CONTAMINATION OF A SOFT-WATER STREAM ECOSYSTEM IN SOUTHWEST VIRGINIA BY HIGHWAY-GENERATED HEAVY METALS

by

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INTRODUCTION

The rising number of vehicles using the nation's highways has generated considerable concern about the distribution of contaminating waste products to the environment adjacent to these highways. Among these contaminants are heavy metals, including lead from gasoline engine exhausts, nickel from diesel fuel and the combustion of lubricating oil, and cadmium and zinc from tire breakdown.

Low concentrations of each of these metals have been demonstrated to be toxic to aquatic organisms. Metallic compounds and elements do not degrade after being introduced into the environment, and in some cases have been shown to be biologically magnifiable. The effects of heavy metal poisoning are not confined to immediate mortality, but may also result in impaired behavior, reduced reproduction, and other sublethal effects. Toxicity is enhanced in soft-water ecosystems, which lack agents to bind and precipitate the metals, and in some instances when more than one of the metals is present. Toxic effects may be caused by accumulation and concentration of heavy metals in internal organs of aquatic organisms either directly from the surrounding environment, or, as has been shown in some instances, via the food chain.

Elevated levels of lead in soil, vegetation, and certain animal species along highways have been well documented. Although there is some indication that heavy metal concentrations in aquatic ecosystems in close proximity to highways may be elevated, information concerning the contamination of aquatic ecosystems by highway-generated heavy metals is very limited, and the relationship of stream contamination to traffic density has not been previously described. Indications of elevated heavy metal levels in aquatic organisms near highways were generally results of comparisons with control organisms.

The goal of this study was to describe highway-generated heavy metal contamination of a soft-water stream ecosystem. Abiotic and biological components were compared at selected sites quarterly with the following specific objectives:

(1) to determine the concentrations of lead, nickel, cadmium, and zinc in surface water, sediments, and the various trophic components of the stream in study areas influenced by different traffic volumes;

(2) to describe variations in the heavy metal burden of invertebrates and fish as related to species differences and duration of exposure;

(3) to determine whether these metals demonstrate trophic-level magnification in aquatic food chains; and

(4) to determine whether seasonal differences in the accumulation of these metals occur.

LITERATURE REVIEW

Highway Generation of Heavy Metals

Heavy metal contamination of roadside ecosystems is a subject which deserves attention because of the size of the affected area. In the United States, the area within 50 m of highways travelled by more than 1000 vehicles/day includes some 306,000 km^2 (Smith 1976). The four major heavy metals generated by highway traffic are lead, zinc, cadmium, and nickel.

Lead is present in leaded gasoline in the form of tetra-alkyl compounds, with concentrations averaging 0.66 g/liter (Getz et al. 1977). This results in 350,000 metric tons of lead combusted annually in the northern hemisphere (Jeffries and French 1972). Figures for the year 1970 show automobile emissions accounted for 140,000,000 kg of lead in the United States--a 95 percent share of total emissions from all sources (Wheeler et al. 1978). A single automobile may release up to 80 mg Pb/km (Smith 1976). Although legislation has caused a gradual changeover in recent years to gasoline containing a maximum of 0.132 g/liter, no assessment has yet been made of the effect of this action on particulate emissions (Friedlander 1977). Of the lead emitted, 90 percent is in the particulate form, with the rest as uncombusted alkyls (Corrin and Natusch 1977). Emitted lead is transferred to the roadside ecosystem by sedimentation, impaction, precipitation, and inhalation (Smith 1976). A number of studies on the residence time of lead in the atmosphere under various conditions have shown that while lead may remain airborne for 7 to 30 days, over 50 percent of all lead is deposited within 50 to 75 m of the highway, with variances due mainly to topography and meteorological conditions; only those particles of size less than 5 μm remain suspended for extended periods of time (National Academy of Sciences 1972a; Smith 1976; Laxen and Harrison 1977). Localized deposition was responsible for increases of up to 400 times over previous concentrations in many regions (National Academy of Sciences 1972a). Laxen and Harrison (1977) reported lead in airborne dust within 48 m of highways averaged 9200 mg/kg, while dust over the eastern Atlantic contained 100 to 1500 mg/kg. Although concentrations of lead in precipitation and runoff may be similar, as much as 95 percent of the lead contributed to a watershed is generally due to runoff, since the vast majority of water contributed to streams is via this source (Getz et al. 1977; Wilber and Hunter 1977). Lead accumulates on streets during dry periods (Newton et al. 1974), but 90 percent will be removed after only two to three millimeters of rainfall (Laxen and Harrison 1977). The lead content of highway runoff is highly variable, ranging from 0.25 to 14.0 mg/liter, with an average of 100 μg/liter present in the soluble form (Laxen and Harrison 1977).

Cadmium and zinc are present in rubber tires. Cadmium occurs as an impurity at concentrations of 20 to 90 mg/kg. Cadmium also occurs in motor oil (0.5 mg/kg), lubricating oil (0.20 to 0.26 mg/kg), and diesel fuel (0.07 to 0.10 mg/kg) (Organisation for Economic Co-operation and Development 1975). Transportation-associated cadmium emissions into the atmosphere in the United States during 1972 included 5200 kg from rubber tires, 830 kg from motor oil, and 54,000 kg from diesel fuel and fuel oil. These sources accounted for less than three percent of the total emissions from all sources, of which metal refineries comprise the majority (U. S. Environmental Protection Agency 1975). Data concerning airborne concentrations, residence times, and runoff contributions of cadmium and zinc are rare. The U. S. Environmental Protection Agency (1975) reported concentrations of airborne cadmium in urban areas to be under 0.1 $\mu g/m^3$. Bowen (1975) stated that heavy metals in general have an average atmospheric residence time of one month, while the residence time of zinc in Lake Washington in the state of Washington was 550 days, compared to only 25 days for lead.

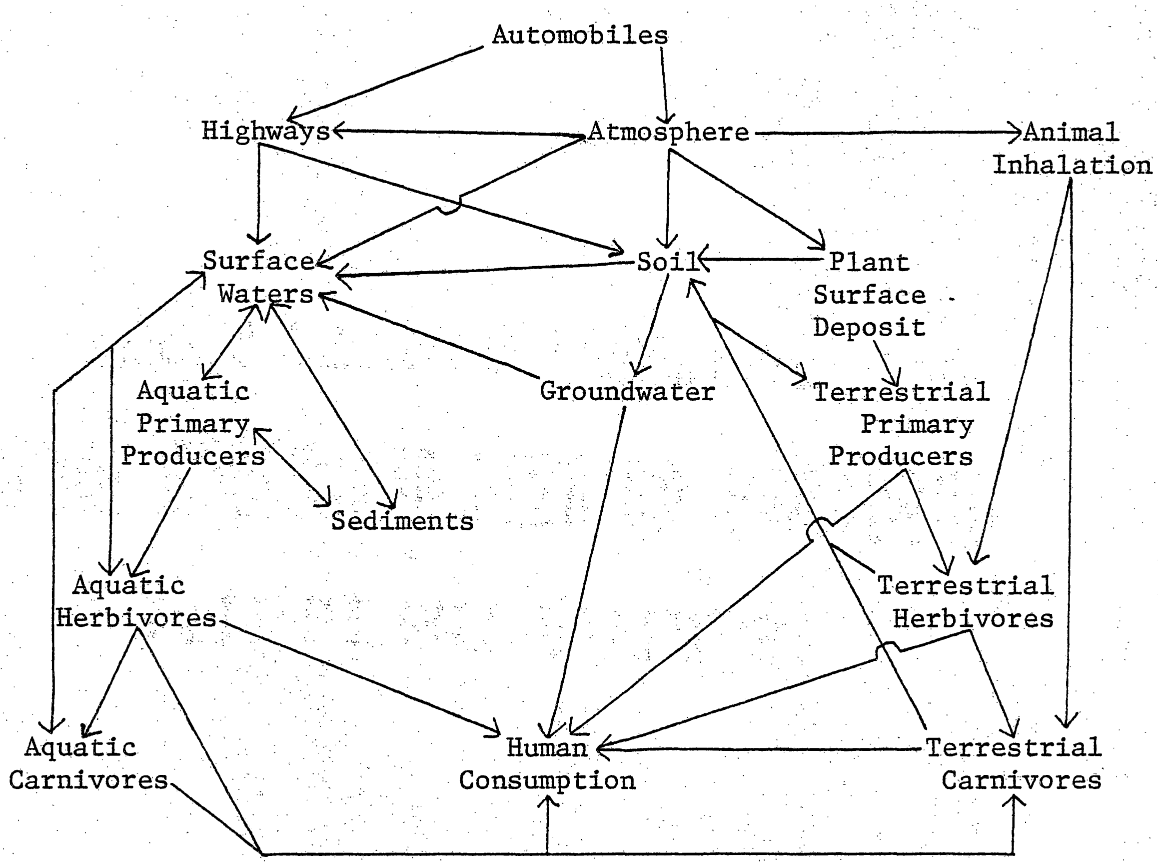
Information concerning nickel is also scarce. Nickel alloys are highly resistant to corrosion in natural environments (National Academy of Sciences 1975). The major highway-generated sources are gasoline and lubricating oil (Gish and Christensen 1973). The National Academy of Sciences (1975) reported that particulate nickel emissions from diesel engine exhausts of 0.65 μg/min resulted in airborne concentrations of 0.021 $\mu g/m^3$ in urban areas, and 0.006 $\mu g/m^3$ in nonurban areas.

A generalized flow-diagram for heavy metal distribution to the environment from automotive sources is given in Figure 1.

Contamination of the Roadside Ecosystem

The effect of heavy metals, particularly lead, on the terrestrial ecosystem has been well-documented. Concentrations of lead and zinc in roadside soil and vegetation increased with traffic volume and decreased with distance from the highway (Motto et al. 1970; Jeffries and French 1972; Hiller et al. 1978). Ganje and Page (1972) found that lead in soil near highways was highest in the top 0 to 15 cm. Similar correlations between lead concentrations and traffic volume and distance from the highway have been observed in earthworms (Goldsmith and Scanlon 1977).

Fig. 1. Highway-generated heavy metals: possible distribution to the environment (modified from Smith 1976; Getz et al. 1977).


Automobiles
Highways
Atmosphere
Animal Inhalation
Surface Waters
Soil
Plant Surface Deposit
Aquatic Primary Producers
Groundwater
Terrestrial Primary Producers
Sediments
Aquatic Herbivores
Terrestrial Herbivores
Aquatic Carnivores
Human Consumption
Terrestrial Carnivores

Ward et al. (1978) found increased blood lead levels in sheep grazing near a highway, which was attributed to inhalation of airborne particulates and ingestion of polluted forage. Concentrations of lead, nickel, cadmium, and zinc in earthworms increased with traffic volume, and decreased with distance from the highway. The levels of lead and zinc in some specimens were at lethal levels for earthworm-eating animals (Gish and Christensen 1973). Blair et al. (1977) reported significant correlations between lead, cadmium, and zinc levels in mammals and associated highway traffic volumes.

Differences in both the seasonal patterns of heavy metal accumulation in vegetation (Hiller et al. 1978) and their uptake by various mammalian species (Blair et al. 1977) along roadsides have been reported. One study also indicated the possibility of biomagnification of lead in terrestrial ecosystems (Goldsmith and Scanlon 1977).

Information concerning the effect of heavy metals on the aquatic roadside ecosystem is limited. Getz et al. (1977) reported that a single watershed with an annual incoming load of 16,000 kg of lead from highways and other urban sources of runoff showed a streamwater output of only 800 kg/yr, with the remainder accumulating somewhere in the system. The largest concentrations of lead were found in sediments, and decreased rapidly with increased distance from highways. Lead levels in both sediments and organisms reflected the relative amount of runoff lead entering a particular sampling location. Other investigators have reported significant differences in the concentrations of lead in benthic invertebrate communities from rural and urban streams (Rolfe and Jennett 1975), and between hatchery rainbow trout (<u>Salmo gairdneri</u>)

and those sampled in a stream near a highway (Pagenkopf and Neuman 1974). Gale et al. (1973) found significantly higher concentrations of lead, zinc, and cadmium in crayfish sampled near a highway than in control specimens. Concentrations of these metals were found to decrease rapidly with distance from highway impacted areas as they were taken up by aquatic vegetation and trapped sediments, returning to control levels approximately 6.5 km downstream.

Heavy Metals in Aquatic Ecosystems

Concentrations of heavy metals in various components of aquatic ecosystems from a large variety of environments have been reported (Appendix Table II). Studies show that heavy metals entering a stream ecosystem collect mainly in aquatic vegetation and sediments (Gale et al. 1973; Cook 1977; Getz et al. 1977). Finer sediments contained higher concentrations than the coarse sediments due to the increased surface area and correspondingly higher adsorptive capacity (Oliver 1973). Concentrations in the water at any one time are generally low, with the highest levels in suspended particles. The highest overall metal load, however, is in the dissolved fraction due to its substantially greater volume (Perhac 1972; U. S. Environmental Protection Agency 1975; Cook 1977; Suzuki et al. 1979).

Sources of Contamination

The relative concentrations of lead, nickel, cadmium, and zinc in polluted aquatic ecosystems have been investigated in a number of studies. Atchison (1975) reported water concentrations of cadmium in an industrially polluted lake in Indiana 25 times greater than those in an unpolluted area, while cadmium in sediments was over 100 times greater in the polluted areas. Fish from industrially contaminated water were found to contain an average of at least 19 times the amount of cadmium and lead, and twice the amount of zinc as fish from unpolluted areas (Atchison et al. 1977). Similar differences in heavy metal concentrations were reported between sediments and water from the Illinois River and from nonindustrial-use streams (Mathis and Cummings 1973), between control fish and those influenced by mill and mine effluents (National Academy of Sciences 1972a; Gale et al. 1973), between hatchery fish and those in streams near a highway (Pagenkopf and Neuman 1974), and between urban versus rural benthic invertebrates and fish communities (Rolfe and Jennett 1975; McNurney et al. 1977).

Heavy Metals in Aquatic Biota

Uptake. Uptake of these metals by aquatic organisms is by active metabolic processes or by simple diffusion down concentration gradients created by surface adsorption and subsequent binding to constituents of the surface cells, body fluids, and internal organs (Warren 1971; Lisk 1972; Bryan 1976). The degree to which heavy metals are accumulated is greatly dependent upon their chemical form, their particle size, the physiological and ecological characteristics of the organism (Delisle et al. 1975; Jenne and Luoma 1977), water hardness, the presence of other metals or complexing agents, changes in temperature and pH, size differences among the organisms, and starvation (Bryan 1976; Jenne and Luoma 1977). The accumulation of heavy metals in aquatic organisms has been found to be greatly influenced by the levels found in the water (Delisle et al. 1975; Benoit et al. 1976; Atchison et al. 1977; Mayes et al. 1977; Spehar et al. 1978; Wheeler et al. 1978), sediments (Delisle et al. 1975;

Atchison et al. 1977; Getz et al. 1977; Jenne and Luoma 1977; Mayes et al. 1977; Anderson and Brower 1978; Wheeler et al. 1978), and to the organism's food supply (Bryan 1976; Jenne and Luoma 1977; Patrick and Loutit 1978). The metals are taken in through the gills and mouth of fish (Bowen 1966), however, homeostatic control of absorption in the gut limits significant toxic input by this route (Brown 1976). Internal regulation of heavy metals is better for essential metals, such as nickel and zinc, than it is for non-essential ones, such as cadmium and lead (Bryan 1976). Accumulation of both cadmium (Kincade and Erdman 1975; Rehwoldt and Karimian-Teherani 1976; Spehar 1976b) and zinc (Spehar 1976b) has been found to reach a plateau in fish. Internal regulation of zinc has also been shown in crayfish (Anderson and Brower 1978). Invertebrates and fish that have been exposed to high concentrations of cadmium and lead can lose a large portion of the metals accumulated when returned to uncontaminated water at a species-specific rate (Holcombe et al. 1976; McIntosh and Bishop 1976; Nehring et al. 1979).

Seasonal differences in the uptake of heavy metals have been investigated, but findings are inconclusive. Studies by Kelso and Frank (1974), Rolfe and Jennett (1975), and Getz et al. (1977) indicated no consistent seasonal differences in the accumulation of lead and cadmium. Hardisty et al. (1974a) and Badsha and Sainsbury (1978) found increases in heavy metal levels in estuarine fish with the progression of winter, but postulated that this could have been due to fish movement in and out of contaminated areas. Marjori et al. (1978) also found that concentrations of zinc in the muscle of Mytilus galloprovincialis in the Gulf of Trieste underwent cyclic variations, reaching a peak during the winter and a low during the summer, but found little variation in the seasonal levels of lead, nickel, or cadmium. Namminga (1977) found stream sediments contained higher levels of zinc in the summer, while water concentrations of zinc were higher in the winter.

Bioaccumulation. Bioaccumulation (an increase in the tissue burden of a substance over time) of lead, nickel, cadmium, and zinc in fish has been demonstrated, and the results of some representative studies are summarized in Appendix Table II. Lead accumulates in bone (Lisk 1972; Einaga 1977) because of its resemblance to calcium ions (Bowen 1966), which may be related to similarities in the valence states and crystalline structure of these two elements. Lead accumulates to a lesser extent in the gills, fins, kidney, liver, heart, and gut (Hardisty et al. 1974a; Leland et al. 1974; Holcombe et al. 1976; McIntosh and Bishop 1976; Brown and Chow 1977; Einaga 1977; Merlini and Pozzi 1977; Ray 1978). Nickel also accumulates in bone, but to a lesser extent than lead (Bowen 1966). Cadmium accumulates most in kidney, liver, gill, and gut tissues, and to a lesser extent in fins, bone, brain, spleen, blood, and swim bladder (Bowen 1966; Mount and Stephan 1967; Hardisty et al. 1974a; Leland et al. 1974; Rowe and Massaro 1974; Atchison 1975; Tafanelli and Summerfelt 1975; Benoit et al. 1976; McIntosh and Bishop 1976; Smith et al. 1976; Brown and Chow 1977; Einaga 1977; Sangalang and Freeman 1979). Zinc also accumulates in liver, kidney, gill, and gut tissues, and to a lesser extent in fins, bone, heart, and brain (Bowen 1966; Hardisty et al. 1974a; McIntosh and Bishop 1976; Einaga 1977; Wiener and Giesy 1979).

The amount of an element accumulated is a function of many factors, including duration of exposure, organism size, and species. Lisk (1972) and Ray (1978) found lead in fish increased as the age of the fish increased. However, Andersen et al. (1973) found lower lead concentrations in age I than age 0 fish, while others found no significant correlation between lead concentrations and age (Pakkala et al. 1972; Hardisty et al. 1974a; Tong et al. 1974). Andersen et al. (1973) found zinc in age I fish higher than that in age 0 fish. However, Hardisty et al. (1974a) and Papadopoulou et al. (1978) found that zinc decreased as fish age increased. Tong et al. (1974) again found no significant correlation between zinc load and age. Jaakkola et al. (1971) found cadmium concentrations higher in older fish, while Lovett et al. (1972) and Tong et al. (1974) found no significant correlation. No significant correlation has been shown between nickel concentrations and fish age.

According to Bryan (1976), the concentration of a metal is dependent upon the size of an organism; however, investigators have found no significant correlation between lead or nickel and organism size (Pakkala et al. 1972; Tong et al. 1974; Anderson and Brower 1978). Cutshall et al. (1977) found that zinc and cadmium concentrations in Pacific hake increased as fish weight increased, while Murphy et al. (1978), Papadopoulou et al. (1978), and Wiener and Giesy (1979) reported that zinc concentrations were negatively correlated with fish length. Other investigators found no significant correlations with size for either zinc or cadmium (Lovett et al. 1972; Tong et al. 1974; Anderson and Brower 1978).

Many investigators have reported species differences in the concentrations of heavy metals (Lucas et al. 1970; Lovett et al. 1972; Delisle et al. 1975; Wiener and Giesy 1979). However, Spehar et al. (1978) found no significant differences in the accumulation of lead and cadmium by various species of aquatic insects, and results of other studies failed to demonstrate differences between species of fish (Pakkala et al. 1972; Giesy and Wiener 1977).

Biomagnification. The possibility of lead, nickel, cadmium, and/or zinc biomagnification (an increase in the tissue burdens of a substance in organisms occupying higher trophic levels) through aquatic food chains has not been confirmed. The National Academy of Sciences (1975) suggested that nickel has the potential for biomagnification, and the U. S. Environmental Protection Agency (1975) hypothesized the same for cadmium, but several studies have so far shown no biomagnification of any of the four metals (Gale et al. 1973; Mathis and Cummings 1973; Mathis and Kevern 1975; Enk and Mathis 1977; Getz et al. 1977; Giesy and Wiener 1977; Murphy et al. 1978; Wiener and Giesy 1979).

Hardisty et al. (1974b) reported that cadmium and lead concentrations were higher in estuarine fish with crustaceans as the major food item than in other fish, but that zinc was not, providing a weak suggestion of biomagnification of those two elements.

Toxicity of Heavy Metals to Aquatic Life

Acute Toxicity

Toxicity of lead, nickel, cadmium, and zinc to aquatic animals occurs through a large variety of pathways, including enzyme inhibition or activation, action as metabolites, formation of precipitates or chelates with essential metabolites, reaction with cell membranes altering permeability, and replacement of structurally or electrochemically important elements in cells (Lisk 1972). Acute fish mortalities due to toxic metals are usually explained by the coagulation-film anoxia theory: precipitation or coagulation of mucoproteins on the gill epithelium interferes with oxygen exchange, secretion of waste products, and osmoregulation, causing suffocation of the fish (Tafanelli and Summerfelt 1975). Acutely toxic concentrations of lead, nickel, cadmium, and zinc for aquatic organisms are summarized in Table 1.

Chronic Toxicity

The effects produced by chronic toxicity levels of metals include: avoidance by fish (Sprague 1968) and chironomids (Wentsel et al. 1977); reduced reproductive capacity of both invertebrates (Biesinger and Christensen 1972; Conway 1978) and fish (Tafanelli and Summerfelt 1975; Benoit et al. 1976; Rehwoldt and Karimian-Teherani 1976; Spehar 1976b; Speranza et al. 1977); abnormal behavior (Weir and Hine 1970; Kwasnik 1977; Ellgaard et al. 1978; Sullivan et al. 1978); reduced growth (Crandall and Goodnight 1962; Benoit et al. 1976; Spehar 1976b; Watson and McKeown 1976); inhibition or activation of enzymes (Hogan and Knowles 1968; Jackim et al. 1970; Hogan 1971; Hodson et al. 1977); cellular and metabolic disorders (Rachlin and Perlmutter 1969; Hiller and Perlmutter 1971; Larsson 1975; Sellers et al. 1975; Christensen et al. 1977); spinal deformity and neurological disorders (Davies et al. 1976; Holcombe et al. 1976); and increased vulnerability to stress (Middaugh et al. 1975; Tafanelli and Summerfelt 1975). Minimum toxic concentrations for various aquatic organisms are summarized in Table 2.

Table 1. Acute toxicity of lead, nickel, cadmium, and zinc to aquatic life. Bioassay type: CF = continuous-flow, ST = static; water hardness: H = hard (>120 mg/l CaCO$_3$), S = soft (≤60 mg/l CaCO$_3$).

Investigator	Conditions	Organism	Metal	Parameter and period measured	Concentration (mg/l)
Biesinger and Christensen (1972)	ST,S	*Daphnia magna*	Pb	48-hr LC$_{50}$	0.45
McKim et al. (1973)	H	Rainbow trout	Pb	96-hr LC$_{50}$	1.38
Biesinger and Christensen (1972)	ST,S	*Daphnia magna*	Ni	48-hr LC$_{50}$	0.51
Pickering (1974)	CF,ST,H	Fathead minnow	Ni	96-hr LC$_{50}$	25.0-32.0
Baudouin and Scoppa (1974)	S	*Daphnia hyalina*	Cd	48-hr LC$_{50}$	0.055
Eaton (1974)	CF,H	Bluegill larvae	Cd	Toxic threshold	0.031-0.080
Baudouin and Scoppa (1974)	S	*Daphnia hyalina*	Zn	48-hr LC$_{50}$	0.040
Pickering and Vigor (1965)	CF,H	Fathead minnow	Zn	1-7 day TL$_m$	0.87-0.95

Table 2. Minimum concentrations of lead, nickel, cadmium, and zinc reported toxic to aquatic life. Bioassay type: CF = continuous-flow, ST = static; water hardness: H = hard (>120 mg/l $CaCO_3$), S = soft ($\leq$60 mg/l $CaCO_3$).

Investigator	Conditions	Organism	Metal	Period of study	Conc. (*μg/l*)	Indicative response
Christensen et al. (1977)	CF,S	Brook trout	Pb	2 *weeks*	0.50	Biochemical inhibition
Davis (1976)	H	Rainbow trout	Pb	19 *months*	31.6	Black tails
Biesinger and Christensen (1972)	ST,S	*Daphnia magna*	Ni	3 *weeks*	30.0	Reproduction impairment
Pickering (1974)	CF,ST,H	Fathead minnow	Ni	--	730	Reduced fecundity
Christensen et al. (1977)	CF,S	Brook trout alevin	Cd	8 *weeks*	0.04	Biochemical inhibition
McIntosh and Bishop (1976)	CF,H	Bluegill	Cd	--	50.0	Coughing
Sprague (1968)	CF,S	Rainbow trout	Zn	20 *minutes*	5.60	Avoidance
Sinley et al. (1974)	H	Rainbow trout juveniles	Zn	--	640	--

Factors Affecting Toxicity to Aquatic Life

Physico-chemical factors. The toxicity of heavy metals to aquatic life is affected by a number of biological and environmental factors which complicate predictive impact assessment. The toxic effect of metals is enhanced in soft waters, while in hard water metal cations can be chelated in large, non-toxic complexes, or prevented from occupying active sites in fish tissues by competition from calcium and magnesium ions (Zitko and Carson 1976). For example, lead is directly toxic in the dissolved state (Davies et al. 1976), but an increase in water hardness from 14 to 53 mg/1 $CaCO_3$ will decrease the lead in solution from 8 mg/1 to 1.6 mg/1 as it is precipitated as carbonate or hydroxide, so that the total concentration of lead in a hard-water system cannot be equated with the amount of toxic lead. Similar correlations between water hardness and toxicity have been shown for other metals (National Academy of Sciences 1972a).

Water temperature also has an effect on heavy metal toxicity to aquatic organisms. In general, as temperature increases, the added stress reduces survival time, although the median tolerance level (TL_m) does not change (Skidmore 1964; Rehwoldt et al. 1972). A 10 C rise in temperature will reduce the survival time of fish exposed to lethal concentrations of lead by 50 percent (National Academy of Sciences 1972a).

Other environmental effects which tend to increase the toxicity of heavy metals include decreased dissolved oxygen (Brown 1968; Cheremisinoff and Habib 1972) and both increases and decreases in pH (Mount 1966; Cheremisinoff and Habib 1972; McKim et al. 1973; Hodson et al. 1978).

The presence of more than one metal may also enhance toxicity. Cadmium and lead at certain concentrations will increase the toxicity of zinc (Organisation for Economic Co-operation and Development 1975; Say and Whitton 1977a; Say and Whitton 1977b). The presence of nickel and zinc together produce a synergistic effect (Skidmore 1964). Antagonistic effects involving lead, nickel, cadmium, and zinc have not been demonstrated.

Biological factors. Many biological factors also influence heavy metal toxicity. Toxic concentrations of metals were shown to be species-specific for fish (Pickering and Henderson 1966; Cearley and Coleman 1974), due possibly to the amount and nature of gill secretions (National Academy of Sciences 1972a) or to relative size differences (Nehring and Goettl 1974). Differences in the toxicity of heavy metals to the different life stages of a species have also been observed (Bengtsson 1974; Christensen et al. 1977; Eaton et al. 1978).

A detoxification function has been proposed for cadmium-binding proteins in the livers of some marine vertebrates by Olafson and Thompson (1974). Other investigators have observed acclimation (both physical and genotypic) in fish (Spehar et al. 1978) and algae (Say and Whitton 1977a) to sublethal concentrations of these metals.

Criteria for Protection of Aquatic Life

Data concerning the toxic and sublethal effects of heavy metals are valuable in determining the concentrations of these metals which are safe for aquatic organisms. Lethal concentrations and "safe" (maximum acceptable toxicant concentrations--MATC) levels determined for various species and life forms are summarized in Appendix Table I.

Concentrations of lead, nickel, cadmium, and zinc that are safe for aquatic life are still being debated. The Aquatic Life Advisory Committee of the Ohio River Valley Water Sanitation Commission (1955) recommended that these concentrations should be no greater than the 48-hr TL_m times an application factor (chronic TL_m value/acute TL_m value). However, application factors are so variable (range: 0.001 to 0.1) and arbitrary that few have been determined. Complete removal of these substances is impossible, since low levels of several metals are required for the maintenance of aquatic life (Katz 1975).

Various criteria have been recommended based on experimental bioassay results. The U. S. Environmental Protection Agency (1976) recommended maximum concentrations of 0.4 μg Cd/l in waters of less than 75 mg/l hardness, 1.2 μg Cd/l in waters of greater than 150 mg/l hardness, and values for lead, nickel, and zinc corresponding to 0.01 times the 96-hr LC_{50} value for sensitive resident species. Table 2 summarizes the minimum toxic concentrations of all four metals for aquatic life forms in hard and soft water, indicating that for the protection of these organisms, the maximum allowable concentrations of these metals must be less than these determinations.

PROCEDURES AND TECHNIQUES

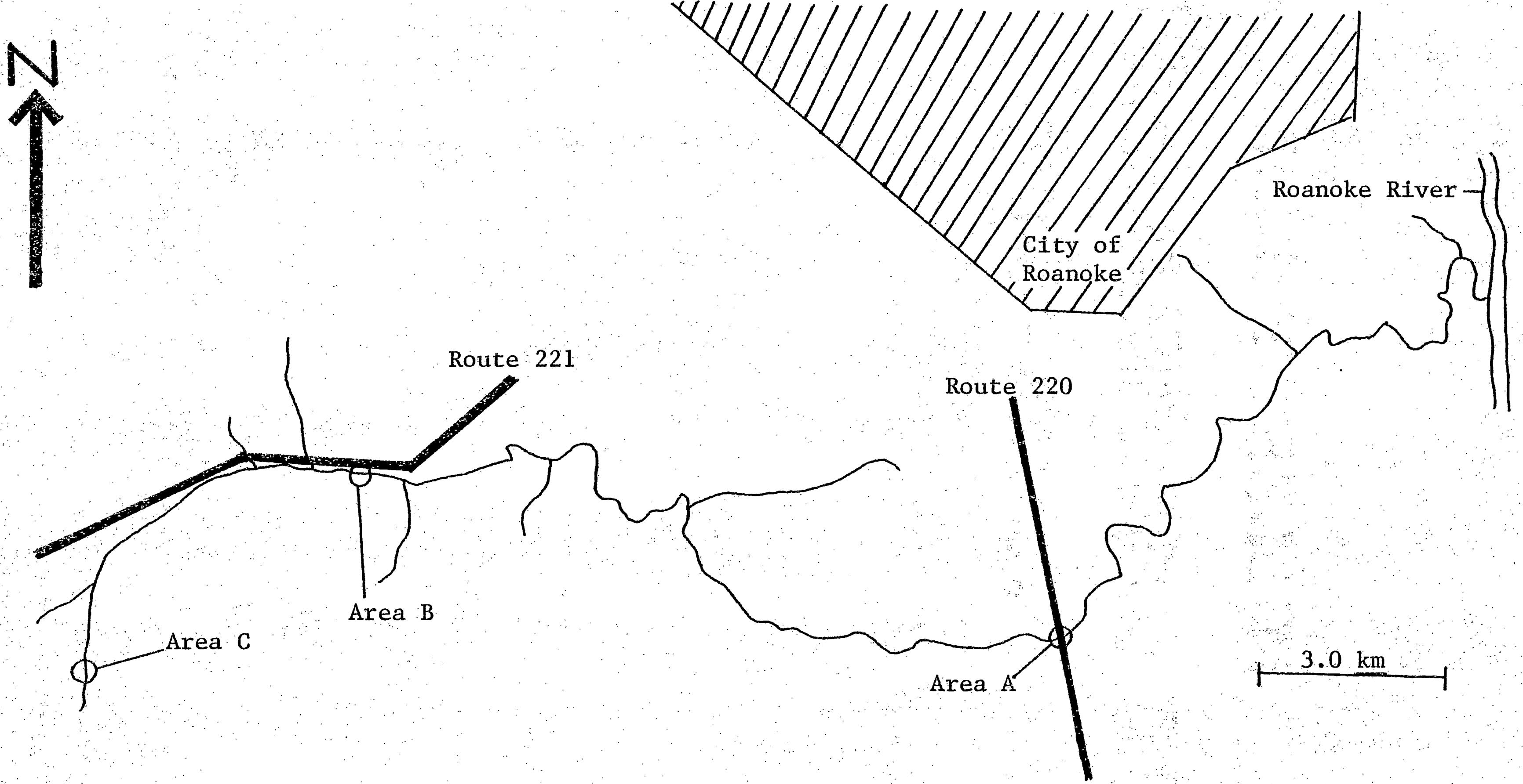
Study Area

The stream chosen for this study was Back Creek, a component of the Roanoke River watershed located southwest of the city of Roanoke, Virginia. This stream lies approximately parallel to the Roanoke River, receiving drainage from the Poor Mountain region encompassing portions of both Roanoke and Franklin counties. The Back Creek area is lightly populated and lacks industrial development.

The mean gradient of the stream was 25 m/km from the most upstream sampling station to that located furthest downstream, a distance of 22 km. The volume of discharge was highly variable, with monthly averages for the years 1969 and 1970 ranging from 12.6 to 79.7 m^3/sec (Waller 1976). The substrate was composed of sediments consisting of small particles ranging in size from 0.004 to 10.0 mm, interspersed with rocks and large stones upstream. Mean stream width increased from an average of 130 to 150 cm upstream to an average of 600 to 640 cm at the most downstream sampling location. Riparian vegetation, consisting of large trees and bushes, was abundant along the entire length of the stream. Instream vegetation was sparse and consisted mainly of scattered patches of periphyton. The stream was characterized by numerous riffles, small falls (average height of approximately 40 cm), and pools (maximum depth of 180 to 200 cm).

Three study areas (Fig. 2) were investigated, chosen on the basis of 1975 traffic volume estimates obtained from the Virginia Department of Highways:

Fig. 2. Map of Back Creek and location of study areas.


N
City of Roanoke
Roanoke River
Route 221
Route 220
Area B
Area C
Area A
3.0 km

Area A: Bridge over Back Creek at Route 220. Traffic volume approximately 15,000 vehicles/day.

Area B: Back Creek along Route 221. Traffic volume approximately 6,550 vehicles/day.

Area C: Reference area located upstream on Back Creek. Less than 20 vehicles/day within 500 m; no traffic upstream.

Areas A and B were sampled within 50 m of the associated highways. Upstream migration of organisms from one sampling location to another, or to area A from downstream, was effectively limited by numerous riffles and at least two falls of over 60 cm.

Field Collections

Collection of water, sediments, macroinvertebrates, and fish were made at each area in April, July, and November of 1978, and in February of 1979. Additional sediments were collected in May of 1979 for verification of results concerning seasonal variation in heavy metal concentrations. Invertebrate samples were collected from riffle areas by qualitative kick-sampling with dip nets. Fish were also sampled qualitatively by electroshocking utilizing a Coffelt Model BP-2 backpack electroshocking unit. Each sample was placed in a plastic bag or polyethylene container, labelled, and put on ice for return to the laboratory.

Laboratory Analyses

Water samples were analyzed immediately upon return to the laboratory for physical and chemical parameters. Concentrations of metals in the water were determined from samples preserved in the field with conc. HNO_3 and held at 4 C until the time of analysis, usually within one month. Sediment, benthic invertebrate, and fish samples were frozen until further preparations were made.

Throughout the entire process of sample collection, preparation, and analysis all containers were washed using the sequence of detergent and tap water, chromic acid, tap water, 1:1 nitric acid, tap water, and deionized distilled water. Samples were never brought into contact with an object containing metal.

Water samples were tested to determine dissolved oxygen, turbidity, hardness, and alkalinity using Hach procedures (Hach Chemical Co. 1975) in the laboratory. Field measurements of temperature and pH, and laboratory measurements of total, dissolved, and suspended metals were performed according to standard methods (Am. Public Health Assoc. et al. 1976).

Sediment samples (Table 3) were dried approximately 72 h at 75 to 80 C, after which they were characterized according to particle size: $<$0.004 mm (clay), 0.004 to 0.062 mm (silt), 0.062 to 0.500 mm (fine), 0.500 to 2.000 mm (medium), $>$2.000 mm (coarse) (Oliver 1973). The dried samples were then weighed to 0.001 g into no. 9860 Pyrex ignition tubes for heavy metal analysis.

Macroinvertebrate organisms were identified to family and characterized by food and feeding habits (Table 3) according to Merritt and Cummins (1978) as either predator-engulfers or shredder-detritivores. These four families were used for the analysis due to their large size, long aquatic life spans (two to three years), and presence in large numbers in the stream. The samples were then measured to the nearest millimeter total length (without tails) and placed in shell vials. Individuals less than 20.0 mm total length were pooled with invertebrates of the same family to provide sufficient tissue for the detection of heavy

Table 3. Sediments, invertebrates, and fish analyzed from each area, including classifications applied for data analysis: predator-engulfer (PE), shredder-detritivore (SD), herbivore (H), omnivore (O), bottom feeder (B), and upper water column feeder (WC).

Sample	Classification	Date of collection	Number analyzed		
			A	B	C
Sediment	silt	4/5/78	0	0	2
	fine	4/5/78	6	7	7
	medium	4/5/78	3	2	0
	silt	7/28/78	1	2	3
	fine	7/28/78	2	1	1
	medium	7/28/78	3	3	2
	silt	11/7/78	0	0	2
	fine	11/7/78	2	2	1
	medium	11/7/78	1	1	0
	silt	2/26/79	0	1	1
	fine	2/26/79	2	0	1
	medium	2/26/79	2	1	1
	coarse	2/26/79	0	1	0
	silt	5/26/79	1	2	3
	fine	5/26/79	8	7	8
	medium	5/26/79	3	3	1
Benthic Invertebrates					
Diptera Tipulidae (crane fly)	SD,H	4/5/78	7	11	14
		7/28/78	1	0	1
		11/7/78	1	2	4
		2/26/79	7	16	6
Megaloptera Corydalidae (hellgrammite)	PE	4/5/78	19	1	0
		7/28/78	36	25	0
		11/7/78	26	8	4
		2/26/79	10	0	0
Plecoptera Perlidae (stonefly)	PE	4/5/78	0	0	16
		7/28/78	3	3	19
		11/7/78	3	9	10
		2/26/79	9	9	5
Plecoptera Pteronarcidae (stonefly)	SD	4/5/78	2	4	18
		7/28/78	24	0	36
		11/7/78	0	2	18
		2/26/79	8	8	7
Fish					
Rhinichthys atratulus (blacknose dace)	O,B	4/5/78	0	3	10
		7/28/78	0	9	16
		11/7/78	0	9	13
		2/26/79	19	3	5

Table 3. Sediments, invertebrates, and fish analyzed from each area, including classifications applied for data analysis: predator-engulfer (PE), shredder-detritivore (SD), herbivore (H), omnivore (O), bottom feeder (B), and upper water column feeder (WC). (continued).

Sample	Classification	Date of collection	Number analyzed A	B	C
Fish (continued)					
Lepomis	O,WC	7/28/78	3	0	0
macrochirus		11/7/78	0	1	0
(bluegill)					
Hybopsis	O,B	4/5/78	14	17	12
leptocephala		7/28/78	15	13	7
(bluehead chub)		11/7/78	12	9	1
		2/26/79	3	6	6
Cyprinus	O,B	4/5/78	3	0	0
carpio		7/28/78	1	0	0
(carp)		11/7/78	1	0	0
Notropis	O,B	4/5/78	14	10	0
cerasinus		7/28/78	7	4	0
(crescent shiner)		11/7/78	11	19	0
		2/26/79	13	8	0
Etheostoma	O,B	4/5/78	0	5	4
flabellare		7/28/78	5	10	10
(fantail darter)		11/7/78	1	12	9
		2/26/79	13	14	1
Micropterus	O,WC	11/7/78	2	0	0
salmoides		2/26/79	1	0	0
(largemouth bass)					
Chrosomus	H,B	4/5/78	0	5	4
oreas		7/28/78	0	1	0
(mountain redbelly		11/7/78	0	0	2
dace)		2/26/79	1	10	8
Hypentelium	O,B	4/5/78	3	4	0
nigricans		7/28/78	0	1	0
(northern hogsucker)		11/7/78	4	0	0
		2/26/79	5	3	0
Lepomis	O,WC	4/5/78	12	0	0
auritus		7/28/78	1	4	0
(redbreast sunfish)		11/7/78	13	0	0
		2/26/79	4	6	0
Ambloplites	O,WC	4/5/78	5	0	0
rupestris		7/28/78	1	0	0
(rock bass)		11/7/78	7	0	0
		2/26/79	1	0	0

Table 3. Sediments, invertebrates, and fish analyzed from each area, including classifications applied for data analysis: predator-engulfer (PE), shredder-detritivore (SD), herbivore (H), omnivore (O), bottom feeder (B), and upper water column feeder (WC). (continued).

Sample	Classification	Date of collection	Number analyzed A	B	C
Catostomus commersoni (white sucker)	O,B	4/5/78	8	13	0
		7/28/78	9	2	0
		11/7/78	6	15	0
		2/26/79	3	5	0

metals (Table 4). Samples were then placed in a Labconco Freeze Dry-5 freeze-drier for approximately 24 h to eliminate moisture, after which they were moved to a drying oven at 75 to 80 C for 48 h. Invertebrate samples were then weighed to 0.001 g into ignition tubes for heavy metal analysis.

All fish collected (Table 3) were measured for total length (mm), weighed (0.1 g), and identified to species (Eddy 1977). Each species was characterized by food and feeding habits according to Carlander (1969, 1977) as herbivores or omnivores, and as bottom feeders or upper water column feeders. Scale samples were removed and age determinations were made from plastic impressions by standard techniques (Lagler 1956). The contents of the digestive tracts of the fish were removed to reduce variations due to heavy metals present in undigested matter. Fish less than 100 mm total length were then placed in shell vials. Larger fish were sectioned and placed in vials. Fish larger than approximately 170 mm total length were utilized for analyses of kidney, liver, spleen, gills, gonads, bone, and muscle tissue. Tissue analyses were restricted to fish taken from areas A and B, since large individuals were not found at area C. The fish samples were then freeze-dried and weighed to 0.001 g into ignition tubes for heavy metal analysis.

Metal Analyses

The ignition tubes containing the dried samples were placed in a Thermolyne Furnatrol II muffle furnace to burn off the organic portion, leaving the inorganic ash content of the sample. A three-hour warm-up period at 200 to 250 C to prevent "flashing" (instantaneous combustion causing emission of both organic and inorganic constituents) was followed

Table 4. Detection limits of lead, nickel, cadmium, and zinc for the experimental range of sample weights and dilutions.

Sample Dry Weight (g)	Dilution Volume (ml)	Minimum Conc. Detectable (µg/g)			
		Pb	Ni	Cd	Zn
Water					
0.010-0.150	1	0.003	0.003	0.003	0.003
--	50	0.0005	0.0005	0.0005	0.0005
Sediment					
0.550-0.650	10	0.091	0.091	0.009	0.091
0.650-0.750	10	0.077	0.077	0.008	0.077
0.750-0.850	10	0.067	0.067	0.007	0.067
0.850-1.000	10	0.059	0.059	0.006	0.059
1.000-1.500	10	0.050	0.050	0.005	0.050
1.500-2.000	10	0.033	0.033	0.003	0.033
Invertebrates					
0.010-0.150	5	2.500	2.500	0.250	2.500
0.150-0.250	10	0.333	0.333	0.033	0.333
0.250-0.350	10	0.200	0.200	0.020	0.200
0.350-0.450	10	0.143	0.143	0.014	0.143
0.450-0.550	10	0.111	0.111	0.011	0.111
0.550-0.650	10	0.091	0.091	0.009	0.091
0.650-0.750	10	0.077	0.077	0.008	0.077
0.750-0.850	10	0.067	0.067	0.007	0.067
0.850-1.000	10	0.059	0.059	0.006	0.059
Fish					
0.010-0.150	5	2.500	2.500	0.250	2.500
0.150-0.250	10	0.333	0.333	0.033	0.333
0.250-0.350	10	0.200	0.200	0.020	0.200
0.350-0.450	10	0.143	0.143	0.014	0.143
0.450-0.550	10	0.111	0.111	0.011	0.111
0.550-0.650	10	0.091	0.091	0.009	0.091
0.650-0.750	10	0.077	0.077	0.008	0.077
0.750-0.850	10	0.067	0.067	0.007	0.067
0.850-1.000	10	0.059	0.059	0.006	0.059
1.000-1.500	10	0.050	0.050	0.005	0.050
1.500-2.000	10	0.033	0.033	0.003	0.033
2.000-2.500	10	0.025	0.025	0.003	0.025
2.500-3.000	10	0.020	0.020	0.002	0.020
3.000-	10	0.017	0.017	0.002	0.017

by 18 h at 525 C. The ashed samples were digested with 5 to 10 ml of a solution of 1 HNO_3:1 HCl:2 H_2O (deionized and distilled) to free all metallic constituents of the ash into solution. Thorough digestion was assured by mixing of each sample with a Vortex-Genie. Particles in the acid solution were removed from suspension by centrifugation at 2800 rpm for 10 min to avoid aspiration into the atomic absorption spectrophotometer. Analyses for lead, nickel, cadmium, and zinc content were then performed with a Perkin-Elmer Model 460 atomic absorption spectrophotometer following standard techniques (Perkin-Elmer 1976). All concentrations are expressed in µg/g dry weight. Detection limits of heavy metals achieved by these procedures are listed in Table 4. The reduced sensitivity for small sample weights did not influence comparisons except in a conservative manner due to the wide range of concentrations included in each increment of the spectrophotometer reading, since measurements were of acid-digested samples, not actual concentrations.

Statistical Interpretation

Statistical analysis of the distribution of the data sets (Hollander and Wolfe 1973) indicated generally nonnormal distributions. The procedures used in subsequent data analysis were therefore nonparametric (Table 5). Nonparametric procedures are usually only slightly less efficient than their parametric counterparts under conditions of normality, while they are generally far more appropriate when the distribution of the data is nonnormal.

Statistical comparisons of heavy metal concentrations were made among study sites and seasons for water, sediments, benthic invertebrates, and fish (Table 3); among taxonomic units of benthos and fish;

Table 5. Statistical procedures applied to analyze various data sets for the three study locations.

Data Set	Wilcoxon Rank Sum	Kruskal-Wallis test	Friedman's test	Jonckheere procedure	Spearman's Rank Correlation
Heavy metal differences among study areas	X		X	X	X
Heavy metal differences among seasons	X	X	X		
Heavy metal differences among taxonomic units	X	X	X		
Heavy metal differences among tissues		X	X		
Heavy metal correlations to length, weight, age, and particle size					X
Heavy metal differences among trophic levels		X	X		

among whole body concentrations in individual benthic invertebrates and fish; among tissue concentrations in fish; and among trophic levels of benthos and fish. Throughout all comparisons, differences were considered statistically significant at the 10 percent level (P = 0.10).

RESULTS AND DISCUSSION

Water Quality

Measurements of the variation in temperature, dissolved oxygen, alkalinity, hardness, and turbidity at each area over the year-long study period are summarized in Table 6. Dissolved oxygen concentrations at each area were well above critical levels necessary for the safety of aquatic life (Brown 1968).

The relatively higher hardness and alkalinity values found at the downstream sampling area indicated a potentially higher degree of complexation of incoming heavy metals, thereby reducing both their toxic effect and their availability for uptake and accumulation by stream organisms (Bryan 1976; Zitko and Carson 1976). However, since all hardness values were well within the soft-water category: ≤ 60 mg/l $CaCO_3$ on a scale of 0 to 360 mg/l $CaCO_3$ (National Academy of Sciences 1972b), this effect was probably minimal. In addition, the higher turbidity downstream indicated increased sediment-water interaction, which increases the availability of heavy metals from sediment pools (Jenne and Luoma 1977).

Ranges of streamwater concentrations of lead, nickel, cadmium, and zinc during normal flow periods are given in Table 7. All are extremely low, not exceeding values for uncontaminated North American streams, and most likely never exceeding baseline concentrations except during runoff periods of roadside metal accumulations (Klein et al. 1974). Levels of each metal in the suspended fraction were up to 9.5 times the concentrations in the dissolved fraction, but had little effect on total water concentrations due to the low particulate content of the water. This

Table 6. Ranges and median values (in parentheses) of water quality parameters at each area recorded quarterly between April 1978 and February 1979.

Study Area	Temperature (C)	D. O. (mg/1)	Alkalinity (mg/1)	Hardness (mg/1)	Turbidity (FTU)
A	8.3-22.8 (17.1)	8.1-9.2 (8.7)	24-51 (33)	28-54 (36)	12-84 (49)
B	7.8-20.0 (14.4)	9.0-9.6 (9.3)	19-43 (31)	20-40 (30)	9-68 (29)
C	5.6-17.8 (11.6)	9.3-10.0 (9.7)	14-32 (19)	16-37 (23)	8-78 (26)

Table 7. Concentrations of lead, nickel, cadmium, and zinc in Back Creek water as compared to normal ranges for other, uncontaminated waters (U. S. Environmental Protection Agency 1971, 1976).

Metal	Component	Concentration (mg/l) Area A	Area B	Area C	Reference Waters
Pb	Dissolved	≤.004	≤.004	≤.003	0.000-0.140
	Suspended	0.038	0.026	0.010	0.010-4.800
	Total	0.004	0.004	0.003	0.000-0.140
Ni	Dissolved	≤.004	≤.004	≤.003	0.000-0.086
	Suspended	0.017	0.011	0.010	0.005-2.600
	Total	0.004	0.003	0.003	0.000-0.130
Cd	Dissolved	≤.003	≤.003	≤.004	0.000-0.010
	Suspended	≤.003	≤.003	≤.003	0.000-1.300
	Total	0.0005	0.0005	0.0005	0.000-0.010
Zn	Dissolved	0.018	0.018	0.015	0.000-1.200
	Suspended	0.110	0.100	0.080	0.050-5.250
	Total	0.020	0.021	0.014	0.000-1.182

disparity between dissolved and suspended concentrations of heavy metals corresponds well to the findings of Perhac (1972) for two Tennessee streams, and to those of Suzuki et al. (1979) for the Tama River in Japan.

Relation of Heavy Metal Concentration to Traffic Volume

Sediments

Sediment loads of lead, nickel, and zinc were all highly correlated ($P<.006$) to the traffic volumes received by each area, respectively (Fig. 3). Only cadmium sediment loads were not significant ($P>.34$), possibly due to extremely low concentrations (range: <0.02 to 0.24 µg/g), in many cases below the detectable limits of the spectrophotometer for sample weights of over one gram (Table 4).

Concentrations of all four metals were up to 9000 times higher in sediments than in water, with the greatest disparity at area A (Fig. 4). The greatest differences between sediment and water concentrations at each area were found in lead, followed in magnitude by zinc and nickel, with cadmium exhibiting the least increase in sediment loads over water concentrations. These findings are consistent with the relative concentration of each metal, which is probably contributed to the stream via runoff (Klein et al. 1974) (i.e. the greater the runoff concentration of a metal, the greater the difference between sediment loads and normal water concentrations of the metal). The only exception to this relationship was zinc, which was present at relatively high natural levels, which reduced the disparity between water and sediment loads of this metal. These findings are similar to those of other investigators (Mathis and Cummings 1973; Enk and Mathis 1977; Anderson and Brower 1978).

Fig. 3. Range, 25th and 75th percentile levels (box), and median concentrations (bar) of lead, nickel, cadmium, and zinc in sediments at each area over a one-year period.

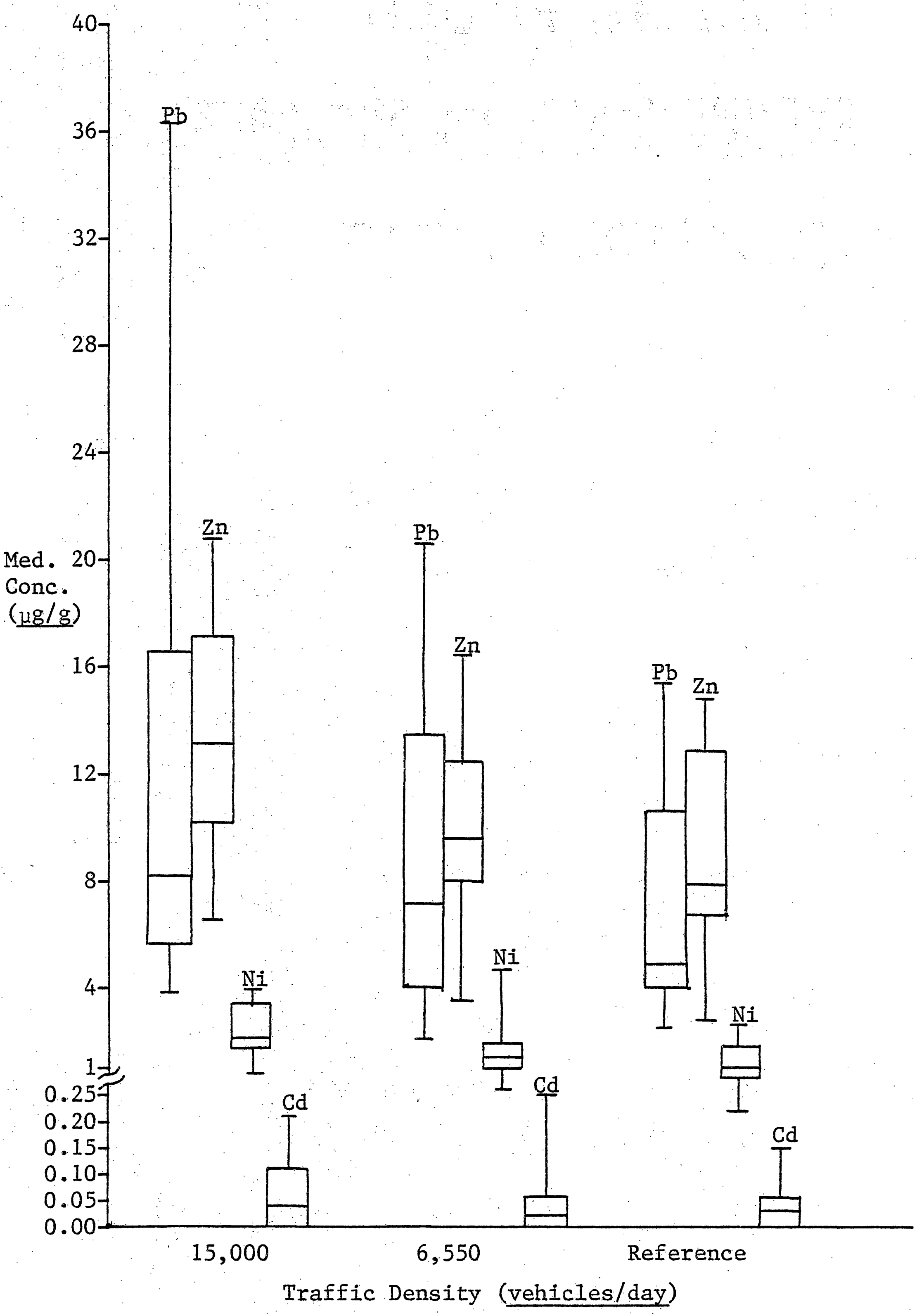
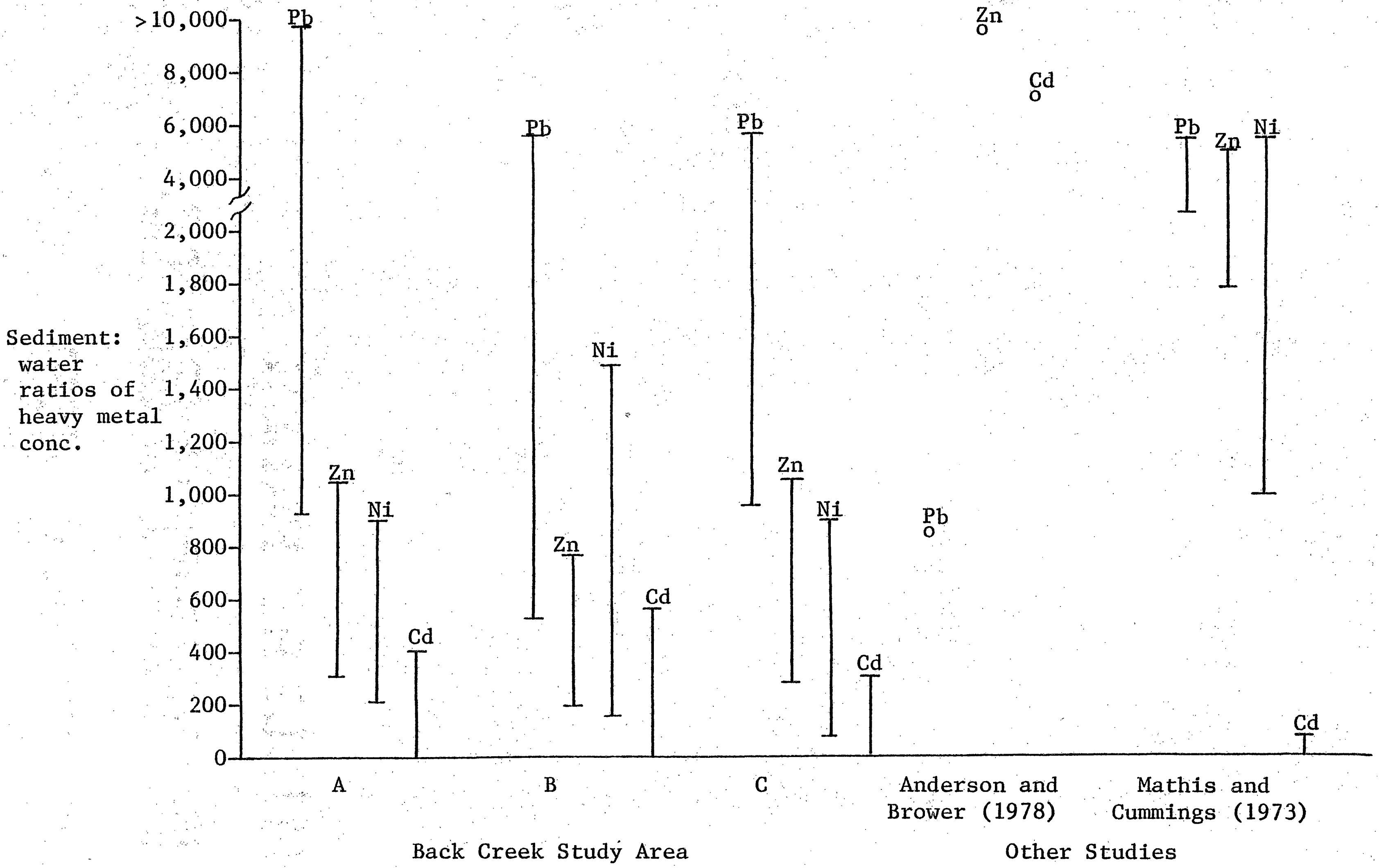

40
36
32
28
24
20
16
12
8
4
1
0.25
0.20
0.15
0.10
0.05
0.00
Med.
Conc.
(µg/g)
Pb
Zn
Ni
Cd
Pb
Zn
Ni
Cd
Pb
Zn
Ni
Cd
15,000
6,550
Reference
Traffic Density (vehicles/day)

Fig. 4. Range of increases in sediment levels of lead, nickel, cadmium, and zinc over streamwater concentrations at each study site over a one-year period, as compared to findings of other investigators.



Sediment: water ratios of heavy metal conc.
>10,000
8,000
6,000
4,000
2,000
1,800
1,600
1,400
1,200
1,000
800
600
400
200
0
Pb
Zn
Ni
Cd
A
B
C
Anderson and Brower (1978)
Mathis and Cummings (1973)
Back Creek Study Area
Other Studies

These results have three important implications: (1) The role of sediments as a sink for heavy metal particulates (Gale et al. 1973; Getz et al. 1977). Natural concentrations in water are low, but any influx of high levels of particulate metals will quickly be adsorbed by the surfaces of both suspended particles in the water and, in particular, sediments (Oliver 1973; Cook 1977). (2) Sediment pools of heavy metals are a constant and highly available source for uptake of these elements by bottom-associated inhabitants of a stream (Delisle et al. 1975; Jenne and Luoma 1977). (3) The direct relationship between the volume of particulate emissions to the roadside stream ecosystem and the levels present in the substrate of that system. This relationship has not been previously documented.

According to Oliver (1973), finer sediment samples will usually contain higher concentrations of metal due mainly to their larger surface area, and correspondingly greater adsorptive capacity. Correlations of lead, nickel, cadmium, and zinc to particle size of Back Creek sediments were not significant ($P>.10$) at any area. This may be a function of inadequate distribution of samples over all particle sizes (Table 3). Visual examination of the data, however, does indicate a possible link between sediment particle size and heavy metal concentration as described by Oliver.

Invertebrates

The results of the heavy metal analysis of benthic macroinvertebrates are summarized in Table 8. Whole body concentrations of lead were highly correlated ($P<.025$) to traffic density in three of the four families sampled; only Corydalidae did not differ significantly between

Table 8. Ranges and median concentrations (in parentheses) of lead, nickel, cadmium, and zinc in macroinvertebrate families sampled over a one-year period. P-value indicates the degree of correlation to traffic density.

Sample	Area	Number Analyzed	Metal Concentration (μg/g) Lead	Nickel	Cadmium	Zinc
Diptera Tipulidae	A	16	5.88-44.2 (17.3)	<.74-10.0 (6.69)	<.07-2.24 (0.97)	64.4-217 (106)
	B	29	<.30-25.9 (12.0)	<.30-9.26 (3.72)	<.03-2.27 (0.90)	50.0-486 (92.4)
	C	25	<.32-36.7 (7.29)	<.32-16.3 (1.79)	<.03-2.61 (0.78)	61.4-170 (83.2)
		-P-	<.005	>0.40	>0.40	<0.08
Megaloptera Corydalidae	A	91	<.15-67.6 (10.1)	<.30-17.2 (2.60)	<.05-3.61 (0.69)	8.42-200 (137)
	B	34	<1.47-23.2 (13.4)	<.31-12.5 (2.48)	<.03-13.6 (0.36)	1.47-250 (132)
		-P-	>.50	>.50	0.20	>.287
Plecoptera Perlidae	A	15	6.52-56.7 (27.6)	1.27-16.7 (11.0)	<.03-2.52 (0.88)	154-287 (204)
	B	21	3.23-36.4 (19.7)	<.52-12.5 (6.45)	<.06-2.50 (0.83)	155-527 (227)
	C	50	2.38-29.2 (17.8)	<.35-14.7 (2.88)	<.03-3.10 (0.69)	<.25-457 (235)
		-P-	0.02	0.0015	0.406	>.50
Plecoptera Pteronarcidae	A	34	<.26-76.5 (20.4)	<.74-15.1 (<2.27)	0.52-3.37 (1.01)	217-341 (240)
	B	14	<.51-26.8 (16.4)	<.40-10.4 (3.39)	0.16-2.11 (0.99)	135-413 (232)
	C	79	<.31-59.5 (7.14)	<.05-13.8 (2.38)	<.03-1.89 (0.63)	41.3-757 (254)
		-P-	<.0005	>.50	0.017	>.50

areas A and B (P>.5). Concentrations of nickel were correlated to traffic density in both Tipulidae (P<.005) and Perlidae (P<.002). Only Pteronarcidae showed a significant correlation (P<.02) between cadmium concentration and traffic density, while Tipulidae demonstrated the only significant correlation (P<.09) between zinc concentration and traffic density. These results indicate that, at least in some benthic invertebrate taxa, heavy metal concentrations may be a function of the volumes of heavy metals emitted to a roadside stream within the effective range of the emission source, which has yet to be determined. Gale et al. (1973) found lead, zinc, and cadmium concentrations in aquatic vegetation and sediments returned to control levels approximately 6.5 km downstream of the source of effluent discharge. The distance upstream affected by aerial deposition has not been studied. Although higher levels of lead, zinc, and cadmium were observed in crayfish near a highway compared to control specimens (Gale et al. 1973), no correlations between increased traffic use and the heavy metal content of associated invertebrate organisms have been reported.

Although invertebrates have been reported to assimilate heavy metals at similar rates (Nehring et al. 1979), the concentration present in a given individual is influenced by a number of factors. Most important is the availability of the metal, which is a function of the form and amount of the metal in both water and ingested solids, the physiological and ecological characteristics of the organism, and the chemical and physical characteristics of the water (Jenne and Luoma 1977). The higher concentrations of lead at area A account for the elevated levels of this metal present in invertebrates at this location. This is also the most likely reason for the comparative lack of significant differences due to associated traffic densities in the nickel and cadmium concentrations of most of the invertebrate families, since the low environmental concentrations of these two metals causes the amount present in a given individual to be extremely time- and location-dependent. There is some indication of increased nickel and cadmium levels in invertebrates associated with highway activity (Table 8), but the increased variability due to limited metal availability, in addition to the reduced spectrophotometer sensitivity due to the combination of low metal concentrations and low sample weights (Table 4), served to obscure any significant differences present. The lack of significant differences among areas in the zinc concentrations of three of the four invertebrate families is an indication of the reported ability of aquatic organisms to maintain some degree of internal regulation of levels of this metal in tissues (Bryan 1976; Anderson and Brower 1978). There were no apparent relationships between heavy metal concentrations and the ecological characteristics of the four invertebrate families (see p. 56).

The fact that the concentrations of each metal were relatively greater (1.25 to 24 times median concentrations) in invertebrates than in sediments is characteristic of stream ecosystems with relatively low sediment concentrations of these metals (Enk and Mathis 1977; Anderson and Brower 1978). This does not necessarily indicate biomagnification; invertebrate animals readily assimilate heavy metals in bodily tissues, where residence times are much greater than those for sediments, which may continually lose and receive heavy metals through interaction with water (Jenne and Luoma 1977), and are therefore good indicators of heavy metal passage through a system. In streams containing extremely high sediment heavy metal concentrations, sediment levels may exceed invertebrate levels due to plateaus in invertebrate heavy metal accumulation (Kincade and Erdman 1975).

Fish

High correlations between traffic volume and heavy metal content were also evident in most fish species analyzed (Table 9). Fish were directly influenced by the quantity of heavy metal input to their habitat, as were the invertebrates. In instances where a significant correlation did not exist, notably in the northern hogsucker (Hypentelium nigricans), an apparent difference existed in the data, but lacked sufficient data points (12 sampled at area A; 8 at area B) to demonstrate statistical significance. No other published research has documented this correlation, although similar increases in whole body heavy metal concentrations in fish attributed to industrial pollutants have been reported (Gale et al. 1973; Atchison et al. 1977; McNurney et al. 1977).

The concentrations of nickel, cadmium, and zinc in Back Creek fish are fairly consistent with those reported by other investigators for a variety of aquatic systems (Gale et al. 1973; McIntosh and Bishop 1976; Giesy and Wiener 1977). Lead, however, was substantially higher (up to five times median concentrations) in fish from areas A and B than for fish from other rural and semi-rural areas (Rolfe and Jennett 1975; Enk and Mathis 1977; Getz et al. 1977), which were comparable to levels in fish from reference area C. This phenomenon is most likely attributable to the fact that 95 percent of all emissions of lead originate from automobile exhausts (Wheeler et al. 1978), so that areas such as those

Table 9. Ranges and median whole body concentrations (in parentheses) of lead, nickel, cadmium, and zinc in fish sampled over a one-year period. P-value indicates the degree of correlation to traffic density.

Sample	Area	Number Analyzed	Metal Concentration (µg/g)			
			Lead	Nickel	Cadmium	Zinc
Blacknose dace	A	19	9.90-26.0	1.88-4.04	0.41-1.09	187-388
			(15.5)	(2.99)	(0.76)	(331)
	B	24	4.87-36.3	0.42-8.82	<.03-0.65	103-588
			(11.3)	(2.87)	(0.33)	(248)
	C	44	2.01-18.4	0.83-3.69	0.37-6.32	111-257
			(7.16)	(1.97)	(0.66)	(151)
		-P-	<.001	<.0015	<.0038	<.0005
Bluehead chub	A	44	2.08-61.0	1.71-15.1	<.08-1.31	82.0-171
			(8.38)	(2.47)	(0.42)	(98.3)
	B	45	2.10-18.6	<.23-6.67	<.02-0.72	84.3-288
			(6.59)	(2.53)	(0.41)	(93.5)
	C	26	3.14-7.40	0.83-3.69	0.09-0.50	41.9-88.4
			(5.38)	(1.97)	(0.32)	(75.9)
		-P-	<.001	<.0005	<.0038	<.001
Crescent shiner	A	45	5.07-19.7	<.36-37.2	<.05-0.95	134-376
			(7.86)	(2.01)	(0.38)	(181)
	B	41	0.96-19.3	<.05-4.90	<.01-0.89	47.6-292
			(8.51)	(2.32)	(0.38)	(178)
		-P-	>.50	>.50	>.50	>.50
Fantail darter	A	19	11.9-28.8	3.70-13.4	0.16-1.02	91.1-224
			(19.5)	(5.93)	(0.60)	(147)
	B	41	<.22-42.4	<.22-10.3	<.02-2.62	92.1-168
			(13.5)	(3.45)	(0.72)	(115)
	C	24	<.17-15.4	<.17-11.8	<.02-1.91	87.0-128
			(9.59)	(2.86)	(0.65)	(112)
		-P-	<.0005	<.0005	>.35	<.05
Mountain red-belly dace	A	1	22.8	3.00	0.63	293
			(22.8)	(3.00)	(0.63)	(293)
	B	38	<.14-25.7	<.14-23.3	<.01-1.30	160-392
			(10.7)	(2.44)	(0.33)	(246)
	C	18	5.03-9.34	0.89-3.98	0.25-1.23	143-209
			(7.16)	(2.05)	(0.40)	(159)
		-P-	<.0005	>.40	>.40	<.0005

Table 9. Ranges and median whole body concentrations (in parentheses) of lead, nickel, cadmium, and zinc in fish sampled over a one-year period. P-value indicates the degree of correlation to traffic density. (continued).

			Metal Concentration (µg/g)			
Sample	Area	Number Analyzed	Lead	Nickel	Cadmium	Zinc
Northern hogsucker	A	12	6.65-13.8	2.10-4.87	0.32-0.50	77.5-111
			(10.4)	(2.76)	(0.40)	(89.4)
	B	8	7.48-13.0	1.63-5.12	0.07-0.57	72.8-139
			(8.34)	(2.75)	(0.35)	(77.8)
		-P-	>.50	>.52	>.235	>.220
Redbreast sunfish	A	30	5.57-11.3	<.29-26.3	0.03-1.05	41.1-117
			(7.15)	(2.51)	(0.40)	(79.0)
	B	10	6.15-9.34	1.64-2.84	0.34-0.47	41.0-96.7
			(7.85)	(2.23)	(0.42)	(72.4)
		-P-	>.50	<.08	>.457	>.140
White sucker	A	26	6.50-27.6	2.10-13.5	0.09-0.62	41.0-114
			(12.1)	(2.80)	(0.44)	(66.6)
	B	35	1.87-11.0	<.03-6.12	<.01-0.75	50.3-123
			(6.69)	(2.07)	(0.27)	(73.0)
		-P-	<.0005	<.003	<.0064	>.50

on Back Creek which come into contact with highways will reflect this association through the high lead content of their various components. Nickel, cadmium, and zinc are emitted in substantial amounts from sources other than automobiles (National Academy of Sciences 1975; U. S. Environmental Protection Agency 1975), so that they are distributed to a variety of other aquatic environments.

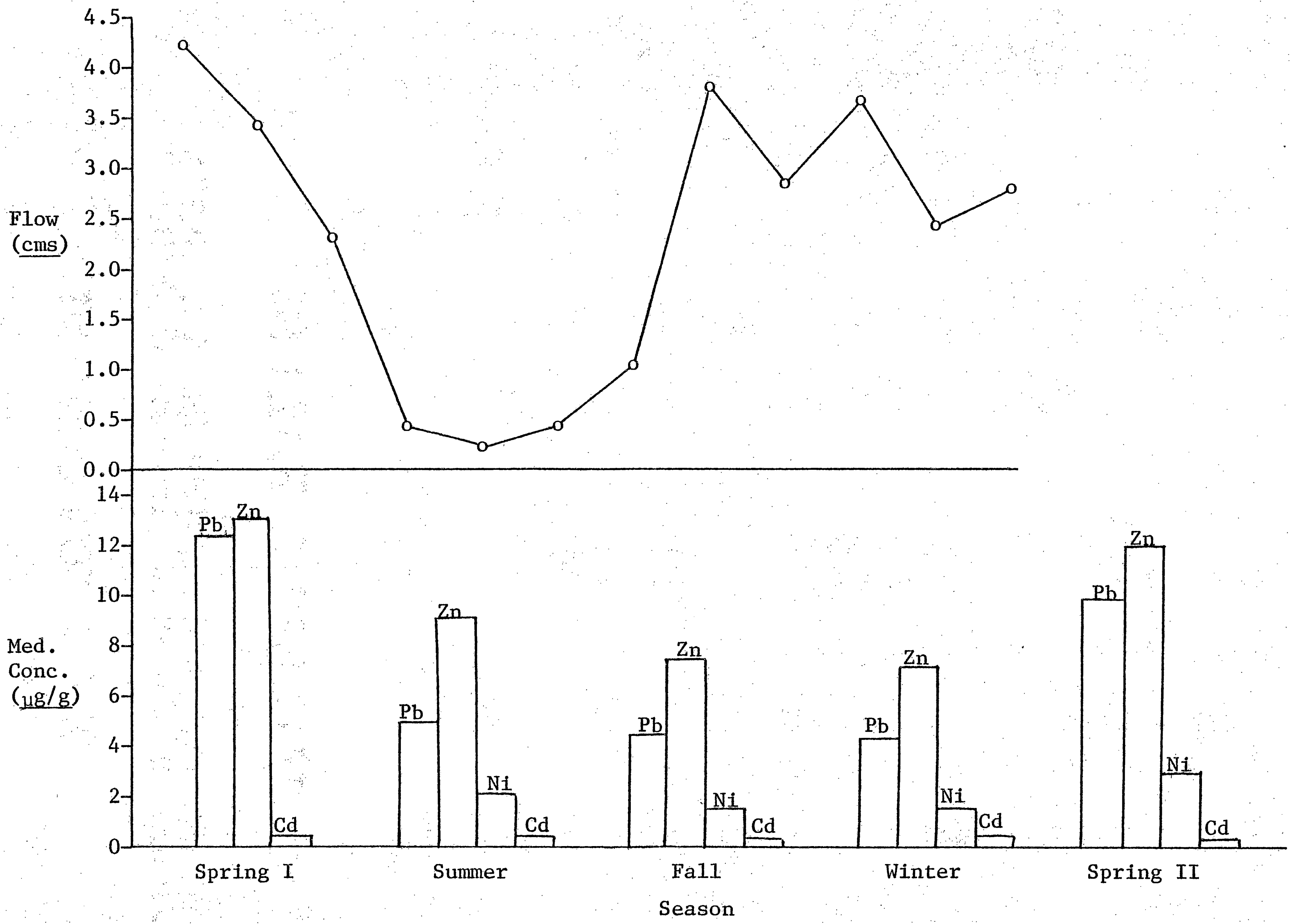
A comparison of data in Tables 8 and 9 reveals that invertebrates generally contained slightly higher (1.2 to 2.4 times) concentrations of all four metals than fish. Although this phenomenon will be discussed more thoroughly in relation to trophic biomagnification, it is appropriate to suggest that this difference was probably the result of at least two factors: (1) all four of the invertebrate families studied were more intimately associated with the high heavy metal content of the substrate than most of the fish; and (2) much of the bulk of fish is composed of tissue with relatively low heavy-metal affinity, e.g. muscle (Jenne and Luoma 1977), while invertebrates consist mostly of viscera and hard parts--both of which are usually high in heavy metals (Anderson and Brower 1978). These two factors are independent of the system under investigation and have been verified by other studies (Mathis and Cummings 1973; Rolfe and Jennett 1975; Enk and Mathis 1977; McNurney et al. 1977; Wheeler et al. 1978).

Seasonality of Heavy Metal Concentrations

Sediments

Sediment concentrations of lead, nickel, and zinc were highest at each area in spring, showing similar decreases through summer, fall, and winter. Figure 5 illustrates the significantly higher ($P<.005$)

Fig. 5. Median concentrations of lead, nickel, cadmium, and zinc in sediments for combined areas over a 14-month period, as compared to U. S. Geological Survey streamflow data for Back Creek.


Flow
(cms)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Med.
Conc.
(µg/g)
14
12
10
8
6
4
2
0
Pb
Zn
Cd
Pb
Zn
Ni
Cd
Pb
Zn
Ni
Cd
Pb
Zn
Ni
Cd
Pb
Zn
Ni
Cd
Spring I
Summer
Fall
Winter
Spring II
Season

concentrations of lead, nickel, and zinc for all areas in spring, the gradual decrease over the remainder of the year, followed by high concentrations the next spring (no data were obtained for nickel in the first spring sample). High spring concentrations (up to four times median concentrations) most likely resulted from runoff of metal-laden snow from roadsides and surrounding regions of particulate deposition, and subsequent adsorption to trapped sediments in the stream. This hypothesis is supported by U. S. Geological Survey monthly streamflow data (Fig. 5), which indicated that during 1978, as well as most preceding years, peaks in both daily and monthly flows on Back Creek occurred in the spring, particularly April, which can be attributed to the increased runoff due to spring rains and melting snow. Uptake by other components in the system and the gradual tendency toward an equilibrium sediment-water interaction may have accounted for the decrease observed in sediment loads of lead, nickel, and zinc during the remainder of the year. Sediment concentrations of heavy metals are simply adsorbed to the surface of individual sediment particles, and are easily lost to water and taken up by other components of the system or transported downstream.

Seasonal variations in cadmium were not significant ($P>.25$) at any site. Concentrations of this element in sediment were so low that no significant variations could be detected in the data (range: <.02 to 0.24 μg/g).

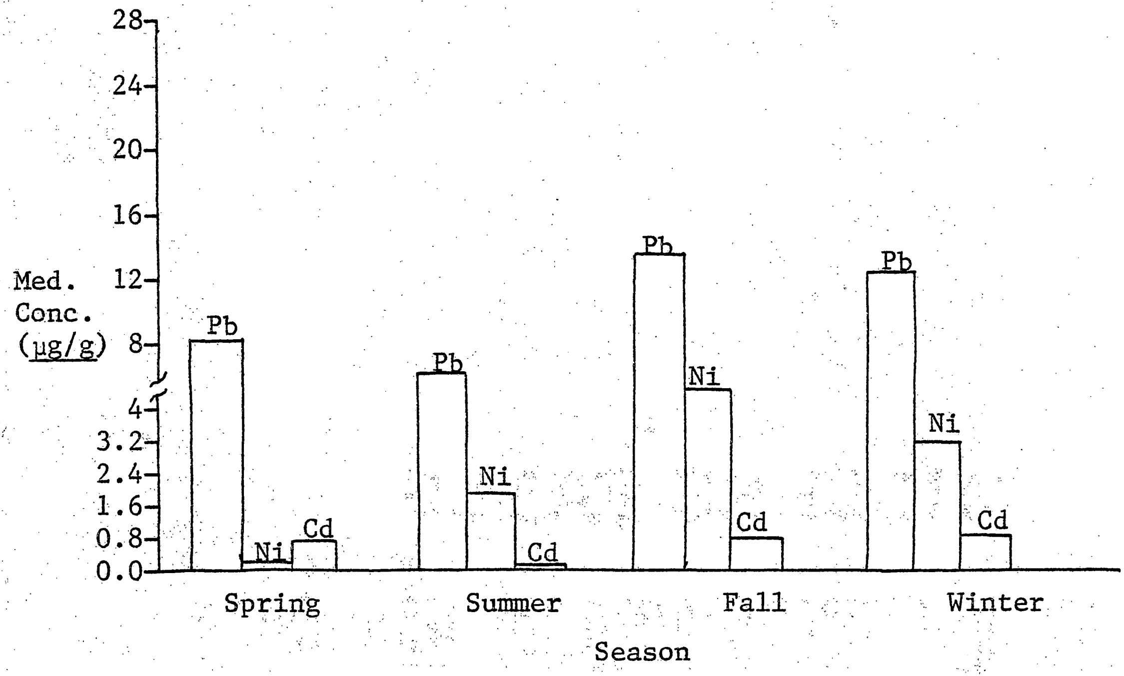
These results again emphasize the potential impact of highway-generated heavy metals on nearby stream ecosystems, especially when runoff is the major contributor of contamination. Namminga (1977) found sediment concentrations of zinc higher in summer, but this result was attributed to decreased scour during low-flow periods and no reference was made to highway runoff.

Invertebrates

Significant seasonal variation did not exist ($P>.10$) in the data for any of the four invertebrate families at areas A or B for any of the four metals. The failure of these organisms to exhibit higher heavy metal concentrations during the spring corresponding to the higher spring sediment levels may be a function of the slow rates of exchange common to muscle and hard body parts (Jenne and Luoma 1977; Anderson and Brower 1978), so that a sudden influx of metals would not be immediately apparent in whole-body concentrations of these metals, but only in tissues such as the gills and kidney.

Significant seasonal differences ($P<.10$) did occur at area C for lead, nickel, and cadmium in the two stonefly families (Fig. 6), but not for zinc ($P>.14$) or for tipulids ($P>.19$) at this site. The only consistent difference at area C was that fall concentrations of the three metals were generally highest. There is no readily apparent reason for this finding, especially since heavy metal availability is lowest at area C, and there is no evidence of similar differences at either of the other two sites. It is possible that there was a period of higher concentrations of these metals, as was apparent in the spring sediments, after which the low heavy metal concentrations that usually prevail at area C allowed soft tissue burdens of these metals to be accumulated in the exoskeleton (Anderson and Brower 1978), reaching a peak in

Fig. 6. Median concentrations of lead, nickel, and cadmium in pteronarcid and perlid stoneflies at area C over a one-year period.


28
24
20
16
12
8
4
3.2
2.4
1.6
0.8
0.0
Med.
Conc.
(µg/g)
Pb
Ni
Cd
Pb
Ni
Cd
Pb
Ni
Cd
Pb
Ni
Cd
Spring
Summer
Fall
Winter
Season

the fall. The consistent input of heavy metals at areas A and B would prevent a similar effect in invertebrates at these areas.

Other investigators have also failed to establish consistent seasonal variations in the accumulation of heavy metals by invertebrate animals (Rolfe and Jennett 1975; Getz et al. 1977).

Fish

Significant seasonal variation did not occur ($P > .10$) in heavy metal levels in fish. However, zinc concentrations appeared slightly higher in the spring samples for many species, which could be related to the high spring sediment levels or internal regulation of this metal by the fish (Bryan 1976). As with invertebrates, the slow rates of exchange in many tissues may cause the bioaccumulation of heavy metals to be spread over extended periods of exposure, so that sudden influxes of high levels of these metals would not be immediately reflected in whole-body burdens. Seasonal trends in the heavy metal content of specific tissues are discussed in a later section devoted to tissue burdens of heavy metals.

The only previously observed seasonal trends were concerned with estuarine fish (Hardisty et al. 1974a; Badsha and Sainsbury 1978), and were not conclusive.

Bioaccumulation of Heavy Metals

Taxonomic Differences

Invertebrates. Taxonomic differences among the four insect families in the whole-body concentrations of each metal are depicted in Figure 7. The breakdown by study area and metal is provided in Table 10. In general, the two stonefly families accumulated up to three times the median

Fig. 7. Range, 25th and 75th percentile levels (box), and median concentrations (bar) of lead, nickel, cadmium, and zinc in macroinvertebrate families from all study areas over a one-year period.

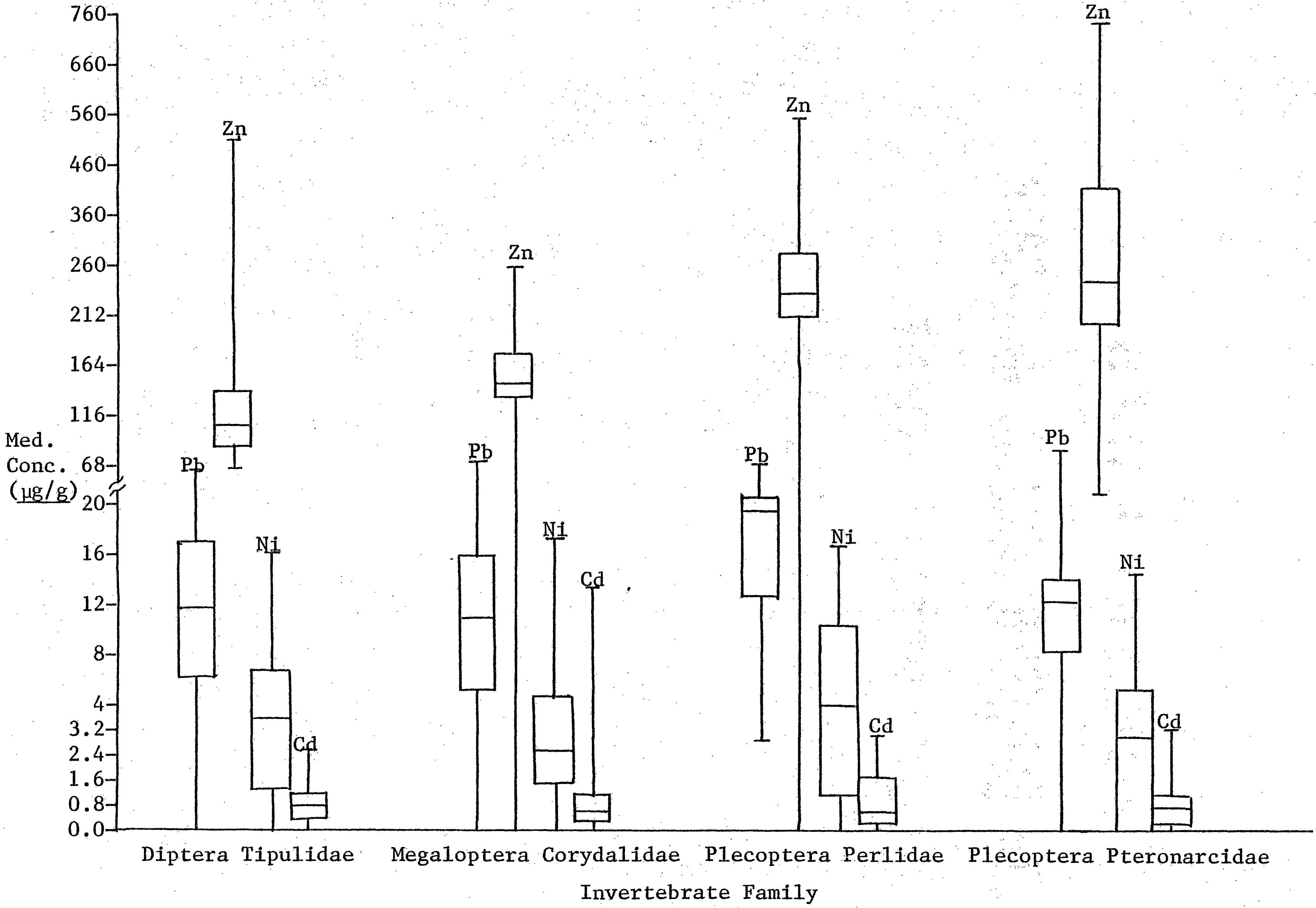

760
660
560
460
360
260
212
164
116
68
20
16
12
8
4
3.2
2.4
1.6
0.8
0.0
Med.
Conc.
(μg/g)
Zn
Pb
Ni
Cd
Zn
Pb
Ni
Cd
Zn
Pb
Ni
Cd
Zn
Pb
Ni
Cd
Diptera Tipulidae
Megaloptera Corydalidae
Plecoptera Perlidae
Plecoptera Pteronarcidae
Invertebrate Family

Table 10. Taxonomic differences among four invertebrate families in lead, nickel, cadmium, and zinc concentrations at each study location. Underlined families did not show a significant difference ($P > .10$). (T = Tipulidae, C = Corydalidae, Pt = Pteronarcidae, Pe = Perlidae).

Study Area	Metal	Level of Significance	Order of Metal Concentrations (from least to greatest)
A	Pb	<.005	C T Pt Pe
B		<.005	T C Pt Pe
C		<.025	Pt T Pe
A	Ni	<.005	Pt C T Pe
B		0.400	Not significant
C		>.500	Not significant
A	Cd	0.060	C Pt T Pe
B		0.300	Not significant
C		0.200	Not significant
A	Zn	<.005	T C Pe Pt
B		<.005	T C Pt Pe
C		<.005	T Pe Pt

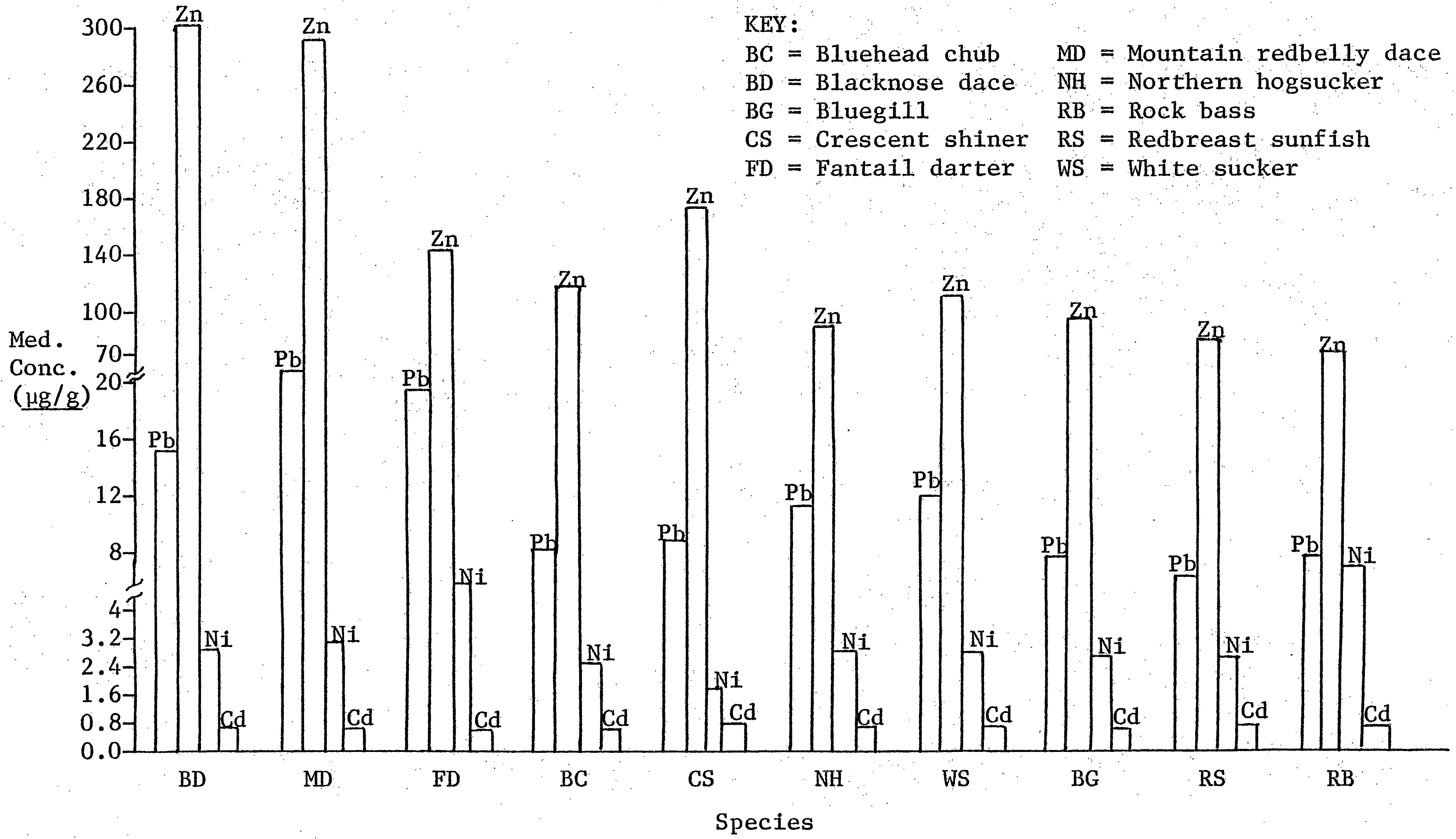
concentrations of lead and zinc found in tipulids and hellgrammites. This trend suggests a physiological basis for the differences, since (1) all individuals have similar life cycles and were collected from basically similar substrate at each area; and (2) no distinctions can be made on the basis of food or feeding habits, since pteronarcid stoneflies (detritivores) and perlid stoneflies (predators) contained higher lead and zinc levels than tipulids (detritivore-herbivores) and hellgrammites (predators). The availability of both lead and zinc was generally the highest of the four metals, so that organisms with a greater percentage of tissue with a high heavy-metal affinity and residence time should accumulate greater quantities of these two metals over an extended period of time. The two stonefly families have a higher percentage of hard body parts, which have been shown to be sinks for lead, in particular (Anderson and Brower 1978), than do hellgrammites or tipulids. Since the aquatic life spans of the four families do not differ substantially (Merritt and Cummins 1978), this was probably the major cause of the differences.

Differences were also evident among the four invertebrate families at area A in the concentrations of both nickel and cadmium. The only consistent pattern was the lower concentrations of both metals in hellgrammites. This pattern is also evident in the data for area B, but was not significant, probably due to the low availability of these two metals. The fact that hellgrammites contain the lowest levels of these two metals at area A, where the availability of cadmium and nickel is generally greatest, indicates that the difference may have been caused as a result of physiological differences. Hellgrammites contain a greater percentage of muscle, which possesses a low affinity for heavy metals, than the other three invertebrate families.

Taxonomic differences among invertebrate groups have also been reported by Enk and Mathis (1977) for lead and cadmium in mayflies, damselflies, and caddisflies, but no reasons were cited. Spehar et al. (1978) in a laboratory study found no differences in lead and cadmium accumulation by stoneflies, mayflies, and caddisflies.

Fish. Fish species also exhibited significant differences ($P < .005$) in the whole-body concentrations of all four metals (Fig. 8). Fantail darters (Etheostoma flabellare) and two species of dace (Rhinichthys atratulus and Chrosomus oreas) contained up to five times greater amounts of all four metals than the other species present at each area. This disparity may have both an ecological and a physiological basis. Darters and dace are sediment-associated fish (Carlander 1969), and may become contaminated directly by elevated metal levels in water interacting with sediment metal loads rather than through assimilation from the upper water column, where metal concentrations are much lower. The relation of sediment interaction to elevated heavy metal content has been reported in both fish and invertebrates (Delisle et al. 1975; Atchison et al. 1977; Getz et al. 1977; Wheeler et al. 1978). A second possible cause which may have produced higher heavy metal concentrations in darters and dace is physiological and similar to that postulated previously for invertebrates, involving differential heavy-metal affinity for specific tissues and residence time, or exchange rate, of heavy metals in these tissues. These fish have a relatively small percentage of muscle (33 to 50 percent) when compared to other resident fish such as suckers or

Fig. 8. Median concentrations of lead, nickel, cadmium, and zinc in fish species from area A over a one-year period.


KEY:
BC = Bluehead chub
BD = Blacknose dace
BG = Bluegill
CS = Crescent shiner
FD = Fantail darter
MD = Mountain redbelly dace
NH = Northern hogsucker
RB = Rock bass
RS = Redbreast sunfish
WS = White sucker
Med. Conc. (µg/g)
300
260
220
180
140
100
70
20
16
12
8
4
3.2
2.4
1.6
0.8
0.0
Pb
Zn
Ni
Cd
BD
MD
FD
BC
CS
NH
WS
BG
RS
RB
Species

centrarchids (55 to 67 percent). Muscle is generally low in heavy metals because it has a low affinity for these elements (Jenne and Luoma 1977). The lack of a large muscle mass in the dace and darters may have caused higher whole body concentrations of these metals. Lack of bulk is an adaptation to the riffle areas that serve as the major habitat for these species (Carlander 1969).

Support for habitat association and body structure as explanations of specific differences in heavy metal concentration is strengthened by a comparison of the remaining species described in Figure 8 and Table 9. The lowest concentrations of lead, nickel, and zinc were found primarily in the centrarchids: bluegill (*Lepomis macrochirus*), redbreast sunfish (*Lepomis auritus*), and rock bass (*Ambloplites rupestris*). Intermediate concentrations of these metals were generally found in the remaining species: white sucker (*Catostomus commersoni*), northern hogsucker, crescent shiner (*Notropis cerasinus*), and bluehead chub (*Hybopsis leptocephala*). The centrarchids should have the lowest concentrations of each metal on the basis of habitat and body form, since they are basically water-column dwellers (Carlander 1977) and contain a large percentage of muscle (55 to 67 percent). The suckers, chubs, and shiners, however, are intermediate in both heavy metal content and the two major determining factors under consideration: they contain a much greater percentage of muscle than the darters and dace species, thereby lowering overall body burdens of these metals, but are more intimately associated with sediments than the centrarchids due to their food and feeding habits (Carlander 1969), and so accumulate more metals by this route. No distinction could be made between the cadmium concentrations of the centrarchids and the catostomids, most likely due to the low availability of this metal, as well as the likelihood of variations in these general patterns of accumulation caused by physiological differences and the forms of trace metals available for uptake.

Other authors have found differences in whole body concentrations of heavy metals among fish species (Lucas et al. 1970; Lovett et al. 1972; Delisle et al. 1975; Wiener and Giesy 1979). While they have suggested that these differences are most likely due to differential metal availability, fish physiology, and feeding habits, no attempt at specific explanations were made. A comparison of the data to values observed by previous workers for both invertebrates and fish is presented in Appendix Table III.

Relationship to Period of Exposure

Invertebrates. If heavy metals are accumulated in quantities which are a direct function of availability, it is reasonable to assume that this accumulation will gradually add to the body burden of an organism over time. This would then be reflected by a dependent relationship of heavy metal concentration with duration of exposure, as indicated by size (length, weight) and/or age--particularly the latter. However, as noted previously in the discussion of taxonomic differences, there are confounding physiological and ecological factors that may serve to mask this bioaccumulation effect.

Correlations between heavy metal content and the length (mm) of invertebrates were not significant ($P > .25$) for any of the families studied. Due to difficulties in aging these organisms, and because of the short aquatic life spans (two to three years) of these invertebrate families, during which growth is fairly rapid, length was the only indicator of the period of exposure utilized in this study. Heavy metals may accumulate in tissues which have a high metal affinity and a relatively slow rate of exchange due to fluctuations in uptake, but this accumulation may not be reflected in whole-body burdens of these metals because it is neutralized by more variable concentrations in other tissues, such as muscle and kidney; and by frequent moltings by these organisms (Merritt and Cummins 1978). These differential tissue metal kinetics could at a given time either lower whole-body burdens due to low concentrations in specific tissues, or elevate them due to high uptake in tissues which rapidly exchange metals, such as kidneys and gills. A more in-depth study of heavy metal accumulation in invertebrates would be required to determine the relationship to size and age.

Another factor which may have contributed to the non-significant results was the use of two-sided correlation procedures, which greatly reduces the power of the procedure, since there could be no a priori assumption of either positive or negative association between the two variables.

A study by Anderson and Brower (1978) also failed to correlate whole body concentrations of heavy metals to size classes of benthic invertebrates.

Fish. Significant negative correlations ($P<.10$) were evident between the total length (mm) and whole body lead and zinc content of most of the species sampled for at least one of the study areas (Table 11). This effect was less pronounced for nickel and cadmium. Bluehead chubs and crescent shiners demonstrated the only significant negative

Table 11. Correlations of length (L), weight (W), and age (A) of fish to whole body lead and zinc concentrations.

Species	Area	Range of Length (mm)	Number in: Sample	Negative Correlations	
				Lead	Zinc
Blacknose dace	C	32-83	44	W-($P<.10$)	L-($P<.08$) W-($P<.055$)
Bluehead chub	A	28-150	44	L-($P<.10$)	L-($P<.007$) W-($P<.005$) A-($P<.02$)
	B	44-138	45		L,W,A-($P<.001$)
	C	38-160	26		L-($P<.10$) W-($P<.03$) A-($P<.02$)
Crescent shiner	A	44-138	45	L-($P<.10$)	L-($P<.002$) W-($P<.001$)
	B	37-98	41	L-($P<.02$) W-($P<.008$)	L,W-($P<.001$) A-($P<.04$)
Fantail darter	B	33-64	41	L-($P<.09$)	
	C	30-68	24		L-($P<.035$) W-($P<.065$)
Northern hogsucker	B	58-118	8	L-($P<.045$) W-($P<.01$)	A-($P<.08$)
Mountain redbelly dace	B	39-67	38	L-($P<.055$) W-($P<.025$)	L,W-($P<.025$)
Redbreast sunfish	A	42-165	30		L,W,A-($P<.001$)
	B	64-126	10	A-($P<.03$)	L-($P<.02$) W,A-($P<.001$)
Rock bass	A	57-177	14	L-($P<.035$) W-($P<.015$) A-($P<.10$)	L-($P<.035$) W-($P<.055$) A-($P<.04$)
White sucker	A	50-299	26		L,W-($P<.001$) A-($P<.002$)
	B	66-314	35	L-($P<.002$) W-($P<.003$) A-($P<.05$)	L,W-($P<.001$) A-($P<.05$)

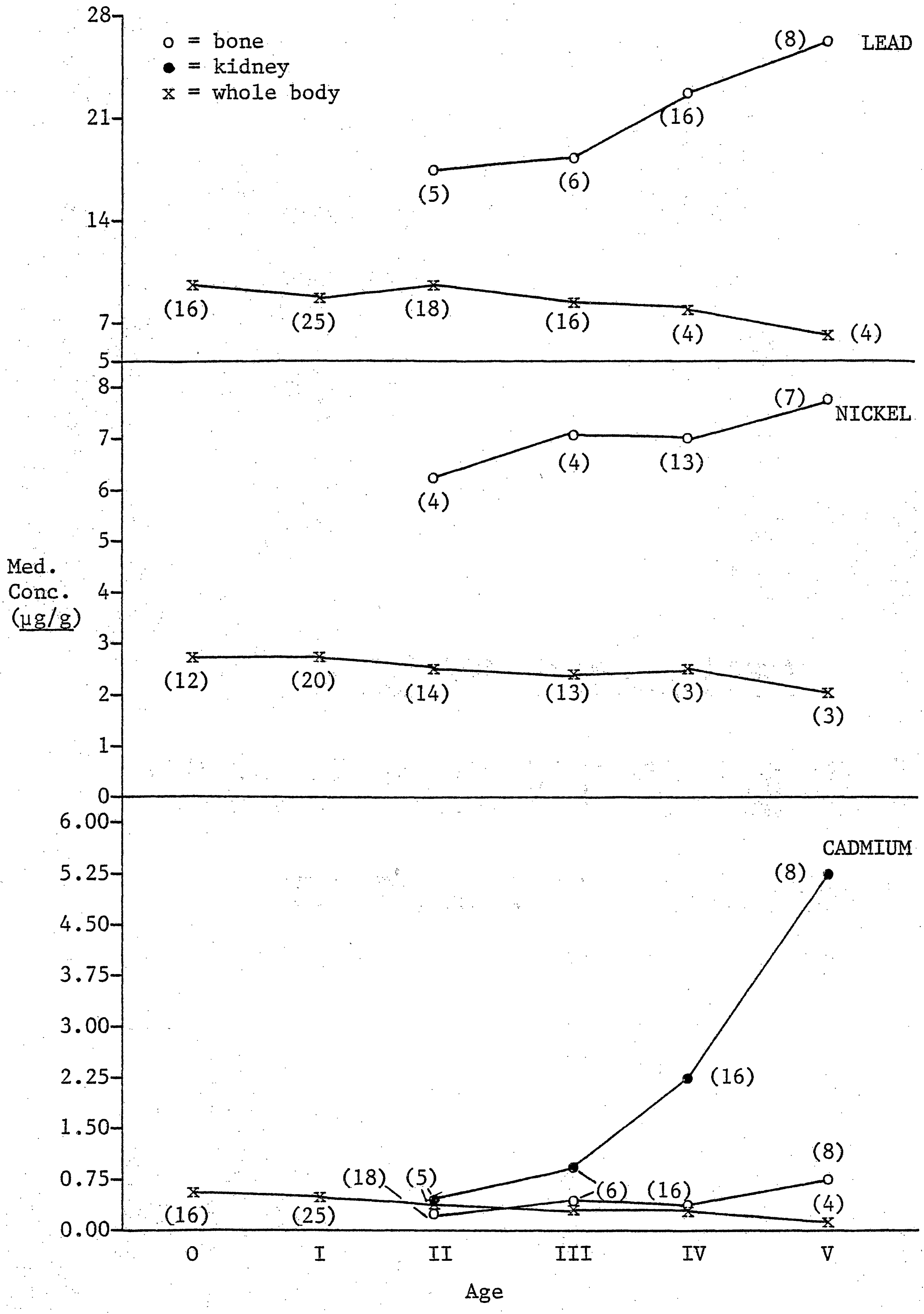
correlations between nickel concentration and size, while crescent shiners, fantail darters, redbreast sunfish, and rock bass demonstrated the only significant negative correlations between cadmium concentration and size. Decreases in whole-body concentrations with increases in size may be attributable to reduced surface-to-volume ratios: as fish get larger, they are adding muscle tissue (Bainbridge 1960) with its low heavy-metal affinity, and therefore tend to reduce whole-body concentrations of these metals. This effect has been previously documented in a number of studies (Murphy et al. 1978; Papadopoulou et al. 1978; Wiener and Giesy 1979). No significant positive correlations were apparent in the data.

The lack of a negative association between heavy metal concentrations and size in some species at some study sites may be a function of: (1) the limited size range of fish lengths within a given species at a particular area; (2) the low availability of nickel and cadmium, which tended to cause their uptake to be very time- and location-dependent, supported by the significant negative correlations for these two metals at area A, where availability is highest; and (3) the interaction of species-specific physiological factors, as described for invertebrates, concerning the different heavy metal loads of the various tissue types.

Tissue Burdens

If heavy metals were accumulating in fish over time, theoretically there should be a significant correlation between age of a fish and the metal content in tissue with high metal affinity. Redbreast sunfish, rock bass, white suckers, and northern hogsuckers from area A showed a significant correlation ($P < .02$) between age and the lead content of bone (Fig. 9), for which it has a high affinity due to its close

Fig. 9. Bioaccumulation of lead, nickel, and cadmium in discrete tissues of redbreast sunfish, rock bass, white suckers, and northern hogsuckers at area A compared to whole-body concentrations in these species. Number in parentheses indicates the number of samples analyzed.


o = bone
● = kidney
x = whole body
LEAD
NICKEL
CADMIUM
Med. Conc. (µg/g)
28
21
14
7
5
8
7
6
5
4
3
2
1
0
6.00
5.25
4.50
3.75
3.00
2.25
1.50
0.75
0.00
(8)
(16)
(6)
(5)
(16)
(25)
(18)
(16)
(4)
(4)
(7)
(4)
(13)
(4)
(12)
(20)
(14)
(13)
(3)
(3)
(8)
(16)
(8)
(18)
(5)
(6)
(16)
(4)
(16)
(25)
O
I
II
III
IV
V
Age

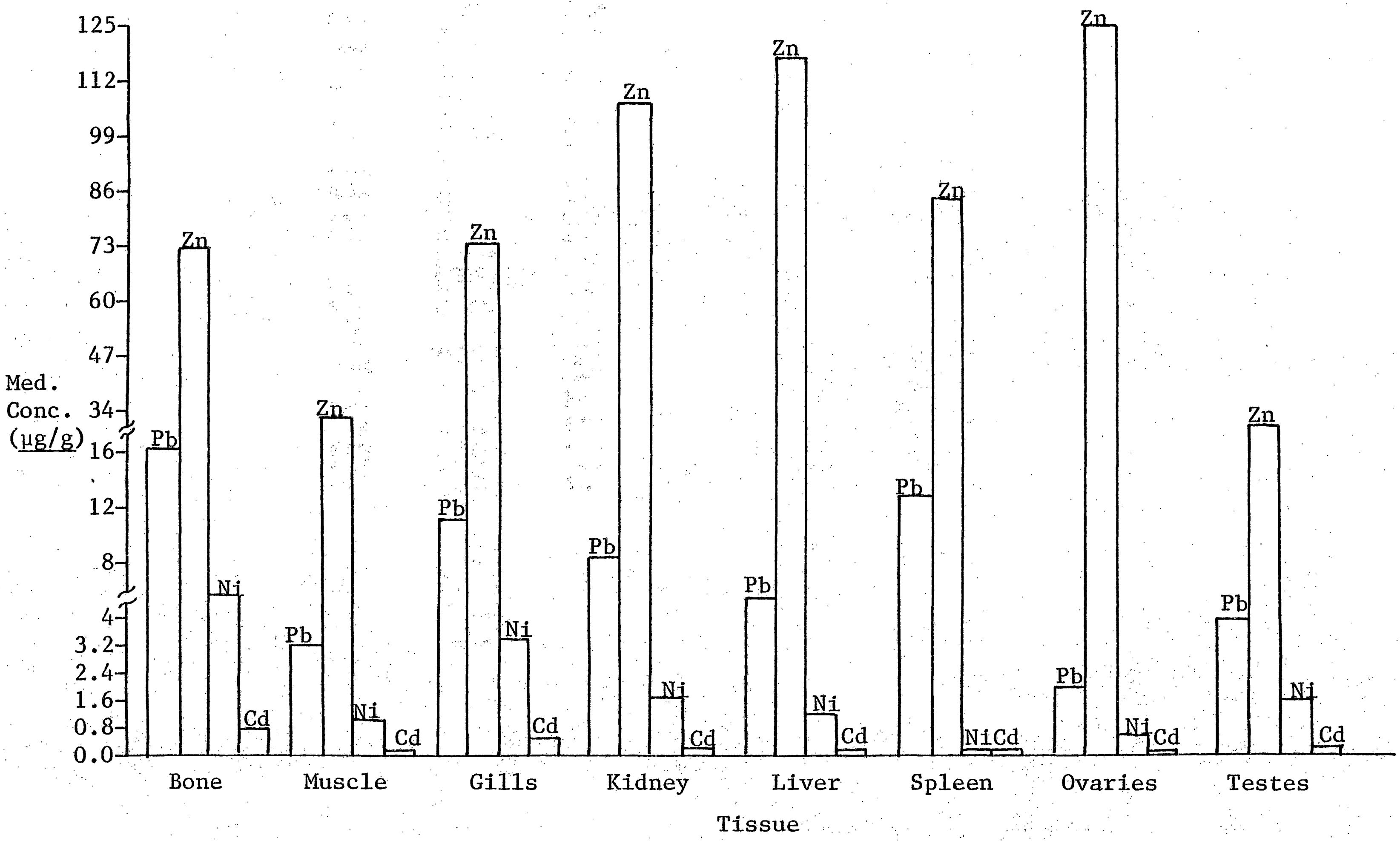
resemblance to calcium ions (Bowen 1966). Nickel concentrations in bone were also correlated ($P<.10$) to the age of white suckers and northern hogsuckers (Fig. 9). Cadmium, although present at low environmental concentrations, was elevated in the bone and kidney of redbreast sunfish, rock bass, white suckers, and northern hogsuckers, and was highly correlated to age ($P<.075$) in both tissues (Fig. 9). Zinc could not be correlated to age, even for tissues which contained high zinc concentrations (up to 1084 μg/g in a carp kidney). Lack of significant correlations could be the result of internal regulation of zinc by fish (Bryan 1976).

There were also no significant correlations between age and heavy metal concentrations in any of the tissues analyzed from white suckers from area B, probably because 70 percent of these fish were of one year-class.

The significant correlation to age indicates that lead, nickel, and cadmium were bioaccumulated in certain tissues, but were not reflected in whole-body burdens of these metals due to their low affinity for muscle tissues. A graphical representation of this effect is given in Figure 9. Other studies have suggested the possibility of bioaccumulation of heavy metals (Lisk 1972; Pagenkopf and Neuman 1974) but there was little other substantive evidence of this phenomenon.

Differences among tissue concentrations of lead, nickel, cadmium, and zinc for white suckers are illustrated in Figure 10. High levels of each metal were present in the gills, kidney, and liver, probably because these tissues represent more rapidly exchanging compartments (Jenne and Luoma 1977), and should be expected to reflect the amount of

Fig. 10. Tissue differences in concentrations of lead, nickel, cadmium, and zinc for 12 white suckers from area B over a one-year period.


Med.
Conc.
(µg/g)
125
112
99
86
73
60
47
34
16
12
8
4
3.2
2.4
1.6
0.8
0.0
Pb
Zn
Ni
Cd
Bone
Muscle
Gills
Kidney
Liver
Spleen
Ovaries
Testes
Tissue

recent metal exposure of an organism. Zinc is an essential metal, so that high liver concentrations may also be an indication of the requirement of zinc as a cofactor in enzymatic reactions (Bryan 1976). High levels of zinc in ovaries are less easily explained, but may be related to the formation of the female sex products. It is also evident from Figure 10 that high levels of lead are present in the spleen, most likely because of its association with blood, where lead levels respond fairly rapidly to environmental fluctuations of this metal (Hodson et al. 1977).

Table 12 summarizes the significant differences in heavy metal accumulation of white sucker tissues between areas A and B (no tissue analysis was performed on fish from area C). All four metals were significantly higher ($P<.0642$) in both bone and muscle tissues from area A fish. Since these two tissues represent slowly exchanging compartments with long heavy metal residence times (Jenne and Luoma 1977), these results are consistent with the respective environmental heavy metal exposure at areas A and B. The significant difference ($P<.0738$) of lead and zinc levels in the gills, kidney, and liver are most likely a reflection of the consistently higher levels of these two metals in the various biotic and abiotic components at area A than in those at area B. Cadmium and nickel, however, were generally in baseline quantities at both areas, except for brief periods of influx from highway runoff, and consequently did not differ substantially ($P>.14$) between the two areas in these organs. The gonads represent tissues with low heavy metal affinity (with the exception of zinc in the ovaries), so that

Table 12. Ranges and median concentrations (in parentheses) of lead, nickel, cadmium, and zinc in selected tissues from white suckers sampled over a one-year period.

Area	Sample	Number Analyzed	Metal Concentration (µg/g) Lead	Nickel	Cadmium	Zinc
A	bone	12	18.1-29.0	5.50-9.88	0.34-1.51	55.6-92.4
			(20.8)	(7.38)	(1.04)	(85.5)
	muscle	12	2.24-54.0	<.12-11.3	<.01-3.08	22.8-77.3
			(4.85)	(1.50)	(0.27)	(45.7)
	gills	12	8.11-156	1.59-8.19	0.37-1.10	64.2-96.7
			(14.7)	(3.35)	(0.72)	(81.3)
	kidney	12	1.25-26.2	<.25-6.70	0.48-2.60	96.4-245
			(13.1)	(4.76)	(0.82)	(194)
	liver	12	5.34-6.17	1.08-2.11	0.09-0.20	176-188
			(6.06)	(1.82)	(0.18)	(180)
	ovaries	7	2.39-3.88	1.04-1.23	0.06-0.16	196-212
			(3.14)	(1.14)	(0.11)	(204)
	testes	5	<.09-3.88	<.06-2.75	<.01-0.36	34.3-45.8
			(2.39)	(<.09)	(0.34)	(42.1)
B	bone	16	5.59-21.0	0.71-8.72	<.03-1.30	16.3-86.1
			(16.3)	(5.77)	(0.80)	(72.3)
	muscle	16	0.73-13.5	<.07-4.35	<.02-0.50	13.0-289
			(3.26)	(0.95)	(0.09)	(33.1)
	gills	16	4.69-18.4	0.58-8.65	0.23-0.95	53.1-104
			(11.5)	(3.27)	(0.52)	(75.1)
	kidney	16	<.46-31.8	<.28-9.52	<.04-52.6	60.1-141
			(8.59)	(1.59)	(0.17)	(105)
	liver	16	0.94-28.6	<.42-11.7	<.03-0.61	72.4-136
			(5.32)	(1.09)	(0.11)	(119)
	ovaries	11	0.41-3.81	0.19-1.92	<.02-1.26	34.6-175
			(1.97)	(0.66)	(0.07)	(126)
	testes	5	1.77-6.25	<.44-2.43	<.04-0.52	25.5-62.8
			(4.31)	(1.56)	(0.18)	(32.4)

concentrations of all metals at both areas are for the most part relatively low and exhibit little variation.

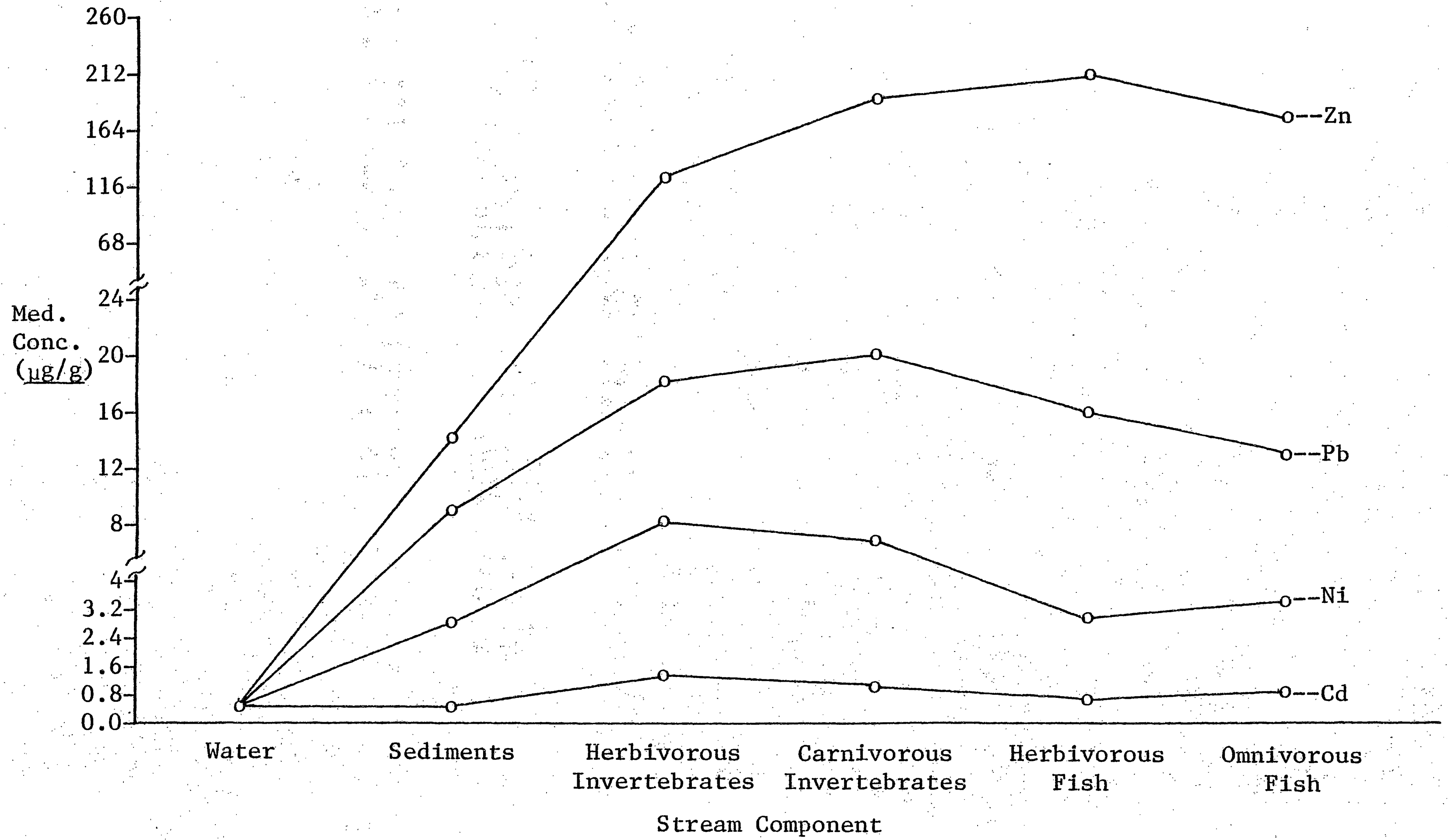
The only other studies which related tissue concentrations of heavy metals to highways (Pagenkopf and Neuman 1974) indicated that lead in the bone and gills was higher in brook trout collected near a highway than in those from a fish hatchery. The concentrations of lead in these tissues were less than 25 percent of the levels found in those of white suckers at both areas A and B in Back Creek, possibly because of the small traffic volume of the highway in the previous study, although species differences cannot be ruled out.

There were no significant seasonal differences ($P>.10$) apparent among any of the tissues analyzed. This result was expected for tissues such as bone and muscle due to their slow rates of heavy metal exchange with the environment. However, tissues such as gills and kidneys should have reflected the recent exposure to elevated metal levels that were evident in the spring sediment samples. The reason metal levels in fish tissues were not significantly elevated during spring may have been the result of the limited sample size of large fish utilized for tissue analyses collected at area A (six fish) and area B (two fish), which reduced statistical power.

Biomagnification of Heavy Metals

Consistent trophic level magnification of lead, nickel, cadmium, and zinc was not observed in this (Fig. 11) or earlier studies (Gale et al. 1973; Mathis and Cummings 1973; Mathis and Kevern 1975; Enk and Mathis 1977; Getz et al. 1977; Giesy and Wiener 1977; Murphy et al. 1978;

Fig. 11. Trophic level comparison of median concentrations of lead, nickel, cadmium, and zinc in components of area A over a one-year period.


260
212
164
116
68
24
20
16
12
8
4
3.2
2.4
1.6
0.8
0.0
Med. Conc. (μg/g)
Zn
Pb
Ni
Cd
Water
Sediments
Herbivorous Invertebrates
Carnivorous Invertebrates
Herbivorous Fish
Omnivorous Fish
Stream Component

Wiener and Giesy 1979). Even discrete tissue levels of these metals in fish generally failed to exceed whole body burdens in macroinvertebrates.

The variations among the different trophic components of the stream have been described as potential functions of physiological and ecological factors, and are summarized in Table 13. These tentative relationships emphasize the variable fate of highway-generated heavy metals in aquatic ecosystems. These findings indicate that most heavy metal uptake in these stream organisms was via water and/or sediment-water interaction. Although metals are absorbed from ingested food across the gut wall (Bowen 1966), assimilation is controlled to a great extent by homeostatic control of absorption (Brown 1976) and the low digestibility of tissues with high heavy metal content (bones of fish and hard parts of invertebrates). Greater than 95 percent of the heavy metals in food may be excreted (Bowen 1966).

These findings for lead, nickel, cadmium, and zinc are directly opposed to those previously reported for the heavy metal mercury, and for many organic pollutants, such as DDT, which concentrate in the soft tissues of aquatic organisms (particularly lipids). Trophic level biomagnification has been documented for many of these substances (Hodkinson 1976).

Table 13. Factors influencing heavy metal concentrations in the biotic and abiotic components of a stream ecosystem.

Component	Heavy Metal Affinity and Residence Time	Degree of Sediment Association	Conc.
Water	Rapid exchange with environment and transport downstream.	Very high	Very low
Sediment	High affinity; short residence time.	—	Moderate
Benthic Invertebrates	a) large % of hard body parts = high affinity; long residence time.	High	High
	b) small % of hard body parts = lower affinity; long or short residence time.	High	Mod. High
Fish	a) large % of muscle = low affinity; long residence time.	1) High 2) Low	Moderate Low
	b) small % of muscle = higher affinity; long or short residence time.	1) High 2) Low	High Moderate

SUMMARY AND CONCLUSIONS

1. Water analysis placed Back Creek in the soft-water category (≤ 60 mg/1 $CaCO_3$), which indicates that heavy metals entering this stream would be readily available for uptake by resident organisms, resulting in accumulation and possible toxic effects.

2. Concentrations of lead, nickel, cadmium, and zinc in Back Creek were low in water, not exceeding values for other uncontaminated streams. These concentrations may become elevated during periods of high runoff from highways. The greatest concentration of these metals occurred in the suspended fraction, which represents a small portion of Back Creek water.

3. Sediment loads of lead, nickel, and zinc were highly correlated to traffic density, and were up to 9000 times higher than water concentrations of these metals. Cadmium concentrations were extremely low at all areas, and did not vary significantly.

4. Benthic macroinvertebrate loads of lead, and to a lesser extent nickel, cadmium, and zinc were correlated to traffic density. The lesser significance of differences among areas in invertebrate zinc concentrations may be attributable to documented internal regulation of this metal.

5. Invertebrate concentrations of heavy metals were 1.25 to 24 times higher than those in sediments. Increase was probably due to assimilation of these metals in invertebrate tissues, where residence times are much greater than those for sediments, which may continually lose and receive heavy metals through interaction with water.

6. Concentrations of lead, nickel, cadmium, and zinc in fish were highly correlated to traffic density. Higher lead concentrations in fish from areas A and B than those in fish from comparable studies may be attributable to automotive emissions of this metal, which account for 95 percent of its distribution to the environment.

7. Invertebrate concentrations of heavy metals were 1.2 to 2.4 times higher than those in fish, probably because of: (a) the greater association of the four invertebrate families with sediment loads of these metals; and (b) the differential tissue affinity for these metals. Fish have a large percentage of muscle, which usually contains low concentrations of heavy metals, while invertebrates consist mainly of viscera and hard parts, both of which have a high affinity for heavy metals.

8. Sediment concentrations of lead, nickel, and zinc were up to four times higher at each area in spring, showing similar decreases through summer, fall, and winter. This may be attributable to spring runoff of snow from roadsides and surrounding areas of aerial deposition.

9. The two stonefly families at area C exhibited the only significant seasonal variation in invertebrate concentrations of lead, nickel, and cadmium. High fall concentrations were possibly a result of a gradual shift of metals taken up from high spring sediment loads into the hard parts of the invertebrates.

10. There was no significant seasonal variation apparent in concentrations of lead, nickel, cadmium, or zinc in fish, probably because of accumulation over extended periods of exposure.

11. The two stonefly families accumulated up to three times the amounts of lead and zinc found in tipulids and hellgrammites, probably due to

the percentage of hard parts with high heavy-metal affinity in the stoneflies. Taxonomic differences in nickel and cadmium were only significant among invertebrate families at the area receiving the highest traffic volume.

12. Heavy metal concentrations in darters and dace were greater than those found in suckers, chubs, and shiners, which in turn were greater than those in bluegills, sunfish, and rock bass. These differences may result from several factors, including: (a) the percentage of tissue with high heavy-metal affinity within a given species; (b) the amount of contact a species has with the substrate; and (c) the degree of internal regulation of a particular heavy metal that a species possesses.

13. There were no significant correlations between length and whole body invertebrate concentrations of lead, nickel, cadmium, or zinc, perhaps because uptake of these metals over time was masked by differential tissue metal kinetics.

14. Significant negative correlations to length, weight, and age were evident in whole body concentrations of lead and zinc, and to a lesser extent nickel and cadmium, in most species of fish. This relationship was most likely a result of decreasing surface-to-volume ratios as individual fish increased in bulk through the addition of muscle, which has a low heavy-metal affinity.

15. Significant positive correlations between age and lead, nickel, and cadmium in bone, as well as cadmium in kidneys were found in tissues of fish from the area receiving the highest traffic volume. Bone and kidney are tissues possessing both high affinities and long residence

times for heavy metals. No bioaccumulation of zinc was observed, probably due to its documented internal regulation.

16. Lead concentrations were high in the bone of fish, probably due to the resemblance of lead to calcium ions; and in the spleen, in association with blood. Zinc concentrations were high in liver, where it is involved in enzymatic reactions; in the kidney, probably because of the regulatory nature of this organ; and in the ovaries for unknown reasons.

17. Heavy metal concentrations in bone and muscle tissues of white suckers from the area receiving the highest traffic volume were higher than those from the other area receiving traffic. Lead and zinc concentrations in the rapidly exchanging tissues (gills, kidney, liver) were also higher in these fish, while nickel and cadmium were not, possibly because of their low environmental levels at both areas.

18. Biomagnification of lead, nickel, cadmium, and zinc did not occur. Variations among trophic components may have been caused by physiological and ecological factors. Most uptake appeared to be by means of water and/or sediment-water interaction.

The combination of chemical and physical parameters in Back Creek, as well as many other soft-water streams, indicate a vulnerability to the influx of heavy metals which is associated with an increase in nearby highway activity. At what point streams of this type can no longer assimilate the pollution generated by increased traffic volume without biological degradation should be determined. Once these contaminants are introduced to an aquatic ecosystem, a multitude of interrelated factors causes uptake and cycling of heavy metals by all resident organisms, and produces an extremely slow rate of elimination and transport out of the system.

The results of this study which have significant ecological implications are threefold. (1) The correlation of heavy metal concentrations in sediments, macroinvertebrates, and fish to associated traffic volumes. At what point is an aquatic ecosystem irreversibly altered? (2) The bioaccumulation of lead, nickel, and cadmium in certain fish tissues. The lack of trophic level magnification and the low accumulations in fish flesh are important positive factors. Although the threat to human health may be limited, there are many fish-eating organisms which do not discriminate between fish flesh and other tissues. (3) The seasonal sediment variations, which indicate the potential for long-term effects if a heavy metal pulse were to occur.

Future investigations of both highway-generated heavy metals and those reaching aquatic communities from other sources should examine both the fate and transport of pollutants in aquatic ecosystems, and their impact on populations inhabiting these systems. This will involve critical analyses of the physiological and ecological factors brought out in this and other studies, and of their influence on the uptake and transport of metals through a system. The digestive physiology of the various forms of heavy metals which are found in aquatic systems, their internal regulation, if any, and their processes and rates of assimilation into various tissues must be understood if their harmful effects are to be understood also. In addition, food and habitat preferences, and the other regular activities of aquatic organisms must be analyzed in terms of both sublethal heavy metal levels in the systems in which these organisms reside, and heavy metal levels in the organisms themselves. This would then provide us with valuable information concerning the effects of heavy metal contamination on the growth, survival, and reproduction of populations of aquatic organisms in natural systems, and allow the development of realistic predictive assessments of their total environmental impacts.

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APPENDIX

Appendix
Table I. Toxic and sublethal concentrations of lead, nickel, cadmium, and zinc as determined by various investigators (CF = continuous-flow, S = static, H = hardness in mg/l $CaCO_3$).

Investigator	Conditions	Organism	Metal	Parameter and Period Measured	Conc. (μg/l)	Indicative Response
Lloyd (1960)	CF,H(320)	Rainbow trout	Zn	LC_{50} 4.75 h	10,000	Death
Crandall and Goodnight (1962)	S,H(165)	Guppy	Zn	LC_{50} 69 d	10,000	Death
			Pb	LC_{50} 76 d	5,000	Death
			Zn	MATC	<5,000	Stunted growth;
			Pb	MATC	<2,000	delayed sexual maturity
Pickering and Vigor (1965)	CF,H(174-198)	Fathead minnow	Pb	TL_m 12 d	1550-1778	Egg mortality
			Zn	TL_m 1-7 d	870-950	Fry mortality
Pickering and Henderson (1966)	S,Soft	Fathead minnow	Cd	TL_m 96 h	630-1050	Death
		Bluegill			1,940	
		Goldfish			2,340	
		Guppy			1,270	
		Green sunfish			2,840	
	S,Hard	Fathead minnow	Cd	TL_m 96 h	72,600-73,500	Death
		Green sunfish			66,000	
	S,Soft	Fathead minnow	Zn	TL_m 96 h	780-960	Death
		Bluegill			4850-5820	
		Goldfish			6,440	
		Guppy			1,270	
	S,Hard	Fathead minnow	Zn	TL_m 96 h	33,400	Death
		Bluegill			40,900	
	S,Soft	Fathead minnow	Ni	TL_m 96 h	4580-5180	Death
		Bluegill			5180-5360	
		Goldfish			9,820	
		Guppy			4,450	

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Table I. Toxic and sublethal concentrations of lead, nickel, cadmium, and zinc as determined by various investigators (CF = continuous-flow, S = static, H = hardness in mg/l $CaCO_3$). (continued).

Investigator	Conditions	Organism	Metal	Parameter and Period Measured	Conc. ($\mu g/l$)	Indicative Response
Pickering and Henderson (cont.)	S,Hard	Fathead minnow	Ni	TL_m 96 h	42,400-44,500	Death
		Bluegill			39,600	
	S,Soft	Fathead minnow	Pb	TL_m 96 h	5580-7330	Death
		Bluegill			23,800	
		Goldfish			31,500	
		Guppy			20,600	
	S,Hard	Fathead minnow	Pb	TL_m 96 h	482,000	Death
		Bluegill			442,000	
Ball (1967)	CF,Soft	Rainbow trout	Cd	TL_m 7 d	8-10	Death
Brown (1968)	H(320)	Rainbow trout	Pb	LC_{50} 48 h	3,500	Death
Patrick et al. (1968)	--	Bluegill	Zn	TL_m 96 h	2860-3780	Death
		Diatom	Zn	TL_m 120 h	4,300	Death
		Snail	Zn	TL_m 96 h	790-1270	Death
Rachlin and Perlmutter (1968)	S,H(166)	Fathead minnow	Zn	TL_m 96 h	7,630	Death
				BSC (calculated)	1,800	
		Platyfish	Zn	TL_m 96 h	12,000	Death
				BSC (calculated)	3,290	
Sprague (1968)	CF,H(13-15)	Rainbow trout	Zn	MATC 20 min	<5.6	Avoidance
				Toxic threshold	570	Death
Brungs (1969)	H(200)	Fathead minnow	Zn	TL_m 96 h	9,200	Death
				MATC	30-180	Reduced fecundity

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Table I. Toxic and sublethal concentrations of lead, nickel, cadmium, and zinc as determined by various investigators (CF = continuous-flow, S = static, H = hardness in mg/l $CaCO_3$). (continued).

Investigator	Conditions	Organism	Metal	Parameter and Period Measured	Conc. (*µg/l*)	Indicative Response
Rachlin and Perlmutter (1969)	S	Rainbow trout	Zn	MATC 96 *h*	<18,000	Mitosis inhibition
Warnick and Bell (1969) (includes literature review)	S,H(44)	*Daphnia magna*	Cd	Toxic threshold	100	Death
		Stonefly	Cd	LC_{50} 14 *d*	32,000	Death
		Mayfly	Cd	TL_m 96 *h*	2,000	Death
		Caddisfly	Cd	LC_{50} 10 *d*	32,000	Death
		Fish	Cd	Toxic threshold	10-73,500	Death
		Daphnia magna	Pb	Toxic threshold	5,000	Death
		Stonefly	Pb	LC_{50} >14 *d*	64,000	Death
		Mayfly	Pb	LC_{50} 7 *d*	16,000	Death
		Caddisfly	Pb	LC_{50} 7 *d*	32,000	Death
		Fish	Pb	Toxic threshold	100-482,000	Death
		Daphnia magna	Ni	Toxic threshold	6,000	Death
		Stonefly	Ni	TL_m 96 *h*	33,500	Death
		Mayfly	Ni	TL_m 96 *h*	4,000	Death
		Caddisfly	Ni	LC_{50} >14 *d*	32,000	Death
		Fish	Ni	Toxic threshold	80-44,500	Death
		Daphnia magna	Zn	Toxic threshold	1,800	Death
		Stonefly	Zn	LC_{50} >14 *d*	32,000	Death
		Mayfly	Zn	Toxic threshold	300	Death
		Caddisfly	Zn	LC_{50} 11 *d*	32,000	Death
		Fish	Zn	Toxic threshold	10-33,400	Death
Weir and Hine (1970)	--	Goldfish	Pb	MATC	<100	Behavior impairment
Hiller and Perlmutter (1971)	S	Rainbow trout	Zn	MATC 96 *h*	10,000-18,000	Mitosis inhibition

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Table I. Toxic and sublethal concentrations of lead, nickel, cadmium, and zinc as determined by various investigators (CF = continuous-flow, S = static, H = hardness in mg/l $CaCO_3$). (continued).

Investigator	Conditions	Organism	Metal	Parameter and Period Measured		Conc. (µg/l)	Indicative Response
Rehwoldt et al. (1971)	S,H(53)	Banded killifish	Ni	TL_m	96 *h*	46,200	Death
		Striped bass				6,200	
		Pumpkinseed				8,100	
		White perch				13,600	
		American eel				13,000	
		Carp				10,600	
		Banded killifish	Zn	TL_m	96 *h*	19,100	Death
		Striped bass				6,700	
		Pumpkinseed				20,000	
		White perch				14,300	
		American eel				14,600	
		Carp				7,800	
Biesinger and Christensen (1972)	S,H(45.3)	*Daphnia magna*	Zn	LC_{50}	48 *h*	100	Death
				MATC	3 *wk*	<70	Reproduction impairment
			Ni	LC_{50}	48 *h*	510	Death
				MATC	3 *wk*	<30	Reproduction impairment
			Pb	LC_{50}	48 *h*	450	Death
				MATC	3 *wk*	<30	Reproduction impairment
			Cd	LC_{50}	48 *h*	65	Death
				MATC	3 *wk*	<0.17	Reproduction impairment
Burton et al. (1972)	CF,H(46)	Bluegill	Zn	TL_m	12 *h*	32,000	Death

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Table I. Toxic and sublethal concentrations of lead, nickel, cadmium, and zinc as determined by various investigators (CF = continuous-flow, S = static, H = hardness in mg/l $CaCO_3$). (continued).

Investigator	Conditions	Organism	Metal	Parameter and Period Measured	Conc. (*µg/l*)	Indicative Response
Pickering and Gast (1972)	--	Fathead minnow	Cd	MATC	37-57	Embryo mortality
Sangalang and O'Halloran (1972)	--	Brook trout	Cd	LT_{50} 21 *d*	10	Death
				MATC	<25	Testicular alteration
Davies and Everhart (1973)	S,H(353.5)	Rainbow trout	Pb	TL_{50} 96 *h*	471,000	Death
				MATC	120-360	Black tails
	CF,H(27.2)	Rainbow trout	Pb	TL_{50} 18 *d*	140	Death
				MATC	6.0-11.9	Black tails
Eaton (1973)	H(200)	Fathead minnow	Zn	Toxic threshold	5,030	Death
			Cd	Toxic threshold	7,200	Death
McKim et al. (1973)	Hard	Rainbow trout	Pb	TL_{50} 96 *h*	1,380	Death
				MATC	18-32	--
	Soft	Rainbow trout	Pb	TL_{50} 18 *d*	140	Death
				MATC	6-12	--
		Fathead minnow	Cd	MATC	37-57	Developmental impairment
Baudouin and Scoppa (1974)	S,Soft	*Daphnia hyalina*	Ni	LC_{50} 48 *h*	1,900	Death
			Pb	LC_{50} 48 *h*	600	Death
			Zn	LC_{50} 48 *h*	40	Death
			Cd	LC_{50} 48 *h*	55	Death
Bengtsson (1974)	Soft	Minnow	Zn	LC_{50} 96 *h*	3,200	Death
				MATC	50-130	Spawning impairment

Appendix
Table I. Toxic and sublethal concentrations of lead, nickel, cadmium, and zinc as determined by various investigators (CF = continuous-flow, S = static, H = hardness in mg/l $CaCO_3$). (continued).

Investigator	Conditions	Organism	Metal	Parameter and Period Measured	Conc. (μg/l)	Indicative Response
Cearley and Coleman (1974)	--	Largemouth bass	Cd	TL_{50} 82 d	80	Death
		Bluegill	Cd	TL_{50} 138 d	850	Death
Eaton (1974)	CF,H(200)	Bluegill larvae	Cd	Toxic threshold	31-80	Death
Nehring and Goettl (1974)	CF,Soft	Rainbow trout	Zn	TL_{50} 14 d	410	Death
		Brown trout			640	
		Cutthroat trout			670	
		Brook trout			960	
Pickering (1974)	CF,S, H(210)	Fathead minnow	Ni	MATC	380-730	Reduced fecundity
				TL_{50} 96 h	25,000-32,000	Death
Sinley et al. (1974)	H(330)	Rainbow trout	Zn	TL_{50} 96 h	7,210	Juvenile death
				MATC	320-640	--
	H(25)	Rainbow trout	Zn	TL_{50} 96 h	430	Juvenile death
				TL_{50} 96 h	2,720	Eyed egg death
				MATC	140-260	--
Thorp and Lake (1974)	S,Soft	Freshw. Shrimp	Cd	LC_{50} 96 h	30-100	Death
		Mayfly			440-2250	
		Damselfly			233,000	
		Freshw. shrimp	Zn	LC_{50} 96 h	770-1700	Death
Clubb et al. (1975a)	CF	Stonefly	Cd	TL_{50} 2 wk	1,000	Death
		Mayfly			<2,500	
		True fly			>5,000	
		Caddisfly			>5,000	

Appendix
Table I. Toxic and sublethal concentrations of lead, nickel, cadmium, and zinc as determined by various investigators (CF = continuous-flow, S = static, H = hardness in mg/l $CaCO_3$). (continued).

Investigator	Conditions	Organism	Metal	Parameter and Period Measured	Conc. (µg/l)	Indicative Response
Clubb et al. (1975b)	CF	True fly	Cd	TL_{50} 7 d	42,500	Death
		Mayfly	Cd	TL_m 96 h	28,000	Death
		Stonefly			18,000	
Larsson (1975)	CF	American eel	Cd	MATC 15 d	<5,000	Anemia, hyperglycemia
		Perch	Cd	MATC 11-12 d	320-3200	
		Flounder	Cd	MATC 9 wk	<5	Anemia
Middaugh et al. (1975)	CF	Spot	Cd	LC_{50} 96 h	200-300	Death
				MATC 96 h	90-500	Decreased resistance
Sellers et al. (1975)	CF,H(51-68)	Rainbow trout	Zn	MATC	<690	Coughing
Tafanelli and Summerfelt (1975)	Soft	Goldfish	Cd	TL_m 96 h	2,300	Death
Benoit et al. (1976)	CF,H(44)	Brook trout	Cd	MATC	1.7-3.4	Reduced growth and fecundity
Davies (1976)	H(353)	Rainbow trout	Pb	LC_{50} 96 h	471,000-542,000	Death
	H(28)	Rainbow trout	Pb	LC_{50} 96 h	1320-1470	Death
	H(353)	Rainbow trout	Pb	MATC 19 mo	18.2-31.6	Black tails
	H(28)	Rainbow trout	Pb	MATC 19 mo	7.2-14.6	Black tails
Davies et al. (1976)	S,H(353)	Rainbow trout	Pb	LC_{50} 96 h	471,000-542,000	Death
				MATC	120-360	Black tails

Appendix
Table I. Toxic and sublethal concentrations of lead, nickel, cadmium, and zinc as determined by various investigators (CF = continuous-flow, S = static, H = hardness in mg/l $CaCO_3$). (continued).

Investigator	Conditions	Organism	Metal	Parameter and Period Measured	Conc. (μg/l)	Indicative Response
Davies et al. (cont.)	CF,H(28)	Rainbow trout	Pb	LC_{50} 96 h	1,170	Death
				MATC	4.1-7.6	Black tails
Holcombe et al. (1976)	CF,H(44.3)	Brook trout	Pb	LC_{50} 96 h	4,100	Death
				MATC	59-119	Scoliosis
McIntosh and Bishop (1976)	S,H(18)	Bluegill	Cd	LC_{50} 96 h	1,900	Death
	CF,H(330-350)	Bluegill	Cd	MATC	<50	Coughing
Rehwoldt and Karimian-Teherani (1976)	S,Soft	Zebrafish	Cd	MATC	<10,000	Reproduction impairment
Sauter et al. (1976)	S,H(35-37.5)	Rainbow trout	Pb	MATC 95-97 d	71-146	Reduction of survival and/or growth
		Lake trout		MATC 111-115 d	48-83	
		Channel catfish		MATC 66-68 d	75-136	
		Bluegill		MATC 62 d	70-120	
		White sucker		MATC 70-73 d	119-253	
		Northern pike		MATC 24 d	253-483	
		Walleye		MATC 39-42 d	237-397	
		Brook trout	Cd	MATC	1-3	Reduction of survival and/or growth
		Channel catfish		MATC	11-17	
		Walleye		MATC	9-27	
	S,H(185-189)	Brook trout	Cd	MATC	7-12	Reduction of survival and/or growth
		Channel catfish		MATC	12-17	
		Walleye		MATC	>87	
Spehar (1976a)	CF,H(44)	Flagfish	Cd	LC_{50} 96 h	2,500	Juvenile death
				MATC	<8.1	Reproduction impairment

Appendix
Table I. Toxic and sublethal concentrations of lead, nickel, cadmium, and zinc as determined by various investigators (CF = continuous-flow, S = static, H = hardness in mg/l $CaCO_3$). (continued).

Investigator	Conditions	Organism	Metal	Parameter and Period Measured	Conc. (*µg/l*)	Indicative Response
Spehar (1976a) (cont.)	CF,H(44)	Flagfish	Zn	LC_{50} 96 *h*	1,500	Juvenile death
				MATC	<51	Reduction of growth
Watson and McKeown (1976)	--	Rainbow trout	Zn	MATC 7 *d*	<214	Hyperglycemia
Christensen et al. (1977)	CF,H(44)	Brook trout alevin	Pb	MATC 8 *wk*	<0.50	Biochemical inhibition
			Cd	MATC 8 *wk*	<0.04	
Hale (1977)	CF,Mod. hard	Rainbow trout	Cd	TL_{50}	6.6	Death
			Pb	TL_{50}	8,000	Death
			Ni	TL_{50}	35,500	Death
			Zn	TL_{50}	550	Death
Hodson et al. (1977)	--	Rainbow trout	Pb	MATC 2 *wk*	<10	Enzyme inhibition
		Brook trout			<90	
		Goldfish			<470	
		Pumpkinseed			<90	
Kwasnik (1977)	--	Fathead minnow	Cd	MATC	<25	Increased vulnerability
Lorz and McPherson (1977)	H(89-99)	Coho salmon	Zn	LC_{50} 96 *h*	4,600	Death
Pascoe and Mattey (1977)	H(103-111)	Stickleback	Cd	Toxic threshold	<1	Death
Rosko and Rachlin (1977)	--	Chlorophyte	Cd	EC_{50}	60	Depressed cell division
			Zn		5,100	
			Pb		>100,000	

Appendix
Table I. Toxic and sublethal concentrations of lead, nickel, cadmium, and zinc as determined by various investigators (CF = continuous-flow, S = static, H = hardness in mg/1 $CaCO_3$). (continued).

Investigator	Conditions	Organism	Metal	Parameter and Period Measured	Conc. (µg/1)	Indicative Response
Speranza et al. (1977)	--	Zebrafish	Zn	MATC 9 d	<5,000	Impaired reproduction
Wentsel et al. (1977)	--	Midge larvae	Cd	MATC	213,000-442,000	Avoidance
			Zn	MATC	4,385,000-8,330,000	Avoidance
Conway (1978)	--	Diatom	Cd	MATC	<2	Reduced population growth
Eaton et al. (1978)	CF,H(45)	Brook trout	Cd	MATC	<3.8	Reduced growth
Ellgaard et al. (1978)	S,H(105)	Bluegill	Cd Zn	MATC MATC	<100 <100	Behavior impairment
McCarty et al. (1978)	S,H(20) S,H(140)	Goldfish Goldfish	Cd Cd	LC_{50} 240 h LC_{50} 240 h	1,780 40,200	Death Death
Sullivan et al. (1978)	--	Fathead minnow	Cd	MATC 21 d	<25	Increased vulnerability
Wentsel et al. (1978)	S	Midge larvae	Cd Zn	MATC MATC	<1,030,000 <17,300,000	Delay in emergence

Appendix
Table II. Concentrations of lead, nickel, cadmium, and zinc in the biotic and abiotic components of various ecosystems.

Investigator	Sample	Study Area	Tissue or Fraction	Concentration (ppm)			
				Pb	Ni	Cd	Zn
Stapleton (1968)	Calico Bass	Steam plant effluent	Dorsal muscle	2.1	5.0	3.0	26
			Ventral muscle	1.1	5.8	4.0	15
			Gonads	2.3	14.7	10.0	172
			Liver	0.7	3.9	11.0	61
			Integument	1.0	9.0	6.0	223
			Heart	1.3	6.1	4.0	82
			Eyeball	2.2	6.4	6.0	700
		Offshore site	Dorsal muscle	1.3	6.4	3.0	10
			Ventral muscle	1.3	6.1	2.0	9.0
			Gonads	2.2	22.2	6.0	119
			Liver	1.5	7.6	24	100
			Integument	1.6	10.2	2.0	190
			Heart	0.9	10.8	2.0	90
			Eyeball	3.4	33.2	4.0	600
Lucas et al. (1970)	Alewife	Great Lakes	Whole body			0.062	
	Trout-perch					0.076	
	Lake herring		Liver			1.6	38
	Lake whitefish					0.3	23
	Bloater					0.7	44
	Round whitefish					0.4	11
	Lake trout					0.06	48
	Smelt					0.07	
	Goldfish					1.4	36
	White bass					0.2	28
	Yellow perch					0.54	
	Walleye					0.2	24

Appendix
Table II. Concentrations of lead, nickel, cadmium, and zinc in the biotic and abiotic components of various ecosystems. (continued).

Investigator	Sample	Study Area	Tissue or Fraction	Concentration (ppm) Pb	Ni	Cd	Zn
Jaakkola et al. (1971)	Seawater	Finland				0-0.0102	
	Sediments					0.29-1.88	
	Fish		Flesh			0.011-0.062	
			Liver			0.014-0.409	
			Kidney			0.108-1.550	
	Lichen					0.05-1.10	
	Elk & Reindeer		Meat			2.35	
			Liver			5.50	
			Kidney			51.9	
			Blood			2.35	
			Backbone			6.90	
U. S. Environ. Protect. Agency (1971)	Drinking water	U. S.		0-0.1	0-0.1	<0.013	0-3.00
	Surface waters			0-1.0	0-0.130	0-0.120	0-1.182
	U.S.P.H.S. Standard			0.05		0.01	5.0
Uthe and Bligh (1971)	Fish	Canada	Whole body (wet wgt.)	<0.5	<0.2	<0.06	<20
Goodyear and Boyd (1972)	Largemouth bass		Whole body				43.3-55.9
Lisk (1972)	Sea foods			0.15-2.50	0.02-1.7	0.05-3.66	
	Vegetables			0-1.26	0-2.59	0.01-0.45	
	Meats			0.0-57	0-4.5	0.19-3.49	
	Dairy products			0-0.79	0-0.03	0.1-0.56	
	River water			0.005	0.010	0.080	
	Seawater			0.00003	0.0054	0.00011	
	Freshwater fish			0.5-2	0.03-3.8	0.02-0.15	

Appendix
Table II. Concentrations of lead, nickel, cadmium, and zinc in the biotic and abiotic components of various ecosystems. (continued).

Investigator	Sample	Study Area	Tissue or Fraction	Concentration (ppm)			
				Pb	Ni	Cd	Zn
Lisk (cont.)	Marine fish			0.5	1.0	3.0	
	Marine plants			6.7	17	0.4	
Natl. Acad. Sci. (1972a)	River water	North America		0.0066			
	Drinking water			0.003-0.040			
	Fish	Unpolluted	Whole body	0.16-0.24			
	Fish	Lead mine	Liver	12			
			Gills	5.7			
			Muscle	1.4			
Pakkala et al. (1972)	Fish	New York waters	Whole body (wet wgt.)	0.3-1.5			
Perhac (1972)	Stream water	Tennessee	Total	11-19	4-11	2-3	10-25
			Dissol. solid	84-96	36-45	9-14	42-141
			Coarse partic.	123-653	46-73	13-24	228-2480
			Colloid	62-2820	222-481	69-1685	<50-1840
Tong et al. (1972)	Fish	New York waters	Whole body (wet wgt.)		0.03-3.8	0.05-0.15	0.9-38
Andersen et al. (1973)	Herring	Norway	Whole body	3.2-12.2		<0.2-0.2	95-140
	Sprat			<0.3-21.8		<0.2-0.7	85-210
	Seawater			0.001-0.0028		0.00067-0.00092	0.0406-0.0725
Gale et al. (1973)	Crayfish	Control	Whole body	22-38		2.6-3.4	74-76
		Highway		155		4.6	86
		Mine & mill effluents		69		3.5	97

Appendix
Table II. Concentrations of lead, nickel, cadmium, and zinc in the biotic and abiotic components of various ecosystems. (continued).

Investigator	Sample	Study Area	Tissue or Fraction	Concentration (ppm)			
				Pb	Ni	Cd	Zn
Gale et al. (cont.)	Fish	Control	Whole body	9-22		1.2-2.9	200-230
		Effluents		24		1.0	130
Mathis and Cummings (1973)	Sediments	Illinois River		28	27	2.0	81
		Other streams		17	16	0.4	80
	Water	Illinois River		0.002	0.002	0.0006	0.030
		Other streams		0.005	0.01	0.08	0.01
	Clams	Illinois River		0.9-7.6	0.4-3.0	0.15-1.41	25-178
	Annelids			17	11	1.1	47
	Northern pike			0.34	0.15	0.022	2.6
	Largemouth bass			0.59	0.11	0.022	3.4
	White bass			0.45	0.08	0.024	4.5
	Shortnose gar			0.74	0.18	0.030	3.6
	Smallmouth bass			0.98	0.13	0.005	3.8
	Lgmth. buffalofish			0.57	0.10	0.032	3.5
	Gizzard shad			0.84	0.28	0.033	4.0
	North. redhorse			0.62	0.14	0.017	3.3
	Quillback carpsuck.			0.64	0.18	0.024	3.4
	Carp			0.56	0.19	0.035	10.2
Oliver (1973)	Sediments	Ottawa &	Silt	33	29		88
		Rideau Rivers	Medium	5	10		24
Windom et al. (1973)	Osteich. fish	N. Atlantic	Whole body			<0.1-1.6	7-81
	Chondrich. fish	N. Atlantic	Muscle			<0.1-2.1	8-20
			Liver			<0.1-5.0	9-44
			Kidney			2.6	25
			Brain			<0.2	10-36
			Gonads			<0.2-0.7	10-36

Appendix
Table II. Concentrations of lead, nickel, cadmium, and zinc in the biotic and abiotic components of various ecosystems. (continued).

Investigator	Sample	Study Area	Tissue or Fraction	Concentration (ppm) Pb	Ni	Cd	Zn
Windom et al. (cont.)	Chondrich. fish	N. Atlantic	Gills			<0.2	24
			Spleen			<0.2-1.4	25-32
Childs and Gaffke (1974)	Groundfish	Oregon	Whole body (wet wgt.)	<0.20		<0.10	
Durum (1974)	Surface water	U. S.		0.002		<0.001	0.02
Kelso and Frank (1974)	Yellow perch	Lake Erie	Whole body (wet wgt.)			0.039-0.063	
	White bass					0.007-0.034	
	Smallmouth bass					0.043-0.047	
Klein et al. (1974)	Surface runoff	New York City			0.15	0.025	1.6
Pagenkopf and Neuman (1974)	Brook trout	Highway	Bone	3.86			
			Liver	1.56			
			Gill	2.43			
		National park	Bone	2.73			
			Liver	1.93			
			Gill	2.12			
		Fish hatchery	Bone	0.95			
			Liver	0.61			
			Gill	0.62			
Atchison (1975)	Sediments	1 (unpollut.)				73	
		2 (industr.				638	
		3 pollut.)				1298	
		4 (mod. pollut.)				205	
		5 (unpollut.)				6	
	Water	1				0.0023	
		5				0.0010	

Appendix
Table II. Concentrations of lead, nickel, cadmium, and zinc in the biotic and abiotic components of various ecosystems. (continued).

Investigator	Sample	Study Area	Tissue or Fraction	Concentration (ppm)			
				Pb	Ni	Cd	Zn
Atchison (cont.)	Water	4 (mod. pollut.)				0.0015	
		2 (industr.				0.052	
		3 pollut.)				0.027	
	Bluegill	1 (unpollut.)	Muscle			0-0.93	
		5 (unpollut.)				0-0.73	
		4				0.10-1.14	
		2				0-1.70	
		1	Gill			0-6.90	
		5				0-0.95	
		4				0-1.81	
		2				0-0.54	
		1	Liver			0-11.69	
		5				0.0	
		4				0-12.1	
		2				6.26-33.36	
		1	GI tract			1.04-12.99	
		5				0.50-1.33	
		4				1.01-1.17	
		2				1.18-51.57	
	Largemouth bass	1	Muscle			0.08-0.33	
		5				0.15-0.48	
		4				0-0.99	
		2				0-15.98	
		1	Gill			0.11-1.39	
		5				0.29	
		4				0.18-1.06	
		2				0-1.89	

Appendix

Table II. Concentrations of lead, nickel, cadmium, and zinc in the biotic and abiotic components of various ecosystems. (continued).

Investigator	Sample	Study Area	Tissue or Fraction	Concentration (ppm) Pb	Ni	Cd	Zn
Atchison (cont.)	Largemouth bass	1 (unpollut.)	Liver			1.01-4.39	
		5 (unpollut.)				2.11	
		4 (mod pollut.)				1.48-5.48	
		2 (industr. pollut.)				0-26.3	
		1	GI tract			0.65-23.11	
		5				0.30	
		4				0.22-2.48	
		2				0.10-7.23	
	Golden shiner		Whole body			0.68-0.79	
Mathis and Kevern (1975)	Largemouth bass	Eutrophic lake	Whole body	0.301		0.036	
	Yellow perch			0.378		0.040	
	Yellow bullhead			0.304		0.034	
	Hybrid sunfish			0.198		0.039	
	Lake chubsucker			0.503		0.036	
	Zooplankton			7.9		0.397	
	Aratophyllum demersum			1.45		0.164	
	Nuphar sp.		Stems	1.043		0.102	
			Roots	0.359		0.041	
			Leaves	1.154		0.291	
	Sediments			31.9		1.85	
	Water			0.017		0.0009	
Natl. Acad. Sci. (1975)	Surface waters	U. S.			0.019		
	Drinking water				0.0048		

Appendix
Table II. Concentrations of lead, nickel, cadmium, and zinc in the biotic and abiotic components of various ecosystems. (continued).

Investigator	Sample	Study Area	Tissue or Fraction	Concentration (ppm) Pb	Ni	Cd	Zn
Rolfe and Jennett (1975)	Fish	Rural	Whole body	1.8-2.4			
	Macrophytes			16.2-24.1			
	Benthos			5.4-18.9			
	Benthos	Urban	Whole body	139.6-518.8			
Stickney et al. (1975)	Fish	Estuary	Muscle	0.01-0.16		0.02-0.22	21-51
	Crustacea		Whole body	0.03-0.94		0.01-0.40	55-290
U. S. Environ. Protect. Agency (1975)	Freshwater					0.08	
	Soils					0.06	
	Plankton					0.4	
	Coelenterata					1.0	
	Mollusca					3.0	
	Echinodermata					1.0	
	Crustacea					0.15	
	Pisces					3.0	
McIntosh and Bishop (1976)	Water	Industrially polluted lake	Dissolved	0.003-0.095		0.0003-0.0658	
			Suspended	0.003-0.014		0.0001-0.0087	
			Total	0.002-0.095		0.0001-0.0658	
	Sediments		0-15 cm	125-1135		29-723	
			15-30 cm	40-226		3-28	
	Periphyton					26.9-34.4	
	Bluegill		Whole body	1.3-42.4		0.094-1.073	93-446
	Pumpkinseed		Whole body	0.1-11.7		0.088-3.072	86-273
	Bluegill		Muscle	0.837-1.069		0.078-1.026	68-89
			Liver	0.0		3.519-10.95	226-807
			Gill	2.139-18.98		0.105-8.781	175-422

Appendix
Table II. Concentrations of lead, nickel, cadmium, and zinc in the biotic and abiotic components of various ecosystems. (continued).

Investigator	Sample	Study Area	Tissue or Fraction	Concentration (ppm)			
				Pb	Ni	Cd	Zn
Atchison et al. (1977)	Water	1 (industri-		0.0		0.0173	0.29
		2 ally pol-		0.0		0.0009	0.05
		3 luted)		0.0201		0.002	0.17
	Sediment	1		0.0		800	12,800
		2		0.0		4.4	320
		3		450		394	7,530
	Bluegill	1	Whole body	0.0		3.4	220
		2		0.0		0.10	140
		3		6.1		0.36	180
		4 (unpollut.)		0.0		0.09	110
Brown and Chow (1977)	Fish	Ontario	Muscle (wet	0.09-2.35		0.01-0.25	2.85-82
			Liver wgt.)	0.05-2.19		0.02-0.50	7.58-240
			Kidney	0.16-61.40		0.05-1.26	6.51-278
Einaga (1977)	Fish	Japan seas	Whole body	0.0-10		0.1	10-100
Enk and Mathis (1977)	Mayflies	Stream	Whole body	6.83		1.19	
	Damselflies			12.59		1.54	
	Caddisflies			6.85-11.00		0.53-0.81	
	River carpsucker			2.55		0.08	
	Fantail darter			2.88		0.15	
	Smallmouth bass			2.47		0.10	
	Sediments			8.30		0.14	
	Snails			13.64			
	Water			<0.5		<0.02	
Getz et al. (1977)	White sucker	Stream	Whole body	2.4			
	Johnny darter			4.1			
	Silverjaw minnow			1.8			

Appendix
Table II. Concentrations of lead, nickel, cadmium, and zinc in the biotic and abiotic components of various ecosystems. (continued).

Investigator	Sample	Study Area	Tissue or Fraction	Concentration (ppm) Pb	Ni	Cd	Zn
Getz et al. (cont.)	Redfin shiner	Stream	Whole body	1.9			
	Bluntnose minnow			2.7			
	Creek chub			1.4			
	Substrate			6.5-13.8			
Giesy and Wiener (1977)	Water	Uncontaminated lake				0.00005	0.014
	Bluegill		Whole body			0.29	173.2
	Blueback herring					0.08	101.8
	Brook silverside					0.19	232.9
	Golden shiner						127.7
	Chain pickerel					0.12	151.5
Anderson and Brower (1978)	Water	Fox River-1		<0.022		<0.00007	<0.002
	Sediment			161.66		5.35	101.90
	Algae			13.17		3.54	30.25
	Benthic insects			28.60		2.27	225.63
	Water	River-2		<0.022		<0.00007	<0.002
	Sediment			19.22		0.54	43.44
	Algae			13.51		2.89	30.16
	Benthic insects			17.98		1.35	132.08
	Crayfish	River-1	Whole body	27.39		2.22	78.30
		Pool-1	Whole body	8.41		1.04	87.07
		River-2	Whole body	11.33		1.09	101.30
			Exoskeleton	23.43		0.78	34.80
			Muscle	<4.0		<0.5	52.50
			Gills	12.93		1.47	81.98
			Viscera	5.31		0.94	82.45

Appendix
Table II. Concentrations of lead, nickel, cadmium, and zinc in the biotic and abiotic components of various ecosystems. (continued).

Investigator	Sample	Study Area	Tissue or Fraction	Concentration (ppm)			
				Pb	Ni	Cd	Zn
Koli et al. (1978)	Shrimp	S. Carolina	Muscle			0.03	5.2
	Silver snapper					<0.01	0.44
	Brown trout					<0.01	0.59
	Mudfish					<0.01	0.40
	White bass					<0.01	0.03
	Catfish					<0.01	0.02
Wheeler et al. (1978)	Sediments	Stream		59			
	Water			0.004			
	Benthos			47			
	Plants			33			
	Fish		Whole body	1.0			
Wiener and Giesy (1979)	Bluegill	Highly organic softwater pond	Whole body	0.3-4.3			56-582
			Muscle				12.3-48.7
			Liver				21.3-55.4

Appendix
Table III. Comparisons of study results with selected literature concentrations of lead, nickel, cadmium, and zinc in sediments, macroinvertebrates, and fish.

Sample	Study Area	Tissue or Fraction	Concentration (ppm)				Investigator
			Pb	Ni	Cd	Zn	
Sediment	Back Creek		2.01-36.2 (7.21)	0.23-4.39 (1.28)	<.02-0.24 (0.04)	3.04-20.6 (9.83)	Present study
	Ottawa & Rideau Rivers		5-33	10-29		24-88	Oliver (1973)
	Stream		8.30		0.14		Enk and Mathis (1977)
Invertebrates	Back Creek	Whole body	<.15-76.5 (15.1)	<.05-17.2 (3.56)	<.03-13.6 (0.87)	<.25-757 (180)	Present study
	Rural stream	Whole body	5.4-18.9				Rolfe and Jennett (1975)
	Stream	Whole body	6.83-12.59		0.53-1.54		Enk and Mathis (1977)
	Fox River (Illinois)	Whole body	17.98-28.60		1.35-2.27	132-226	Anderson and Brower (1978)
Fish	Back Creek	Whole body	<.14-61.0 (8.42)	<.03-37.2 (2.49)	<.01-6.32 (0.37)	41.0-588 (104)	Present study
	Rural stream	Whole body	1.8-2.4				Rolfe and Jennett (1975)
	Indiana lake	Whole body	0.1-42.4		0.088-3.0	86-446	McIntosh and Bishop (1976)
	Stream	Whole body	2.47-2.88		0.08-0.15		Enk and Mathis (1977)
	Stream	Whole body	1.4-4.1				Getz et al. (1977)
	Uncontaminated lake	Whole body			0.08-0.29	102-233	Giesy and Wiener (1977)
	Softwater pond	Whole body	0.3-4.3			56-582	Wiener and Giesy (1979)

Appendix
Table III. Comparisons of study results with selected literature concentrations of lead, nickel, cadmium, and zinc in sediments, macroinvertebrates, and fish. (continued).

Sample	Study Area	Tissue or Fraction	Concentration (ppm) Pb	Ni	Cd	Zn	Investigator
Fish	Back Creek	Muscle	0.73-54.0 (4.67)	<.07-22.3 (1.48)	<.01-3.08 (0.19)	13.0-289 (43.0)	Present study
		Gills	4.69-156 (11.6)	<.05-23.8 (3.48)	<.01-1.12 (0.56)	53.1-1040 (81.8)	
		Liver	0.93-28.6 (5.12)	<.05-11.7 (1.08)	<.08-2.03 (0.19)	72.4-905 (136)	
	Indiana lake	Muscle			0.0-1.70		Atchison (1975)
		Gills			0.0-6.90		
		Liver			0.0-33.4		
	Indiana lake	Muscle	0.84-1.07		0.08-1.03	68-89	McIntosh and Bishop (1976)
		Gills	2.14-19.0		0.11-8.78	175-422	
		Liver	0.0		3.52-11.0	226-807	
	Softwater pond	Muscle				12.3-48.7	Wiener and Giesy (1979)
		Liver				21.3-55.4	

CONTAMINATION OF A SOFT-WATER STREAM ECOSYSTEM IN SOUTHWEST VIRGINIA BY HIGHWAY-GENERATED HEAVY METALS

by

John Herbert VanHassel

(ABSTRACT)

Study of two sections of a stream associated with highways of different traffic densities and a third section used as a reference area demonstrated that concentrations of lead, nickel, cadmium, and zinc in sediments, benthic macroinvertebrates, and fish were highly correlated to the amount of traffic associated with the respective sampling site.

Highest sediment concentrations of lead, nickel, and zinc occurred in spring, most likely due to accumulation and subsequent runoff in snow. Accumulation of these metals in invertebrates and fish is probably a more time-dependent process. Concentrations in these organisms were related to physiological and ecological factors as well as the relative availability of each metal at each study site.

The major physiological factor influencing accumulation of heavy metals in stream organisms seemed to be the relative amount of tissue with a high affinity for these metals in each organism. The major ecological factor influencing accumulation of heavy metals seemed to be the relative amount of sediment association characteristic of each species. Invertebrates contained the highest levels of each metal, followed by bottom-oriented fish with a small proportion of muscle, while species of fish with a large percentage of muscle inhabiting the upper water column contained the lowest concentrations.

Biomagnification of these four metals was not demonstrated. Significant bioaccumulation of lead, nickel, and cadmium in bone, and cadmium in the kidneys of fish was found to occur. Major biological uptake of lead, nickel, cadmium, and zinc in these organisms was via water and/or sediment-water interaction.