 1990. Recent influxes of metals into Lake Pepin, a natural lake on the Upper Mississippi River. Arch. Environ. Contam. Toxicol. 19:712-716.

Marcus, A.H., and J. Schwartz. 1987. Dose-response curves for erythrocyte protoporphyrin vs blood lead: Effects of iron status. Environ. Res. 44:221-227.

Santos, MA., and A. Hall. 1990. Influence of inorganic lead on the biochemical blood composition of the eel, Anguilla anguilla L. Ecotox. Environ. Safety 20:7-9.

Schmitt, C.J., F.J. Dwyer, and S.E. Finger. 1984. Bioavailability of Pb and Zn from mine tailings as indicated by erythrocyte ∫-aminolevulinic acid dehydratase (ALA-D) activity in suckers (Pisces: Catostomidae). Can. J. Fish. Aquat. Sci. 41:1030-1040.

Schmitt, C.J., M.L. Wildhaber, J.B. Hunn, T. Nash, M.N. Tieger, and B.L. Steadman. 1993. Biomonitoring of lead-contaminated Missouri streams with an assay for erythrocytes ∫-aminolevulinic acid dehydrase (ALA-D) in fish blood. Archives of Environmental Contamination and Toxicology In revision.

Smart, M.M., R.G. Rada, D.N. Nielsen, and T.O. Claflin. 1985. The effect of commercial and recreational traffic on the resuspension of sediment in Navigation Pool 9 of the upper Mississippi River. Hydrobiologia 126:263-274.

Sparks, R.E. 1984. The role of contaminants in the decline of the Illinois River: Implications for the Upper Mississippi. In: Wiener, J.G., Anderson R.V., McConville, D.R. (eds). Contaminants in the Upper Mississippi River. Butterworth Publ. Boston, MA, pp 25-66.

Spry, D.J., and J.G. Wiener. 1993. Metal bioavailability and toxicity to fish in low alkalinity lakes: A critical review. Environ. Poll., 71:243-304.

Sullivan, J.F. 1993. Weaver Bottoms resource analysis program interim report. Wisconsin Department of Natural Resources, La Crosse, WI.

United States Environmental Protection Agency. 1977. Guidelines for the pollutional classification of Great Lakes harbor sediments. Chicago, IL.

United States Environmental Protection Agency. 1989. Sediment classification methods compendium. Washington, D.C.

Weber, D.N. 1993. Exposure to sublethal levels of waterborne lead alters reproductive behavior patterns in fathead minnows (Pimephales promelas). Neurotoxicology 14:347-358.

Wiener, J.G., G.A. Jackson, G.A., T.W. May, and B.P. Cole. 1984. Longitudinal distribution of trace elements (As, Cd, Cr, Hg, Pb, and Se) in fishes and sediments in the Upper Mississippi River. In *Contaminants in the Upper Mississippi River*, ed. J.G. Wiener, R.V. Anderson and D.R. McConville, Boston, Massachusetts, Butterworth Publishers, pp. 139-170.

Wigfield, D.C., and S.C. Wright, C.L. Chakrabarti, and R. Karwowska. 1985. Evaluation of the relationship between chemical and biological monitoring of low level poisoning. J. Appl. Toxicol. 6:231-235.

Young, M. 1991. Survey for contaminants in sediments at selected sites on the Upper Mississippi River (RM 579 to RM 3) including the Mark Twain National Wildlife Refuge. U.S. Fish and Wildlife Service. Rock Island Field Office. Rock Island, IL.

TABLES

Table 1. Daily test chamber rotation schedule for the 28-d suspended sediment test.

Time				
Begin	End	Simulated navigation event	Duration (min)	Days ommitted
00:00:00	00:30:00	Commercial	30	------
02:00:00	02:30:00	Commercial	30	------
04:00:00	04:30:00	Commercial	30	------
06:00:00	06:30:00	Commercial	30	------
06:41:15	06:56:15	Recreational	15	------
07:07:30	07:22:30	Recreational	15	------
07:33:45	07:48:45	Recreational	15	------
08:00:00	08:30:00	Commercial	30	------
08:41:15	08:56:15	Recreational	15	------
09:07:30	09:22:30	Recreational	15	------
09:33:45	09:48:45	Recreational	15	------
10:00:00	10:30:00	Commercial	30	------
10:41:15	10:56:15	Recreational	15	------
11:07:30	11:22:30	Recreational	15	------
11:33:45	11:48:45	Recreational	15	------
12:00:00	12:30:00	Commercial	30	SaSu
12:41:15	12:56:15	Recreational	15	SaSu
13:07:30	13:22:30	Recreational	15	SaSu
13:33:45	13:48:45	Recreational	15	SaSu
14:00:00	14:30:00	Commercial	30	SaSu
14:41:15	14:56:15	Recreational	15	SaSu
15:07:30	15:22:30	Recreational	15	SaSu
15:33:45	15:48:45	Recreational	15	SaSu
16:00:00	16:30:00	Commercial	30	SaSu
16:41:15	16:56:15	Recreational	15	SaSu
17:07:30	17:22:30	Recreational	15	SaSu
17:33:45	17:48:45	Recreational	15	SaSu
18:00:00	18:30:00	Commercial	30	SaSu
18:41:15	18:56:15	Recreational	15	------
19:07:30	19:22:30	Recreational	15	------
19:33:45	19:48:45	Recreational	15	------
20:00:00	20:30:00	Commercial	30	------
20:41:15	20:56:15	Recreational	15	------
21:07:30	21:22:30	Recreational	15	------
21:33:45	21:48:45	Recreational	15	------
22:00:00	22:30:00	Commercial	30	------
12:00:00	18:30:00	Commercial, recreational	390	MTWThF

Table 2. Cumulative mass of wet sediment placed in each exposure chamber during the 28-d suspended and bedded sediment tests.

Treatment	Cumulative Mass of Wet Sediment (g)
Pool 10	218
Pool 12	172
Pool 14	157
Pool 25	197
Open River	189

Table 3. Location of Upper Mississippi River sample sites where grab samples of sediment were collected for bedded and suspended sediment testing.

Treatment	River Mile	Sample Site Coordinates		No. Grabs per Composite
		Latitude	Longitude	
Pool 10	625.8-626.5	NA	NA	4
Pool 12	561.5	42°19.23'-42°19.95'	90°24.34'-90°24.34'	5
Pool 14	510	41°44.54'-41°44.57'	90°17.04'-90°17.07'	7
Pool 25	246.7-248.0	39°03.87'*	90°41.01'*	5
Open River	150.7-151.6	38°12.93'-38°15.78'	90°20.85'-90°22.26'	6

NA - Loran-C navigation system non-functional at this site throughout sampling efforts

* - Loran-C navigation system non-functional at this site during a portion of sampling efforts

Table 4. Physical properties of surficial Upper Mississippi River (UMR) sediments used in bedded and suspended sediment toxicity tests.

UMR Sediment Treatment	Residue		Total Solids (%)	Bulk Density (g/cm^3)	Grain Size		
	Fixed (%)	Volatile (%)			>62 m (% sand)	5-62 m (% silt)	< 5 m (% clay)
Pool 10 (Methodist Lake)	90.0	10.0	29.4	1.19	1.3	54.5	44.2
Pool 12 (Wise Lake)	92.5	7.5	43.4	1.44	4.5	63.6	31.9
Pool 14 (Swan Slough)	96.2	3.8	59.8	1.76	4.3	60.7	35.0
Pool 25 (Batchtown Middle Pool)	94.0	6.0	37.9	1.39	1.5	60.0	38.5
Open River (Herculeneum)	94.4	5.6	47.3	1.52	18.1	50.6	31.3

Table 5. Chemical properties of surficial Upper Mississippi River (UMR) sediments used in bedded and suspended sediment toxicity tests.

UMR Sediment Treatment	Total Organic Carbon (%)	Metal Concentration (μg/g dry weight)					
		Al	As	Cd	Cu	Pb	Zn
Pool 10 (Methodist Lake)	2.9	13000	3.5	0.53	20	20	83
Pool 12 (Wise Lake)	2.1	13800	5.3	1.1	18	87	627
Pool 14 (Swan Slough)	1.7	6190	2.4	0.21	9.2	10	50
Pool 25 (Batchtown Middle Pool)	1.6	13300	4.0	0.33	17	17	68
Open River (Herculeneum)	1.5	11900	4.5	0.58	15	30	82

Table 6. Mass of wet sediment placed in replicate exposure chambers at the start of the 28-d suspended sediment test.

Treatment	Cumulative Mass of Wet Sediment (g)
Pool 10	43.51
Pool 12	34.33
Pool 14	31.47
Pool 25	39.40
Open River	37.70

Table 7. Mean water quality characteristics in treatment exposure chambers during the 28-d bedded and suspended sediment tests (range in parentheses).

Treatment	Dissolved Oxygen (mg/L)		pH		Temperature (°C)	
	Bedded	Suspended	Bedded	Suspended	Bedded	Suspended
Water Control	7.9 (7.5-8.3)	7.9 (7.1-9.5)	8.2 (8-8.5)	8.2 (7.1-8.6)	22.8 (22.3-23.3)	22.8 (22.4-23.3)
Pool 10	7.9 (7.3-8.3)	7.6 (7.1-9.0)	8.3 (8-8.5)	8.1 (7.2-8.6)	22.8 (21.6-23.4)	23.0 (22.4-23.3)
Pool 12	8.0 (7.4-8.3)	7.7 (7.2-9.0)	8.3 (8-8.5)	8.1 (7.2-8.6)	22.8 (22.2-23.4)	22.8 (22.5-23.2)
Pool 14	7.9 (7.2-8.3)	7.7 (7.1-9.1)	8.3 (8-8.5)	8.1 (7.2-8.6)	22.8 (21.6-23.4)	22.9 (22.5-23.3)
Pool 25	8.0 (7.4-8.3)	7.7 (7.1-9.5)	8.3 (8.1-8.5)	8.1 (7.2-8.6)	22.8 (21.9-23.3)	22.8 (22.4-23.2)
Open River	7.9 (7.4-8.4)	7.6 (7.0-8.6)	8.3 (8.1-8.4)	8.0 (7.0-8.6)	22.8 (21.4-23.4)	22.9 (22.4-23.4)

Table 7. Mean water quality characteristics in treatment exposure chambers during the 28-d bedded and suspended sediment tests (range in parentheses; continued).

Treatment	Total hardness (mg $CaCO_3$/L)		Total alkalinity (mg $CaCO_3$/L)		Conductivity (µS/cm)	
	Bedded	Suspended	Bedded	Suspended	Bedded	Suspended
Water Control	196 (186-207)	160 (150-166)	138 (129-144)	119 (107-136)	447 (433-460)	422 (160-600)
Pool 10	196 (185-208)	161 (152-168)	145 (136-163)	119 (106-130)	443 (429-456)	418 (160-580)
Pool 12	196 (185-208)	164 (150-168)	149 (139-163)	121 (108-130)	444 (430-459)	419 (160-600)
Pool 14	195 (180-206)	161 (148-166)	148 (146-163)	119 (106-136)	445 (428-461)	417 (165-590)
Pool 25	196 (180-208)	159 (150-166)	147 (146-161)	118 (101-134)	443 (427-462)	413 (165-580)
Open River	196 (180-208)	162 (149-166)	142 (148-160)	119 (105-138)	443 (427-459)	415 (170-590)

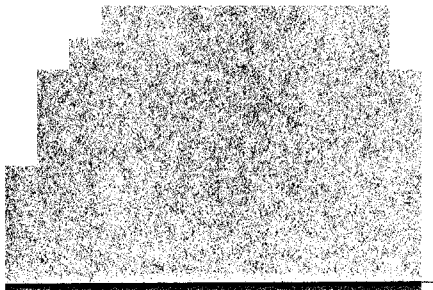


Table 7. Mean water quality characteristics in treatment exposure chambers during the 28-d bedded and suspended sediment tests (range in parentheses; continued).

Treatment	Total non-filterable residue (mg/L)				Turbidity (NTU)		Ammonia (mg/L)	
	Bedded	Suspended			Bedded	Suspended	Bedded	Suspended
		R*	S†	T§				
Water Control	5 (0-15)	8 (0-25)	6 (0-16)	7	3.9 (2.1-8.7)	4.1 (2.0-9.0)	0.30 (0.09-0.69)	0.70 (0.1-1.4)
Pool 10	9 (0-22)	217 (106-290)	55 (2-141)	141	11.0 (2.6-27)	107 (60-136)	0.30 (0.1-0.52)	0.13 (0.1-0.26)
Pool 12	10 (0-39)	250 (102-374)	43 (9-105)	152	10.4 (1.9-33)	114 (76-157)	0.30 (0.1-0.65)	0.12 (0.1-0.29)
Pool 14	3 (0-13)	233 (142-380)	28 (5-77)	136	2.8 (1.9-5.8)	68 (57-96)	0.30 (0.1-0.65)	0.16 (0.1-0.4)
Pool 25	10 (0-30)	215 (87-362)	40 (11-126)	132	9.2 (4.6-26)	119 (76-162)	0.30 (0.13-0.52)	0.15 (0.1-0.4)
Open River	12 (0-35)	204 (100-273)	28 (4-80)	121	13.1 (2.4-33)	94 (51-112)	0.20 (0.1-0.45)	0.24 (0.1-0.96)

*concentration while chambers were rotating

†concentration while chambers were stationary

§time-weighted concentration (i.e., the product of the mean TNFR value during daily periods of chamber rotation/non-rotation and relative duration of these events over the 28-d test)

Table 8. Mean concentration of metals in unfiltered (U) and filtered (F) water samples collected from bedded sediment test treatment exposure chambers (n=3) before the introduction of fish on day 0.

		Concentration (µg/L)							Concentration (µg/L)				
Treatment	Sample	As	Cd	Cu	Pb	Zn	Treatment	Sample	As	Cd	Cu	Pb	Zn
Water control	U	0.13	0.02	2.9	1.0	8.0	Pool 14	U	0.44	0.03	1.3	0.2	8.9
	F	0.3	0.00	1.3	0.0	9.4		F	0.37	0.03	1.3	0.8	4.3
	U-F	< 0	0.02	1.6	1.0	< 0		U-F	0.07	0.00	0.0	< 0	4.6
Pool 10	U	0.45	0.00	0.3	0.0	8.7	Pool 25	U	0.48	0.00	1.5	0.3	6.2
	F	0.3	0.08	1.4	0.0	4.7		F	0.74	0.12	1.0	0.0	4.7
	U-F	0.15	< 0	< 0	0.0	4.0		U-F	< 0	< 0	0.5	0.3	1.5
Pool 12	U	0.54	0.05	2.0	0.8	8.2	Open river	U	0.57	0.00	0.6	0.0	6.0
	F	0.72	0.02	2.5	0.3	7.2		F	0.68	0.03	1.9	0.9	5.3
	U-F	< 0	0.03	< 0	0.5	1.0		U-F	< 0	< 0	< 0	< 0	0.7

Table 9. Mean concentration of metals in unfiltered (U) and filtered (F) water samples collected from suspended sediment test treatment exposure chambers (n=3) before the introduction of fish on day 0.

		Concentration (µg/L)							Concentration (µg/L)				
Treatment	Sample	As	Cd	Cu	Pb	Zn	Treatment	Sample	As	Cd	Cu	Pb	Zn
Water control	U	0.53	0.10	2.0	0.2	4.3	Pool 14	U	0.44	0.09	2.3	1.6	21.3
	F	0.50	0.03	2.3	0.3	4.7		F	0.36	0.02	0.7	0.0	5.3
	U-F	0.03	0.07	< 0	< 0	< 0		U-F	0.08	0.07	1.6	1.6	16.0
Pool 10	U	0.76	0.45	3.3	2.2	17.0	Pool 25	U	2.02	0.20	6.3	6.6	29.0
	F	0.34	0.05	0.3	0.0	0.7		F	0.73	0.09	1.9	0.0	4.5
	U-F	0.42	0.40	3.0	2.2	16.3		U-F	1.29	0.11	4.4	6.6	24.5
Pool 12	U	1.63	0.39	3.8	21.9	156	Open river	U	1.57	0.22	5.4	7.2	25.0
	F	0.69	0.06	0.3	0.3	4.8		F	1.00	0.20	3.1	0.3	1.0
	U-F	0.94	0.33	3.5	21.6	151		U-F	0.57	0.02	2.3	6.9	24.0

Table 10. Mean recovery of metals from certified reference materials (sample size in parentheses).

Metal	Mean Percent Recovery			
	Oyster Tissue[§]	Buffalo River Sediment[§§]	Bovine Liver[§§§]	Water[†]
Aluminum	----	21 (1)	----	----
Arsenic	94[*] (7)	73 (1)	----	87[*] (4)
Cadmium	97[*] (7)	107 (1)	----	90[*] (4)
Copper	98[*] (7)	95[*] (1)	----	95[*] (4)
Lead	99[*] (10)	93[*] (1)	59 (3)	104[*] (4)
Zinc	97[*] (10)	91 (1)	92 (3)	97[*] (4)

[§]National Institute of Standards and Technology Standard Reference Material 1566a.
[§§]National Institute of Standards and Technology Standard Reference Material 2704.
[§§§]National Institute of Standards and Technology Standard Reference Material 1577a.
[†]Environmental Resource Associates WasteWatR™ trace metals standard (lot #01032).
[*]Within the 95% confidence interval of the certified value.

Table 11. Mean recovery of metals from spiked samples (sample size in parentheses).

Metal	Mean Percent Recovery				
	Blood	Feed	Sediment	Tissue	Water
Arsenic	----	88 (1)	102 (1)	91 (10)	94 (8)
Cadmium	----	82 (1)	92 (1)	92 (10)	83 (8)
Copper	----	89 (1)	96 (1)	93 (10)	97 (8)
Lead	97 (3)	120 (1)	97 (1)	99 (15)	101 (8)
Zinc	97 (3)	96 (1)	97 (1)	97 (15)	99 (7)

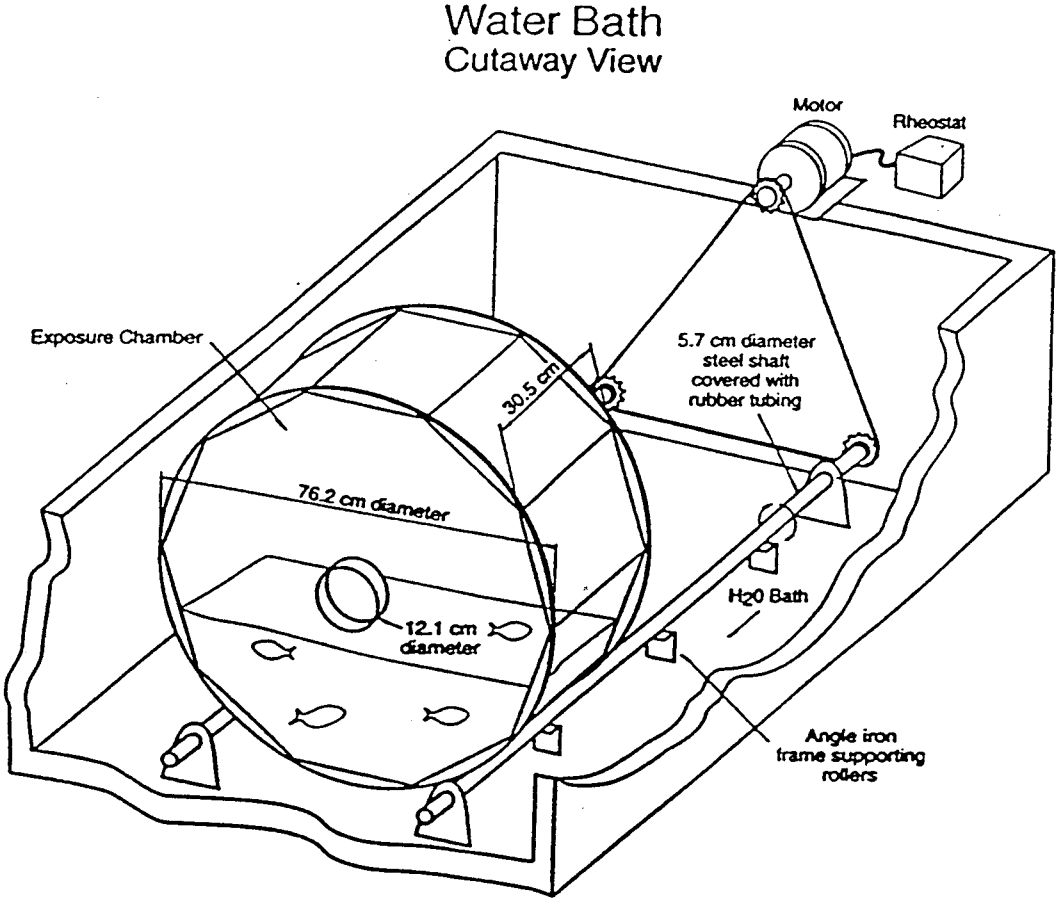
Table 12. Mean percent difference in metal concentration for duplicate sample analyses (number in parentheses represents samples above the detection limit).

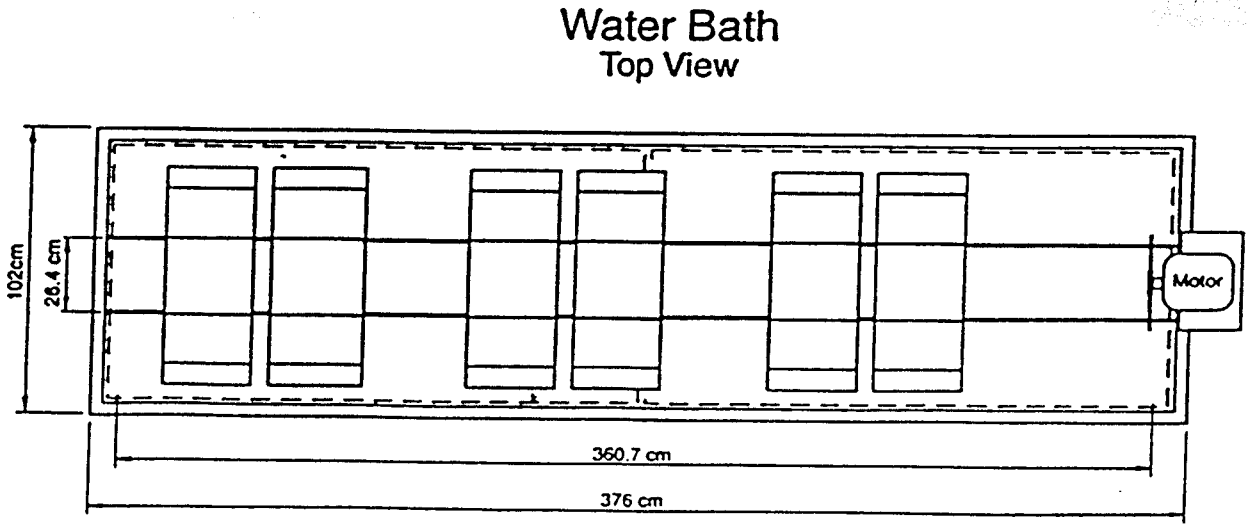
Metal	Mean Percent Difference				
	Blood	Feed	Sediment	Tissue	Water
Arsenic	----	0.(1)	7.8 (1)	3.5 (10)	29[†] (6)
Cadmium	----	0 (1)	0.91 (1)	BDL[*] (10)	0[‡] (1)
Copper	----	1.4 (1)	5.7 (1)	4.0 (10)	60[§] (6)
Lead	BDL[*] (3)	11 (1)	2.3 (1)	41[†] (7)	BDL[*] (8)
Zinc	0.17 (3)	1.8 (1)	1.9 (1)	4.2 (15)	28[§] (6)

[*]Results of all duplicate analyses were below the detection limit.
[†]Results of 8 duplicate analyses were below the detection limit.
[‡]Results of 7 duplicate analyses were below the detection limit.
[§]Results of 2 duplicate analyses were below the detection limit.

FIGURES

Figure 1. Diagrams of exposure chambers in a water bath. The sediment suspension system consisted of glass exposure chambers that revolved in a water bath on motor-driven supports to suspend the sediments.





Each water bath was capable of containing seven 30.5 cm wide exposure chambers

Figure 2. Arsenic (As) concentrations (ng/g, dry weight) in whole green sunfish after 28-d bedded and suspended tests of Water Control (WC) and sediment from treatment sites Pool 10 (P10), Pool 12 (P12), Pool 14 (P14), Pool 25 (P25), and Open River (OR). Lines are standard error and sample size is nine fish for each site of each test.

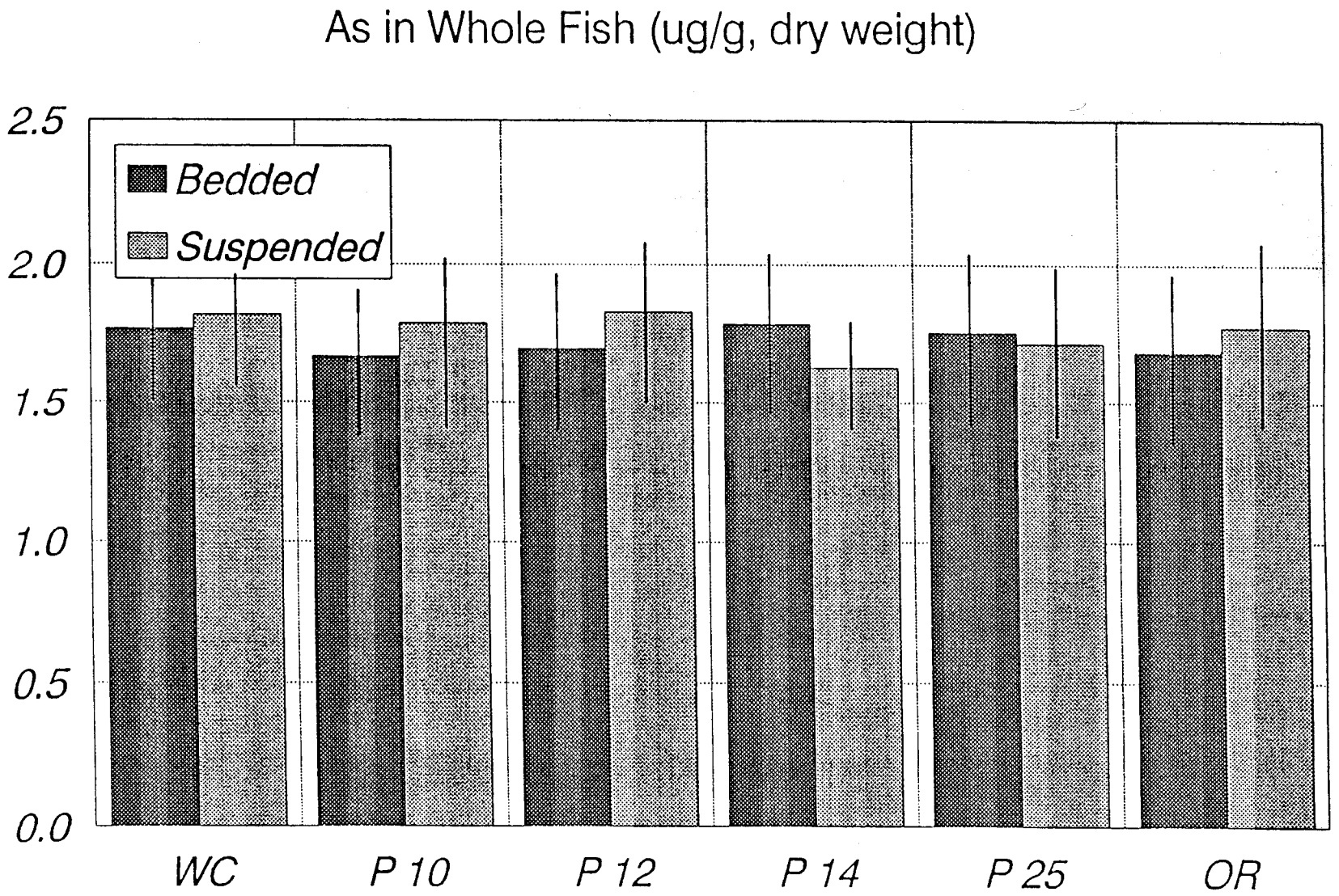


Figure 3. Cadmium (Cd) concentrations (ng/g, dry weight) in whole green sunfish after 28-d bedded and suspended tests of Water Control (WC) and sediment from treatment sites Pool 10 (P10), Pool 12 (P12), Pool 14 (P14), Pool 25 (P25), and Open River (OR). Lines are standard error and sample size is nine fish for each site of each test.

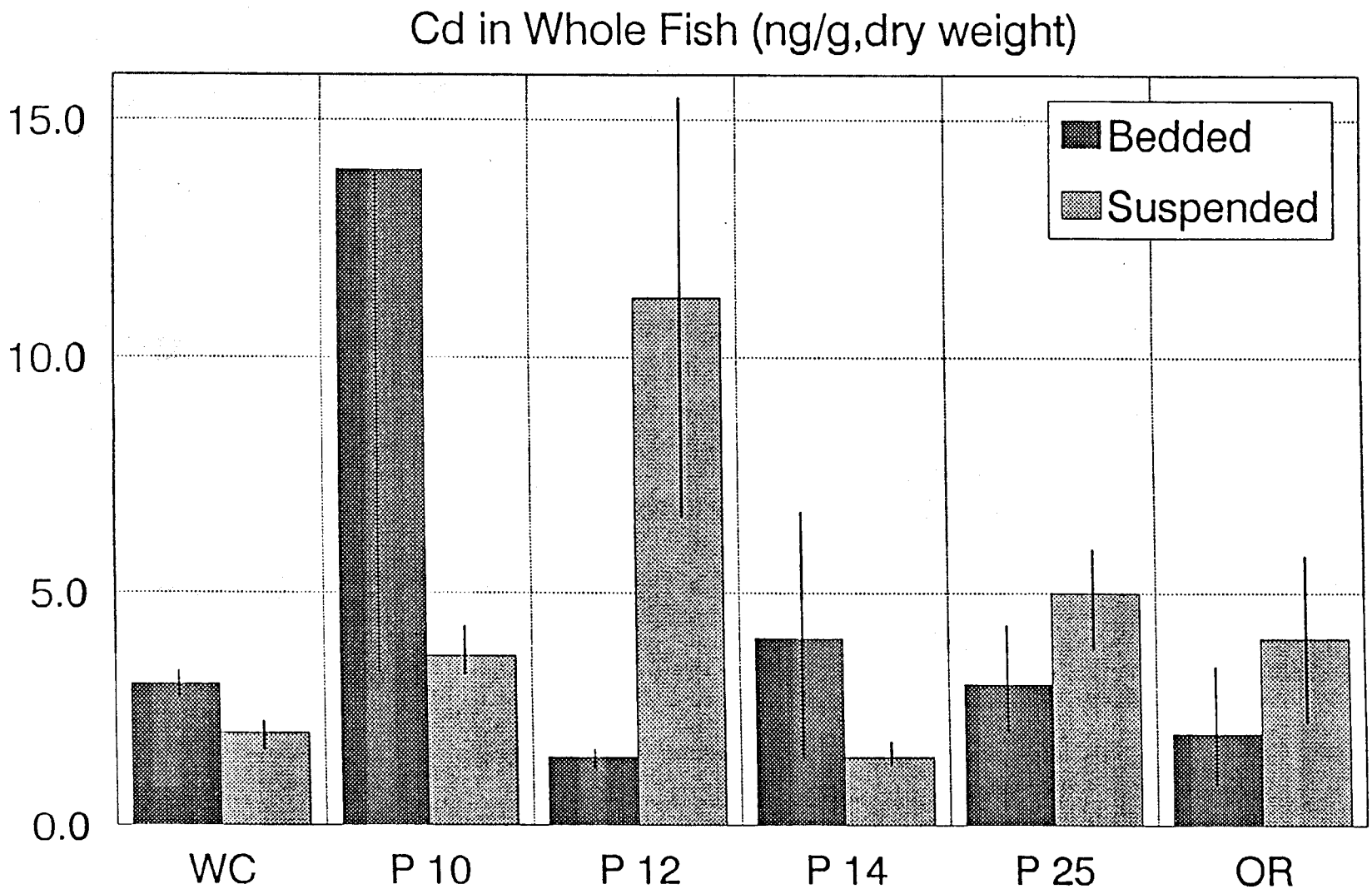


Figure 4. Copper (Cu) concentrations (ug/g, dry weight) in whole green sunfish after 28-d bedded and suspended tests of Water Control (WC) and sediment from treatment sites Pool 10 (P10), Pool 12 (P12), Pool 14 (P14), Pool 25 (P25), and Open River (OR). Lines are standard error and sample size is nine fish for each site of each test.

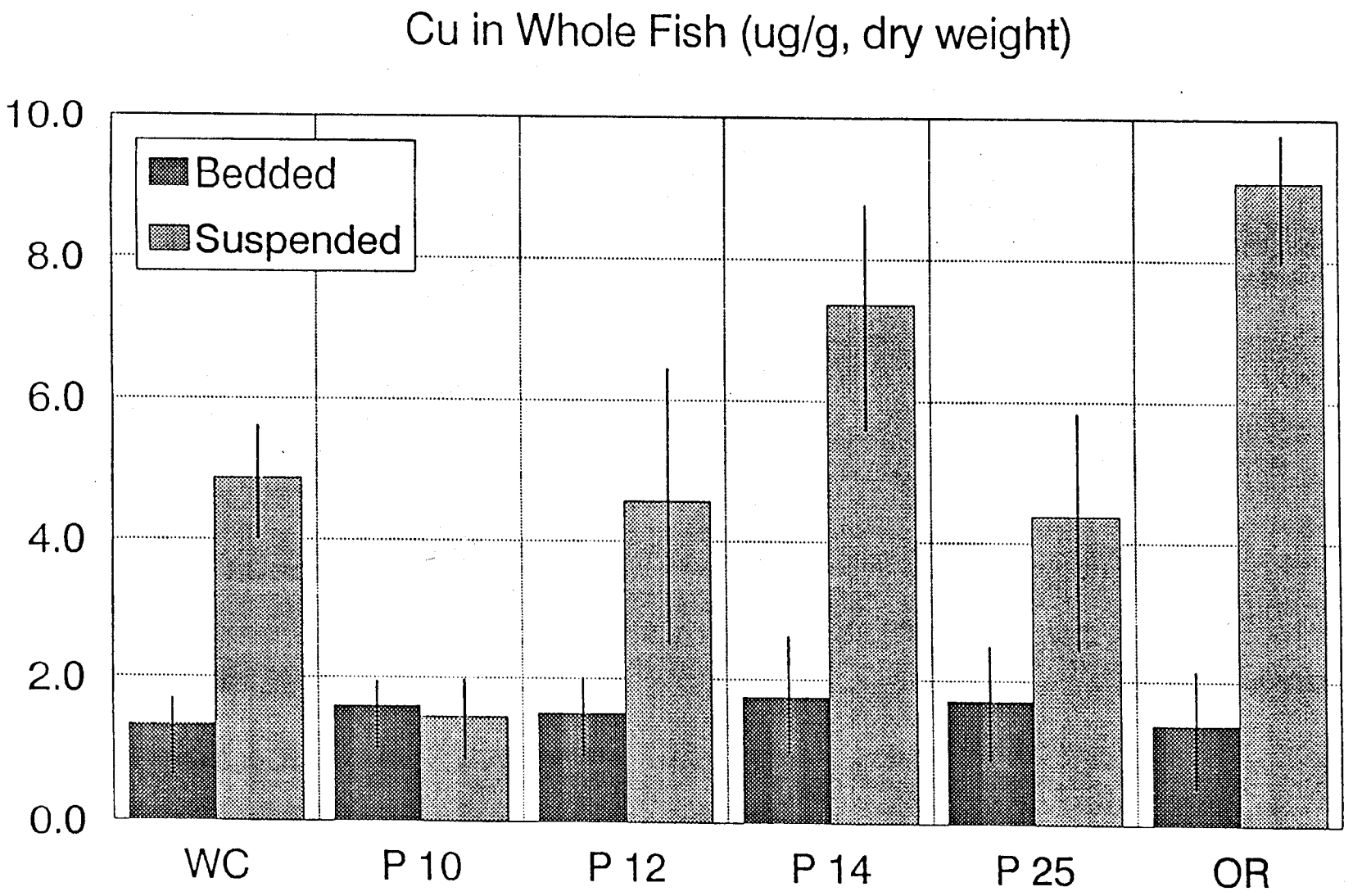


Figure 5. Lead (Pb) concentrations (ug/g, dry weight) in whole green sunfish after 28-d bedded and suspended tests of Water Control (WC) and sediment from treatment sites Pool 10 (P10), Pool 12 (P12), Pool 14 (P14), Pool 25 (P25), and Open River (OR). Lines are standard error and sample size is nine fish for each site of each test.

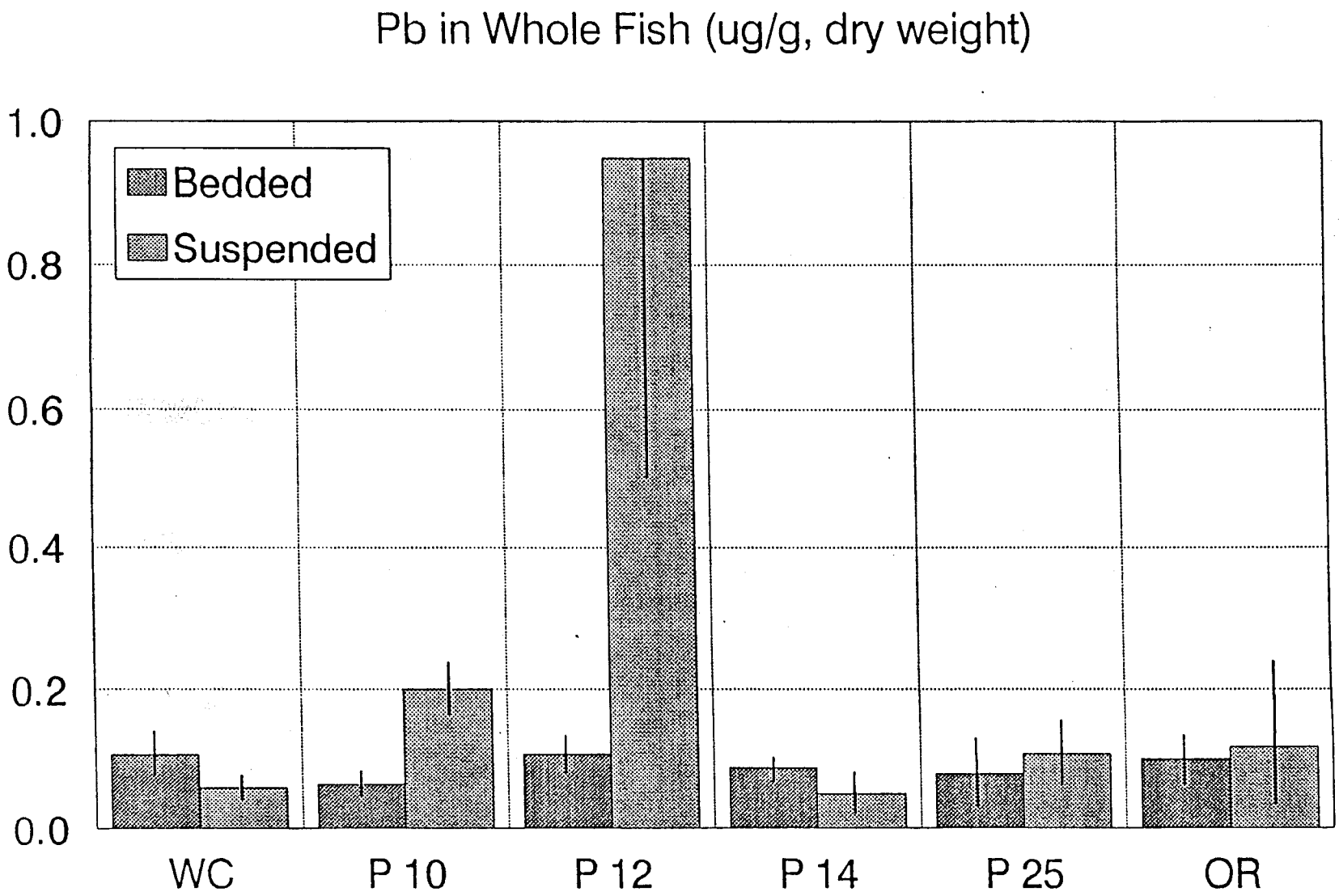


Figure 6. Zinc (Zn) concentrations (ug/g, dry weight) in whole green sunfish after 28-d bedded and suspended tests of Water Control (WC) and sediment from treatment sites Pool 10 (P10), Pool 12 (P12), Pool 14 (P14), Pool 25 (P25), and Open River (OR). Lines are standard error and sample size is nine fish for each site of each test.

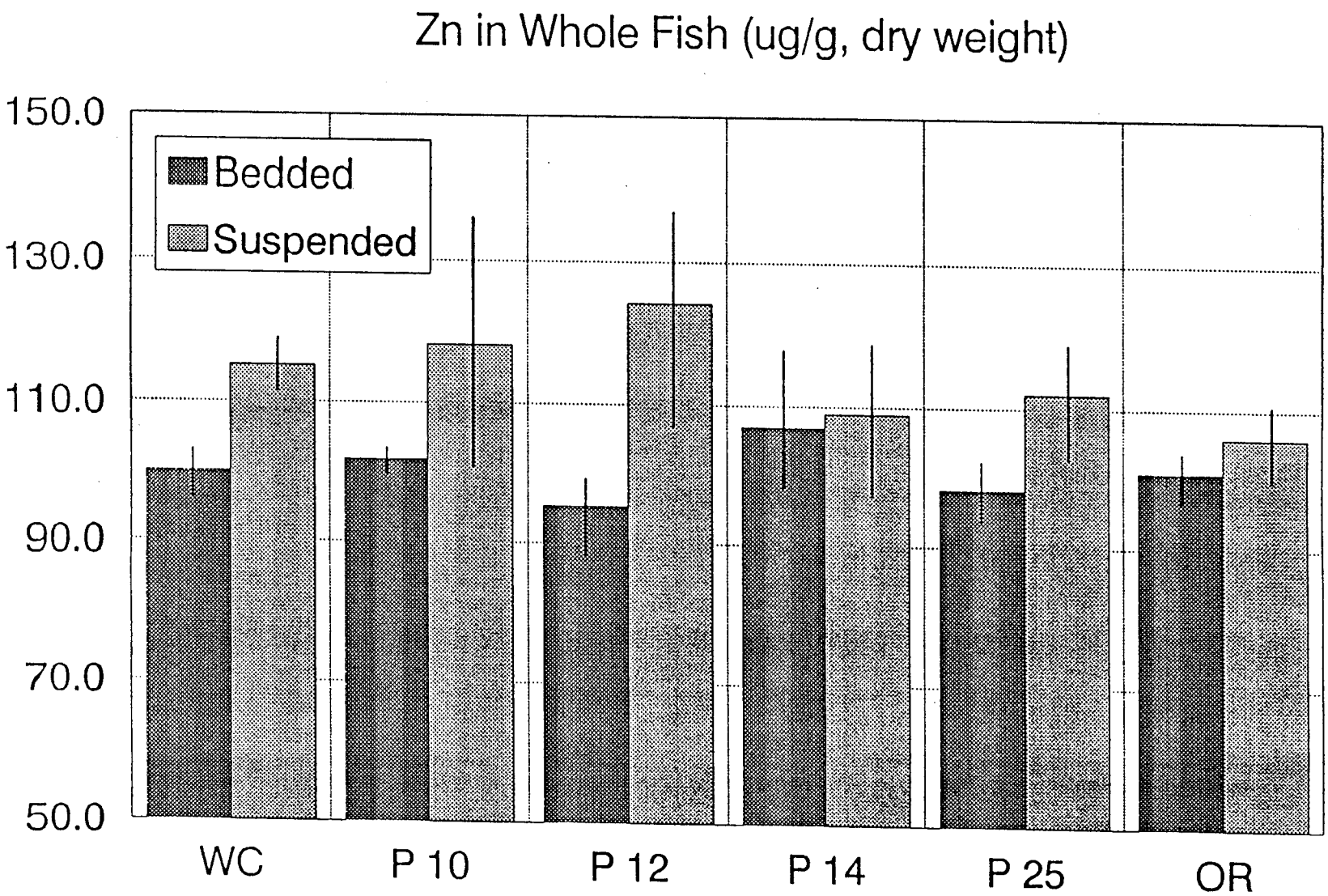


Figure 7. Blood Lead (ng/ml, wet weight) in green sunfish after 28-d bedded and suspended tests of Water Control (WC) and sediment from treatment sites Pool 10 (P10), Pool 12 (P12), Pool 14 (P14), Pool 25 (P25), and Open River (OR). Lines are standard error and sample size is three except for sample size of two as indicated by n=2.

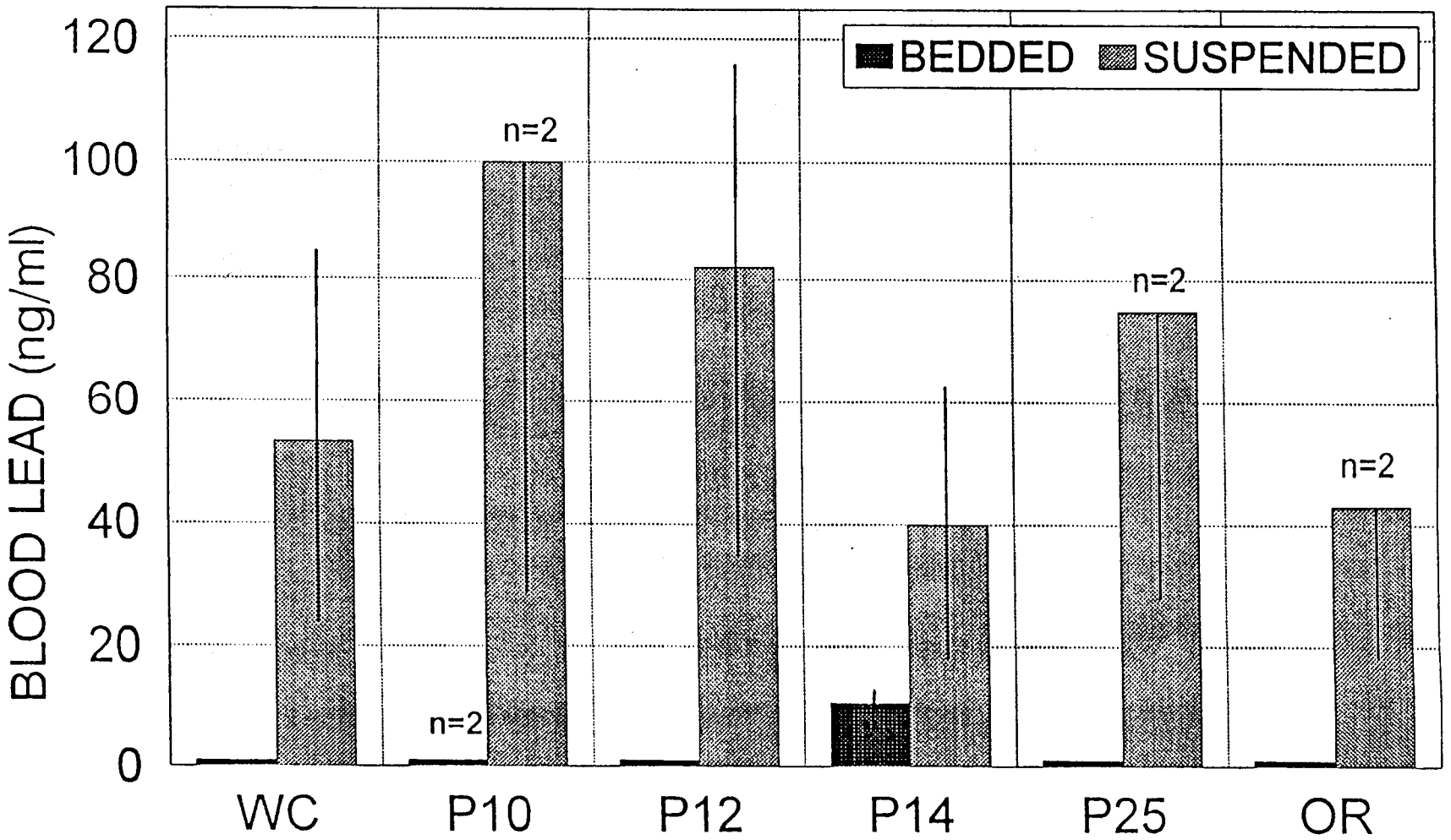


Figure 8. Blood Zinc (ug/ml, wet weight) in green sunfish after 28-d bedded and suspended tests of Water Control (WC) and sediment from treatment sites Pool 10 (P10), Pool 12 (P12), Pool 14 (P14), Pool 25 (P25), and Open River (OR). Lines are standard error and sample size is three except for sample size of two as indicated by n=2.

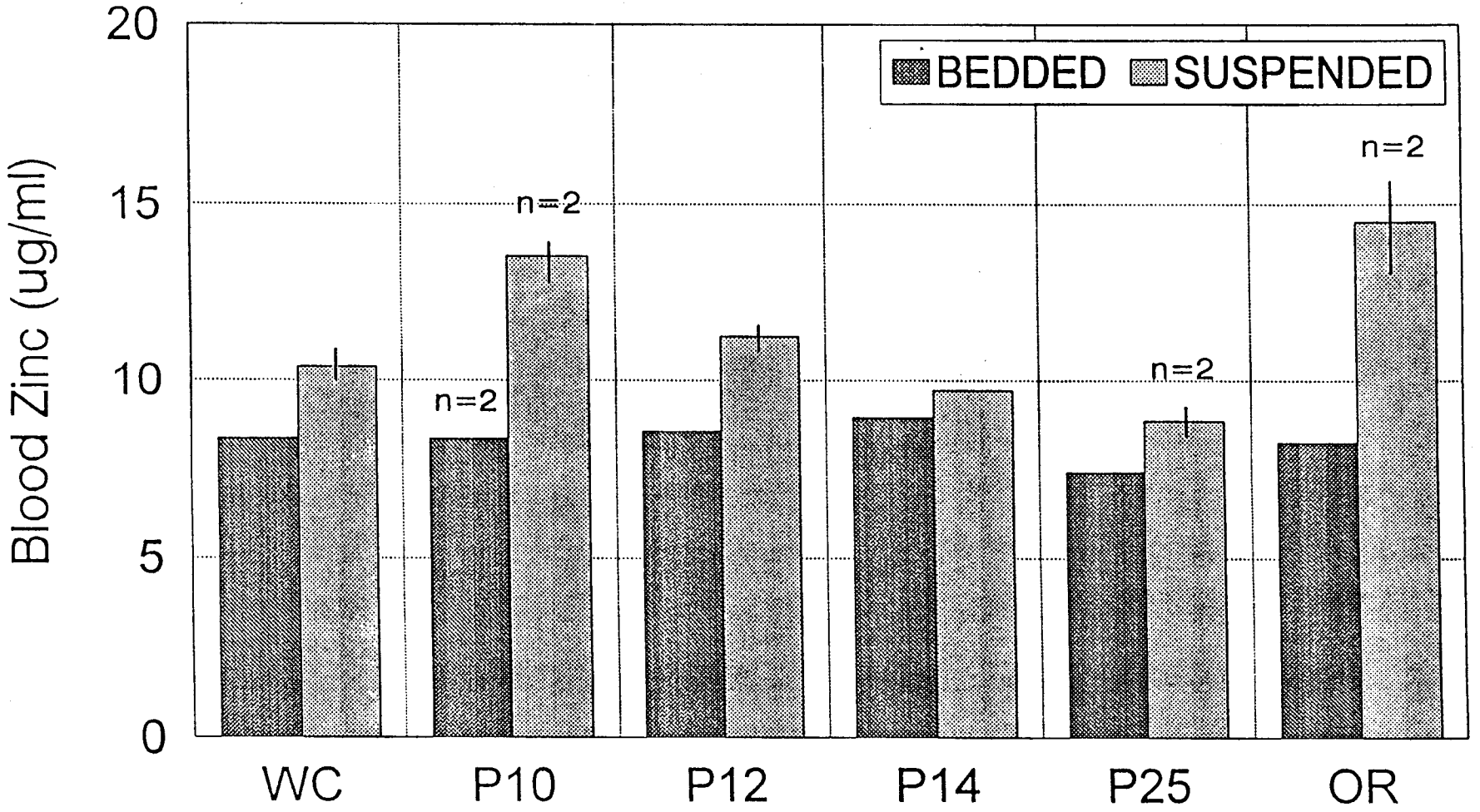


Figure 9. -aminolevulinic acid dehydratase (ALA-D) activity (nmoles PBG/g/hr) in green sunfish after 28-d bedded and suspended tests of Water Control (WC) and sediment from treatment sites Pool 10 (P10), Pool 12 (P12), Pool 14 (P14), Pool 25 (P25), and Open River (OR). Lines are standard error and sample size is nine for each site of each test.

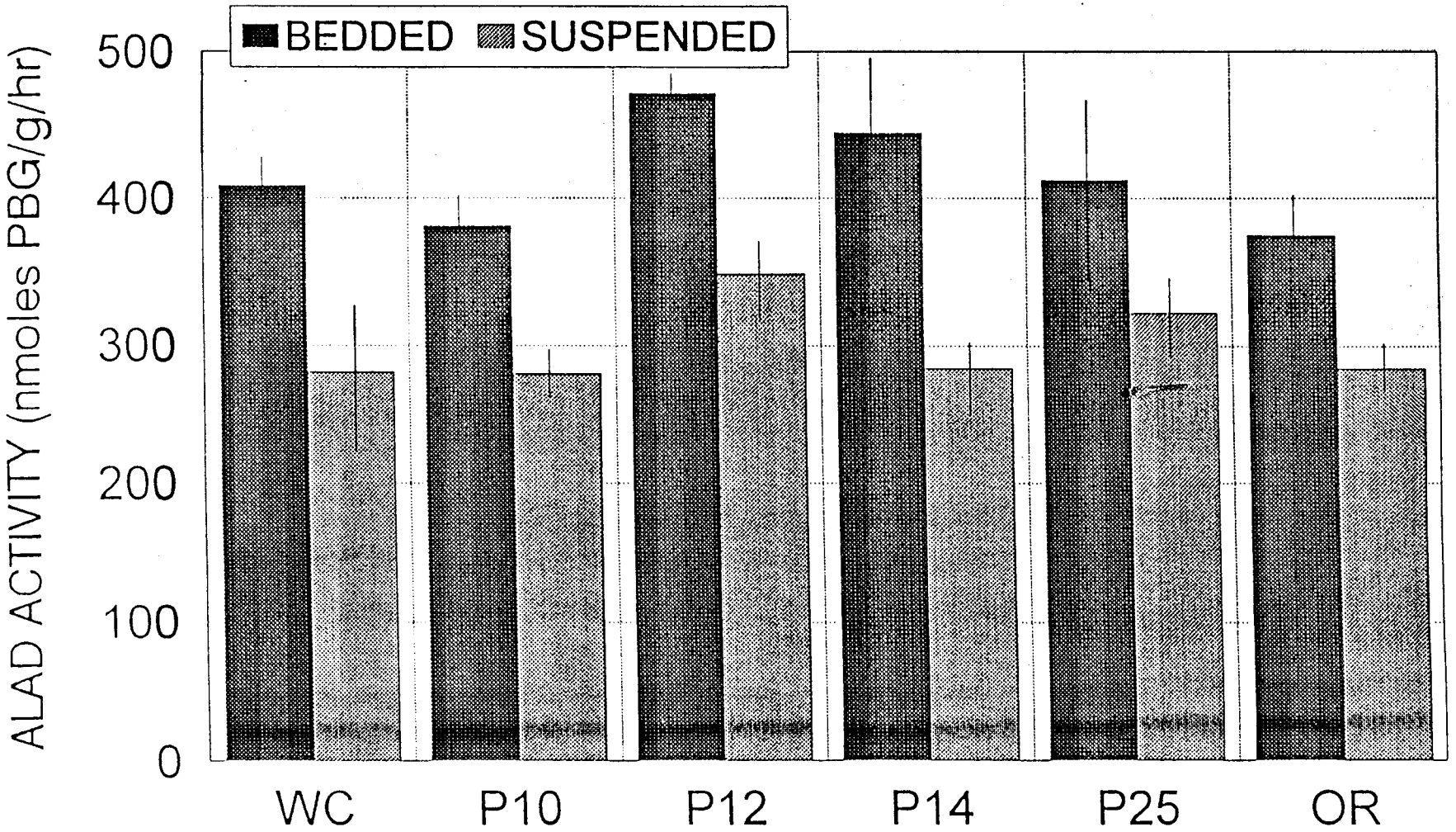
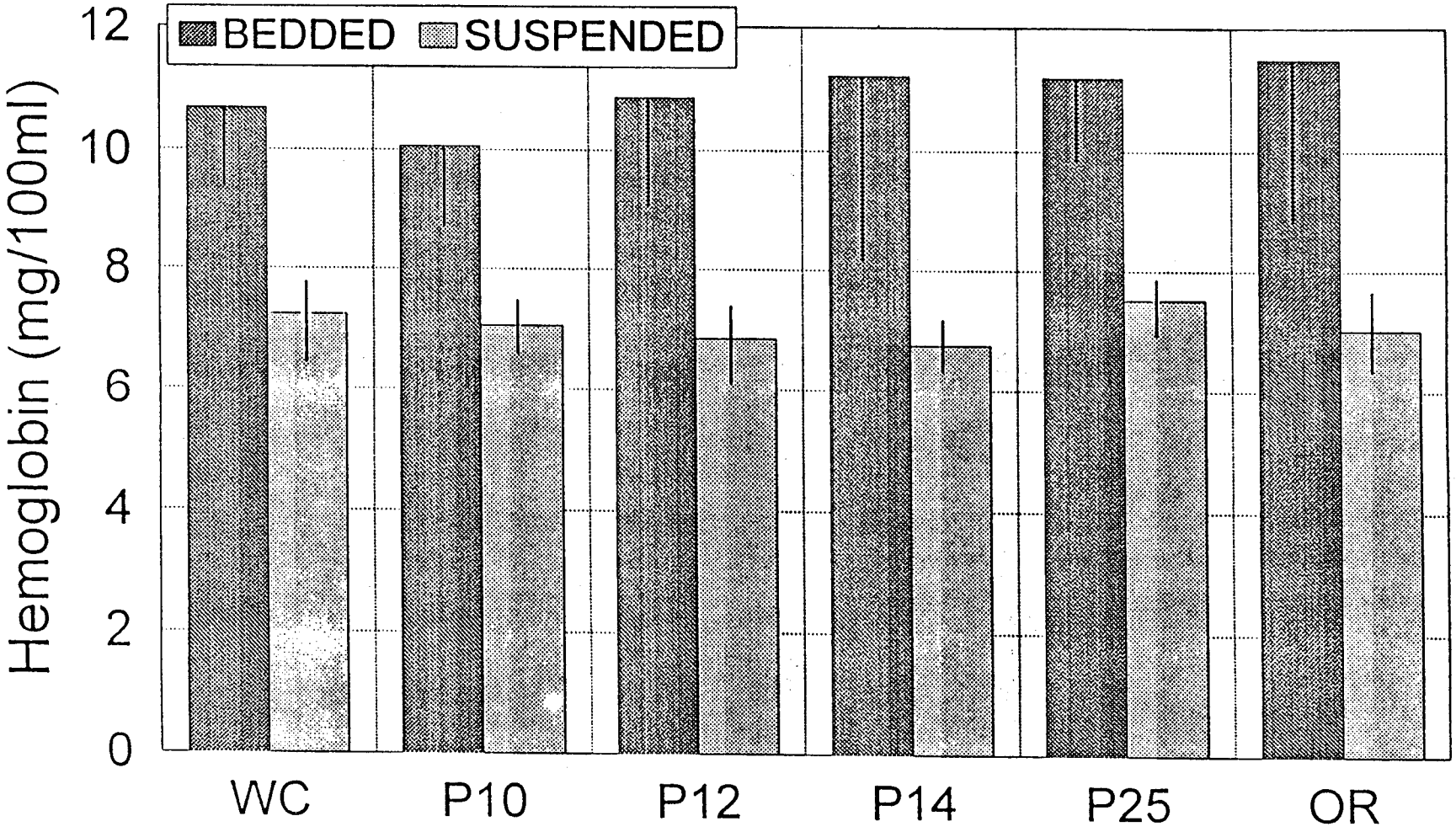


Figure 10. Hemoglobin concentrations (mg/100 ml) in green sunfish after 28-d bedded and suspended tests of Water Control (WC) and sediment from treatment sites Pool 10 (P10), Pool 12 (P12), Pool 14 (P14), Pool 25 (P25), and Open River (OR). Lines are standard error and sample size is nine for each site of each test.



Appendix A
Test Matrices, Variables, and Analytical Methods

I. SEDIMENT

Variable	Method
Bulk density	Gravi-volumetric (Steingraeber 1992)
Residues	Gravimetric-ignition (Plumb 1981)
Total solids	Gravimetric-ignition (Plumb 1981)
Total organic carbon	Gravimetric-ignition (Plumb 1981)
Grain size	Sieve-pipet (Guy 1969; Plumb 1981)
Arsenic	Digestion*, hydride generation graphite furnace atomic absorption spectroscopy
Aluminum and Zinc	Nitric-perchloric-hydrochloric acid digestion**, inductively coupled plasma analysis††
Cadmium, Copper, and Lead	Nitric acid digestion***, graphite furnace atomic absorption spectroscopy†††

II. FISH TISSUE & FEED

Variable	Method
Arsenic	Digestion*, hydride generation graphite furnace atomic absorption spectroscopy
Cadmium, Copper, and Lead	Nitric acid digestion***, graphite furnace atomic absorption spectroscopy†††
Zinc	Nitric-perchloric-hydrochloric acid digestion**, inductively coupled plasma analysis††

III. FISH BLOOD

Variable	Method
ALA-D	Schmitt et. al. 1984
Glucose	Sigma Chemical Co., St. Louis, MO
Chloride	Buchler Chloridometer, AgCl Method, Buchler Instruments
Hemoglobin	Larson and Snieszko, 1961. Sigma Chemical Co., St. Louis, MO
Lead	Nitric acid digestion[***], graphite furnace atomic absorption spectroscopy[†††]
Zinc	Nitric-perchloric-hydrochloric acid digestion[**], inductively coupled plasma analysis[††]

IV. WATER

Variable	Method
pH	Electrometric (150.1[§])
Temperature	Thermometric (170.1[§])
Dissolved oxygen	Membrane electrode (360.1[§])
Total non-filterable residue	Gravimetric, 105°C (160.2[§])
Alkalinity	Titrimetric, pH 4.5 (310.1[§])
Hardness	Titrimetric EDTA (130.2[§])
Turbidity	Nephelometric (180.1[§])
Conductivity	Specific conductance @ 25°C (120.1[§])
Ammonia, unionized	Ion selective electrode (350.3[§])

Appendix A (continued).

Test Matrices, Variables, and Analytical Methods

IV. WATER (Continued)

Variable	Method
Arsenic	Digestion[*], hydride generation graphite furnace atomic absorption spectroscopy
Cadmium, Copper, and Lead	Nitric acid digestion[***], graphite furnace atomic absorption spectroscopy[†††]
Zinc	Nitric-perchloric-hydrochloric acid digestion[**], inductively coupled plasma analysis[††]

[*] Environmental Trace Substance Research Center method #09
[**]Environmental Trace Substance Research Center method #03
[***]Environmental Trace Substance Research Center method #13
[††]Environmental Trace Substance Research Center method #04
[†††]Environmental Trace Substance Research Center method #14
[§]U.S. Environmental Protection Agency 1981

APPENDIX B

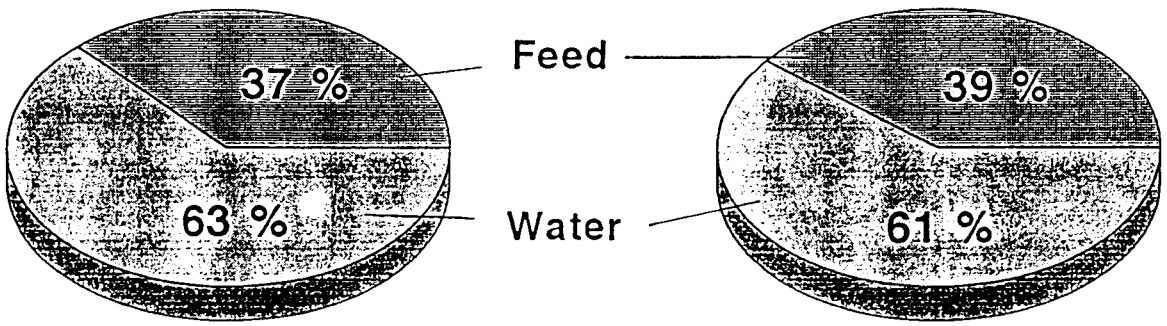
Contributions of Metals from Sediments, Feed, and Test Water

As (ug) present in:

Bedded Sediment Tests

Suspended Sediment Tests

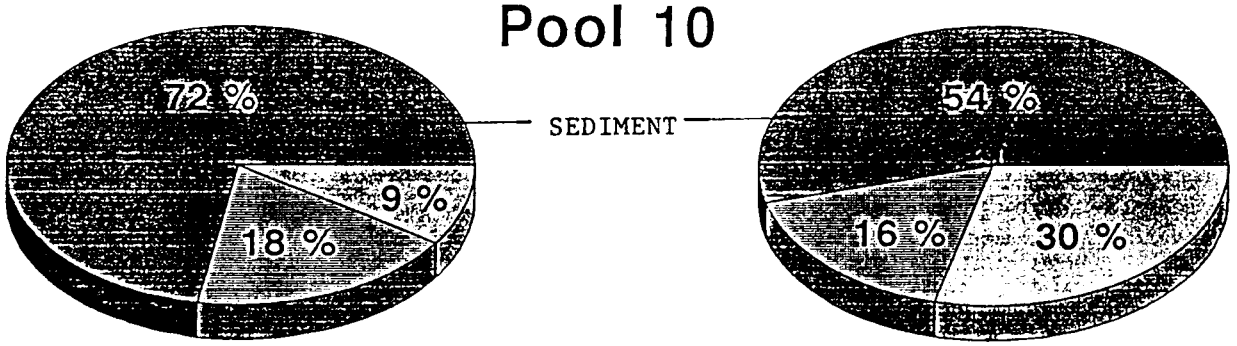
Water Control (no sediment)



Total metal present: 46 ug As

Total metal present: 196 ug As

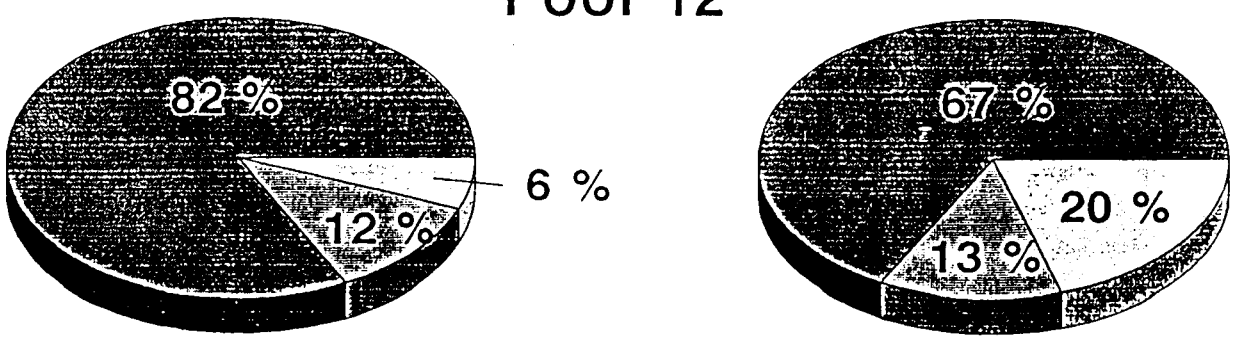
Pool 10



Total metal present: 310 ug As

Total metal present: 413 ug As

Pool 12



Total metal present: 480 ug As

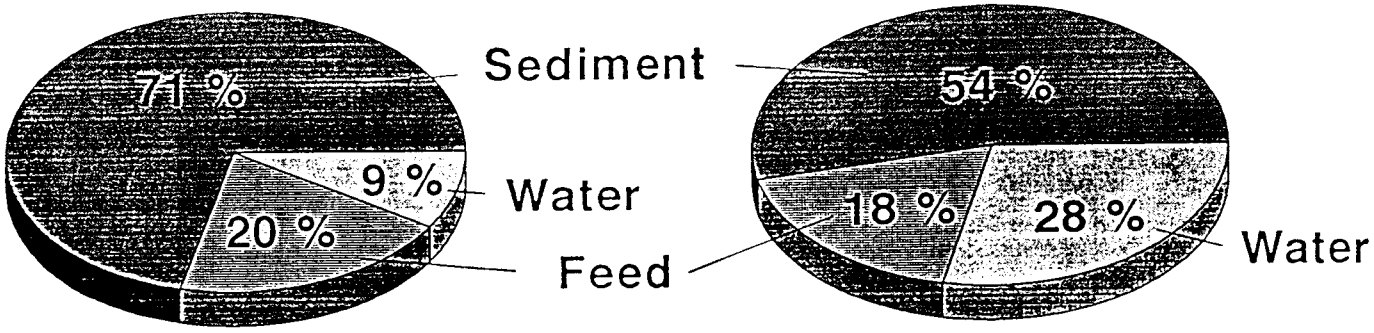
Total metal present: 587 ug As

As (ug) present in:

Bedded Sediment Tests

Suspended Sediment Tests

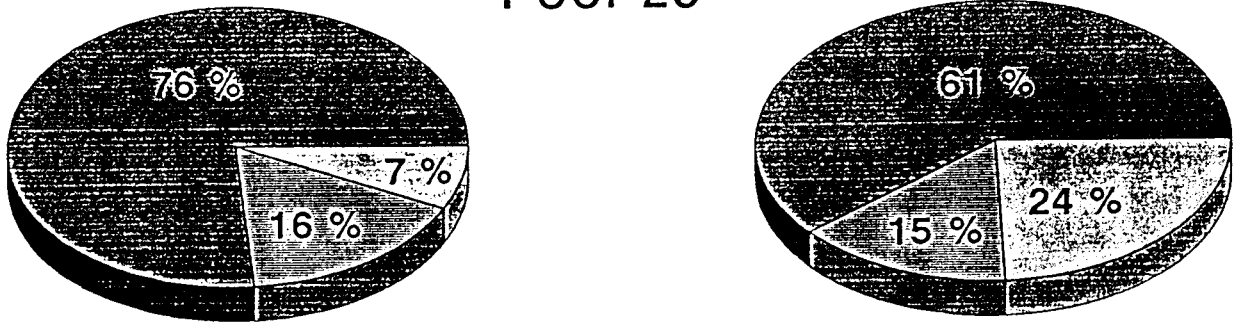
Pool 14



Total metal present: 317 ug As

Total metal present: 421 ug As

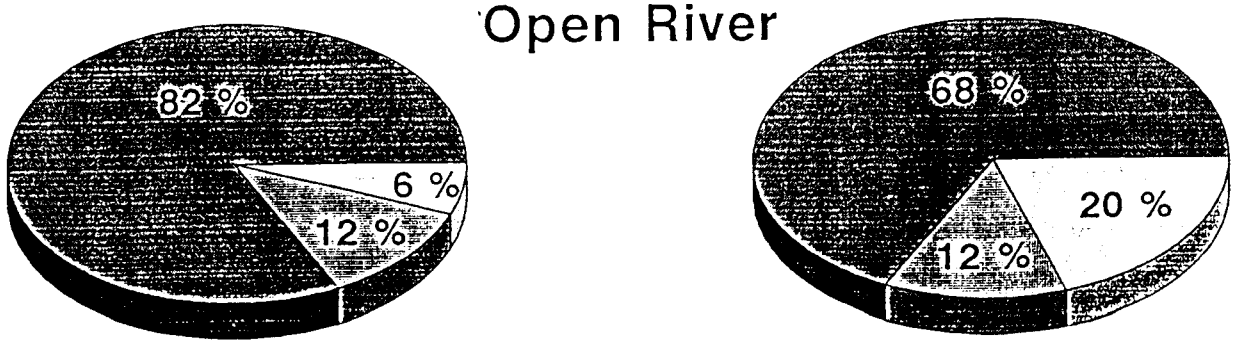
Pool 25



Total metal present: 391 ug As

Total metal present: 490 ug As

Open River



Total metal present: 487 ug As

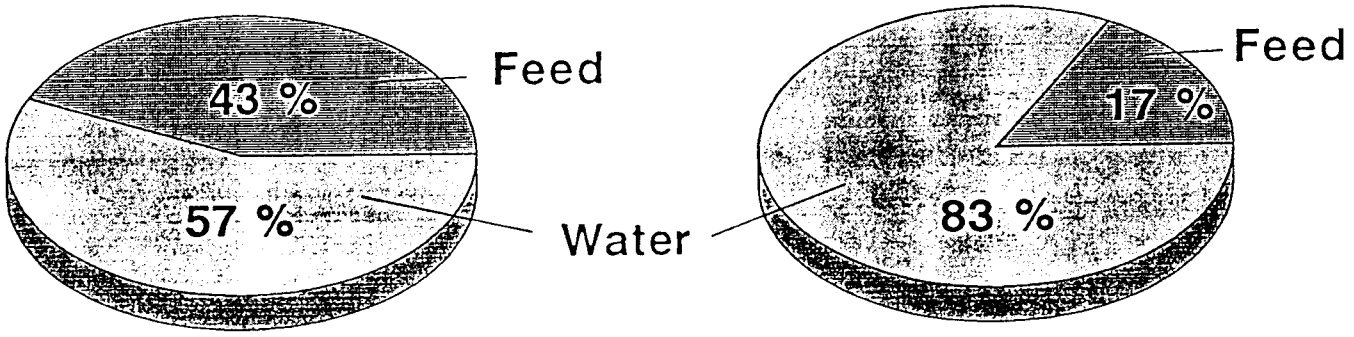
Total metal present: 594 ug As

Cd (ug) present in:

Bedded Sediment Tests

Suspended Sediment Tests

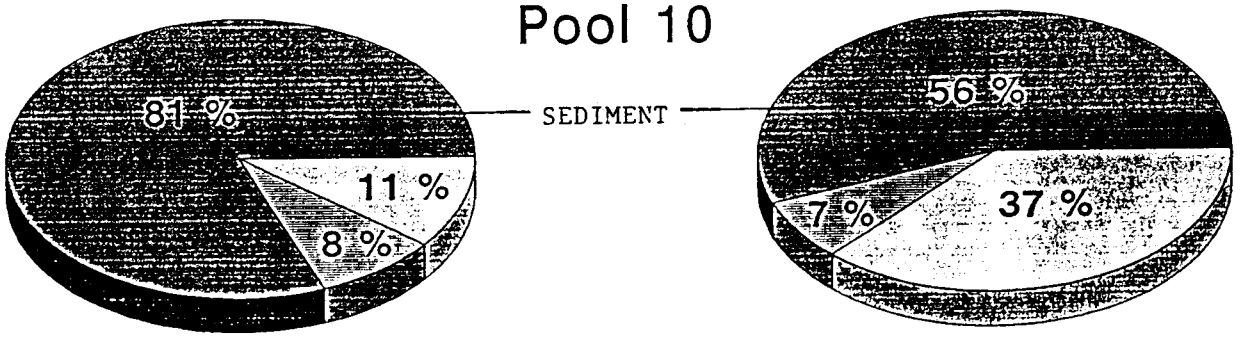
Water Control (no sediment)



Total metal present: 8 ug Cd

Total metal present: 27 ug Cd

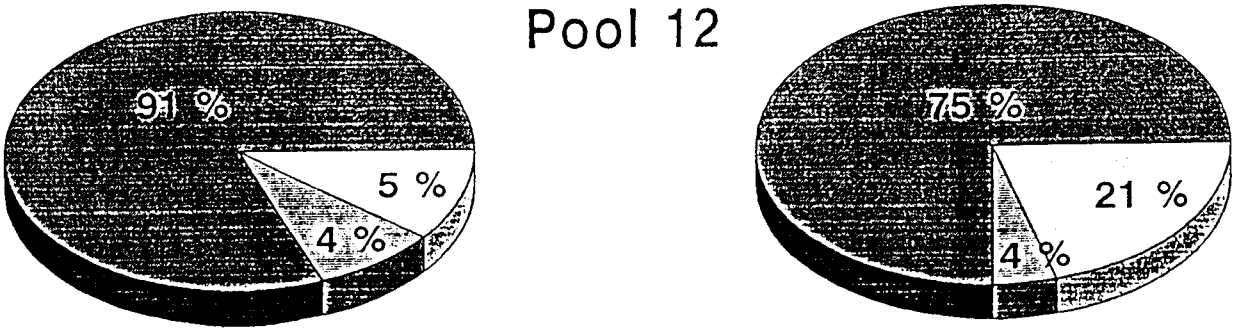
Pool 10



Total metal present: 42 ug Cd

Total metal present: 61 ug Cd

Pool 12



Total metal present: 89 ug Cd

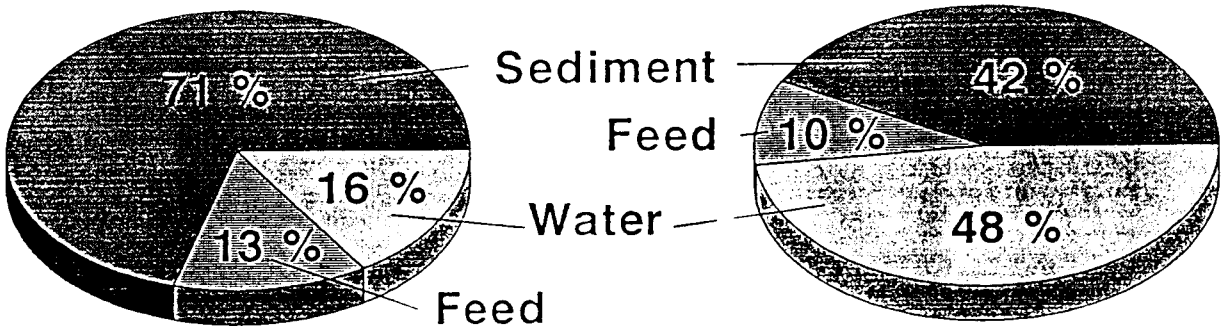
Total metal present: 108 ug Cd

Cd (ug) present in:

Bedded Sediment Tests

Suspended Sediment Tests

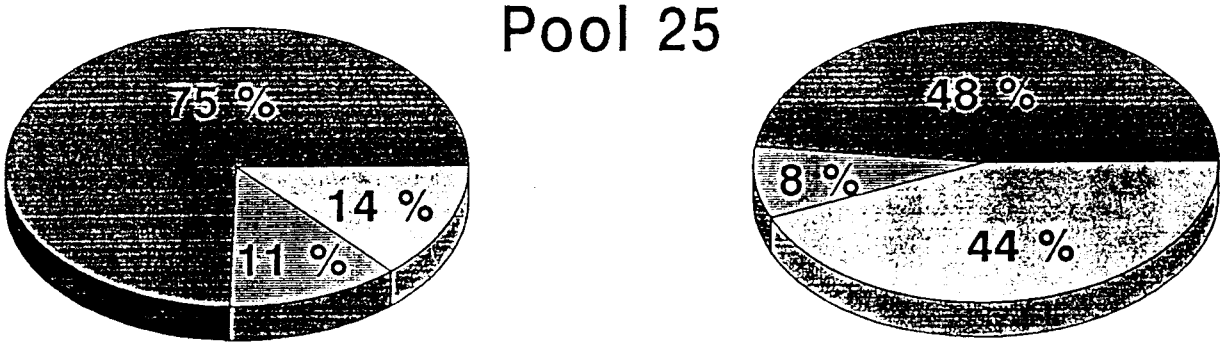
Pool 14



Total metal present: 28 ug Cd

Total metal present: 47 ug Cd

Pool 25



Total metal present: 33 ug Cd

Total metal present: 52 ug Cd

Open River

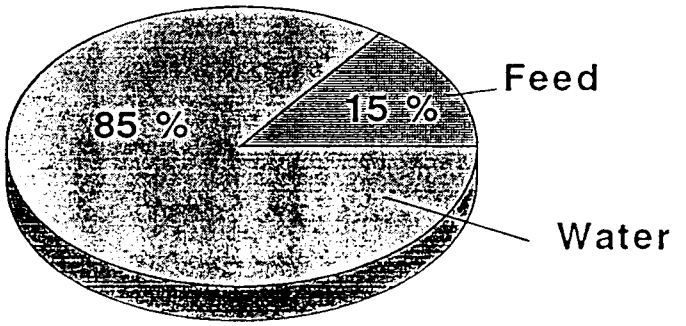
Total metal present: 60 ug Cd

Total metal present: 79 ug Cd

Cu (ug) present in:

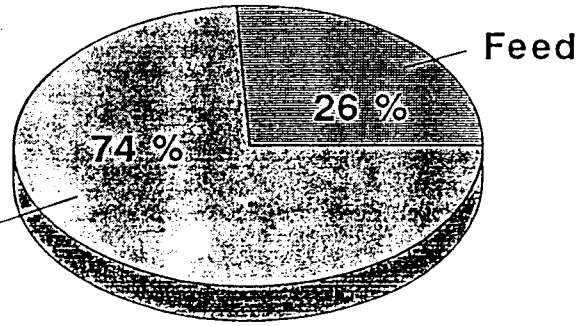
Water Control (no sediment)

Bedded Sediment Tests



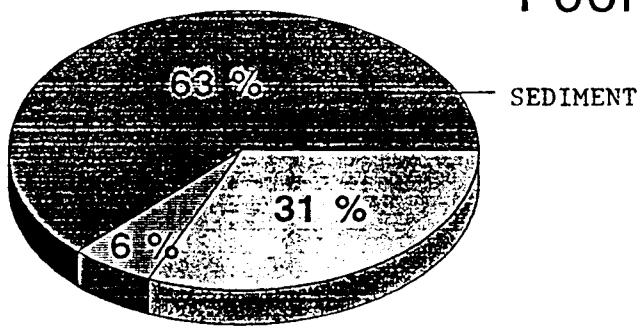
Total metal present: 768 ug Cd

Suspended Sediment Tests

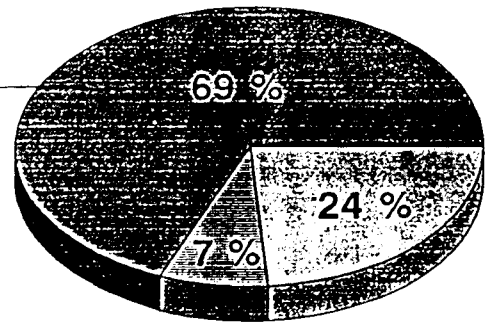


Total metal present: 608 ug Cd

Pool 10

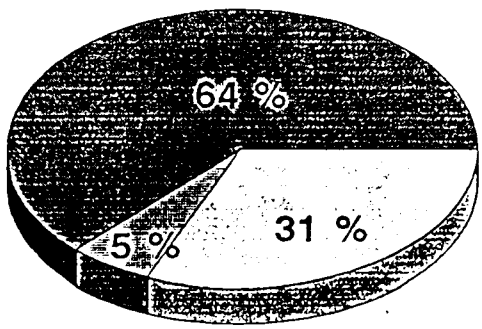


Total metal present: 2077 ug Cu

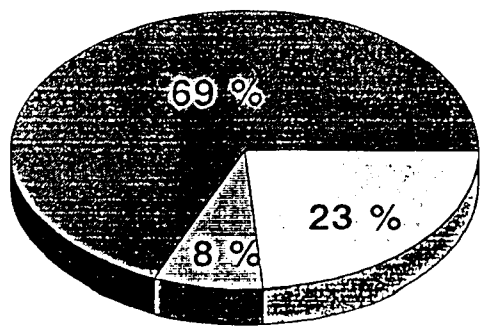


Total metal present: 1900 ug Cu

Pool 12



Total metal present: 2128 ug Cu

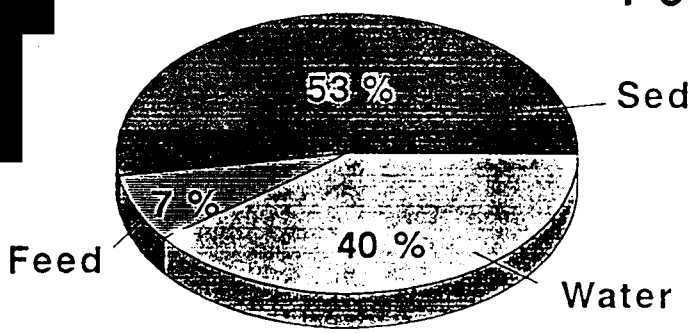


Total metal present: 1942 ug Cu

Cu (ug) present in:

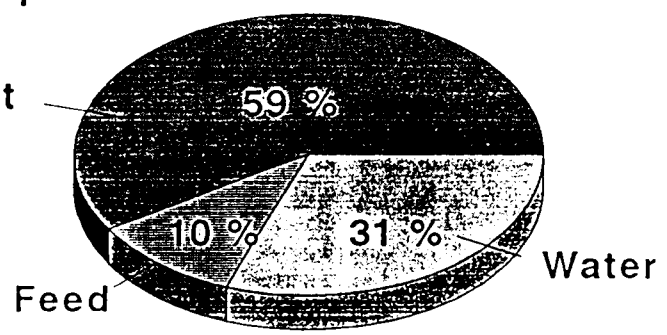
Pool 14

Bedded Sediment Tests



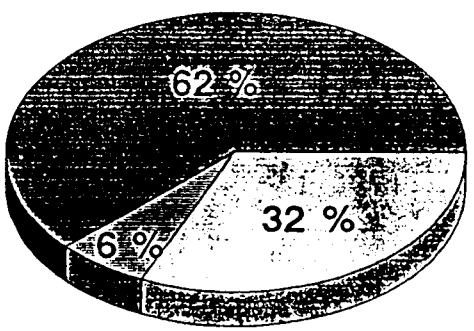
Total metal present: 1648 ug Cu

Suspended Sediment Tests

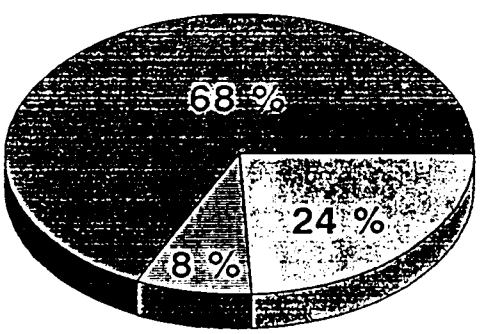


Total metal present: 1473 ug Cu

Pool 25

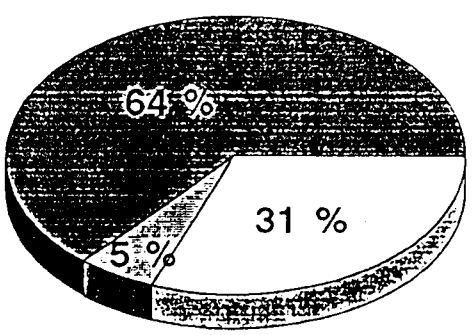


Total metal present: 2054 ug Cu

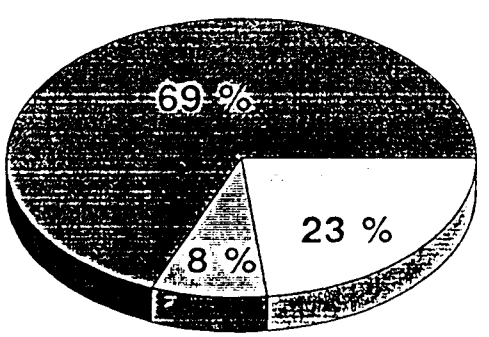


Total metal present: 1868 ug Cu

Open River



Total metal present: 2108 ug Cu



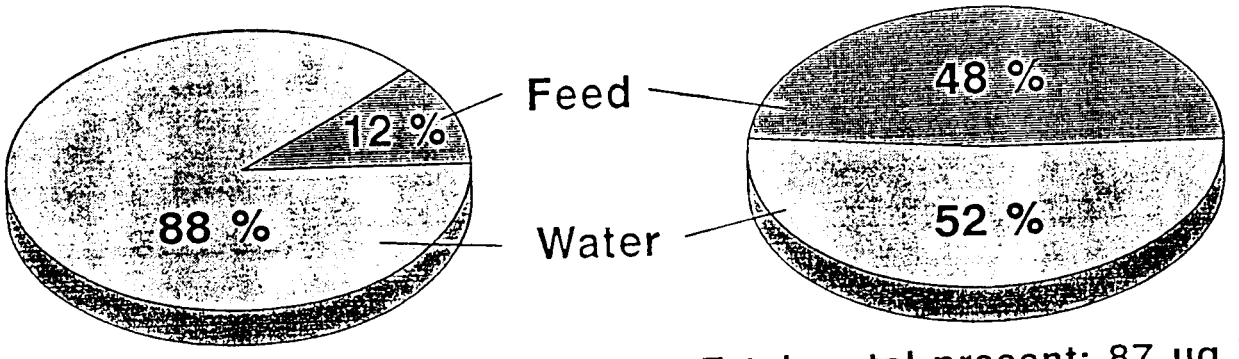
Total metal present: 1939 ug Cu

Pb (ug) present in:

Bedded Sediment Tests

Suspended Sediment Tests

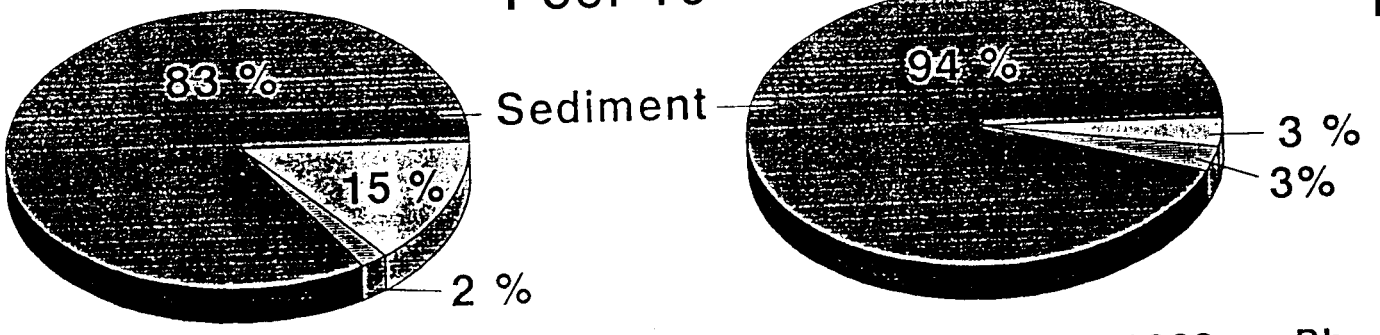
Water Control (no sediment)



Total metal present: 256 ug Pb

Total metal present: 87 ug Pb

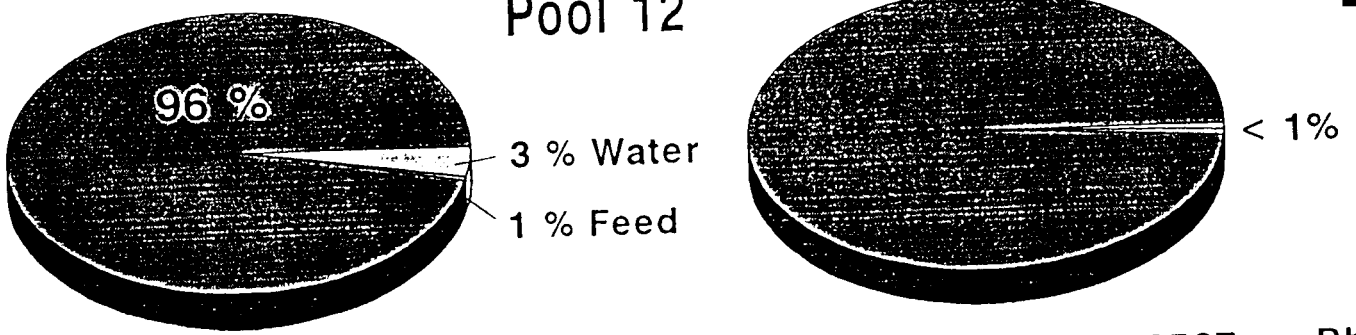
Pool 10



Total metal present: 1536 ug Pb

Total metal present: 1363 ug Pb

Pool 12



Total metal present: 6737 ug Pb

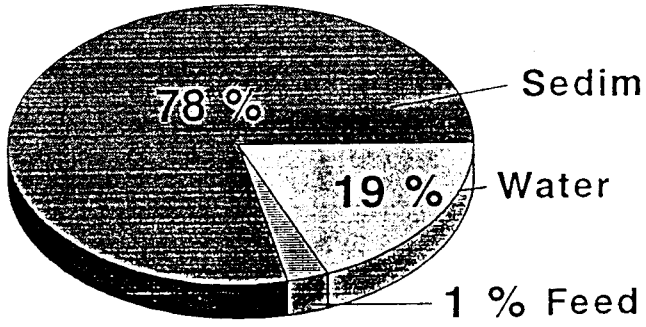
Total metal present: 6567 ug Pb

Pb (ug) present in:

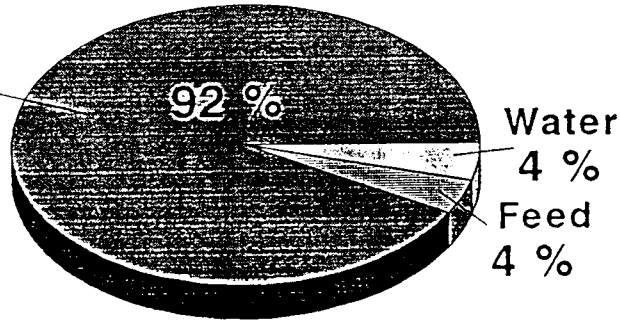
Bedded Sediment Tests

Suspended Sediment Tests

Pool 14

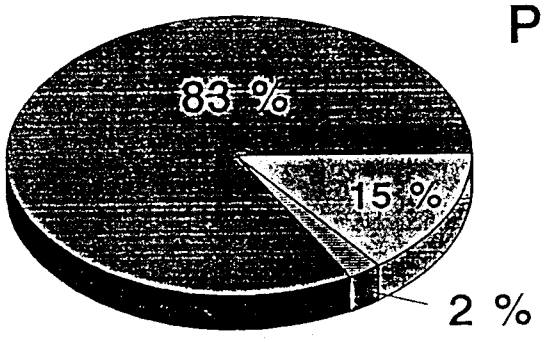


Total metal present: 1200 ug Pb

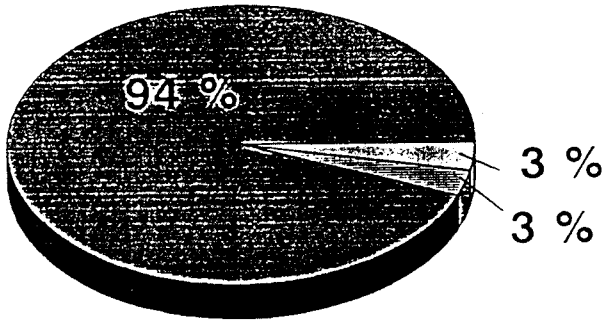


Total metal present:1028 ug Pb

Pool 25

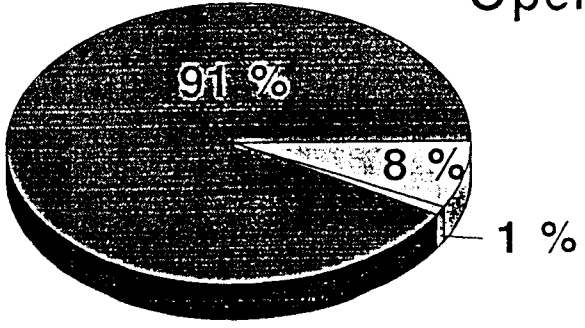


Total metal present: 1530 ug Pb

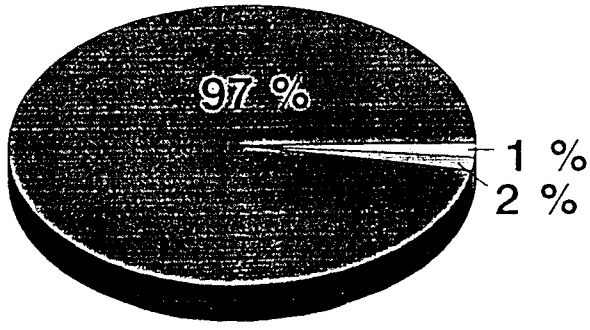


Total metal present: 1354 ug Pb

Open River



Total metal present: 2932 ug Pb



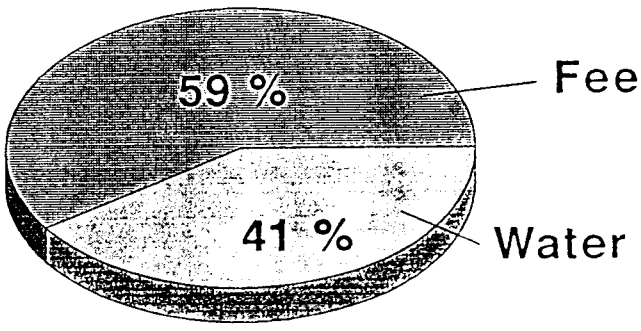
Total metal present: 2761 ug Pb

Zn (mg) present in:

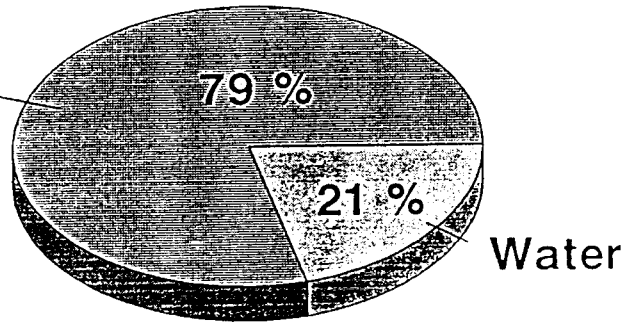
Bedded Sediment Tests

Suspended Sediment Tests

Water Control (no sediment)

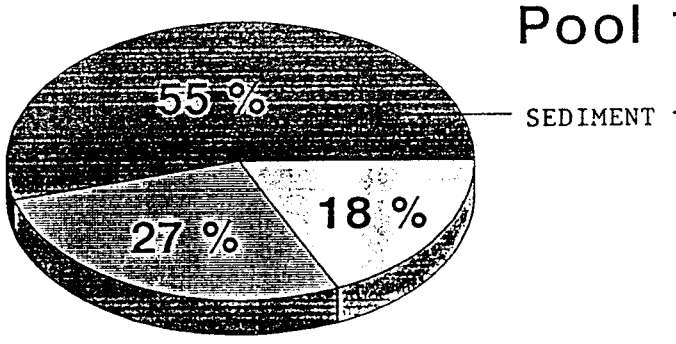


Total metal present: 4.4 mg Zn

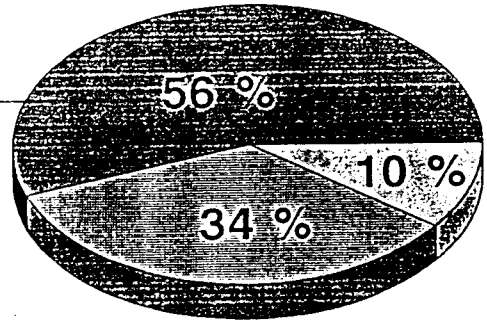


Total metal present: 4.6 mg Zn

Pool 10

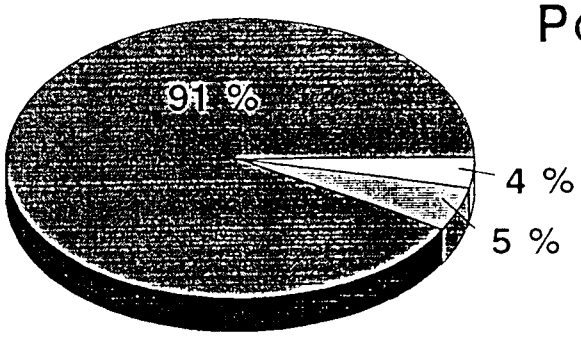


Total metal present: 9.8 mg Zn

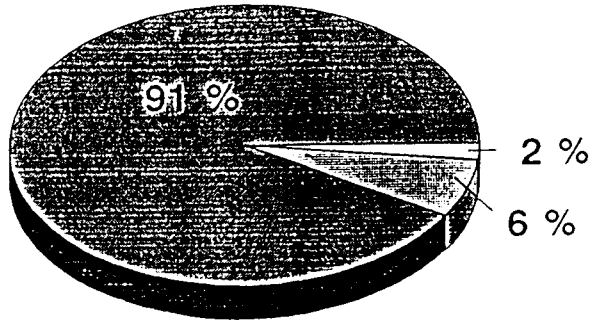


Total metal present: 9.6 mg Zn

Pool 12



Total metal added: 51 mg Zn

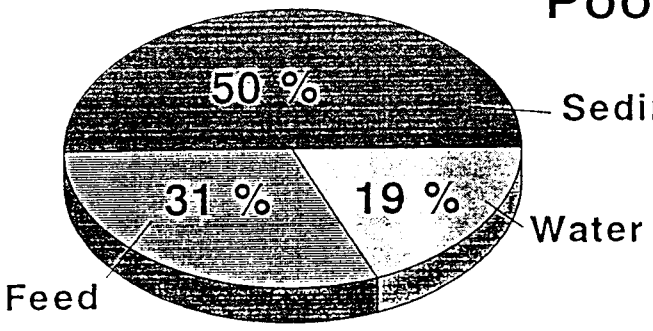


Total metal added: 51 mg Zn

Zn (mg) present in:

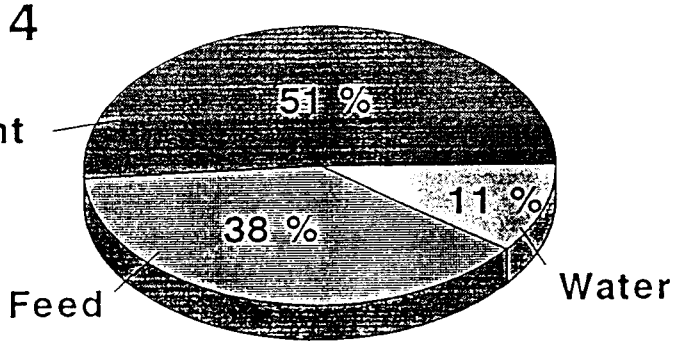
Pool 14

Bedded Sediment Tests



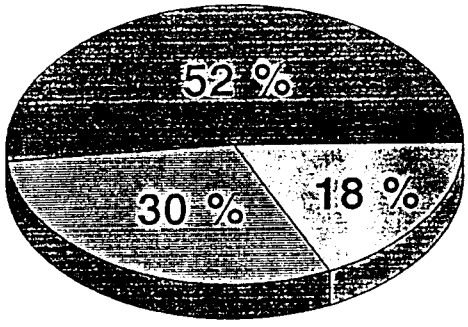
Total metal present: 9.5 mg Zn

Suspended Sediment Tests

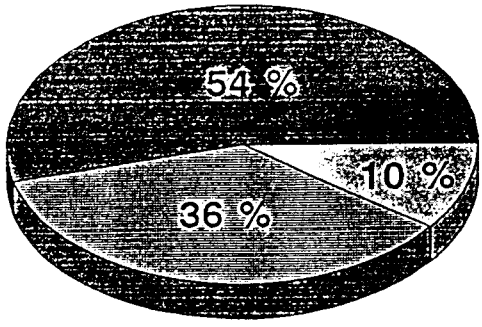


Total metal present: 9.3 mg Zn

Pool 25

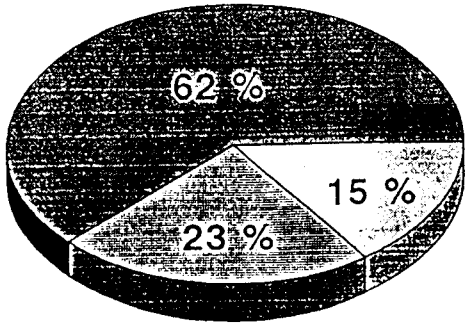


Total metal present: 9.9 mg Zn

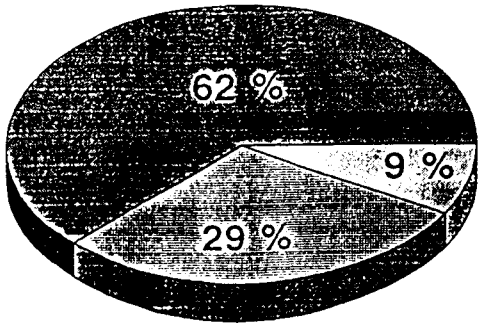


Total metal present: 9.5 mg Zn

Open River



Total metal present: 11.8 mg Zn



Total metal present: 11.7 mg Zn