National Water-Quality Assessment Program

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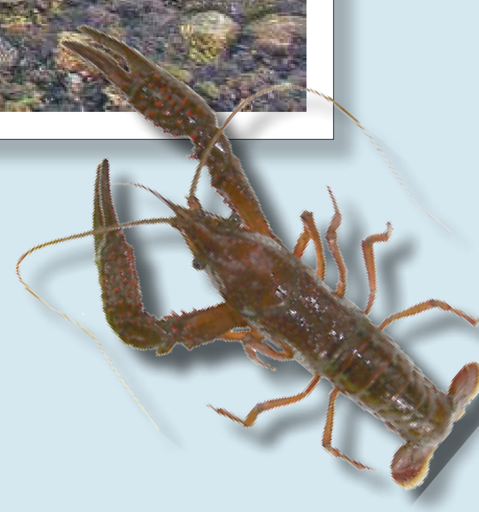

Scientific Investigations Report 2012–5272

U.S. Department of the Interior
U.S. Geological Survey

Cover art: Collecting a macroinvertebrate sample on Big Wood River in southern Idaho, November 2010. Photo credit: Terry Maret

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By Angela P. Paul, Nicholas V. Paretti, Dorene E. MacCoy, Anne M.D. Brasher

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Foreword

The U.S. Geological Survey (USGS) is committed to providing the Nation with reliable scientific information that helps to enhance and protect the overall quality of life and that facilitates effective management of water, biological, energy, and mineral resources (http://www.usgs.gov/). Information on the Nation's water resources is critical to ensuring long-term availability of water that is safe for drinking and recreation and is suitable for industry, irrigation, and fish and wildlife. Population growth and increasing demands for water make the availability of that water, measured in terms of quantity and quality, even more essential to the long-term sustainability of our communities and ecosystems.

The USGS implemented the National Water-Quality Assessment (NAWQA) Program in 1991 to support national, regional, State, and local information needs and decisions related to water-quality management and policy (http://water.usgs.gov/nawqa). The NAWQA Program is designed to answer: What is the quality of our Nation's streams and ground water? How are conditions changing over time? How do natural features and human activities affect the quality of streams and ground water, and where are those effects most pronounced? By combining information on water chemistry, physical characteristics, stream habitat, and aquatic life, the NAWQA Program aims to provide science-based insights for current and emerging water issues and priorities. From 1991 to 2001, the NAWQA Program completed interdisciplinary assessments and established a baseline understanding of water-quality conditions in 51 of the Nation's river basins and aquifers, referred to as Study Units (http://water.usgs.gov/nawqa/studyu.html).

In the second decade of the Program (2001–2012), a major focus is on regional assessments of water-quality conditions and trends. These regional assessments are based on major river basins and principal aquifers, which encompass larger regions of the country than the Study Units. Regional assessments extend the findings in the Study Units by filling critical gaps in characterizing the quality of surface water and ground water, and by determining water-quality status and trends at sites that have been consistently monitored for more than a decade. In addition, the regional assessments continue to build an understanding of how natural features and human activities affect water quality. Many of the regional assessments employ modeling and other scientific tools, developed on the basis of data collected at individual sites, to help extend knowledge of water quality to unmonitored, yet comparable areas within the regions. The models thereby enhance the value of our existing data and our understanding of the hydrologic system. In addition, the models are useful in evaluating various resource-management scenarios and in predicting how our actions, such as reducing or managing nonpoint and point sources of contamination, land conversion, and altering flow and (or) pumping regimes, are likely to affect water conditions within a region.

Other activities planned during the second decade include continuing national syntheses of information on pesticides, volatile organic compounds (VOCs), nutrients, trace elements, and aquatic ecology; and continuing national topical studies on the fate of agricultural chemicals, effects of urbanization on stream ecosystems, bioaccumulation of mercury in stream ecosystems, effects of nutrient enrichment on stream ecosystems, and transport of contaminants to public-supply wells.

The USGS aims to disseminate credible, timely, and relevant science information to address practical and effective water-resource management and strategies that protect and restore water quality. We hope this NAWQA publication will provide you with insights and information to meet your needs, and will foster increased citizen awareness and involvement in the protection and restoration of our Nation's waters.

The USGS recognizes that a national assessment by a single program cannot address all water-resource issues of interest. External coordination at all levels is critical for cost-effective management, regulation, and conservation of our Nation's water resources. The NAWQA Program, therefore, depends on advice and information from other agencies—Federal, State, regional, interstate, Tribal, and local—as well as nongovernmental organizations, industry, academia, and other stakeholder groups. Your assistance and suggestions are greatly appreciated.

Matthew C. Larsen
Associate Director for Water

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Conversion Factors

Inch/Pound to SI

Multiply	By	To obtain
	Length	
inch (in.)	2.54	centimeter (cm)
inch (in.)	25.4	millimeter (mm)
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)
	Area	
square mile (mi^2)	2.590	square kilometer (km^2)
	Volume	
cubic-foot (ft^3)	28.316	liter (L)
	Precipitation rate	
inch per year (in/yr)	25.4	millimeter per year (mm/yr)
	mass	
pound, avoirdupois (lb)	0.4536	kilogram (kg)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

°F=(1.8×°C)+32

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows:

°C=(°F-32)/1.8

Vertical coordinate information is referenced to North American Vertical Datum of 1988 (NAVD 88).

Horizontal coordinate information is referenced to North American Datum of 1983 (NAD 83).

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (μS/cm at 25 °C).

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L) or micrograms per liter (μg/L).

Abbreviations

As	Arsenic
AVS	Acid Volatile Sulfide
ANOVA	Analysis of Variance
Cd	Cadmium
CCU	Cumulative Concentration Units
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
Cr	Chromium
CTU	Cumulative Toxic Units
Cu	Copper
EPT	Ephemeroptera, Plecoptera, Trichoptera
GIS	Geographic Information System
HI	Hawaiian Ecoregion
Hg	Mercury
IDAS	Invertebrate Data Analysis System
LC50	Lethal Concentration (50 percent)
MDL	Method detection limit
MDS	Multidimensional Scaling
MT	Mountain Ecoregion (MT-NROCK, MT-SROCK, MT-SWEST, MT-PNW)
MT-NROCK	Mountain Northern Rockies Ecoregion
MT-PNW	Mountain Pacific Northwest Ecoregion
MT-SROCK	Mountain Southern Rockies Ecoregion
MT-SWEST	Southwestern Mountains Ecoregion
NAWQA	National Water Quality Assessment Program
NLCDE	Enhanced National Land Cover Data
NMS	Non-metric multidimensional scaling
MRDS	Mineral Resources Dataset
Ni	Nickel
NWQL	National Water Quality Laboratory
OH	Ozark Highlands Ecoregion
Pb	Lead
PCA	Principal Components Analysis
PCO	Polar Coordinate Ordination
PEC	Probable-Effect Concentration
PEL	Probable-Effect Level
PL	Plains Ecoregion (PL-NCULT, PL-RANGE)
PL-NCULT	Cultivated Northern Plains Ecoregion
PL-RANGE	Rangeland Plains Ecoregion
SIMPROF	Similarity Profile Test
Se	Selenium
TDS	Total Dissolved Solids
TEC	Threshold-Effect Concentration
TEL	Threshold-Effect Level
USEPA	U.S. Environmental Protection Agency
XE	Xeric Ecoregion (XECALIF, XE-NORTH, XE-SOUTH, XE-EPLAT)
XE-CALIF	Xeric California Ecoregion
XE-NORTH	Northern Xeric Basins Ecoregion
XE-SOUTH	Southern Xeric Basins Ecoregion
XE-EPLAT	Eastern Xeric Plateau Ecoregion
Zn	Zinc

The Occurrence of Trace Elements in Bed Sediment Collected from Areas of Varying Land Use and Potential Effects on Stream Macroinvertebrates in the Conterminous Western United States, Alaska, and Hawaii, 1992–2000

By Angela P. Paul, Nicholas V. Paretti, Dorene E. MacCoy, and Anne M.D. Brasher

Abstract

As part of the National Water-Quality Assessment Program of the U.S. Geological Survey, this study examines the occurrence of nine trace elements in bed sediment of varying mineralogy and land use and assesses the possible effects of these trace elements on aquatic-macroinvertebrate community structure. Samples of bed sediment and macroinvertebrates were collected from 154 streams at sites representative of undeveloped, agricultural, urban, mined, or mixed land-use areas and 12 intermediate-scale ecoregions within the conterminous western United States, Alaska, and Hawaii from 1992 to 2000. The nine trace elements evaluated during this study—arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), selenium (Se), and zinc (Zn)—were selected on the basis of potential ecologic significance and availability of sediment-quality guidelines. At most sites, the occurrence of these trace elements in bed sediment was at concentrations consistent with natural geochemical abundance, and the lowest concentrations were in bed-sediment samples collected from streams in undeveloped and agricultural areas. With the exception of Zn at sampling sites influenced by historic mining-related activities, median concentrations of all nine trace elements in bed sediment collected from sites representative of the five general land-use areas were below concentrations predicted to be harmful to aquatic macroinvertebrates. The highest concentrations of As, Cd, Pb, and Zn were in bed sediment collected from mined areas. Median concentrations of Cu and Ni in bed sediment were similarly enriched in areas of mining, urban, and mixed land use. Concentrations of Cr and Ni appear to originate largely from geologic sources, especially in the western coastal states (California, Oregon, and Washington), Alaska, and Hawaii. In these areas, naturally high concentrations of Cr and Ni can exceed concentrations that may adversely affect aquatic macroinvertebrates. Generally, Hg concentrations were below the sediment-quality guideline for this trace element but appeared elevated in urbanized areas and at sites contaminated by historic mining practices. Lastly, although there was no distinctive pattern in Se concentrations with land use, median bed-sediment concentrations were slightly elevated in urbanized areas.

Macroinvertebrate community structure was influenced by topographic, geologic, climatic, and in-stream characteristics. To account for inherent distribution patterns resulting from these influences, samples of macroinvertebrates were stratified by ecoregion to assess the influence of trace elements on community structure. Cumulative toxic units (CTUs) were used to evaluate gradients in trace-element concentrations in mixture. Correlation analyses among the trace elements under different land-use conditions indicate that trace-element mixtures vary among bed sediment and can have a marked influence on CTU composition. Macroinvertebrate response to bed-sediment trace-element exposure was evident only at the most highly contaminated sites, notably at sites classified as contaminated by the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) as a result of historic mining activities. Results of this study agree with the findings of other studies evaluating trace-element exposure to in-stream macroinvertebrate community structure in that generally lower richness metrics and taxa dominance occur in streams where high trace-element enrichment occurs; however, not all streams in all areas have the same characterizing taxa. In the mountain and xeric ecosystems, the mayfly, *Baetis* sp.; the Diptera, *Simulium* sp.; caddisflies in the family Hydropsychiidae; midges in the family Orthocladiinae; and the worms belonging to Turbellaria and Naididae all demonstrated resilience to trace-element exposure and, in some cases, possible changes in physical habitat within stream ecosystems. The taxa characteristics within the Ozark Highland ecoregion were different than other ecoregions as evidenced by generally more diverse mayfly populations. In addition, *Baetis* sp. was common and dominated many of the mayfly populations found in the Rocky Mountain streams within the Mountain Southern Rockies and Mountain Northern Rockies ecoregions; however, within the Ozark Highland ecoregion, *Tricorythodes* sp. appeared to be more common than *Baetis* sp.

Introduction

Local, regional, and national studies have examined effects of agriculture, urbanization, and mining on trace-element occurrence in bed sediment (Horowitz and others, 1993; Rice, 1999; Seiler and others, 2003; Horowitz and Stephens, 2008) and changes in macroinvertebrate community structure along pollution and (or) other stress gradients (Ourso and Frenzel, 2003; Brasher and others, 2004; Cuffney and others, 2005; Carlisle and Hawkins, 2008). Changes in macroinvertebrate community structure can result from the invasion of non-indigenous fauna, alterations in physical habitat, and contamination, all of which can be influenced by human activities, making it difficult to ascertain cause and effect relations. In addition, it is oftentimes problematic to extrapolate from effects on individual taxa to the community level (Sheehan and Winner, 1984; Paul and Meyer, 2001; Griffith and others, 2004; Mahler and others, 2005; Pollard and Yuan, 2006; Strayer, 2006). Evaluating benthic-macroinvertebrate community structure is an important component of assessing the health of stream ecosystems because stream invertebrates respond to water quality, sediment quality, and physical-habitat characteristics of the system; as a result, biological assessments have become an integral component of water-quality and resource-management programs throughout the United States (Cain and others, 1992; Meador and Gurtz, 1994; Hickey and Golding, 2002; U.S. Environmental Protection Agency, 2009). Understanding the interplay between aquatic assemblages and the influences of various land uses on ecosystem dynamics within the Nation's rivers is important in assessing the health of streams and how to most effectively implement best-management practices; however, elucidating cause and effect between land use(s) and potential ecological impairments using data evaluated herein is not within the scope of this work.

Data in this report were collected as part of the National Water Quality Assessment (NAWQA) Program of the U.S. Geological Survey and are used to describe associations among physical, chemical, and biological components in stream ecosystems (Gilliom and others, 1995). The current report summarizes bed-sediment trace-element concentrations and macroinvertebrate assemblages in 208 samples from 154 streams, representative of 12 ecoregions, across the conterminous western United States, Alaska, and Hawaii from 1992 to 2000. The sites included in this evaluation varied in mineralogy, land use (urbanization, agriculture, and mining), and macroinvertebrate communities. Physiochemical influences of urbanization, agriculture, mining, and grazing of livestock on stream environments have been well characterized (Collins and Dunne, 1990; Tucker and Burton, 1999; Hawkins and others, 2000; Griffith and others, 2001; Paul and Meyer, 2001). General influences of these different land uses on occurrences of trace elements and stream conditions are briefly summarized below.

In the western United States, most freshwater withdrawal is used for irrigated agriculture (National Research Council, 1989). Through leaching, erosion, and runoff, agricultural irrigation practices can introduce trace elements, nutrients, and sediment into streams and wetlands (Presser and Ohlendorf, 1987; Hoffman, 1994; National Research Council, 1989; Seiler and others, 2003). Streams in agricultural areas can also be susceptible to pesticide contamination (Gilliom and others, 2006). However, geology can also have a marked influence on the occurrence of trace-element concentrations. For instance, Seiler and others (2003) found that in the western United States surface-water samples with the highest median As concentrations were collected near Tertiary volcanic rocks. Elevated Se concentrations have been correlated to Upper Cretaceous marine shales, and high concentrations of boron and molybdenum are associated with evaporate deposits (Seiler and others, 2003).

Streams within urban centers are influenced by point and nonpoint sources, such as treated wastewater discharge, urban runoff, and interbasin transfers of water that can introduce sediment, trace elements, nutrients, pesticides, and other contaminants into stream ecosystems (Paul and Meyer, 2001; Mahler and others, 2005; Short and others, 2005). Dry and wet deposition originating from industrial emissions, burning of fossil fuels, and dust can transport contaminants, including trace elements (Wilber and Hunter, 1977; Puckett, 1994; Coupe and others, 2000; Davis and others, 2001). Davis and others (2001) found that the amount of Cd, Cu, and Pb contained in precipitation contributed notably to the total trace-element loadings carried in urban streams. Since its elimination from gasoline in the late 1970s, the amount of Pb entering waterways has decreased; however, bed-sediment data collected from streams in high-traffic areas continue to show high levels of Zn (Callender and Rice, 2000; Davis and others, 2001; Councell and others, 2004). Impervious surfaces within urban environments can alter stream geomorphology and hydrology, and it has been estimated that 15 to 20 percent of the streams in the United States are ecologically impaired by roads (Schueler, 1994; Forman and Alexander, 1998; Paul and Meyer, 2001; Ourso and Frenzel, 2003).

Smelting and production of waste tailings can provide a source for airborne particulates that can contribute to the atmospheric deposition of trace elements to surrounding areas near and far from the sources (Evans, 1986; Maret and others, 2003; Peterson and others, 2007). Changes in physiochemical habitat caused by mining include changes in water chemistry and coating of streambed substrates resulting from the precipitation of metal oxides from solution (Theobald and others, 1963; Moore and others, 1991; Griffith and others, 2001). Noted alterations in water chemistry have included decreased pH where acid mine drainage occurs, increased specific conductance and water hardness, and higher concentrations of sulfate, phosphate, and many trace elements (Moran and Wentz, 1974; McKnight and Feder, 1984; Clements and others, 2000). The effects from mining typically attenuate at some point downstream from the mining-related activities as a result of inflow of unaffected tributaries and (or) groundwater seepage, chemical precipitation, and particle settling (Moran and Wentz, 1974).

Environmental concentrations of contaminants, such as trace elements, provide some indication of exposure, but the actual dose received by an organism depends on the amount actually assimilated (Forbes and Forbes, 1994). Landrum and Robbins (1990) define bioavailability as the amount of a contaminant that can be assimilated by an organism through all routes of uptake. Routes of uptake include absorption through the cuticle and respiratory membranes and (or) by ingestion (Selby and others, 1985; Hare, 1992; Michaud and others, 2005). These processes are all influenced by taxa characteristics, including physiology, trophic status, and habit. In streams and lakes, the partitioning of contaminants between the sediment matrix (solids) and interstitial (pore space) water, through a process called sorption, is important in determining bioavailability to aquatic biota (Luoma, 1989; Landrum and Robbins, 1990). The partitioning of a contaminant depends on the physiochemical characteristics of the environment and the properties of the contaminant (Krantzberg, 1989; Landrum and Robbins, 1990). Sediment characteristics important to sorption reactions include surface charge, exchange capacity, organic-carbon content, and particle-size distribution (Elder, 1989; Landrum and Robbins, 1990). Acid-volatile sulfide (AVS), typically composed mostly of iron sulfide, has been studied as a possible protective mechanism against exposure of benthic organisms to heavy metals in interstitial pore water and is considered in the determination of protective benchmarks for Cd, Cu, Pb, Ni, silver, and Zn by the U.S. Environmental Protection Agency (USEPA) (Hansen and others, 2005). Recent investigations have shown that ingested particulate-bound trace elements are of biological importance (Griscom and others, 2000; Lee and others, 2000). Where ingestion is the primary route of exposure to an organism, analysis of fine-grained material can give a reasonable approximation of the amount of contaminant exposure (Landrum and Robbins, 1990). Identifying the biologically available fraction of trace elements associated with sediment particles is problematic when measured concentrations represent the lithogenic contribution (Luoma, 1989); however, the potential bioavailability of some trace elements has been shown to increase with decreasing sediment grain size (Stone and Droppo, 1996).

Some macroinvertebrate taxa possess various mechanisms of protection that enhance their ability to either inhabit or escape from contaminated areas, such as the ability to drift from contaminated areas or burrow into less-contaminated sediment (Cuffney and others, 1984; Courtney and Clements, 1998). Some taxa known to drift include the mayflies, *Ameletus* sp., *Baetis tricaudatus*, *Drunella doddsi*, *Ephemerella infrequens*, and *Rithrogena hageni*; the caddisfly, *Lepidostoma* sp.; and the dipteran, *Atherix pachypus* (Courtney and Clements, 1998). Avoidance behaviors, such as burrowing and drift, and acclimation to site-specific concentrations can obscure results pertaining to contaminant toxicity (Krantzberg and Stokes, 1989; Beltman and others, 1999; Clements, 2004). Pre-exposure to metals has been shown to increase metal tolerance in some aquatic insects such as the mayfly, *Rithrogena* sp., and the midge, *Chironomus riparius* (Miller and Hendricks, 1996; Clements, 1999). Some invertebrate taxa possess a metal-binding glycoprotein that has a relatively high affinity for metals thereby enhancing their excretion from the organism (Clubb and others, 1975; Rainbow and Scott, 1979; Yamamura and others, 1983; Petering and others, 1990; Cain and others, 2006). Cd-binding glycoproteins have been isolated from select stream macroinvertebrates including the mayfly, *Hexagenia lambata*; the midge, *Chironomus yoshimatsui*; the caddisfly, *Hydropsyche californica*; and the stonefly, *Pteronarcys californica* (Clubb and others, 1975; Yamamura and others, 1983; Michaud and others, 2005; Cain and others, 2006; Janssens and others, 2007). Possessing metal-sequestering enzymes, such as this glycoprotein, can potentially give certain taxa an advantage over others when inhabiting metal-enriched areas (Clubb and others, 1975; Yamamura and others, 1983).

The interactions of trace elements in mixtures are important to consider when examining the biological effects of trace elements in natural systems (Oakden and others, 1984; Preston and others, 2000). Cd, Cu, and Zn have been shown to act synergistically (greatly enhance effects) on macroinvertebrate community structure (Moran and Wentz, 1974; Clements, 2004). Antagonistic (inhibitory) interactions among contaminants have been observed in some studies. Se has been shown to influence the manner in which Hg is assimilated by aquatic organisms including the marine crab, *Carcinus maenas*, and the freshwater northern pike, *Esox lucius* (Rudd and others, 1980; Turner and Swick, 1983; Larsen and Bjeeregaard, 1995). To account for trace elements in mixture, researchers have used indices such as cumulative concentration units (CCUs) and cumulative toxic units (CTUs) to quantify effects of aqueous and bed-sediment trace-element concentrations, respectively (Clements and others, 2000; McGuire, 2001; Maret and others, 2003; Clements, 2004). However, the use of these types of indices may overestimate or underestimate effects because this approach assumes additive toxicity among trace elements (Clements and others, 2000; Clements, 2004).

Chelating agents (e.g., dissolved organic carbon) and water hardness can influence the toxicity of some metals, such as Cu and Zn (Stiff, 1971; Oakden and others, 1984; Gauss and others, 1985; Santos and others, 2008). An inhibitory effect of water hardness has been attributed to the reduced effective concentration (activity) of trace elements in solution in relation to other solutes in hard water (Stiff, 1971; Drever, 1988). Humic and fulvic acids are components of the dissolved organic material in natural waters, and the affinity of these acids to metal ions varies by metal, the ratio of acid to metal, water hardness, and pH (Morel and Hering, 1993). Cu toxicity to the cladoceran, *Ceriodaphia silvestrii*, was reduced in the presence of humic substances (Santos and others, 2008); however, in acute tests with the cladoceran, *Simocephalus serrulatus*, in soft water, the 24-hour LC_{50} (the concentration lethal to 50 percent of organisms exposed) for Cu was lower in the presence of organic matter than without (Giesy and others, 1983). Other organic molecules in natural waters may be capable of chelating trace elements and, in some cases, are necessary to facilitate the transport of these trace elements across cellular membranes (Luoma, 1983).

Purpose and Scope

The purpose of this study was to describe, in a regional context, the differences in macroinvertebrate community structure in relation to concentrations of trace elements in bed sediment collected from selected streams in the conterminous western United States, Alaska, and Hawaii from 1992 to 2000. The nine trace elements targeted for investigation (As, Cd, Cr, Cu, Pb, Hg, Ni, Se, and Zn) were selected on the basis of their potential ecological significance and available sediment-quality guidelines (Van Derveer and Canton, 1997; MacDonald and others, 2000).Concentrations of trace elements were measured in 208 samples of bed sediment collected from 154 streams in 15 states and 12 ecoregions. Watersheds associated with the 208 sampling sites had varying mineralogy and were influenced by different human-related activities (such as urbanization, mining, and agriculture). Land use was used to characterize the occurrence of trace elements as well as account for other potential influences (such as habitat alteration) that may affect macroinvertebrate communities. It is outside the scope of this report to evaluate cause-and-effect relations between land use and trace-element concentrations or effects of land use directly on macroinvertebrate populations. Ecoregions, as defined by other researchers (Omernick, 1987; Whittier and others, 2007), were used to reduce the inherent variability among aquatic-macroinvertebrate community distributions. This report evaluates:

- Trace-element concentrations at undeveloped (reference) sites and those representative of four generalized land uses (agricultural, urban, mined, and mixed use);
- Co-occurrence of trace elements in bed-sediment samples collected at sites representative of undeveloped, agricultural, urban, mining, and mixed land uses;
- Relations of trace-element concentrations in bed sediment to established probable-effect and threshold-effect concentrations;
- Macroinvertebrate community structure along bed-sediment trace-element (CTU) gradients;
- Regional evaluation of taxa resilience to trace-element concentrations.

Description of Study Areas

Study areas described in this report generally represent the diverse nature of groundwater and surface-water systems throughout the conterminous western United States, Alaska, and Hawaii (Shelton and Capel, 1994; Gilliom and others, 1995). The 154 streams targeted for examination in this investigation are in the states of Alaska, Arizona, California, Colorado, Hawaii, Idaho, Missouri, Montana, Nebraska, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming (fig. 1).

Carlisle and Hawkins (2008) identified season, latitude/longitude, mean annual precipitation, basin area, channel slope, and mean annual air temperature as important environmental gradients explaining a substantial amount of the biological variability among macroinvertebrate samples collected from undeveloped (reference) areas. Study areas and sampling locations were examined within an ecoregional framework to ascertain whether or not ecoregional differences could be influencing distributional patterns among aquatic communities. The regional study area consisted of 5 large-scale (10 intermediate-scale) ecoregions (table 1). Large-scale ecoregions were delineated by Omernick (1987) and Whittier and others (2007) by considering stream order, slope/relief, soil types, water quality, physical-habitat characteristics, forested/agricultural land uses, and climate. Mountain ecoregions (MT) are generally those with steep slopes, shallow soil, and cooler climates, whereas xeric (XE) and plains (PL) ecoregions are flat and have deep soils and warmer temperatures (Stoddard and others, 2005).The intermediate-scale ecoregions, delineated by Whittier and others (2007), represent areas with greater similarities in climate, topography, and in-stream characteristics relative to the large-scale ecoregions. The characteristics of the intermediate-scale ecoregions in this dataset are summarized in table 1; the extent of the large-scale ecoregions is shown in figure 1. Assessment of general water-quality characteristics for the large-scale ecoregions is summarized in the section "Ecoregion and Land Use Characterization."

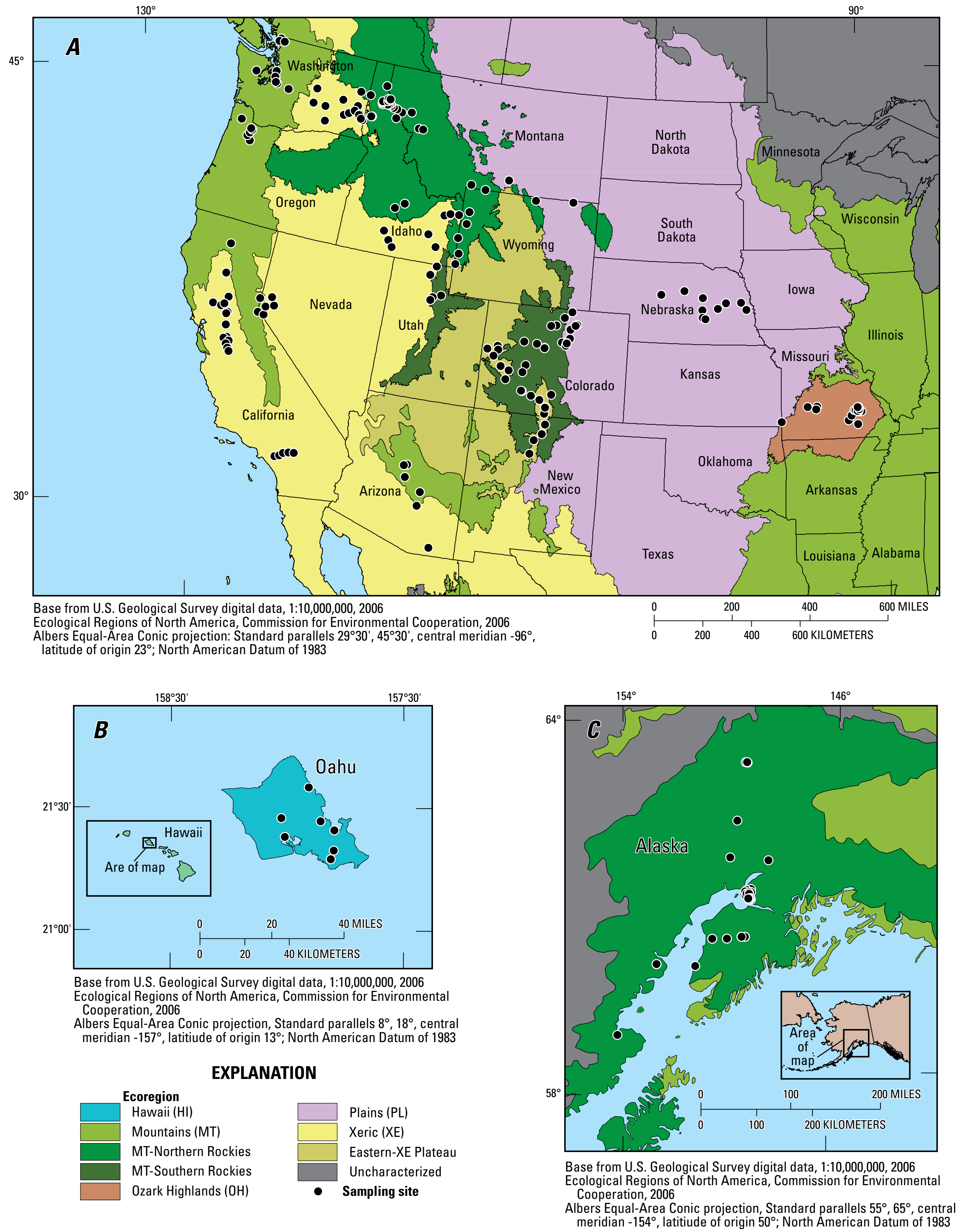


Figure 1. Ecoregions and bed-sediment and macroinvertebrate sampling locations in the *A*, conterminous western United States; *B*, island of Oahu, Hawaii; and *C*, Cook Inlet area near Anchorage, Alaska, 1992–2000.

Table 1. Average basin drainage area and slope and ranges in sampling-site elevation and average annual air temperature, precipitation, and potential evapotranspiration for sites within each intermediate-scale ecoregion included in this investigation for the conterminous western United States, Alaska, and Hawaii.

[**Abbreviations:** °F, degrees fahrenheit; mi^2, square miles; in, inches; yr, year; ±, plus or minus; NA, not available]

Large-scale ecoregion	Intermediate-scale ecoregion	Number of sites	Average basin drainage area ± standard deviation (mi^2)	Average annual watershed air temperature (°F)[1]	Average annual watershed precipitation (in/yr)[2]	Average annual potential evapotrans-piration (in/yr)[3]	Sampling-site elevation (feet)[4]	Average basin slope (percent)[4]
Conterminous Western United States and Alaska								
Mountain (MT)	Mountain Pacific Northwest (MT-PNW)	47	280 ± 1,757	40–53	8–125	17–6	7–6,244	0.2–53.2
	Mountain Northern Rockies (MT-NROCK)	32	1,026 ± 1,894	32–45	22–65	14–22	1,627–7,405	13.3–44.6
	Mountain Southern Rockies (MT-SROCK)	15	611 ± 1,192	32–47	21–37	13–24	5,230–9,505	18.0–38.7
	Southwestern Mountains (MT-SWEST)	6	2,420 ± 2,544	43–54	19–40	18–28	2,097–6,267	9.6–32.9
Ozark Highlands (OH)	NA	15	265 ± 417	55–56	44–46	31–32	456–1,024	1.6–10.8
Plains (PL)	Cultivated Northern Plains (PL-NCULT)	10	2,023 ± 2,364	40–49	15–29	18–28	1,109–5,673	1.8–20.3
	Rangeland Plains (PL-RANGE)	10	1,163 ± 1,224	41–50	15–27	20–28	1,444–3,442	0.5–18.5
Xeric (XE)	Eastern Xeric Plateaus (XE-EPLAT)	17	2,578 ± 2,756	34–50	9–31	14–27	4,521–7,989	1.4–36.6
	Northern Xeric Basins (XE-NORTH)	22	665 ± 995	36–51	8–48	16–29	535–5,850	1.6–27.8
	Southern Xeric Basins (XE-SOUTH)	9	2,024 ± 2,431	41–62	17–38	18–35	1,752–4,928	6.2–36.1
	Xeric California (XE-CALIF)	18	1,789 ± 1,983	50–65	10–63	24–37	16–981	0.2–29.8
Hawaii								
Hawaii (HI)	NA	7	9 ± 16	76	63.7	NA	19–280	NA

[1] PRISM Data, 30 years (1961–1990), 2-kilometer grid size for temperature and relative humidity; Alaska temperature data are averages for Anchorage, Alaska (Western Regional Climate Center 2009a); Hawaii temperature data are averages for Honolulu, Hawaii (Western Regional Climate Center 2009a).

[2] PRISM Data, 30 years (1971–2000), 800-meter grid size; Alaska precipitation is statewide average, 1971–2000 (Western Regional Climate Center 2009b); Hawaii precipitation is statewide precipitation 1971–2000 (Western Regional Climate Center 2009b).

[3] PRISM Data, 30 years (1961–1990), 1-kilometer grid size for potential evaporation; data unavailable for Alaska and Hawaii.

[4] Elevation and basin slope data from National Elevation Dataset, 100-meter resolution; average basin slope data for Hawaii were unavailable.

Methods

Site Selection

A total of 208 bed-sediment and macroinvertebrate samples were collected from 154 streams and rivers across 15 states and 12 ecoregions (fig. 1) to assess the occurrence and influence of trace elements on macroinvertebrate communities. Data used for this analysis were obtained from sites where macroinvertebrate and bed-sediment samples were collected within approximately 1 year (less than or equal to 450 days) of each other during the period 1992 to 2000 (appendix 1, table 1-1; appendix 2, table 2-2). Sites with basin areas greater than about 25,000 km^2 were not included in this study because these were considered to be large rivers (non-wadeable), requiring modified sampling protocols. In addition, stream sites influenced by wastewater outfalls and those containing the invasive New Zealand mudsnail were excluded from the dataset used in this investigation. The number of sites varied across intermediate ecoregions, ranging from 6 in the Southwestern Mountains to 47 in the Mountain Pacific Northwest (table 1).

Bed-Sediment and Surface-Water Collection and Chemical Analyses

Samples of surface water and bed sediment were collected from streams and rivers in areas of differing land use and mineralogy using standard U.S. Geological Survey protocols (Shelton, 1994; Shelton and Capel, 1994; Mueller and others, 1997). Samples for water quality were not collected at all sites. General water-quality characteristics (suspended-sediment concentration, pH, specific conductance, and hardness) are summarized where available (appendix 1, table 1–2). These water-quality characteristics are important in considering distribution patterns of aquatic macroinvertebrates because they influence the colonization of taxa with different physiological characteristics and in-stream habitat preferences (Hynes, 1979; Merrit and Cummins, 1996; Hawkins and others, 2000). The ecoregional delineations used in this study have been described elsewhere (Whittier and others, 2007) and are evaluated in the section of this report "Ecoregion and Land Use Characterization."

Trace-element concentrations were assessed in bed-sediment samples representing undeveloped (reference) conditions and areas influenced by human-related activities (urbanization, agriculture, and mining). Although data were available for 45 trace elements, the 9 targeted for investigation (As, Cd, Cr, Cu, Pb, Hg, Ni, Se, and Zn) were selected on the basis of their potential ecological significance and available sediment-quality guidelines (Van Derveer and Canton, 1997; MacDonald and others, 2000). Bed sediment at each sampling site was collected from 5 to 10 depositional areas, wet-sieved in the field through a 63-micron nylon mesh with ambient stream water, composited, and kept chilled until analysis (Shelton and Capel, 1994). Samples were analyzed by the U.S. Geological Survey Geologic Discipline, Colorado Minerals Program Laboratory, in Denver, Colo., using standardized analytical techniques for total (lithogenic) concentrations (Shelton and Capel, 1994; Horowitz, 1991; Arbogast, 1996). In circumstances where bed-sediment trace-element concentrations are sufficiently low to be considered below a detectable quantity, the Colorado Minerals Program Laboratory reports these concentrations as less than the method detection limit (MDL). The MDL is determined as three times the standard deviation of eight analyses for each trace element in method-blank water (Paul Lamothe, Research Chemist, U.S. Geological Survey, Geologic Discipline, Colo., written commun., 2011). When trace-element concentrations were below the MDL, representative values were calculated as half the MDL and that value used in all analyses. Concentrations of Cr, Cu, Ni, and Zn in bed-sediment samples were above their respective MDLs in all samples used in this study. Concentrations of As, Cd, Pb, Hg, and Se in bed-sediment samples were below the respective MDLs for these constituents in 1 to 13 samples (0.5 to 6 percent of the samples) assessed in this study. Given the relatively low frequency of these occurrences, it is unlikely that the substitution of half the MDL in these cases substantially biased any conclusions made using this technique. Labile contaminants associated with bed sediment are generally considered more bioavailable to sediment-dwelling organisms than those contained in the lithogenic fraction (Luoma, 1989; Luoma and Rainbow, 2008). Weak-acid digestion methods have been employed to approximate the labile fraction of trace elements associated with bed sediment; however, of the 208 sites evaluated in this study, only 21 (10 percent) had labile trace-element concentration information. Given the limited availability of this data, this information was not used in the analyses for this report.

Trace-element concentrations were evaluated for bed sediment collected from streams within undeveloped (reference) and developed (agricultural, urban, mined, and mixed land use) areas. Consensus-based Probable Effect Concentrations (PECs), the concentration of a substance above which adverse effects may likely occur, have been used to evaluate in-stream chemical conditions in aquatic ecosystems (MacDonald and others, 2000; Maret and others, 2003; Mebane and others, 2003). In most cases, trace-element concentrations were compared to a PEC and threshold effect concentration (TEC). A TEC is the concentration below which adverse effects are unlikely to occur (MacDonald and others, 2000). TEC and PEC values were not available for Se; however, a probable effect level (PEL), similar to a PEC, of 4 µg/g was available from Van Derveer and Canton (1997) (table 2).

Two different methods were used to assess the reference and regional baseline concentrations for each of the nine trace elements assessed in this study. Reference bed-sediment concentrations of trace elements were determined from samples collected from undeveloped areas; regional baseline concentrations were determined without considering land use. These two methods were used because although sediment samples collected from areas of agriculture, urbanization, and mining can contain trace-element concentrations above those considered baseline, it does not necessarily follow that bed sediment from these areas will be above baseline. The seven sites in Hawaii were not included in the determination of either the regional reference or baseline trace-element concentrations in bed sediment because the concentrations of Cr, Cu, and Ni were 6 to 11 times higher in Hawaiian bed sediment than in bed sediment from other areas. The method used to determine regional reference trace-element concentrations was a summary of concentrations in bed sediment collected from streams at the 57 sites, in the conterminous western United States and Alaska, minimally influenced by human-related activities. Regional baseline concentrations were determined using methods similar to Velz (1984) and Rice (1999). To determine the regional baseline concentrations of the trace elements, probability distributions were used and trace-element concentrations were evaluated for each of 19 general study areas distributed among the 15 states (fig. 2). Trace-element baseline concentrations were considered those within each study area exhibiting a lower-linear range. Trace-element concentrations lying outside this lower-linear range, as indicated by a break in linearity, were considered above baseline for that area. This approach has two limitations: (1) the sometimes relatively limited number of data points available to determine the chemical baseline condition of bed sediment collected from some

Table 2. Threshold Effect Concentration (TEC) and Probable Effect Concentration (PEC) values for trace elements of ecological significance and evaluated as part of this study.

[TEC and PEC values were obtained from MacDonald and others (2000); Threshold-effect level (TEL) and probable-effect level (PEL) values obtained from Van Derveer and Canton (1997). **Abbreviations:** µg/g, microgram per gram]

Trace element	Threshold-effect concentration (TEC) (µg/g)	Probable-effect concentration (PEC) (µg/g)
Arsenic (As)	9.79	33
Cadmium (Cd)	0.99	4.98
Chromium (Cr)	43.4	111
Copper (Cu)	31.6	149
Lead (Pb)	35.8	128
Mercury (Hg)	0.18	1.06
Nickel (Ni)	22.7	48.6
Selenium (Se)	2.5 [1]	4 [2]
Zinc (Zn)	121	459

[1] Threshold-effect level.

[2] Probable-effect level.

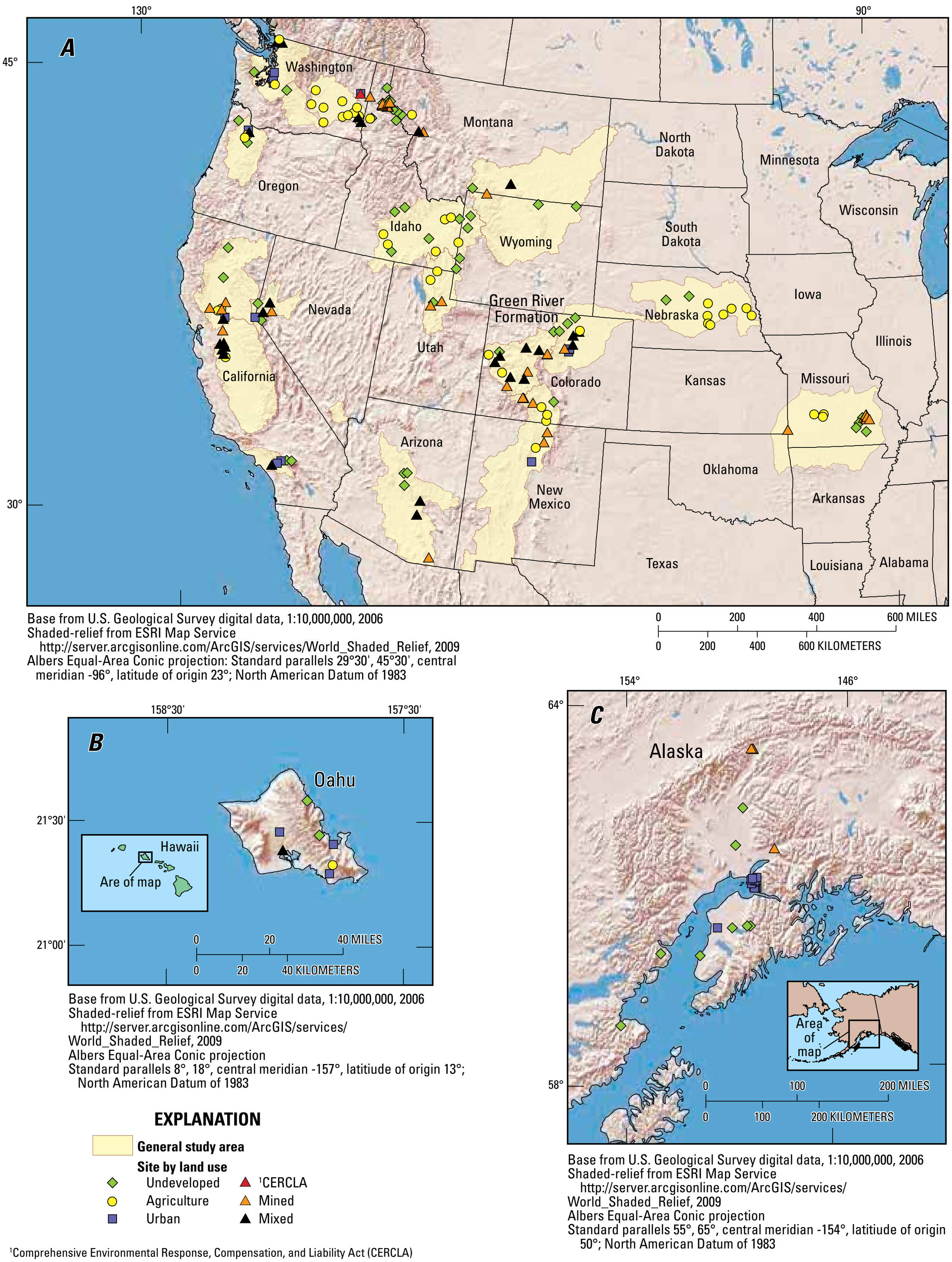


Figure 2. Locations of bed-sediment and macroinvertebrate sampling sites representative of undeveloped and developed (agricultural, urban, mined, and mixed) land uses for the *A*, conterminous western United States, *B*, Island of Oahu, Hawaii, and *C*, Cook Inlet area near Anchorage, Alaska, 1992–2000.

of the study areas, and (2) the visual assessment employed to ascertain the break in linearity. Trace-element concentrations reported among the various land-use categories were not adjusted for baseline concentrations. Baseline adjustment was not used because the purpose of this assessment is not to show a cause and effect between land use and trace-element concentrations but to indicate the relative trace-element exposure to macroinvertebrates at a given site. These results are discussed in further detail within the "Trace Element Concentrations in Bed Sediment" section of this report where regional baseline and reference concentrations derived from these assessments were compared to national baseline trace-element concentrations (Horowitz and Stephens, 2008).

Macroinvertebrate Collection and Taxonomic Resolution

Benthic-macroinvertebrate samples were collected following standard U.S Geological Survey NAWQA protocols (Cuffney and others, 1993). Sampling reaches for wadeable streams were typically between 150 and 300 m in length. Invertebrates were sampled during low-flow conditions and at targeted habitats expected to support the faunistically richest assemblages. In most cases, samples were collected from coarse-grained substrates in riffles, but occasionally, samples were collected from woody snags. Samples were collected using a 0.5 by 0.5 m (0.25-m^2 area) rectangular net with 425-micron mesh. Five discrete samples were collected from representative riffle or snag habitats in the sampling reach. After collection, macroinvertebrate samples were cleaned of extraneous material, composited into a single sample, preserved with 10-percent buffered formalin, and sent to the U.S. Geological Survey National Water Quality Laboratory (NWQL) Biological Unit in Denver, Colo., for identification and enumeration. A quantitative fixed-count (300 organisms) processing method was used to identify and estimate the abundance of each taxon sorted in the sample, and results were reported as relative abundance (the number of individuals per square meter; Moulton and others, 2000). Verification of problematic taxa and routine quality-assurance checks on taxonomic identification were performed. Taxonomic ambiguities were resolved by distributing ambiguous parents among children using the U.S. Geological Survey Invertebrate Data Analysis System (IDAS; Cuffney, 2003); the lowest taxonomic level considered was genus. IDAS was also used to generate a broad suite of macroinvertebrate metrics commonly used in water-quality assessment.

Land-Use Categorization

Land-use classifications were determined using enhanced U.S. Geological Survey National Land Cover Data (NLCDE) with a 30-m by 30-m grid dataset (Gilliom and others, 2006) and comparison with land-use characterizations by Horowitz and Stephens (2008). Land-use information was not available for Alaska; however, in this study, information regarding predominant land use near Alaskan stream sites was obtained from Timothy Brabets (U.S. Geological Survey, Alaska Science Center, Anchorage, Alaska, written commun., 2007). Land use was further refined to 250-m buffer areas along each bank of the stream segment from which macroinvertebrate and bed-sediment samples were collected (Maret and others, 2003; King and others, 2005). Available U.S. Geological Survey Mineral Resource Dataset (MRDS) data were sparse and varied by site, resulting in limited characterization of mining operations and associated mineralogy and dominant ore deposits within the regional study area (U.S. Geological Survey, 2004). Of the 208 sampling locations, which include the 7 sites in Hawaii, 33 were in urbanized, 48 in agricultural, 41 in mined, and 27 in mixed (urban, agriculture, and (or) mined) land-use areas. The remaining 59 sampling locations were in undeveloped areas considered to be relatively unaffected by human-related activities (fig. 2).

Trace Elements and Macroinvertebrate Communities

CTU values were used to assess the relation between trace-element exposure and macroinvertebrate community structure in this study. CTU values were used in order to provide a means of addressing the occurrence of trace-element mixtures and to describe concentration gradients of these mixtures. Researchers have used indices such as CCUs and CTUs to quantify effects of aqueous and sediment trace-element concentrations, respectively (Clements and others, 2000; McGuire, 2001; Maret and others, 2003; Clements, 2004); however, the use of these types of indices may overestimate or underestimate effects because this approach assumes additive toxicity (Clements and others, 2000; Clements, 2004). Although actual toxicity of bed sediment may not be directly elucidated, this technique has provided resource managers with a measure of the relative severity of trace-element contamination in streams (Maret and others, 2003). CTUs were determined for each site by calculating the ratio of each measured trace-element concentration to the appropriate PEC value and then summing the resulting ratios (Maret and others, 2003).

Previous investigations have shown that total counts (abundance) of macroinvertebrate individuals and taxa and abundance of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies), or EPT individuals and taxa, are sensitive indicators of trace-element effects on aquatic communities (Maret and others, 2003; Carlisle and others, 2007). To examine the relation between mixtures of bed-sediment trace-element concentrations and macroinvertebrate community structure, taxa occurring at 30 percent of the sites within each ecoregion and CTUs were evaluated (appendix 2, table 2-1). Using taxa occurring at a minimum of 30 percent of the sites within a given ecoregion was implemented to reduce the complexity of macroinvertebrate communities by de-emphasizing rarer taxa. Attributing a causal effect between trace-element exposure and community structure must be made with caution because habitat characteristics also play an important role in structuring macroinvertebrate communities (Vannote and others, 1980; Plafkin and others, 1989; Hawkins and others, 2000). In this study, ecoregions delineated previously by Omernick (1987) and Whittier and others (2007) were used to reduce the effects of varying gross-level habitat characteristics on the occurrence of macroinvertebrate taxa (table 1).

Statistical Analyses

Relations among bed-sediment trace elements were evaluated using Spearman correlation analysis (SYSTAT, 2004), and correlations were considered significant at the 95-percent confidence level ($\alpha = 0.05$). Significant differences in trace-element concentrations among land-use categories and general ecoregion characteristics were tested using Analysis of Variance (ANOVA) Fisher Least-Significant Difference Test with pairwise comparisons on ranked data (SYSTAT, 2004). The Fisher Least-Significant Difference Test procedure was selected because of the inconsistency in sample sizes within each land-use category and ecoregion (Ott and Longnecker, 2001; Helsel, 2005).

Ordination (multivariate analysis) was used to describe similarities and differences in macroinvertebrate community structure among sites. Ordination is a way of transforming original data into a form that can be interpreted in two or three dimensions (low dimensional space) whereby data points are assessed for similarity (or dissimilarity) along gradients (trajectories or eigenvectors) of interrelated explanatory variables (McGarigal and others, 2000; Clarke and Warwick, 2001). Gradients are described by using scores, calculated by weighting possible explanatory variables, with which trajectories are created in multidimensional space. Each gradient is related to principal components representing the amount of variability (data scatter) accounted for by those components within the dataset. Ideally, only a few variables are necessary to account for most of the variability observed. The components most effective at describing the variability within the dataset are identified as those with higher eigenvalues (McGarigal and others, 2000).

Non-metric (nonparametric) multidimensional scaling (MDS) was the primary multivariate ordination technique used to examine the relative patterns in macroinvertebrate community structure. This method is similar to Principal Components Analysis (PCA); however, PCA is limited in its ability to handle dissimilarities among samples and maintain Euclidean Distances, the measurement of straight lines between data points in multidimensional space (Clarke and Warwick, 2001). Unlike many of the available ordination methods, MDS preserves the relative distance in multivariate space by retaining the rank order of among-sample dissimilarities. Therefore, interpretation of the MDS plot in two-dimensional space is relatively straight forward; samples close in proximity are more similar in taxonomic composition than those that are farther apart (Clarke, 1993).

In nonparametric MDS, if the rank order of the data points is perfectly preserved from high to low dimensional space, a perfect fit would be observed between the data points and the regression line portraying the distance between the most dissimilar samples. It is the deviation from this perfect fit that is termed “stress” within MDS analyses (Clarke and Warwick, 2001). The measure of stress is provided from the MDS analysis as a diagnostic to determine how well the data fit the MDS ordination. Lower stress values are desired, and stress values below 0.20 generally indicate that the MDS ordination is providing an accurate representation of the data in multivariate space. In other words, lower stress values generally indicate that the MDS ordination is producing a true distribution that is not the result of random chance (Clarke and Warwick, 2001).

Transforming original data prior to analysis using MDS can help alleviate bias in similarity (or dissimilarity) that may arise from variables potentially dominating the system(s) evaluated. In this study, a square-root transformation was used to normalize the macroinvertebrate relative-abundance data before calculating the Bray-Curtis dissimilarity matrix. A Bray-Curtis dissimilarity matrix is a type of resemblance matrix that is well suited for species abundance data because of the flexibility to include zero taxa abundance information into the matrix. Dissimilarities are determined by comparing data points to a trajectory drawn between the two most dissimilar data points within the dataset. Data points falling away from the trajectory line indicate the presence of factors, other than those described by the trajectory, influencing the distribution of data within the dataset. This method of analysis is also known as polar coordination (PCO) or Bray-Curtis ordination (McGarigal and others, 2000; Anderson, 2008). MDS analysis was performed on the Bray-Curtis dissimilarity matrix and plotted in two-dimensional space to reduce misinterpretation of multivariate patterns.

PCO can use any resemblance (or dissimilarity) matrix making it similar to but more flexible than PCA. PCO is a re-projection of the sample data in the form of axes that distinguish groupings on the basis of the maximum variation in the sample data. The number of PCO axes used is chosen to minimize the probability of misclassifying a new point to the wrong group. In this study, correlations between the compositions of macroinvertebrate communities were evaluated along trajectories generated within the two-dimensional space of the MDS. Characterizing taxa were identified as those that separated the communities along particular trajectories (gradients) from other communities lying along other similarly generated trajectories. To simplify the results of the MDS, only those taxa occurring at a minimum of 30 percent of the sites within a given ecoregion and found to be correlated to community distribution ($\alpha = 0.05$) were identified within the MDS ordinations. Hawaii was included in the initial MDS ordination to demonstrate the ability of the MDS to identify aquatic communities distinctly different from the other communities included in this study. Unlike macroinvertebrate assemblages in the MT, PL, and XE ecoregions, mayflies and stoneflies are not found in Hawaiian streams. The absence of these taxa is not attributed to contaminant exposure but to the geographic isolation of the Hawaiian Islands (Brasher and others, 2004).

While ordination was used to discern patterns of macroinvertebrate community distribution along trace-element concentrations gradients, cluster analysis was performed to identify groups of similar communities along those gradients. Cluster analysis identifies groups of similar communities by evaluating minimal within-group differences and maximum differences among groups. Similar to the MDS, a Bray-Curtis similarity matrix (the complement to the dissimilarity matrix described above) was used to assess similarities among the various macroinvertebrate assemblages. An agglomerative approach was used within the cluster analysis where similar macroinvertebrate communities were grouped together forming a cluster (McGarigal and others, 2000). To further evaluate the difference among clusters of similar communities, the similarity profile test (SIMPROF) was used (PRIMER v.6; Clarke and Gorley, 2006).

A SIMPROF was used to test for statistically meaningful groups displayed in the dendrogram of the cluster analysis. SIMPROF is a permutation test that evaluates whether or not a specified set of samples, which are not *a priori* assigned into groups, do not differ from each other in multivariate structure (Clarke and others, 2008). SIMPROF first ranks the sample resemblances from smallest to largest and then plots these similarities against their ranks, serving as the basis for the shape of an expected curve. One-thousand curves were generated in this manner by randomly rearranging the variable entries across samples. The 1,000 iterations are averaged to generate a simulated curve. It is the departure from the expected shape of the curve that is used to determine the likelihood that individual groups were generated purely by chance. The sum of the absolute distances between the real similarity profile and the simulated mean profile of the 1,000 iterations is used as the test statistic. The significance of the groupings within the ordination was evaluated at 95-percent confidence ($p = 0.05$).

Vector overlays were added to MDS graphical outputs as an additional exploratory tool to visualize potential linear (monotonic) relations between influential taxa and the ordination axes. Spearman rank correlations were restricted to vectors with lengths of plus or minus 0.50 or greater; plus or minus 1.0 indicates complete correlation. The length and direction of each vector indicates the strength and sign, respectively, of the relation between the taxa and the MDS axes (Anderson and others, 2008). As an additional exploratory tool, bubble plots of sediment CTUs were superimposed onto the MDS plot to better visualize patterns between the macroinvertebrate assemblages and trace-element concentrations. Each bubble is a site assemblage where the size of the bubble represents the magnitude of the CTUs for bed sediment collected from that site. All multivariate analyses were performed using PRIMER v.6 and PERMANOVA v. 1.1 (Clarke and Gorley, 2006; Anderson and others, 2008).

Data Limitations

At large geographic scales, macroinvertebrate community structure varies substantially with natural and anthropogenic disturbance gradients (Whittier and others, 2006). The interrelated environmental and chemical factors occurring at multiple scales can confound the interpretation of a community response to a known pollution gradient (LaPoint and others, 1984; Griffith and others, 2001; Feld and Hering, 2007; Whitter and others, 2007). The regional study area was broken into ecoregions, previously described by Omernick (1987) and Whittier and others (2007), in order to minimize some of the natural habitat-related variability during data analyses. Although delineation into ecoregions did mitigate, to some degree, the variability in macroinvertebrate distributions patterns not associated with contamination, these groupings did not account for site-specific habitat characteristics which, in some cases, may have been influencing aquatic assemblages.

Trace elements are commonly transported in streams in association with suspended particulates, and in areas where flow velocities are sufficiently slow, suspended material can settle out (Moran and Wentz, 1974; Gibbs, 1977). Bed-sediment samples collected from depositional areas containing large quantities of fine-grained materials commonly have higher trace-element concentrations than areas predominantly composed of coarse-grained substrates (Horowitz, 1991). Accordingly, bed-sediment samples collected from depositional areas may contain higher trace-element concentrations than bed sediment in erodible areas within the stream channel where macroinvertebrates sampling was largely targeted. As a result, the actual exposure of macroinvertebrates collected from riffles and woody snags to bed-sediment trace elements collected from depositional areas, where finer-grained material accumulates, remains uncertain. Additionally, data regarding the portion of bioavailable trace elements in sediment to the aquatic organisms were not available for most of the sites included in this assessment.

Table 3. Minimum, maximum, average, and median baseline concentrations for nine selected trace elements determined using two methods of analysis for bed-sediment samples collected from streams and rivers within the conterminous western United States and Alaska, 1992 to 2000, and national median baseline concentrations.

[**Abbreviations:** µg/g, microgram per gram; <, less than; ±, plus or minus]

Trace element	Number of sites	Percent of sites [1, 2]	Minimum concentration (µg/g)	Maximum concentration (µg/g)	Average concentration ± standard deviation (µg/g)	Median concentration (µg/g)	National median baseline concentration, (µg/g) [5]
Concentrations in Bed-Sediment Samples from Undeveloped (Reference) Sites [3]							
Arsenic	57	28	1.9	50	11 ± 10	7.2	6.6
Cadmium	57	28	0.1	1.8	0.4 ± 0.4	0.3	0.4
Chromium	57	28	26	190	64 ± 33	55	58
Copper	57	28	8.0	79	31 ± 17	26	20
Lead	57	28	< 4	200	29 ± 37	20	20
Mercury	57	28	< 0.02	0.34	0.06 ± 0.06	0.04	0.04
Nickel	57	28	10	72	26 ± 14	21	23
Selenium	57	28	0.1	2.5	0.7 ± 0.5	0.53	0.7
Zinc	57	28	34	430	112 ± 72	93	91
Regional Baseline Concentrations [4]							
Arsenic	145	72	<0.1	33	7.4 ± 4.8	6.3	6.6
Cadmium	140	70	<0.1	3.8	0.47 ± 0.64	0.3	0.4
Chromium	153	76	7.0	190	60 ± 30	53	58
Copper	160	80	7.0	80	32 ± 15	29	20
Lead	146	73	<4	37	18 ± 7	17	20
Mercury	139	69	<0.02	0.15	0.04 ± 0.03	0.03	0.04
Nickel	144	72	2.0	90	25 ± 14	21	23
Selenium	120	62	<0.1	1.30	0.50 ± 0.29	0.44	0.7
Zinc	145	72	34	210	100 ± 34	95	91

[1] Total number of sites is 201; Hawaii bed-sediment samples (n=7) were not included in these analyses because concentrations of chromium, copper, and nickel were four to eight times higher in these samples than all other samples included in this study.

[2] 193 sites were sampled for selenium.

[3] Concentrations of the nine selected trace elements reported in this table were determined as those concentrations in bed-sediment samples collected from the 57 undeveloped sites included in this study. These undeveloped sites are in the following states: Alaska, Arizona, California, Idaho, Missouri, Montana, Nebraska, Nevada, Oregon, Utah, Washington, and Wyoming.

[4] Regional baseline concentrations for the nine selected trace elements reported in this table were determined using methods similar to Rice (1999) and Velz (1984). Regional baseline concentrations reported here were calculated from bed-sediment trace-element concentrations for sites in the following states: Alaska, Arizona, California, Colorado, Idaho, Missouri, Montana, Nebraska, Nevada, New Mexico, Oregon, Utah, Washington, and Wyoming.

[5] National baseline concentrations from Horowitz and Stephens, 2008.

Trace-Element Concentrations in Bed Sediment

Reference and regional baseline trace-element concentrations in bed sediment were determined by two methods: evaluating concentrations for 57 undeveloped sites and using methods similar to Rice (1999) and Velz (1984), respectively. Reference and regional baseline trace-element concentrations in bed-sediment samples used for purposes of this study were similar to the national baseline concentrations described by Horowitz and Stephens (2008) (table 3). The following discussion pertains to each of the 9 selected trace elements, their association with other trace elements, and occurrence within each of the 5 general land-use categories for samples collected from within the conterminous western United States and Alaska. Trace elements that are discussed in groups are discussed as such on the basis of moderate to strong correlations among these trace elements in bed sediment collected from undeveloped areas (table 4). Moderate and strong correlations were found among concentrations of Cd, Pb, and Zn ($0.544 \leq rho \leq 0.626$) and between Cr and Ni (*rho* = 0.842) in reference bed-sediment samples. Although moderate correlations were also found between Zn and Cu (*rho* = 0.552) and Hg (*rho* = 0.541), moderate to strong correlations were not found between Cu and Hg (*rho* = 0.470) or between Cu or Hg with either Cd and Pb, with which Zn was moderately correlated (table 4).

Table 4. Correlations among the nine trace elements in bed sediment samples collected at reference and non-reference (agricultural, urbanized, mined, and mixed) streams and rivers in the conterminous western United States and Alaska, 1992–2000.

[A mixed land-use classification indicates a combination of agricultural, urbanization, and (or) mining-related activities present at or near the sampling site. Because of the high concentrations of chromium, copper, and zinc beyond those of other areas included in this study, trace-element concentrations in bed-sediment samples collected from Hawaii were not included in the correlation analysis (n=7). Spearman rho values less than 0.5 were considered weak, between 0.5 and 0.7 considered moderate, and greater than 0.7 considered strong. Correlations greater than 0.5 are emphasized (shown in bold) in the table]

Trace element	Arsenic	Cadmium	Chromium	Copper	Lead	Mercury	Nickel	Selenium	Zinc
Correlation Among Trace Elements in Bed Sediment Collected from Undeveloped (Reference) Sites, n=57									
Arsenic	1.0								
Cadmium	0.438	1.0							
Chromium	0.238	0.027	1.0						
Copper	0.265	0.164	0.381	1.0					
Lead	0.311	**0.626**	–0.108	0.047	1.0				
Mercury	0.339	0.274	0.049	0.470	0.219	1.0			
Nickel	0.276	0.050	**0.842**	0.488	–0.041	0.156	1.0		
Selenium	–0.190	0.373	0.201	0.055	0.145	–0.064	0.222	1.0	
Zinc	0.430	**0.592**	0.170	**0.552**	**0.544**	**0.541**	0.202	0.307	1.0
Correlation Among Trace Elements in Bed Sediment Collected from Agricultural Areas, n=47									
Arsenic	1.0								
Cadmium	0.237	1.0							
Chromium	0.351	0.168	1.0						
Copper	0.423	–0.048	**0.522**	1.0					
Lead	0.225	0.474	0.053	–0.058	1.0				
Mercury	0.244	0.088	**0.544**	**0.665**	0.086	1.0			
Nickel	0.477	0.151	**0.778**	**0.789**	0.053	**0.581**	1.0		
Selenium	0.233	**0.506**	–0.048	0.050	0.257	0.046	0.220	1.0	
Zinc	0.473	0.373	0.337	**0.608**	0.372	0.388	0.476	0.294	1.0
Correlation Among Trace Elements in Bed Sediment Collected from Urban Areas, n=29									
Arsenic	1.0								
Cadmium	0.187	1.0							
Chromium	**0.575**	–0.185	1.0						
Copper	0.051	0.263	0.351	1.0					
Lead	0.138	**0.794**	–0.031	0.476	1.0				
Mercury	0.007	–0.277	0.488	0.305	–0.362	1.0			
Nickel	**0.556**	–0.141	**0.936**	0.425	0.004	0.438	1.0		
Selenium	–0.231	–0.233	0.071	0.137	–0.454	**0.666**	–0.003	1.0	
Zinc	–0.015	**0.783**	–0.045	**0.522**	**0.783**	–0.070	–0.033	–0.207	1.0
Correlation Among Trace Elements in Bed Sediment Collected from Mined Areas, n=41									
Arsenic	1.0								
Cadmium	**0.559**	1.0							
Chromium	–0.097	–0.399	1.0						
Copper	**0.540**	0.395	0.195	1.0					
Lead	**0.503**	**0.889**	**–0.584**	0.301	1.0				
Mercury	**0.585**	0.379	0.266	**0.669**	0.299	1.0			
Nickel	–0.418	**–0.520**	**0.691**	–0.090	**–0.570**	–0.042	1.0		
Selenium	0.136	0.297	0.055	0.130	0.083	0.106	0.088	1.0	
Zinc	**0.568**	**0.917**	–0.341	0.484	**0.851**	0.423	–0.423	0.279	1.0
Correlation Among Trace Elements in Bed Sediment Collected from Mixed Land Use Areas, n=27									
Arsenic	1.0								
Cadmium	0.256	1.0							
Chromium	–0.193	**–0.558**	1.0						
Copper	0.053	–0.024	0.419	1.0					
Lead	–0.018	0.370	–0.297	0.001	1.0				
Mercury	0.336	–0.038	0.342	**0.581**	0.134	1.0			
Nickel	–0.048	–0.313	**0.744**	**0.572**	–0.461	0.206	1.0		
Selenium	0.329	0.434	–0.233	–0.198	0.191	–0.133	–0.161	1.0	
Zinc	0.196	**0.738**	–0.396	0.162	0.475	0.227	–0.194	0.302	1.0

Arsenic

Natural sources of As include weathering of As-bearing rocks (such as arsenopyrite), volcanic activity, and geothermal water (Boyle and Jonasson, 1973; Wilkie and Hering, 1998; Glass and Frenzel, 2001). The median As concentration at undeveloped (reference) sites was 7.2 µg/g, similar to the concentration determined by Horowitz and Stephens (2008) and the regional baseline concentration determined using methods similar to Rice (1999) and Velz (1984) (table 2). Seventy-two percent of the bed-sediment samples collected had As concentrations within regional baseline limits (<0.1 to 33 µg/g, table 2). As concentrations in reference bed sediment were not correlated to any appreciable extent (*rho* < 0.5) with the other eight trace elements included in this investigation; however, in bed sediment collected from streams in developed areas, there were stronger correlations between As and other select trace elements (0.503 ≤ *rho* ≤ 0.585) depending on the area from which the bed-sediment samples were collected (urban or mined) (table 3). Most moderate correlations between As and other trace elements were in bed-sediment samples collected from mined areas.

In this study, median As concentrations in bed sediment from streams within urbanized (9.10 µg/g) and mined (22.70 µg/g) areas were higher compared to bed sediment collected from areas that were largely undeveloped (7.20 µg/g) or influenced by either agriculture (5.59 µg/g) or a mixture (7.20 µg/g) of land uses (p ≤ 0.024, fig. 3). Of the 16 sites at which bed-sediment samples exceeded the PEC for As (33 µg/g), 75 percent of these sites were in mined areas. Eight of these 12 sites are associated with CERCLA areas listed as a result of historic mining activities in Colorado, Idaho, Montana, and Utah (U.S. Environmental Protection Agency, 2010); As concentrations at these eight sites ranged from 42 to 170 µg/g. Bed sediment collected from five of these eight sites also exceeded the PEC values for Cd, Cu, Pb, and Zn (table 2). Concentrations in bed sediment sampled from the other eight sites not associated with CERCLA areas, but where As concentrations exceeded 33 µg/g, ranged from 37 to 93 µg/g; these eight sites were representative of undeveloped and mined areas. The median concentrations of As in bed sediment collected from areas influenced by human-related activities, other than mining, were below the TEC for this trace element (table 2; fig. 3).

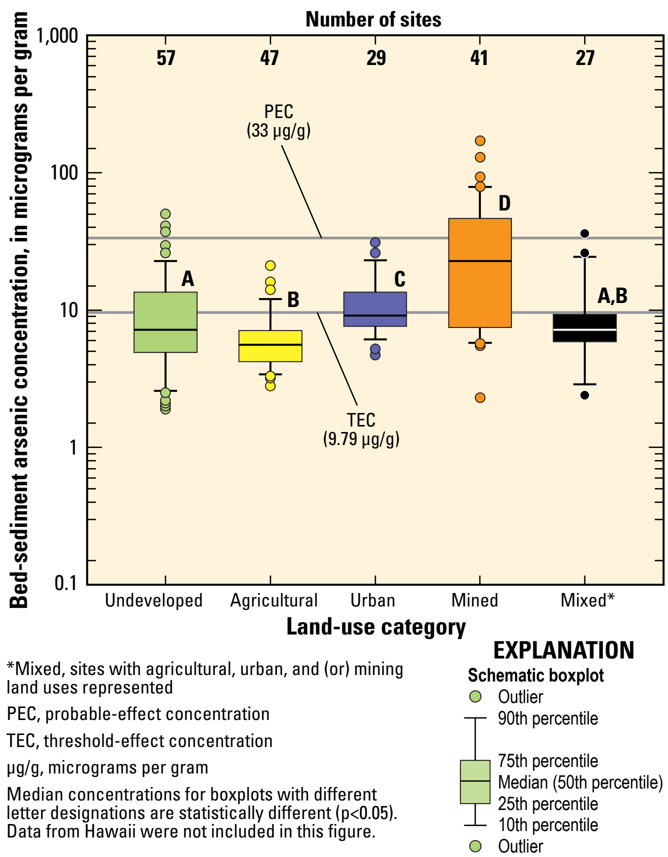


Figure 3. Concentrations of arsenic (As) in bed sediment collected from 201 sites representative of undeveloped and developed (agricultural, urban, mined, and mixed land use) areas in the conterminous western United States and Alaska, 1992–2000.

Cadmium, Lead, and Zinc

The most common sources of Pb and Zn are the sulfide (S) ores of these trace elements that commonly occur together—PbS (galena) and ZnS (sphalerite). Although not widely distributed, the most common Cd-containing mineral, greenockite (CdS), can occur as a coating on sphalerite (Berry and Mason, 1959; Hurlbut, 1971; Helmuth, 1973). Median concentrations of Cd, Pb, and Zn in bed sediment collected from undeveloped areas were 0.3, 20, and 93 µg/g, respectively (table 3, figs. 4*A*–4*C*). Regionally, these reference concentrations are similar to those determined as part of a national study by Horowitz and Stephens (2008) and to the regional baseline concentrations determined using methods similar to Rice (1999) and Velz (1984) (table 2). Most bed-sediment samples (70 to 73 percent) were within Cd, Pb, and Zn regional baseline concentrations (table 3). Cd, Pb, and Zn were moderately correlated in bed sediment collected from reference areas (0.544 ≤ *rho* ≤ 0.626) and strongly correlated at sites influenced by urbanization and mining (*rho* ≥ 0.783, table 4). Zn was the only one of these three trace elements moderately correlated to other trace elements (Cu and Hg) in bed sediment under undeveloped conditions. Cd (-0.558 ≤ *rho* ≤ 0.559), Pb (-0.570 ≤ *rho* ≤ 0.503), and Zn (0.522 ≤ *rho* ≤ 0.608) were correlated to other trace elements in bed-sediment samples collected from developed areas (table 4). The negative moderate correlations between Cd and Pb were for the correlations between these two trace elements and Cr and (or) Ni in bed sediment collected from streams in mined or mixed land-use areas, suggesting these trace elements do not co-occur at the regional scale used during this study.

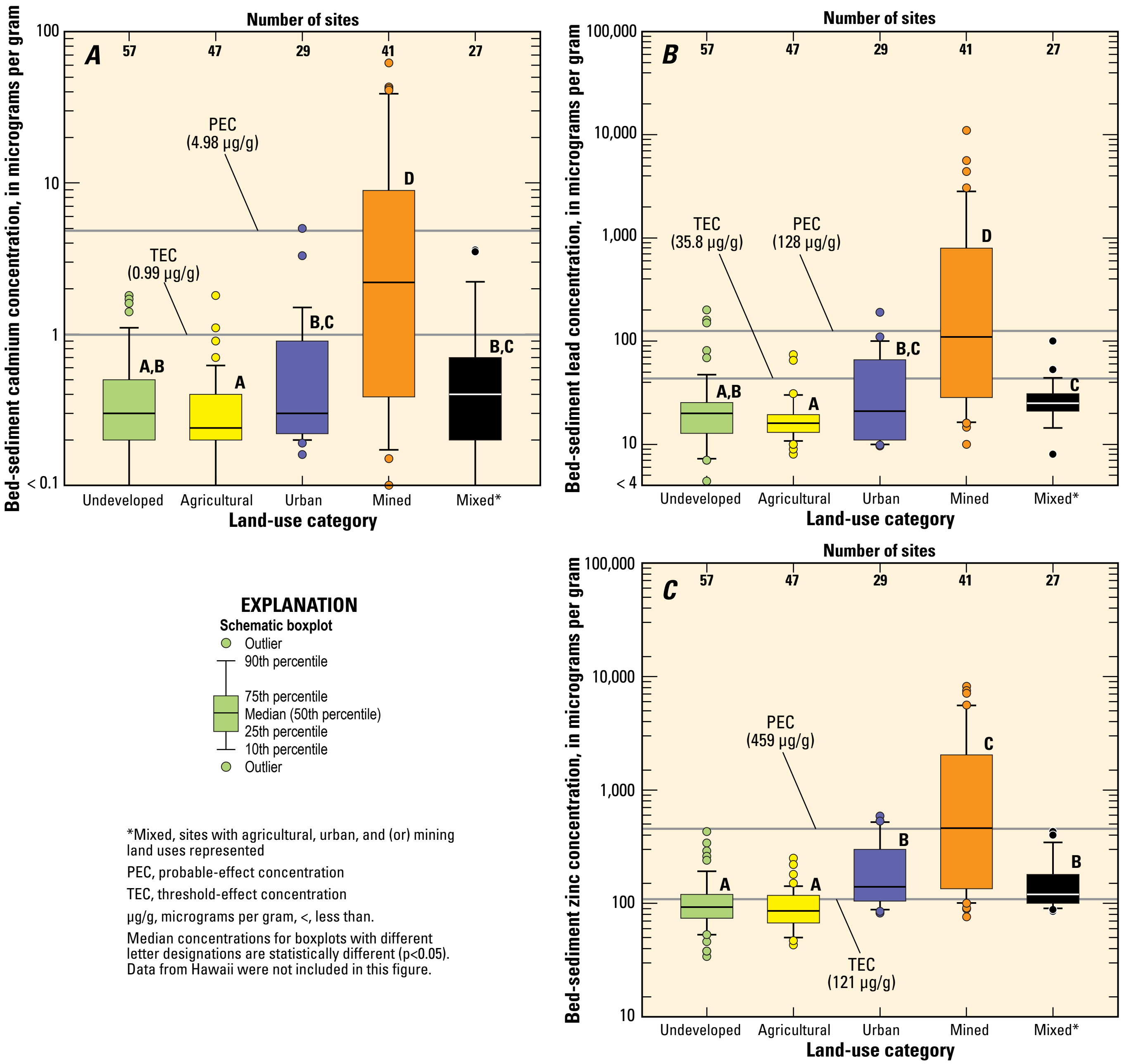


Figure 4. Concentrations of *A*, cadmium, *B*, lead, and *C*, zinc in bed sediment collected from 201 sites representative of undeveloped and developed (agricultural, urban, mined, and mixed land use) areas in the conterminous western United States and Alaska, 1992–2000.

The concentrations of Cd, Pb, and Zn in bed sediment collected from undeveloped and agricultural areas were not significantly different ($0.070 \leq p \leq 0.290$). Concentrations of Cd were similar among undeveloped and urbanized ($p = 0.085$) and mixed land-use ($p = 0.229$) areas, whereas Pb and Zn showed some degree of enrichment in mixed ($p \leq 0.016$) and urbanized ($p < 0.05$) landscapes, respectively (figs. 4*A*–4*C*). The highest concentrations of all three of these trace elements were in bed-sediment samples collected from streams influenced by historic mining-related activities ($p \leq 0.004$). Median concentrations of Cd, Pb, and Zn determined for bed sediment in mined areas were 2.2, 110, and 460 µg/g, respectively. The median concentrations of Pb and Zn at these sites were approaching and equal to the respective PEC values for these trace elements (table 2; figs. 4*B*–4*C*).

The PEC values for Cd, Pb, and Zn were exceeded in bed sediment collected from 12, 24, and 25 sites, respectively (table 2; figs. 4*A*–4*C*). Most of these exceedences (83–92 percent) were in bed sediment collected from streams in mined areas. The other bed-sediment samples (8 to 17 percent), where the PEC value for at least one of these trace elements was exceeded, were collected from undeveloped and urban areas. Eleven bed-sediment samples contained concentrations of Cd, Pb, and Zn that exceeded the PEC values for all three trace elements, all within mined landscapes. Of these 11 sites, 7 exceeded the PEC value for As, 6 exceeded the PEC for Cu, and 4 exceeded the PEC for Hg (table 2). Of these 11 sites, 9 are associated with CERCLA areas in Colorado, Idaho, and Utah listed as a result of trace-element contamination from historic mining activities (U.S. Environmental Protection Agency, 2010).

Chromium and Nickel

Coastal mountain ranges in California, parts of the Sierra Nevada, and mountains in southern Oregon were formed by upward faulting of oceanic crustal deposits containing ophiolite-sequence formations and ultramafic rocks (Bateman and Wahrhaftig, 1966; Irwin, 1966; Kosanke and Harper, 1997; Godfrey and Dilek, 2000). Ni has been found to be enriched in ultramafic rocks and in chromite, which are important sources of naturally occurring Cr and the primary ore targeted by Cr mining operations (Hancock, 1991; Papp, 1994; Salminen and others, 2004). Serpentine soils, derived from the weathering of ultramafic rock, contain relatively large amounts of Cr and Ni. Although not widely distributed, areas noted for having serpentinitic rocks and soils are in California and Oregon (Oze and others, 2004). The high concentrations of Cr in bed sediment collected from streams in California are likely the result of the weathering and transport of material originating from the ultramafic serpentine rock exposed along the Coast Ranges and Sierra Nevada (Morrison and others, 2009). Areas within Washington and Alaska also have geologically derived Cr and (or) Ni (Fuhrer and others, 1999; Frenzel, 2002; Ourso and Frenzel, 2003). Median concentrations of Cr (55 µg/g) and Ni (21 µg/g) in bed sediment collected from streams in undeveloped areas were similar to median concentrations of these trace elements in bed sediment throughout the Nation (Horowitz and Stephens, 2008) and to regional baseline concentrations determined for this study. Most bed-sediment samples had concentrations of Cr (76 percent) and Ni (72 percent) within regional baseline limits (table 3). Concentrations of Cr and Ni in bed sediment collected from streams in undeveloped and developed areas were strongly correlated (*rho* ≥ 0.691, table 3), a condition observed only for these two trace elements.

Median concentrations of Cr and Ni in bed sediment collected from undeveloped and agricultural areas ($p \geq 0.386$) were not significantly different. Median concentrations of Cr in bed sediment from areas influenced by mining (48 µg/g) were statistically similar to those in bed sediment from undeveloped (55 µg/g) and agricultural (53 µg/g) areas ($p \geq 0.380$); median concentrations of Cr were elevated but statistically similar in bed sediment from streams in urbanized (78 µg/g) and mixed (65 µg/g) land-use areas ($p = 0.286$, fig. 5*A*). Of the 19 bed-sediment samples exceeding the PEC for Cr (111 µg/g), the fewest exceedences were in agricultural (10 percent) and urban (10 percent) landscapes; the most exceedences

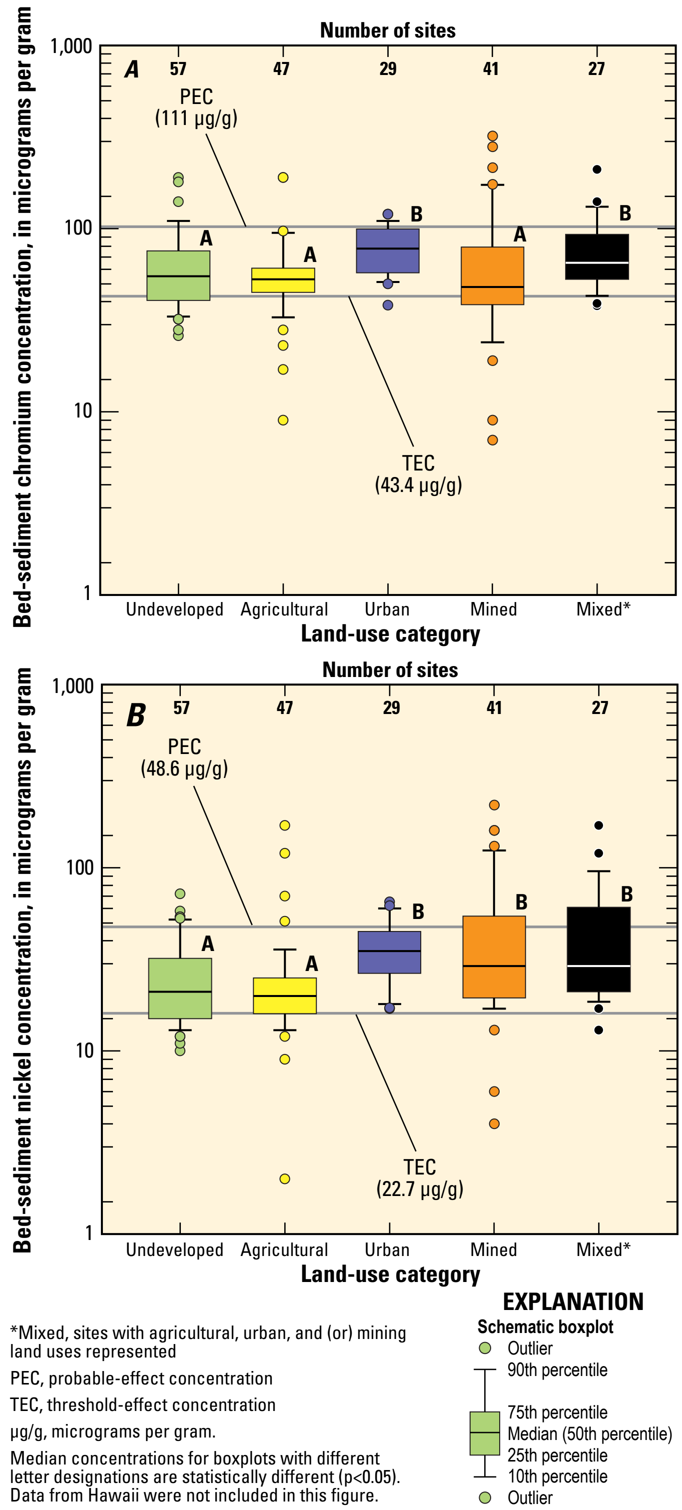


Figure 5. Concentrations of *A*, chromium, and *B*, nickel in bed sediment collected from 201 sites representative of undeveloped and developed (agricultural, urban, mined, and mixed land use) areas in the conterminous United States and Alaska, 1992–2000.

were in mined (38 percent) areas. These results indicate that relatively high concentrations of Cr are encountered in many bed-sediment samples from urban and mixed land-use areas; however, these concentrations are generally below the PEC for this trace element. In contrast, Cr concentrations in bed sediment collected from streams in mined landscapes contain generally lower concentrations than in urban and mixed land-use areas but have occasional samples with high concentrations exceeding the PEC for Cr. Although Cr can be mobilized when mined, Cr contained in waste tailings is relatively immobile (Papp, 1994; Salminen and others, 2004). Median concentrations of Cr and Ni in bed sediment collected from all land-use areas were between the TEC and PEC concentrations for these trace elements (figs. 5*A*-5*B*). Ninety-five percent of the bed-sediment samples exceeding the PEC for Cr also exceeded the PEC for Ni (48.6 µg/g), 10 percent for As (33 µg/g), and 5 percent each for Pb (128 µg/g) and Zn (459 µg/g).

Concentrations of Ni were elevated, and median concentrations similar in bed sediment collected from streams flowing through areas influenced by urbanization (35 µg/g), mining (29 µg/g), and mixed (29 µg/g) land uses ($p \geq 0.277$, fig. 5*B*). The PEC for Ni was exceeded in 37 bed-sediment samples from all five land-use categories (undeveloped, agricultural, urban, mined, and mixed); the lowest number of exceedences was in streams in agricultural areas (11 percent), and the highest in streams influenced by historic mining-related activities (32 percent). Where PEC exceedences occurred for Ni, 47 percent of those samples also exceeded the PEC for Cr; the PECs for As, Pb, and Zn were exceeded in 5 to 11 percent of these 37 samples. These results show a connection between Cr and Ni where sediment-quality guidelines generally exceeded for one of these trace elements may be exceeded for the other. There were a few instances where the PECs for Cr and (or) Ni were exceeded and concentrations of the other seven trace elements considered in this evaluation were also greater than respective PEC values. Of the 18 bed-sediment samples where concentrations of Cr and Ni exceeded their respective PEC values, 7 of these samples (39 percent) were collected from streams that have segments listed as impaired; none were associated with areas influenced by known CERCLA areas or considered impaired as a result of either Cr and (or) Ni contamination (table 2; Alaska Department of Environmental Conservation, 2010; California Environmental Protection Agency, 2010; State of Washington, Department of Ecology, 2010).

Copper

Chalcopyrite ($CuFeS_2$) is commonly associated with galena (PbS) and sphalerite (ZnS), which were all relatively common in areas where bed-sediment samples were collected and mineralogic data were available (Berry and Mason, 1959; Hurlbut, 1971); other researchers have noted the common co-occurrence of Cd, Cu, Pb, and Zn (Baker and Proctor, 1990). Available U.S. Geological Survey Mineral Resource Dataset (MRDS) data were sparse and varied by site, resulting in limited characterization of the mineralogy associated with dominant ore deposits throughout the study area (U.S. Geological Survey, 2004). However, of the 117 sites for which MRDS information was available, chalcopyrite was identified at 77 sites, and chalcopyrite, galena, and sphalerite co-occurred at 61 (79 percent) of these 77 sites. The median Cu concentration in bed sediment collected from streams in undeveloped areas was 26 µg/g, similar to the regional baseline concentration and the national baseline concentration determined by Horowitz and Stephens (2008). Concentrations of Cu in 80 percent of the samples collected throughout the regional study area were within regional baseline concentration limits (7 to 80 µg/g, table 3). In undeveloped areas, bed-sediment Cu concentrations were moderately correlated to Zn concentrations (*rho* = 0.552). Concentrations of Cu in bed-sediment samples collected from streams in developed areas were at least moderately correlated to various other trace elements ($0.522 \leq rho \leq 0.789$) depending on the land use (table 4). These results show that Cu commonly co-occurred with other trace elements in the bed-sediment samples.

The median Cu concentration in bed sediment collected from agricultural areas (21 µg/g) was statistically lower than concentrations in bed sediment collected from undeveloped areas (26 µg/g, $p = 0.008$, fig. 6). Although the median Cu

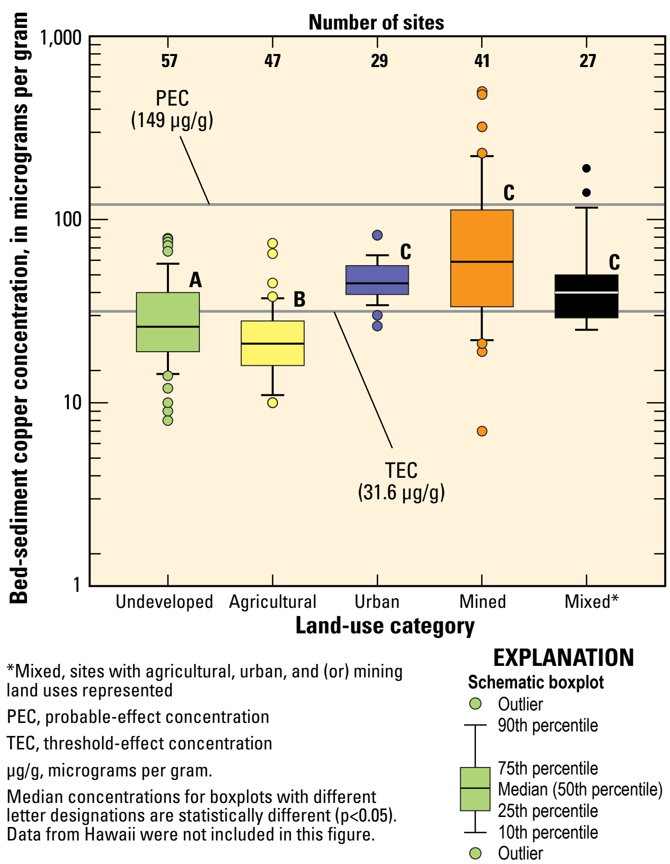


Figure 6. Concentrations of copper (Cu) in bed sediment collected from 201 sites representative of undeveloped and developed (agricultural, urban, mined, and mixed land use) areas in the conterminous western United States and Alaska, 1992–2000.

concentration appears to be the highest in bed sediment collected from areas influenced by mining, there was no strong statistical difference among median Cu concentrations in bed-sediment samples collected from areas of mining (58.7 µg/g), urban (45 µg/g), and mixed (40 µg/g) land use ($p \geq 0.055$, fig. 6). The PEC (149 µg/g) for Cu was exceeded in nine bed-sediment samples of which eight were associated with CERCLA areas listed because of historic mining activities in Colorado, Idaho, Montana, and Utah (U.S. Environmental Protection Agency, 2010). Concentrations of Cu at these eight sites ranged from 170 to 500 µg/g. In the nine instances where the PEC for Cu was exceeded, 44 to 89 percent of these samples also exceeded a PEC value for one or more of the other trace elements—As, Cd, Pb, Hg, and (or) Zn.

Mercury

Cinnabar (HgS) is the most important Hg-containing mineral and is commercially mined in only a few locations in the United States (Bailey and others, 1973). In the western United States, Hg deposits are found in Alaska, California, Idaho, Nevada, Oregon, and Utah (Hurlbut, 1971; Bailey and others, 1973). Hg is a global-scale pollutant, widely distributed by atmospheric transport (Fitzgerald and others, 1998; Eisler, 2000). Through atmospheric transport mechanisms, elevated Hg concentrations have been occurring in otherwise relatively “pristine” areas (Fitzgerald and others, 1998; Krabbenhoft and others, 1999). The use of Hg to facilitate the extraction of gold and silver from milled ore by a process called amalgamation can release large quantities of Hg to adjacent streams and rivers (Bevans and others, 1998; Alpers and others, 2005). Activities related to historic amalgamation operations continue to be an environmentally important source of Hg contamination within the western United States (Maret and others, 2003; Norman and others, 2008). Scudder and others (2009) found that concentrations of total Hg were higher in bed-sediment samples collected from streams in mined areas relative to samples collected from unmined areas in the western United States.

The median Hg concentration in bed sediment from undeveloped areas was 0.04 µg/g, similar to the regional baseline concentration determined as part of this study (0.03 µg/g) and the same as the national baseline value determined by Horowitz and Stephens (2008). Sixty-nine percent of the bed-sediment samples collected and analyzed for Hg contained concentrations of Hg within regional baseline limits (<0.02 to 0.15 µg/g, table 3). Concentrations of Hg in bed sediment were correlated with Zn concentrations in samples collected from streams at undeveloped sites (*rho* = 0.541, table 4). At developed sites, Hg was moderately correlated with bed-sediment concentrations of Cr, Cu, Ni, and Se ($0.544 \leq rho \leq 0.669$) depending on the predominant land use (table 4). Concentrations of Hg in bed sediment exceeded the PEC (1.06 µg/g) at seven sites, all influenced by historic mining-related activities (fig. 7). At these seven sites, 57 to 86 percent of these samples also exceeded the PECs for As, Cd, Pb, and Zn. Six of these seven sites were associated with three CERCLA areas in Idaho, Montana, and Nevada. Concentrations of Hg associated with bed-sediment samples from these CERCLA areas ranged from 1.7 to 17 µg/g; the highest concentration was in bed sediment collected from a river in Nevada. Scudder and others (2009) indicate that a more accurate estimate of Hg toxicity in bed sediment would be represented by concentrations of methyl mercury (methyl-Hg), the more toxic form of Hg; however, sediment-quality guidelines for methyl-Hg are currently unavailable.

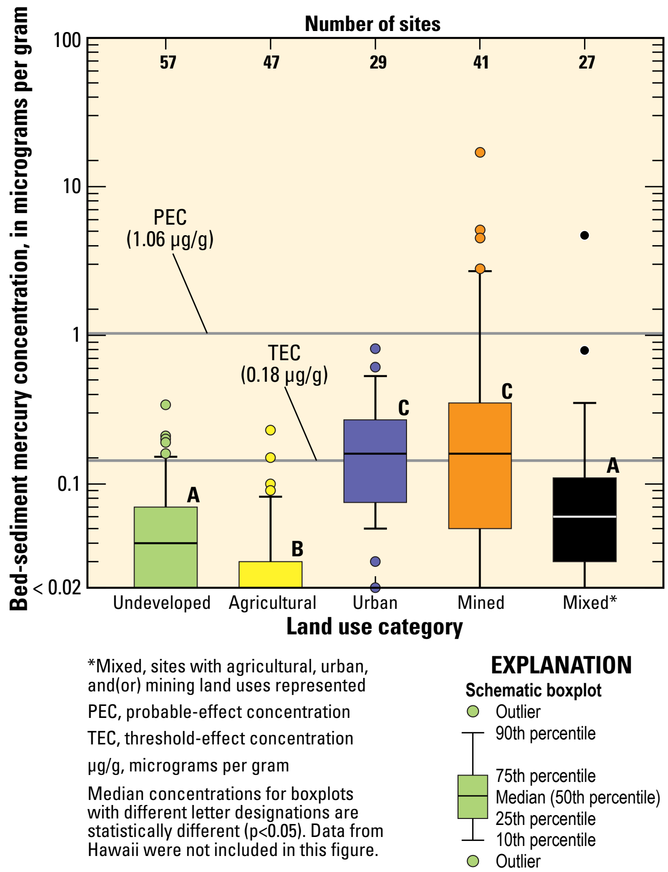


Figure 7. Concentrations of mercury (Hg) in bed sediment collected from 201 sites representative of undeveloped and developed (agricultural, urban, mined, and mixed land use) areas in the conterminous western United States and Alaska, 1992–2000.

Selenium

The most common Se-containing mineral is clausthalite (PbSe); however, Upper Cretaceous marine sedimentary deposits are the most prominent Se-containing material in the western United States (Bear, 1957; Berry and Mason, 1959; Seiler and others, 2003; Mineral Data Publishing, 2005). Cutter and Bruland (1984) suggest a biogeochemical cycle of Se in the marine environment that involved Se uptake by marine organisms and subsequent deposition of biogenic organic-selenide particulate matter. Areas within the western United States with noted Upper Cretaceous marine shales include California, Colorado, eastern Utah, Montana, parts of New Mexico, and Wyoming (Byers, 1935; Seiler and others, 2003). Concentrations of Se in seleniferous soils derived from these deposits have been found to range from 5 to 80 µg/g (Bear, 1957). The world's largest deposit of oil shale, a bituminous sedimentary rock containing trace elements including Se, is the Green River Formation in Colorado, Utah, and Wyoming (Stollenwerk and Runnells, 1981; Spackman and others, 1990). The median Se concentration in bed sediment collected from undeveloped sites was 0.53 µg/g, similar to the regional baseline concentration of 0.44 µg/g and that determined in the national study by Horowitz and Stephens (2008) (table 3). Of the 201 sites within the conterminous western United States and Alaska included in this study, 193 bed-sediment samples were analyzed for Se; 62 percent of those samples had concentrations within baseline limits (<0.1 to 1.3 µg/g, table 3). Concentrations of Se were not substantially correlated (*rho* ≤ 0.373) to any of the other eight trace elements evaluated in bed-sediment samples collected from undeveloped areas (table 4). Concentrations of Se in bed sediment from streams in agricultural areas were moderately correlated to Cd (*rho* = 0.506), and in urban areas, there was a moderate correlation between Se and Hg (*rho* = 0.666); all other correlations between Se and the remaining five other trace elements within developed areas were weak (*rho* ≤ 0.339).

Compared to undeveloped (reference) sites, significantly higher concentrations of Se in bed sediment were observed only in streams in urban land-use settings (p = 0.012, fig. 8). Sources of Se to receiving streams include municipal wastewater discharges and runoff from agricultural lands, coal and metal mining operations, and oil refineries (Renner, 1998). The highest concentrations of Se observed in this study, and the only two instances where concentrations exceeded the PEL (4 µg/g; Van Derveer and Canton, 1997), were in bed-sediment samples collected from a wash influenced by agricultural practices in Colorado (5.6 µg/g) and a creek in an urbanized landscape in Alaska (5.8 µg/g). Geological sources, such as coal deposits, exist within the Cook Inlet area near Anchorage, Alaska, and could be contributing to elevated Se concentrations in the area (Frenzel, 2000). In all land-use categories, median Se concentrations were below the threshold-effect level (TEL) of 2.5 µg/g.

Cumulative Toxic Units

The interactions among trace elements in mixture are important to consider when examining the effects of trace elements in natural systems (Dunlop and Chapman, 1981; Oakden and others, 1984; Preston and others, 2000; Griffith and others, 2004). Enhanced trace-element interactions can be additive, where toxicity among trace elements increases in proportion to the number of trace elements present, or synergistic, where a trace element enhances the toxicity of others by a factor greater than the number of trace elements present.

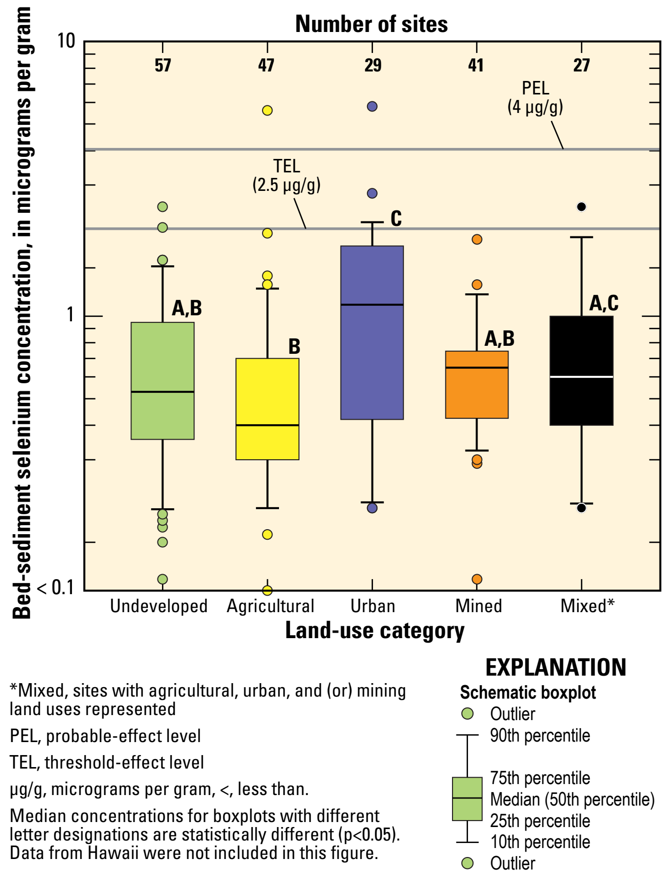


Figure 8. Concentrations of selenium (Se) in bed sediment collected from 201 sites representative of undeveloped and developed (agricultural, urban, mined, and mixed land use) areas in the conterminous western United States and Alaska, 1992–2000.

Alternatively, trace-element interactions can be antagonistic (inhibitory), whereby a trace element can decrease the expressed toxicity of another (Larsen and Bjeeregaard, 1995; Andrewes and others, 2000). The use of CTUs assumes additive toxicity among trace elements. The use of CTUs to characterize the degree of bed-sediment impairment can potentially underestimate or overestimate the harmful quality of the sediment being evaluated and should be considered an indicator of impairment, not an actual measure of impairment.

CTUs were used to evaluate trace-element concentrations in bed sediment in mixture with respect to available sediment-quality guidelines (MacDonald and others, 2000). CTU values were calculated using available bed-sediment concentration data for eight of the nine trace elements examined in this study. Se was not used in the determination of CTUs because data were not available for all bed-sediment samples collected. There were only two instances where Se concentrations exceeded the PEL of 4 µg/g, and with the exception of two instances, Se was at best weakly correlated with other trace elements. Median CTU values for bed sediment collected from streams in undeveloped (2.04) and agricultural (1.57) landscapes were below the 50th percentile for the entire study area (2.5). Bed sediment collected from urban and mixed land-use areas had median CTU values (2.9 and 2.7, respectively) exceeding the 50th percentile for the entire regional dataset (fig. 9). All median CTU values were statistically different ($p \leq 0.002$) except those for bed sediment from urban and mixed ($p = 0.35$) landscapes. The highest CTU values were calculated for bed sediment collected from streams in mined areas. The median CTU value for mined landscapes (7.5) was equal to the 90th percentile for the entire regional dataset. Only bed sediment from mined landscapes had CTU values exceeding the 90th percentile (fig. 9). For the purposes of this study, sediment with CTU values less than or equal to the 25th percentile for the regional dataset (1.71) were considered unenriched in trace elements (table 5). Sediment with CTU values ranging between the 26th and 50th percentiles (1.72 to 2.52) were considered to have low trace-element enrichment. Moderately and moderately-high enriched sediment were classified as those sediment samples with CTU values ranging from the 51st to 75th percentiles (2.52 to 3.9) and from the 76th and 89th percentiles (4.1 to 7.2), respectively. Sediment samples with CTU values greater than the 90th percentile of the regional dataset (less than or equal to 7.5) were considered highly enriched.

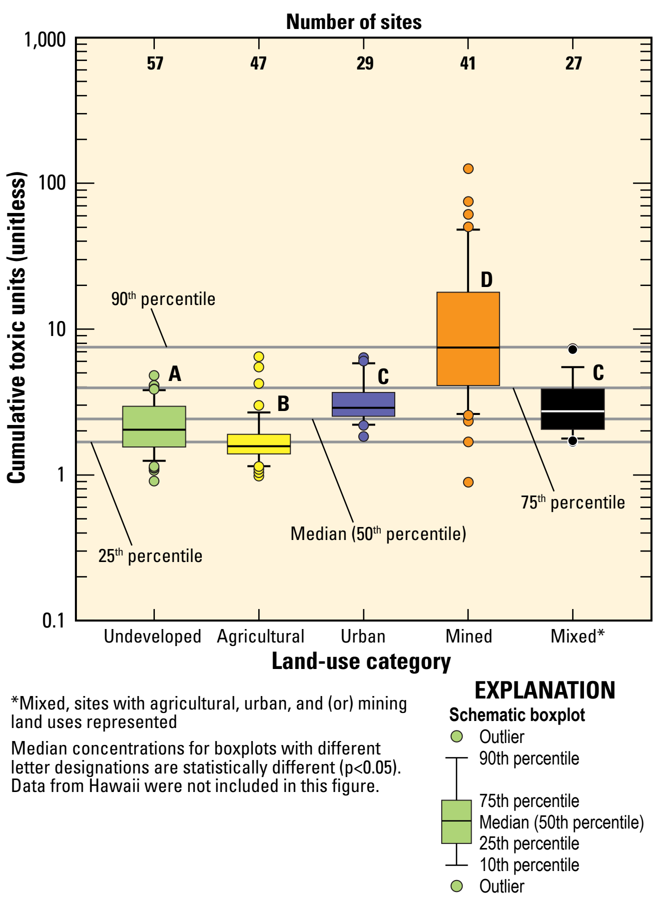


Figure 9. Cumulative toxic units (CTUs) calculated for 201 bed-sediment samples collected from streams in undeveloped, agricultural, urban, mined, and mixed land-use areas within the conterminous western United States and Alaska, 1992–2000.

Table 5. Cumulative toxic unit (CTU) percentile enrichment thresholds used to characterize bed sediment samples collected from selected streams in the conterminous western United States and Alaska, 1992–2000.

[**Abbreviations:** ≤, less than or equal to; ≥, greater than or equal to]

Percentile threshold	Value (unitless)	Description
≤ 25	1.71	No enrichment
26–50	1.72–2.52	Low enrichment
51–75	2.53–3.9	Moderate enrichment
76–89	4.1–7.2	Moderately-high enrichment
≥ 90	≥ 7.5	High enrichment

Bed sediment collected from different locations can have different trace-element compositions. Generally, Hg contributed the least to CTU values determined for bed sediment collected from undeveloped, agricultural, mined, and mixed land-use areas (figs. 10*A*–10*E*). Hg contributed over 50 percent to the calculated CTU value in two instances; both were associated with bed sediment collected from a river in Nevada where contamination of bed sediment resulted from historic mining practices involving the use of Hg to amalgamate gold from ore (figs. 10*D*–10*E*). Hg contributions to overall CTU values in bed sediment in urban areas showed greater variability than in other landscapes evaluated as part of this study (fig. 10*C*). Cd contributed the least to CTU values determined for bed sediment collected from streams in urbanized areas (fig. 10*C*). With a few subtle differences, the relative contribution of each of the eight trace elements to respective CTU values calculated for undeveloped, agricultural, urban, and mixed land uses were similar (figs. 10*A*–10*E*). Given the regional perspective of the analysis, the most important point here is the relative pattern(s) in trace-element contributions among these various land uses. These analyses do not take into account site-specific differences in actual CTU composition that occur and should be addressed on a case-by-case basis. In samples evaluated as part of this study, Cr and Ni were identified as contributing substantially more to the overall CTU values than the remaining six trace elements (figs. 10*A*–10*E*). The pattern observed for samples collected from mined areas differs markedly from the other land-use areas (fig. 10*D*). In these samples, median contributions of Pb and Zn are of greater significance than those for either Cr or Ni.

Concentrations of Cr and Ni were strongly correlated in all bed-sediment samples evaluated in this study ($rho \geq 0.691$). Of the 29 bed-sediment samples collected from urbanized areas, Cr and Ni comprised 24 to 61 percent of the CTU values (range 1.8 to 6.4). Bed sediment from streams in areas of mixed land use had CTU values similar to those in urbanized areas (range 1.7 to 7.3); Cr and Ni contributed 12 to 84 percent. The general pattern observed in CTU contributions among the various land uses, except mined, shows that Cr and Ni contribute variable and oftentimes the most to the overall CTU values in undeveloped, agricultural, urban, and

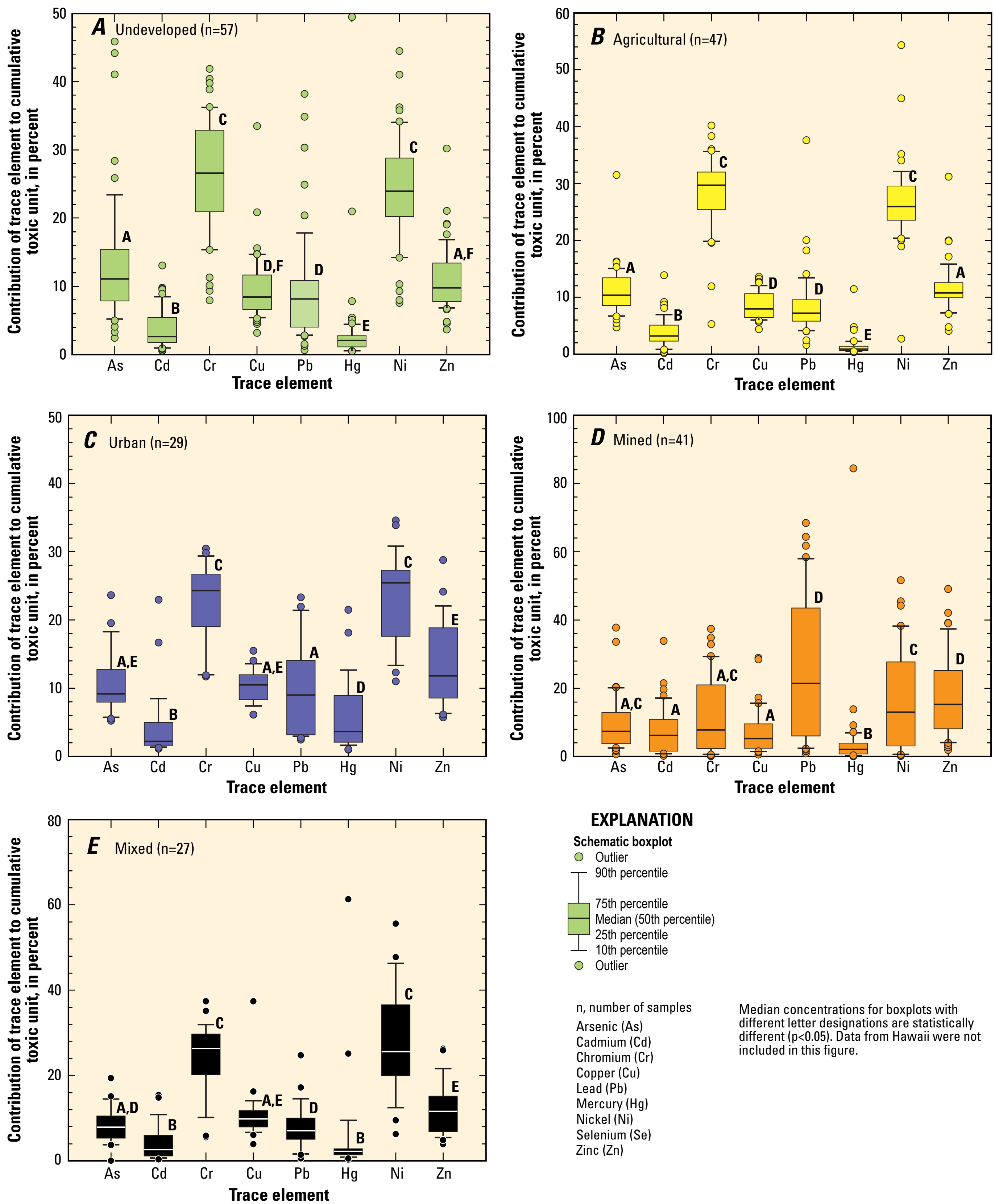


Figure 10. Contribution of each of the eight trace elements used to determine the cumulative toxic units (CTUs) for bed sediment collected from selected streams and rivers in *A*, undeveloped; *B*, agricultural; *C*, urban; *D*, mined; and *E*, mixed land-use areas in the conterminous western United States and Alaska, 1992– 2000.

mixed land-use areas. Given the essentially unwavering strong correlation between Cr and Ni, areas where PEC values are exceeded for these trace elements are of particular interest. Median concentrations of Cr and Ni were relatively low in bed sediment collected from streams in undeveloped and agricultural areas; concentrations were elevated in samples of bed sediment from urban and mixed land-use areas and, in the case of Ni, also in mined landscapes (figs. 5*A*–5*B*). Most of the PEC exceedences for Cr and Ni were in bed-sediment samples collected from mined areas.

Regionally, As, Cd, Pb, and Zn were moderately correlated ($rho \geq 0.503$) in bed sediment collected from mined areas. The CTUs determined for bed sediment collected from the mined landscapes varied in composition from those in undeveloped, agricultural, urban, and mixed land-use areas where the contribution of As, Cd, Pb, and Zn (as a group) ranged from 7 to 98 percent, commonly exceeding the relative contributions of Cr and Ni. Generally, CTUs determined for bed sediment in mined areas were primarily composed of Pb and Zn (fig. 10*D*). In the mined landscapes, CTU values ranged from 0.89 to 125.7 units. Non-CERCLA associated mined areas (23 sites) had CTU values ranging from 1.7 to 28.8 units. The trace elements As, Cd, Pb, and Zn comprised from 7 to 95 percent of these CTU values, and Cr and Ni comprised 3 to 85 percent. Mined areas associated with CERCLA sites had CTU values ranging from 0.89 to 125.7 units. The trace elements As, Cd, Pb, and Zn comprised from 9 to 98 percent of these CTU values, and Cr and Ni comprised 0.3 to 37 percent. It is apparent from these results that a combination of CTU composition, PEC exceedences, and site-by-site variability exists, and although composition patterns may be similar, relative contributions of trace elements vary.

Macroinvertebrate Communities and Habitat Characteristics

To assess the relation between bed-sediment trace-element concentrations and macroinvertebrate community structure, habitat characteristics of the sampling site must be taken into account. For example, macroinvertebrate taxa have different habitat preferences for water velocity, substrate, and general water-quality characteristics. Additionally, the introduction of contaminants to streams may coincide with physical alterations that themselves can cause changes in aquatic communities (Sheehan and Winner, 1984; Landrum and Robbins, 1990; Cuffney and others, 2005). A preliminary assessment of aquatic-macroinvertebrate community structure using MDS showed little distinction among macroinvertebrate assemblages at sampling sites representing the large-scale PL and XE ecoregions; however, the Hawaii (HI), MT, and Ozark Highlands (OH) ecoregions were distinctly different from each other and the PL and XE ecoregions (fig. 11). Stratification of macroinvertebrate communities by ecoregion for statistical analysis can, at least to some degree, minimize inherent variability in aquatic community composition that results from differences in climate, topography, geology, and general in-stream characteristics.

The grouping of Hawaiian macroinvertebrate communities illustrates the ability of MDS analysis to distinguish these very different macroinvertebrate communities from those in the other ecoregions within the conterminous western United States and Alaska. A primary distinguishing feature of the aquatic communities in Hawaiian streams is that taxa belonging to the insect orders Ephemeroptera (mayflies) and Plecoptera (stoneflies) are absent. Bed sediment collected from the seven Hawaiian streams also had a different chemical signature; median concentrations of Cu, Cr, and Ni ranged from about 6 to 11 times higher than in bed sediment sampled from streams in the other ecoregions (see Hawaii Box; figs. 5*A*–5*B* and 6).

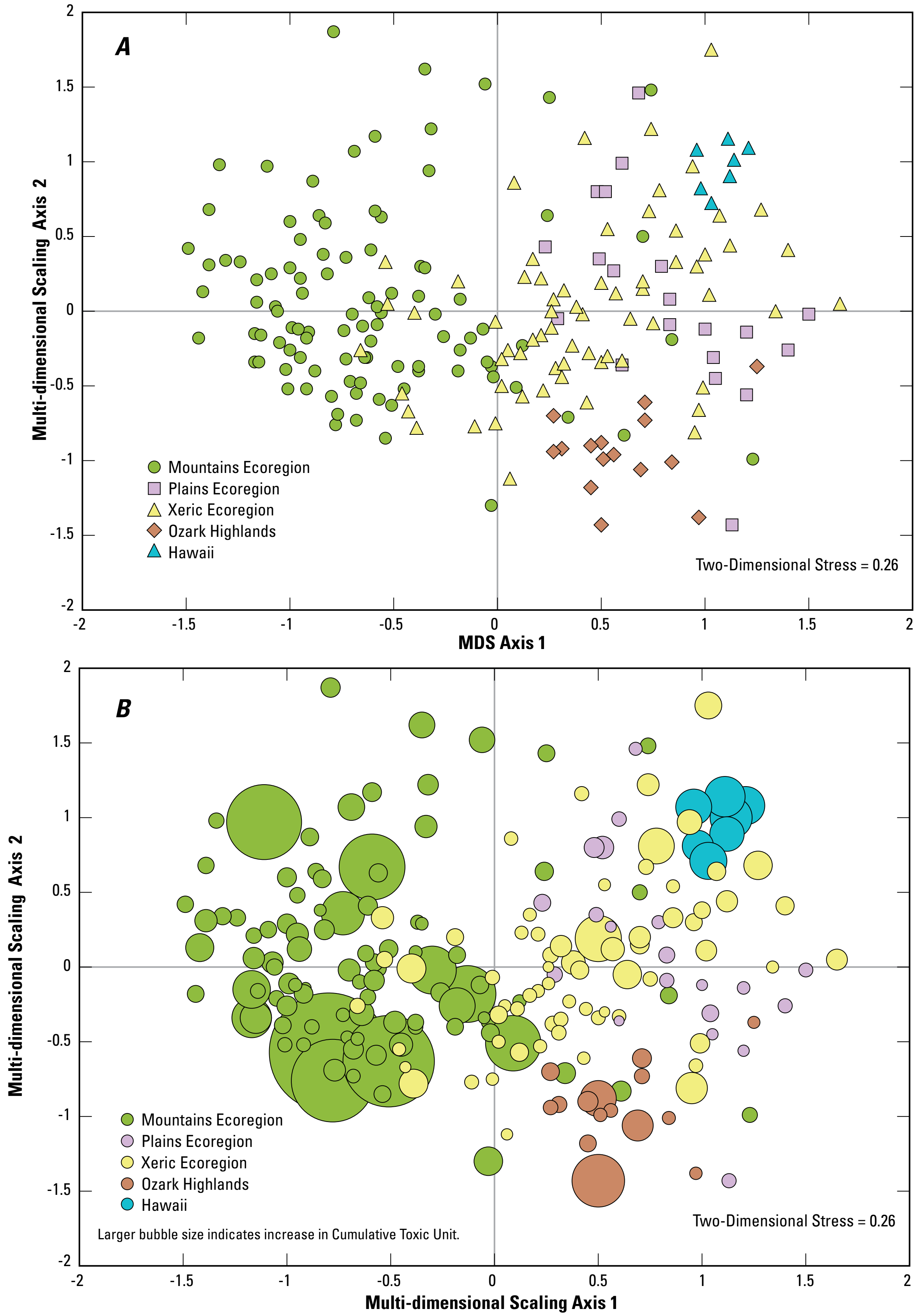
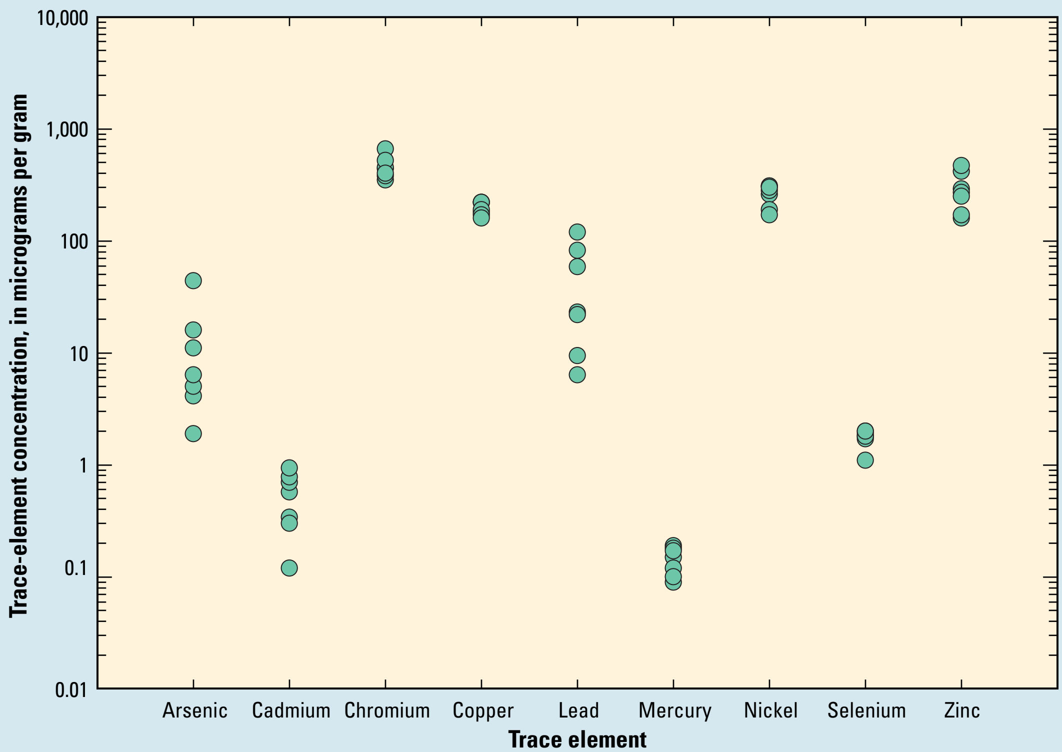


Figure 11. Non-metric multidimensional scaling ordination representing *A*, aquatic-macroinvertebrate communities within the large-scale ecoregions; and *B*, associated distribution patterns in trace-element cumulative toxic units for the 208 samples collected from streams in the conterminous western United States, Alaska, and Hawaii, 1992–2000.

Trace Elements and Macroinvertebrates in the Hawaiian Islands

Seven stream sites on the island of Oahu, Hawaii, representing undeveloped, urban, and agricultural landscapes were sampled for bed sediment and macroinvertebrates. In general, median concentrations of As (6.4 µg/g), Cd (0.57 µg/g), Pb (23 µg/g), Hg (0.15 µg/g), Se (1.8 µg/g), and Zn (270 µg/g) in bed sediment collected from the seven Oahu sites were similar to those determined for bed-sediment samples collected from streams in the conterminous western United States and Alaska; however, median concentrations of Cr (440 µg/g), Cu (190 µg/g), and Ni (280 µg/g) were about 6 to 11 times higher in the Oahu bed sediment. The relatively high concentrations of Cr and Ni in Hawaiian sediment originate from the volcanic rocks and soils of the islands (De Carlo and others, 2005). De Carlo and others (2004) suggest an urban source of Cu in sediment samples from Oahu. Concentrations of As, Cd, Pb, and Zn in sediment from urban areas were substantially higher than concentrations measured in undeveloped areas on Oahu (Brasher and Wolff, 2007). Cr, Cu, and Ni in bed-sediment samples from these seven Oahu sites exceeded the sediment-quality guidelines for these trace elements.

Because of the extreme isolation of the Hawaiian islands, macroinvertebrate assemblages in these streams are relatively depauparate and considerably different than in the other areas included in this study (Brasher and others, 2004). Most notably, none of the three insect orders, Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies), that are routinely used as indicators of water quality in the continental United States occur naturally in Hawaii. Only caddisflies, which were introduced, now occur at high abundance in most Hawaiian streams (Brasher and others, 2004). The non-metric MDS model (fig. 11) shows how different the Hawaii macroinvertebrate samples are in comparison to the other samples in this study and consequently helps illustrate how an MDS performs.



Ecoregion and Land-Use Characterization

To characterize some of the differences among the ecoregions (HI, MT, OH, PL, and XE), general water quality (pH, specific conductance, total dissolved solids (TDS), hardness, and suspended-sediment concentrations) was evaluated (figs. 12*A*–12*D*). These water-quality characteristics are important in considering distribution patterns of aquatic macroinvertebrates because they influence the colonization of taxa with different physiologic characteristics and in-stream habitat preferences (Hynes, 1979; Merrit and Cummins, 1996; Hawkins and others, 2000). With the exception of the HI ecoregion, the MT ecoregion had lower median pH, specific conductance, TDS, and hardness than the other ecoregions (figs. 12*A*–12*D*). The OH ecoregion had similar general water-quality characteristics to the XE ecoregion (figs. 12*A*–12*D*); however, suspended-sediment concentrations of the OH ecoregion closely resembled those observed in the MT ecoregion (fig. 12*E*). Sites within the PL ecoregion had the highest median specific conductance, TDS, and suspended-sediment concentration (fig. 12*A*–12*B* and 12*E*).

Impairments in habitat can result in conditions unfavorable to sensitive taxa, resulting in similar macroinvertebrate community structure to that found at sites with elevated trace-element concentrations (another type of impairment). Sampling sites in streams within the PL (45 percent) and XE (44 percent) ecoregions were primarily characterized as agriculture (fig. 13). Agricultural runoff has been found to transport higher quantities of nitrogen and phosphorus than forested areas, and elevated concentrations of these constituents can cause increased algal growth, reduced light permeability, and depleted dissolved oxygen concentrations, resulting in gradual changes in downstream aquatic communities (Stevenson and others, 1986; Staver and others, 1988). Additional physiochemical alterations downstream from agricultural drainage inputs include increased salinity, siltation of streambed materials, and turbidity (National Research Council, 1989; Tucker and Burton, 1999). Grazing cattle can create streambank instability and reduced riparian vegetation resulting in increased erosion, siltation, nutrient input, and fecal coliform bacteria (Forest Practices Board, 2002; Murray, 2007). Streams influenced by urban and mixed land-use-related activities accounted for 6 to 23 percent of the sites within the PL and XE ecoregions (fig. 13). Mined areas were not represented in the PL ecoregion; however, samples collected from the XE ecoregion included some sites representative of areas associated with mining-related activities (17 percent, fig. 13).

Streams within the MT ecoregion were representative of mainly undeveloped (42 percent), urbanized (22 percent), and mined (22 percent) conditions (fig. 13). The agricultural landscape is represented in about 6 percent of the sites. Streams within urban centers are influenced by point and nonpoint sources, such as treated wastewater discharge, urban runoff, and interbasin transfers of water that can introduce sediment, trace elements, nutrients, pesticides, and other contaminants into stream ecosystems (Paul and Meyer, 2001; Mahler and others, 2005; Short and others, 2005). Impervious surfaces within urban environments can alter stream geomorphology and hydrology, and it is estimated that 15 to 20 percent of the streams in the United States are ecologically affected by roads (Schueler, 1994; Forman and Alexander, 1998; Paul and Meyer, 2001; Ourso and Frenzel, 2003). In addition, losses of pools and riffles, reduced vegetative cover, greater fluctuations in stream temperature, diminished base flows, channel enlargement, and siltation resulting from stream-channel erosion have been recognized as ecological issues in urbanized watersheds (Wolman and Schick, 1967; Schueler, 1994; Trimble, 1997; Tucker and Burton, 1999). Short and others (2005) found that streambed instability, remnant bed armoring, channel incision, periods of drought and high-flow conditions, and basin slope can all influence streambed embeddedness.

Fifty-three percent of the stream sites sampled in the OH ecoregion were in areas that have been mined (fig. 13). Changes in physiochemical habitat caused by mining can include changes in water chemistry and coating of streambed substrates from the precipitation of metal oxides from solution (Theobald and others, 1963; McKnight and Feder, 1984; Moore and others, 1991; Clements and others, 2000; Griffith and others, 2001). Changes in water chemistry have included increased specific conductance and water hardness and higher concentrations of sulfate, phosphate, and many trace elements (Moran and Wentz, 1974; Clements and others, 2000). Where acid mine drainage occurs, receiving waters can also undergo acidification (Moran and Wentz, 1974; McKnight and Feder, 1984). The effects from mining typically attenuate at some point downstream from mining activities as a result of inflow of unaffected tributaries and (or) groundwater seepage, chemical precipitation, and particle settling (Moran and Wentz, 1974). Trace elements are commonly transported in streams and rivers in association with suspended organic and inorganic particulates (Moran and Wentz, 1974; Gibbs, 1977). In areas where flow velocities are sufficiently slow, suspended material can settle out (Moran and Wentz, 1974). Sediment samples collected from depositional areas containing large quantities of fine-grained materials commonly have high trace-element concentrations (Horowitz, 1991). In-stream dredging can cause physical alterations to stream channels such as bank instability, lowering of stream channels, increased erosion, and sedimentation (Collins and Dunne, 1990).

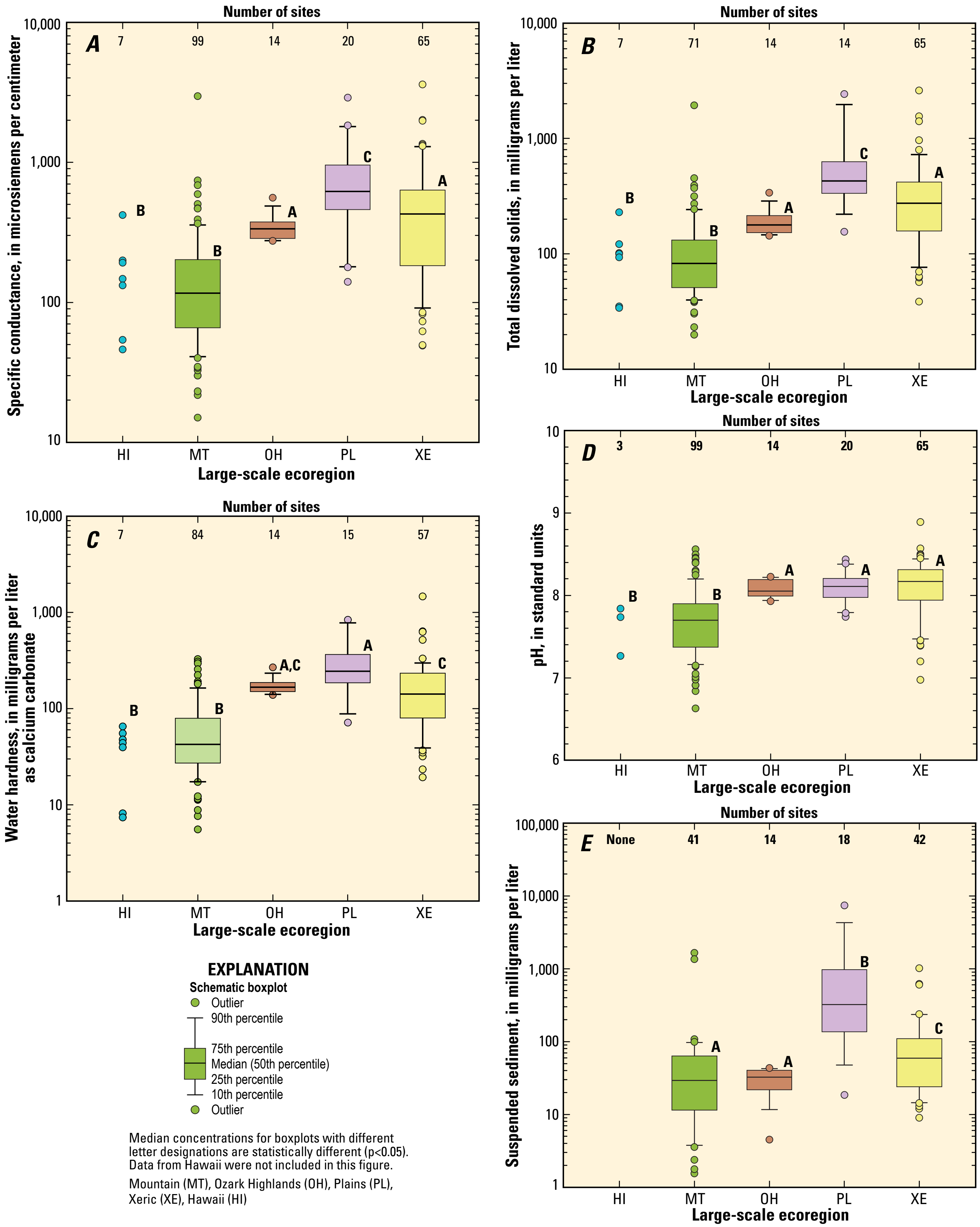


Figure 12. General water-quality characterization of *A*, specific conductance; *B*, total dissolved solids concentration; *C*, water hardness; *D*, pH; and *E*, suspended-sediment concentration by large-scale ecoregion in the conterminous western United States, Alaska, and Hawaii, 1992–2000.

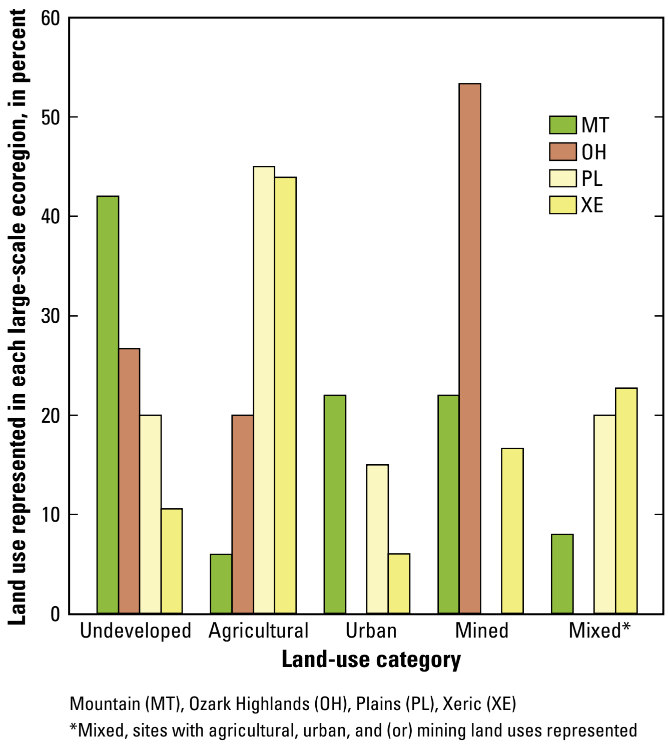


Figure 13. General land-use categories (undeveloped, agricultural, urban, mined, and mixed) represented within the large-scale ecoregions within the conterminous western United States and Alaska, 1992–2000.

Macroinvertebrate Community Response to Trace Elements

Cuffney and others (2005) found that climate, topography, geology, and chemistry all influence macroinvertebrate community structure, making it difficult to separate out the independent effects of land use on macroinvertebrate assemblages. These factors, which vary across the regional study area, could also make it difficult to evaluate independent effects of trace elements on macroinvertebrate communities investigated as part of this study. To help alleviate some of the abiotic variability among the different areas of the regional study (fig. 1), community structure was examined within individual ecoregions. To account for known differences in climatic, topographic, and in-stream characteristics, an intermediate-scale ecoregion was chosen as the regional scale at which the possible influences of trace elements on macroinvertebrate community patterns were evaluated (Whittier and others, 2007) (fig. 14). The intermediate-scale ecoregional designation allowed for maximizing trace-element gradients while minimizing variability in land uses within a given ecoregion (fig. 14).

Table 6. Range in cumulative toxic unit (CTU) values for each of the intermediate-scale ecoregions used to evaluate the influence of trace elements on macroinvertebrate community structure in selected streams in the conterminous western United States and Alaska, 1992–2000.

[**Abbreviations:** MT-NROCK, Mountain Northern Rockies ecoregion; MT-SROCK, Mountain Southern Rockies ecoregion; OH, Ozark Highlands ecoregion; XE-EPLAT, Eastern Xeric Plateau ecoregion]

Intermediate-Scale Ecoregion	Number of samples included in the intermediate-scale ecoregion	Range in CTU values for bed sediments in each intermediate-scale ecoreigon
MT-NROCK	32	1.1–126
MT-SROCK	15	0.89–50
OH	15	1.3–25
XE-EPLAT	17	1.0–7.5

The evaluation of CTUs described previously within this report showed that the CTU values calculated for bed sediment collected from streams in mined areas not only exhibited the widest range in values (0.89 to 126) but included the highest values (fig. 9). Four of the 12 intermediate-scale ecoregions are discussed in detail in this report because bed sediment collected from these areas varied in their ranges in CTU values and, for the most part, also represented the highest values. Samples were evaluated from the four intermediate-scale ecoregions, MT-SROCK, MT-NROCK, OH, and XE-EPLAT (table 6).

Given the complex interactions among physical, chemical, and biological characteristics in an ecosystem, MDS ordinations developed for the four intermediate-scale ecoregions were used in conjunction with richness and relative-abundance assessments. The error associated with the MDS ordinations, as evidenced by the stress levels, ranged from 0.11 to 0.2. These stress levels result from bringing the distribution patterns of the macroinvertebrate community from multidimensional space into two-dimensional space and indicate that the observed distributions are unlikely due to random chance. Multiple lines of evidence from the MDS plots and the richness and relative-abundance data were used to develop data interpretations. Associations between CTU values and macroinvertebrate community structure were evaluated using two-dimensional MDS analysis and are presented with average taxonomic characteristics described in bar charts with standard deviation error bars.

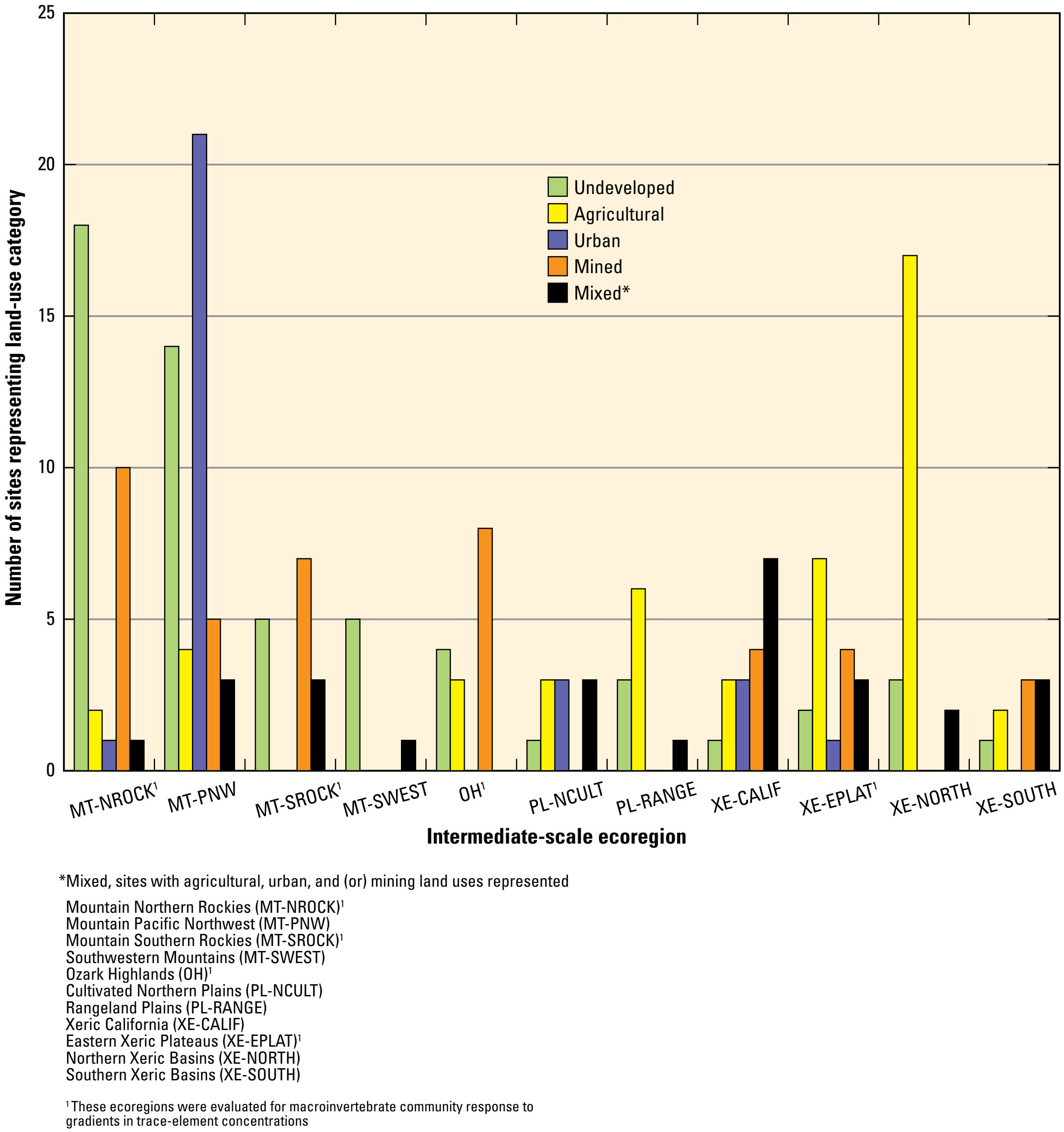
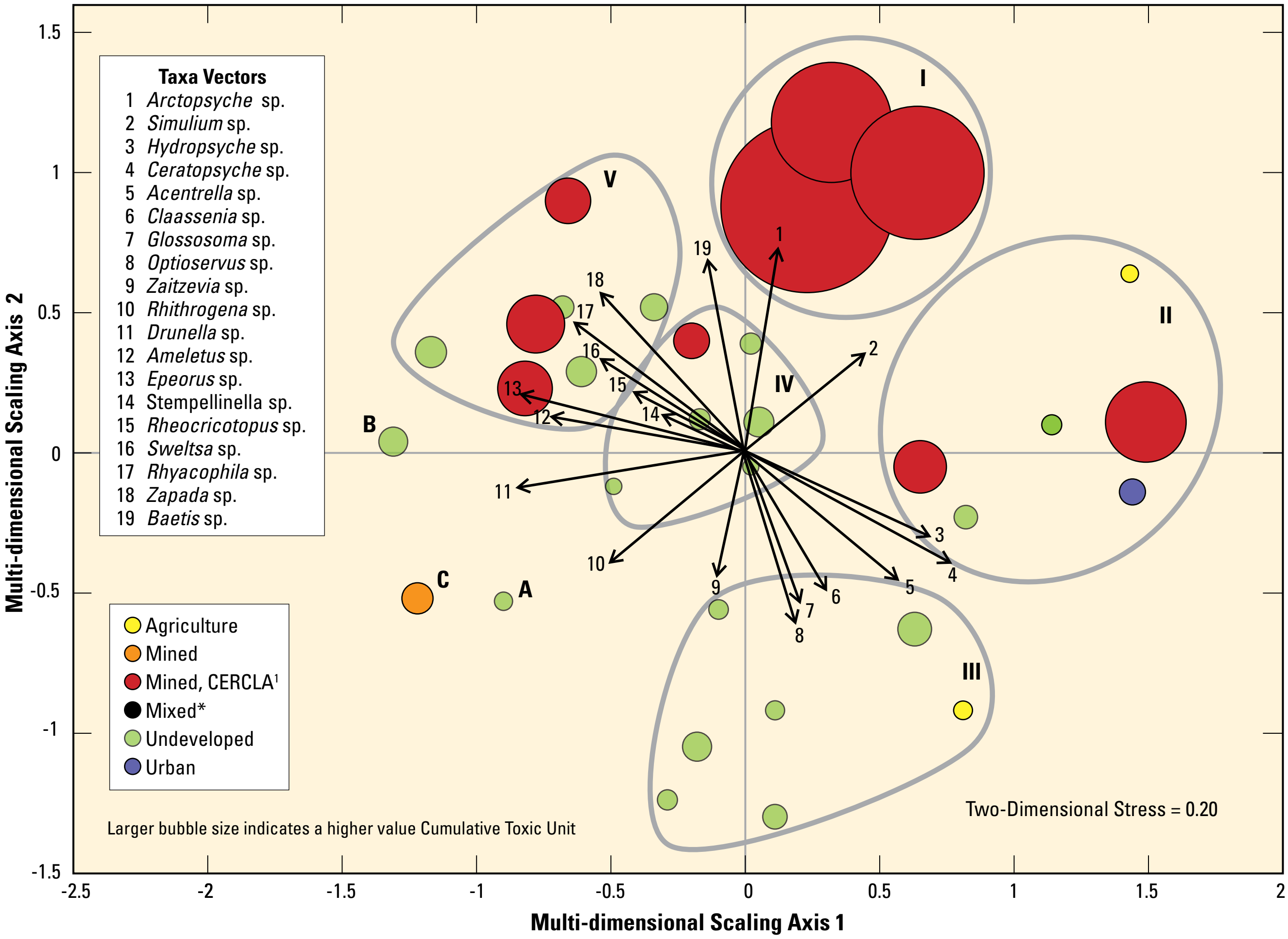


Figure 14. General land-use categories (undeveloped, agricultural, urban, mined, and mixed) represented in intermediate-scale ecoregions in the conterminous western United States and Alaska, 1992–2000.

Mountain Northern Rockies Ecoregion (MT-NROCK)

Samples collected from streams in the MT-NROCK ecoregion represented undeveloped (56 percent), agricultural (6 percent), urban (3 percent), mined (31 percent), and mixed (3 percent) land uses with CTU values ranging from 1.1 to 125.7, the greatest range in values of bed-sediment CTUs in this study (figs. 14 and 15). The highest CTU values were associated with bed-sediment samples collected from streams in mined areas. Using agglomerative hierarchical cluster analysis for data from the intermediate-scale ecoregion MT-NROCK, the MDS ordination shows five distinct clusters (I–V) and three unclustered Samples (A–C) (fig. 15). Generally, richness characteristics in all macroinvertebrate communities (clustered and unclustered) consisted predominantly of EPT and dipteran taxa (fig. 16*A*)

Cluster I consists of three macroinvertebrate communities inhabiting streams with very high CTU values for bed sediment (range 61.1–126). The median CTU value (74.9) is well above the 90th percentile (7.5) for the entire regional dataset (fig. 9). These CTU values indicate that these bed-sediment samples are highly enriched in trace elements (table 5). Pb and Zn, together, contributed from 74 to 83 percent to the CTU



[1] Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA)

*Mixed, sites with agricultural, urban, and (or) mining land uses represented

Figure 15. A non-metric multidimensional scaling (MDS) ordination of macroinvertebrate communities in selected streams in the Mountain Northern Rockies (MT-NROCK) ecoregion and distribution of cumulative toxic units (CTUs), 1992–2000.

values, and Cr and Ni, together, contributed about 1 percent. The three sites from which these samples were collected were on streams associated with a designated CERCLA area (listed as a result of historic mining-related activities) where mine tailings are known to have contaminated bed sediment with high concentrations of As, Cd, Cu, Pb, Hg, and Zn (Horowitz and others, 1993; Maret and others, 2003). In all three samples, concentrations of As, Cd, Cu, Pb, Hg, and Zn exceeded their respective PEC values; concentrations of Cr and Ni remained below their respective PEC values (table 2). Generally, macroinvertebrate communities included in Cluster I had the lowest overall richness (average 17 taxa) of all clusters within the MT-NROCK ecoregion (fig. 16*A*). Most of the relative abundance (percent contribution of taxa to total number of organisms) was composed of mayflies (41 percent), dipterans (31 percent), and caddisflies (26 percent) (fig. 16*B*). The macroinvertebrate communities included in Cluster I were largely characterized and dominated by one to two taxa—a black fly (*Simulium sp.*), caddisflies (*Arctopsyche sp.* or *Ceratopsyche* sp.), and (or) a mayfly (*Baetis sp.*). Ordination results from the MT-NROCK ecoregion support the findings of other studies that have shown elevated trace elements from mining activities influence macroinvertebrate community structure (Canfield and others, 1994; Clements, 1994; Maret and others, 2003) and that these taxa are likely tolerant to elevated concentrations of trace elements (Clements, 1994; Clements and others, 2000).

Six samples with bed-sediment CTU values ranging from 1.3 to 27.5 are included in Cluster II. The sites from which these samples were collected included streams in undeveloped (reference) areas and areas representing various land-use activities (fig. 15). The bed-sediment samples included in Cluster II have trace-element concentrations representing unenriched to highly enriched conditions (table 5, fig. 15). The median CTU value for bed-sediment samples in this cluster (2.6) is slightly higher than the 50th percentile (2.5) for the entire regional

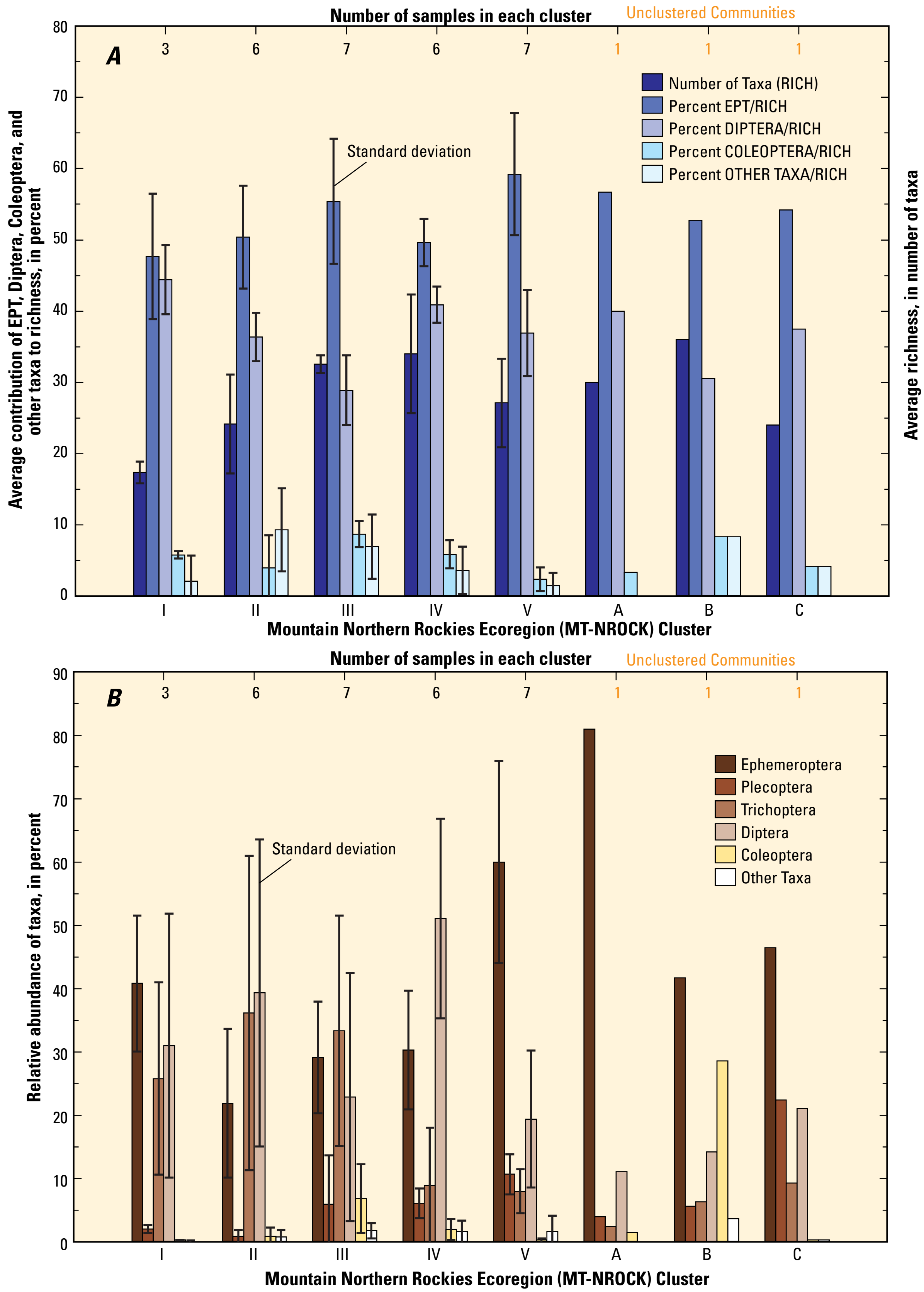


Figure 16. *A*, Average number of taxa (richness) and percent contribution from each of Ephemeroptera, Plecoptera, and Trichoptera (EPT), Diptera, Coleoptera, and other taxa to the overall richness; and *B*, average relative abundances of Ephemeroptera, Plecoptera, Trichoptera, Diptera, Coleoptera, and other taxa for macroinvertebrate communities in selected streams of the Northern Rocky Mountains ecoregion, 1992–2000.

dataset (fig. 9). Together, Pb and Zn contributed from 15 to 72 percent to the CTU values; Cr and Ni contributed about 4 to 63 percent for the six bed-sediment samples. These results indicate no clear pattern in regional trace-element composition of CTU values that is characteristic of a particular land use. Concentrations of As, Cd, Cu, Pb, and Hg exceeded their respective PEC values in at least one bed-sediment sample collected from the sites included in Cluster II; however, only occasionally did exceedences occur in the same sample. The PEC value for Zn (table 2) was exceeded in two bed-sediment samples. The stream included in Cluster II that was influenced by historic mining-related activities with the highest CTU value (27.5) was likely enriched in trace elements (Antimony, As, Cd, Cu, Pb, Hg, silver, and Zn) from mine tailings transported downstream from the CERCLA area in Idaho mentioned above in Cluster I (Horowitz and others, 1993; Maret and others, 2003). The second mined area in this cluster was associated with a different CERCLA site listed as a result of elevated As, Cd, Cu, Pb, and Zn resulting from historic mining and smelting operations in the watershed (U.S. Environmental Protection Agency, 2010). In both cases, bed sediment from these two rivers in mined areas is highly enriched with trace elements. It is unlikely that trace-element exposure is the sole cause of the clustering of these macroinvertebrate samples within Cluster II because the other CTU values (1.3 to 2.9) within the cluster indicate unenriched and moderately enriched conditions (table 5). Generally, the overall richnesses of Cluster II (24 taxa), Cluster V (27 taxa), and unclustered Sample C (24 taxa) were similar (fig. 16*A*). The most abundant taxa comprising the macroinvertebrate communities included in Cluster II were Diptera (39 percent), caddisflies (36 percent), and mayflies (22 percent) (fig. 16*B*). The macroinvertebrate communities included in Cluster II are primarily characterized by Diptera (*Simulium sp.*), Orthocladiinae midges (*Cardiocladius sp.*, *Eukiefferiella sp.*, and *Tvetenia sp.*), and caddisflies (*Hydropsyche sp.*, *Ceratopsyche sp.*, and *Cheumatopsyche sp.*). These taxa have been found to not only show tolerance to trace-element concentrations but have also exhibited tolerance to physical-habitat alterations such as sedimentation (Carlisle and others, 2007). On average, the mayfly communities in the macroinvertebrate samples in Cluster II were dominated by *Baetis* sp. (50 percent) (appendix 3, fig. 3-2).

Overall, Cluster III had bed sediment with the lowest overall range of CTU values (1.5–4.8) of all the clusters in the MT-NROCK ecoregion. The median CTU value (1.7) is equal to the 25^{th} percentile for the entire dataset (fig. 9). Trace-element enrichment in bed sediment ranged from unenriched to moderately-high at these sites (table 5). Together, Pb and Zn contributed from 8 to 52 percent to the CTU values; Cr and Ni contributed about 22 to 64 percent in these seven bed-sediment samples. The regional trace-element composition of CTU values indicated no clear pattern found to be characteristic of a particular land use, although bed-sediment samples were collected from streams predominantly in undeveloped (reference) areas (fig. 15). Concentrations of Pb, Cr, and Ni exceeded their respective PEC values in one bed-sediment sample collected from the sites included in Cluster III; in two samples, concentrations of As exceeded the PEC for this trace element (table 2). On average, the overall richness of macroinvertebrate communities represented in Cluster III (33 taxa) was similar to those in cluster IV (34 taxa) and in the unclustered Samples A (30 taxa) and B (36 taxa), indicating that overall richness is unlikely a defining characteristic for these communities (fig. 16*A*).

The relative abundance of taxa in macroinvertebrate samples included in Cluster III showed a fairly even distribution among mayflies (29 percent), caddisflies (33 percent), and dipterans (23 percent) (fig. 16*B*). Macroinvertebrate communities in Cluster III were largely characterized by three caddisfly (*Ceratopsyche sp., Glossosoma sp.,* and *Hydropsyche sp.*), two beetle (*Optioservus sp.* and *Zaitzevia sp.*), two mayfly (*Drunella sp.* and *Rhithrogena sp.*) and one stonefly (*Claassenia sp.*) taxa. The caddisfly, *Glossosoma sp.*, and the Heptageniidae mayflies, *Drunella sp.* and *Rhithrogena sp.*, are frequently reported as trace-element sensitive taxa (Clements and others, 2000; Carlisle and others, 2007). Beetle abundances have been reported as being reduced at locations with high trace-element concentrations (Clements and others, 2000).

Macroinvertebrate communities in Clusters IV and V have similar taxa composition, contributing to their proximity to each other in the MDS ordination (fig. 15). The sites included in Cluster IV are from predominantly undeveloped (reference) sites; however, one site is associated with a CERCLA area and is listed as impaired because of water temperature and concentrations of Cd, Pb, and Zn (U.S. Environmental Protection Agency, 2010; Idaho Division of Environmental Quality, 2008). The CTU values for Cluster IV ranged from 1.1 to 5.4; the median CTU value was 1.9. These results indicate that enrichment of trace-element concentrations in these bed-sediment samples range from unenriched to moderately-high (table 5). Pb and Zn contributed from 11 to 54 percent to the CTU values; Cr and Ni contributed about 17 to 62 percent for the six bed-sediment samples. The regional trace-element composition of CTU values indicated no clear pattern found to be characteristic of a particular land use. Concentrations of trace elements in bed-sediment samples associated with communities represented in Cluster IV seldom exceeded their respective PEC values. Concentrations of As, Ni, and Zn exceeded their respective PEC values (table 2) in one sample each.

Generally, Cluster IV had a similar overall taxa richness (34 percent) to Cluster III (33 percent) and the unclustered Samples A and B (fig. 16*A*), suggesting that, on average, overall richness is not necessarily a defining characteristic for these communities. The macroinvertebrate communities included in Cluster IV were primarily composed of dipterans (51 percent) and mayflies (30 percent) (fig. 16*B*). Taxa characterizing the macroinvertebrate communities in Cluster IV were somewhat similar to Cluster V; however, the differences in the relative abundances between mayflies and dipterans appear to be the features distinguishing these two clusters. In general, Cluster IV had lower relative abundances of *Baetis* sp., *Drunella* sp., *Epeorus* sp. mayflies, lower relative abundances of *Zapada* sp. stoneflies, and higher relative abundances of *Simulium* sp. flies than did Cluster V.

Cluster V included macroinvertebrate communities collected from seven sites associated with the CERCLA area mentioned above, in Clusters I and II, representing undeveloped (reference) and mined landscapes (fig. 15). The CTU values (range 2.2–14.1) were not as high as those in Cluster I, but the median CTU value for Cluster V (4.1) was above the 75th percentile (3.9) for the entire regional dataset. Regionally, the range in CTU values indicates low to high trace-element enrichment at these sites (table 5, fig. 9). Together, Pb and Zn contributed from 35 to 75 percent to the CTU values; Cr and Ni contributed about 5 to 30 percent for the seven bed-sediment samples. These results show a distinctive pattern in the contribution of these trace elements to the CTU values representative of a regional mining signature (fig. 10*D*). Five of the seven bed-sediment samples in Cluster V had Pb concentrations exceeding the PEC for this trace element. The PECs for As and Zn were exceeded in 14 and 43 percent, respectively, of the bed-sediment samples collected from these seven sites. The PECs for the other trace elements (Cd, Cr, Cu, Hg, and Ni) (table 2) were not exceeded in any of the bed-sediment samples associated with Cluster V. On average, Cluster V had a similar overall richness (27 percent) to Cluster II (24 percent) and the unclustered Sample C (fig. 16*A*). The most abundant macroinvertebrates in Cluster V were mayflies (60 percent) and dipterans (19 percent) (fig. 16*B*). Macroinvertebrate communities grouped in Cluster V were characterized by several taxa of mayflies (*Baetis sp.*, *Epeorus sp.*, and *Ameletus sp.*), two midges (*Stempellinella sp.* and *Rheocricotopus sp.*), a caddisfly (*Rhyacophila sp.*), and two stoneflies (*Sweltsa sp. and Zapada sp.*) (fig. 15). Heptageniidae mayflies (e.g., *Epeorus sp.*) are generally regarded as very sensitive to elevated trace-element concentrations (Mize and Deacon, 2002; Maret and others, 2003; Clark and Clements, 2006); however, this family of mayflies comprised more than 10 percent of the relative abundance of the three macroinvertebrate communities collected from streams associated with the CERCLA area (CTU values ranging from 4.1 to 14.1). Stoneflies were an important component of these macroinvertebrate communities; the relative abundance of these taxa (about 11 percent) was higher than the other Clusters I–IV and the unclustered Samples A and B. These results suggest that these taxa are at least moderately tolerant to the trace elements present in the bed sediment. Previous studies by Clements and others (2000) and Pollard and Yuan (2006) found several of these taxa at sites with CCUs in the moderate range of 2 to 10.

Three sites did not significantly group during the cluster analysis (A, B, and C; fig. 15); unclustered Samples A and B represented undeveloped (reference) sites with CTU values of 1.4 and 3.5 indicative of unenriched and moderately enriched conditions, respectively (table 5, fig. 9). Sample C represented a site influenced by historic mining practices with a CTU value of 4.1, exceeding the 75th percentile (3.9) for the entire regional dataset. The contribution of Pb and Zn, together, to the bed-sediment sample collected from unclustered Sample C was only 8 percent; Cr and Ni contributed 82 percent to this CTU value. This pattern in CTU composition deviates from the general pattern for trace-element contributions to CTU values for bed-sediment samples collected from streams in mined areas (fig. 10*D*). Together, Cr and Ni contributed more (58 and 82 percent) to the CTU composition in reference Samples B and C than did Pb and Zn (18 and 8 percent), characteristic of undeveloped landscapes (fig. 10*A*).

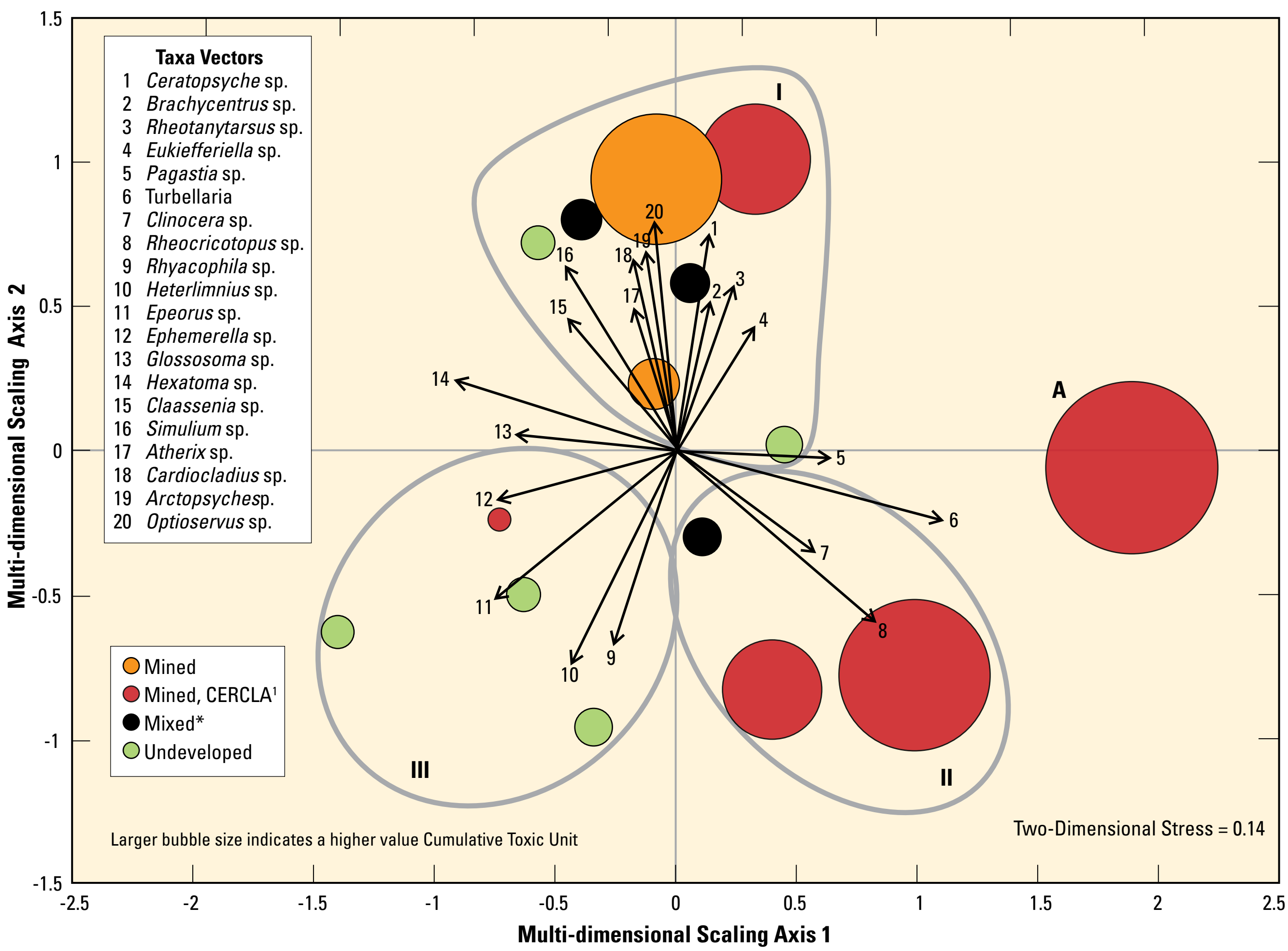
A distinguishing feature of the macroinvertebrate community represented by unclustered Sample C is the relative abundance of stoneflies. Three taxa (*Zapada* sp., *Megarcys* sp., and those belonging to the family Chloroperlidae) accounted for 92 percent of the stoneflies present in the sample (fig. 16*B*). The CTU value of 3.5 associated with the unclustered Sample B, representing an undeveloped (reference) condition, approached the 75th percentile for the entire dataset, suggesting that this bed sediment is naturally enriched in trace elements. The communities in unclustered Samples A and B represent reference conditions and show similar overall richnesses (30 and 36 taxa, respectively) to those of Clusters III and IV (33 and 34 taxa, respectively). However, the macroinvertebrate community for unclustered Sample A is markedly different from Sample B and Clusters III and IV in that about 80 percent of the community is composed of mayflies (fig. 16*B*). The mayfly community in unclustered Sample A is primarily composed of the Heptageniidae mayflies, *Epeorus* sp. (36 percent) and *Rhithrogena* sp. (17 percent), and the Ameletidae mayfly, *Ameletus* sp. (20 percent). The macroinvertebrate community of unclustered Sample B had about half the number of mayflies as Sample A. One of the distinguishing characteristics of unclustered Sample B was the number of Coleoptera (beetle) individuals. Beetles from the family Elmidae (riffle beetles) comprised almost 30 percent of the relative abundance in Sample B, the highest of all samples collected from the MT-NROCK ecoregion (fig. 16*B*).

The structure of macroinvertebrate communities clustering within the MT-NROCK ecoregion appears to be associated with trace-element concentration and land-use activities. Generally, macroinvertebrate samples collected from streams where historic mining-related activities greatly enriched bed sediment with trace elements (Cluster I) showed communities dominated by a few resilient taxa resulting in a lower richness metric. Trace-element enrichment in bed-sediment samples associated with macroinvertebrate communities grouped together in Cluster II is not strongly evident. The diverse nature of land uses in this group suggests that something other than exposure to trace elements is contributing to the macroinvertebrate community structure in these samples. Cluster II communities exhibit a slight depression in taxa richness relative to the other clusters, except Cluster I, and are dominated largely by tolerant mayfly, caddisfly, and dipteran taxa. The richness and relative abundance metrics determined for Clusters IV and V are interesting in that generally more trace-element sensitive taxa (e.g., Heptageniidae mayflies) were in macroinvertebrate communities included in Cluster V where bed sediment had CTU values exceeding the 75th percentile for the entire regional study area, indicating moderately-high enrichment at these sites. It is unlikely that trace-element exposure is driving the macroinvertebrate community structure at these sites. Macroinvertebrate communities included in Cluster III, with the lowest bed-sediment CTUs representing predominantly undeveloped (reference) conditions, are distinguished by a relatively diverse community of taxa including tolerant and sensitive mayflies and caddisflies.

Mountain Southern Rockies Ecoregion (MT-SROCK)

Samples collected from streams in the MT-SROCK ecoregion represented undeveloped (33 percent), mined (47 percent), and mixed (20 percent) landscapes. Within this ecoregion, CTU values ranged from 0.89 to 50.3 units; the highest CTU values were associated with bed sediment collected from streams in mined areas (fig. 17). Using hierarchical agglomerative clustering, three significant Clusters (I-III) of macroinvertebrate communities emerged in the MT-SROCK MDS ordination, each containing from three to seven samples (fig. 17).

The highest CTU value (50.3) was at a site (Sample A) that did not cluster with others and had a macroinvertebrate community characteristically different from the rest in the MT-SROCK ecoregion (fig. 17). This site is on a stream associated with a CERLA area listed as a result of historic mining-related activities. The contamination of bed sediment with As, Cd, Pb, and Zn as a result of these mining-related activities resulted in the listing of this area on the National Priorities List in 2008 (U.S. Environmental Protection Agency, 2010). The contribution of Pb and Zn to the bed-sediment sample associated with this unclustered sample was 78 percent; Cr and Ni contributed 0.3 percent to this CTU value. These results show a distinctive



[1] Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA)

*Mixed, sites with agricultural, urban, and (or) mining land uses represented

Figure 17. A non-metric multidimensional scaling (MDS) ordination of macroinvertebrate communities in selected streams in the Mountain Southern Rockies (MT-SROCK) ecoregion and distribution of cumulative toxic units (CTUs), 1992–2000.

pattern in the contribution of these trace elements to the CTU values representative of a regional mining signature (fig. 10*D*). Cr, Cu, Hg, and Ni did not exceed their respective PEC values in this bed-sediment sample (table 2). The overall taxa richness (16 taxa) of Sample A was the lowest in the MT-SROCK ecoregion (fig. 18*A*). Mayflies (42 percent) and dipterans (42 percent) accounted for about 84 percent of the individuals in this sample (fig. 18*B*). The mayfly community in this sample was dominated by the family Ephemerellidae (99 percent). Eighty percent of the dipteran individuals were midges, mainly in the family Orthocladiinae (88 percent) (appendix 3, fig. 3-4). Orthocladiinae midges have been identified as being tolerant to trace elements and other physical-habitat disturbances; however, mayflies belonging to the family Ephemerellidae have exhibited varied responses to elevated trace-element exposure. Clements (1994) showed that Ephemerellidae mayflies were sensitive to aqueous Zn concentrations, and Carlisle and others (2007) associated low (less tolerant) indicator values to this family of mayflies with respect to chemical and physical stressors. Results from this study are more similar to the findings from Beasley and Kneale (2003), which identified Ephemerellidae mayflies as tolerant to trace-element exposure.

CTU values for bed-sediment samples from the seven sites included in Cluster I ranged from 1.92 to 28.85 (fig. 17). These sites are predominantly influenced by historic mining-related activities and mixed (urban and agriculture) land uses. The median CTU value (2.8) is only slightly higher than the 50th percentile (2.52) for the entire dataset (fig. 9). The CTU values indicate that bed-sediment samples collected from the sites included in Cluster I were minimally enriched in trace elements, and others were highly enriched (table 5, fig. 9). The contribution of Pb and Zn to the bed-sediment samples ranged from 21 to 69 percent; Cr and Ni contributed about 3 to 59 percent to these values. The regional trace-element composition of CTU values indicated no clear pattern found to be characteristic of a particular land use. Concentrations of As, Cd, Cu, Pb, Hg, and Zn exceeded their respective PEC values (table 2) in one to three samples collected from these seven sites but not necessarily in the same samples; there were no PEC exceedences for either Cr or Ni in these samples.

The overall richness of macroinvertebrate communities represented in Cluster I (36 taxa) was the highest in the MT-SROCK ecoregion (fig. 18*A*). On average, Diptera (49 percent), mayflies (24 percent), and caddisflies (15 percent) accounted for most of the relative abundance (fig. 18*B*). Most of the taxa in Cluster I are known to be tolerant to elevated trace elements and other physical disturbances (Beltman and others, 1999; Mize and Deacon, 2002; Clements, 2004; Carlisle and others, 2007). The EPT taxa were dominated by a resilient mayfly, *Baetis* sp., and caddisflies belonging to the family Hydropsychidae (*Arctopsyche* sp. and *Ceratopsyche* sp.) (appendix 3, fig. 3-4); however, *Baetis* sp. was not found to be characteristic of the macroinvertebrate communities within the MT-SROCK ecoregion. It is possible there were not enough differences among the *Baetis* sp. contributions among the clusters to elucidate a significant difference among them, thereby resulting in the lack of correlation with this taxon. Other characteristic taxa included two Diptera (*Atherix* sp. and *Simulium* sp.), two Orthocladiinae midges (*Cardiocladius* sp. and *Eukiefferiella* sp.), and the caddisfly, *Brachycentrus* sp. The stonefly, *Claassenia* sp., accounts for 3.7 percent of the relative abundance at the only undeveloped site in Cluster I and 0.2 percent or less at the other two sites. *Rheotanytarsus* sp., a member of the Tanytarsini midges, was characteristic of communities in Cluster I; however, Swansburg and others (2002) indicated that the Tanytarsini midges are sensitive to trace-element exposure. Alternatively, Canfield and others (1994) found that Tanytarsini midges were somewhat tolerant to trace-element exposure in bed sediment. Carlisle and others (2007) listed high indicator values (tolerance) for *Rheotanytarsus* sp. to concentrations of suspended sediment and nutrients. The results found in this study are similar to those of Canfield and others (1994) in that it appears *Rheotanytarsus* sp. displays some degree of resilience to trace-element exposure in bed sediment.

Cluster II consists of three sites; two sites are in a watershed associated with a CERCLA site (listed as a result of past dredge-mining practices) with Cd and Zn enrichment in bed sediment as well as impaired habitat conditions (U.S. Environmental Protection Agency, 2010); the other site is predominantly undeveloped but is influenced by a combination of urban and mining-related activities. CTU values in Cluster II range from 2.4 to 38.8 (median 16.7). Compared to regional values, these bed-sediment samples range from low enrichment to high enrichment conditions (table 5, fig. 9). The contribution of Pb and Zn to the bed-sediment samples collected from streams in mined areas included in Cluster II was substantially different that those collected from streams in areas with mixed landscapes. Pb and Zn contributed 69 and 80 percent to the CTU values in bed sediment in the mined areas but only contributed 27 percent to the CTU value in bed sediment collected from the area of mixed land use. Cr and Ni contributed less than 10 percent (3 and 8 percent) to the CTU values for bed sediment collected from the streams in mined areas; these trace elements contributed 53 percent of the CTU value in samples collected from the mixed land-use site. Concentrations of As, Cd, Pb, and Zn exceeded their respective PEC values (table 2) in bed-sediment samples from the mined area. No trace-element concentrations from the mixed land-use site exceeded their respective PEC values.

On average, the overall richness of macroinvertebrate communities in Cluster II (25 taxa) was the same as that for Cluster III (25 taxa); however, Cluster II had less EPT taxa relative to dipteran taxa than did Cluster III (fig. 18*A*). Although variable, on average, 63 percent of the organisms in Cluster II were flies (Diptera). The dipteran community was composed of about 95 percent midges of which taxa belonging to the family Orthocladiinae dominated (77 percent) (appendix 3, fig. 3-4). Flatworms (Turbellaria), midges (*Rheocricotopus* sp.), and diptera belonging to the genus *Clinocera* sp. distinguished the macroinvertebrate communities in Cluster II

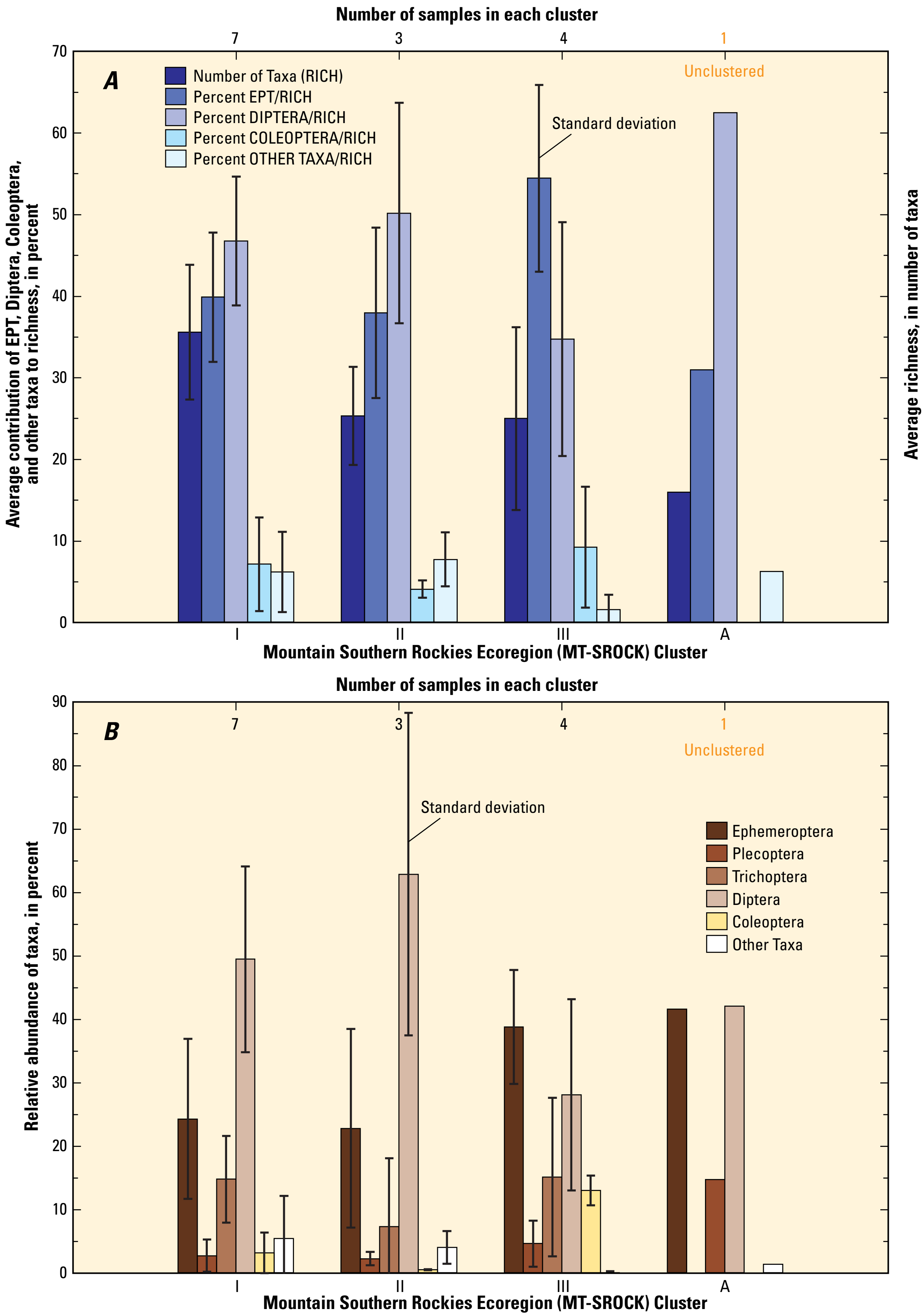


Figure 18. *A*, Average number of taxa and percent contribution from each of Ephemeroptera, Plecoptera, and Trichoptera (EPT), Diptera, Coleoptera, and other taxa to the overall richness; and *B*, average relative abundances of Ephemeroptera, Plecoptera, Trichoptera, Diptera, Coleoptera, and other taxa for macroinvertebrate communities in selected streams of the Mountain Southern Rockies ecoregion, 1992–2000.

(fig. 17). Turbellaria worms and Orthoclaniinae midges were the most abundant taxa in Cluster II. Studies have found these taxa tolerate a wide range of physical and chemical stressors (Carlisle and others, 2007). The habitat impacts from dredge-related mining activities in combination with higher CTUs may be influencing the community structure in Cluster II and may be playing a role in the separation of these communities from the other clusters. These results are consistent with other investigations that have documented the loss of richness corresponding with an increase in the abundance of fewer, but tolerant, taxa at sites affected by trace-element pollution (Canfield and others, 1994; Clements and others, 2000; Maret and others, 2003).

Overall, Cluster III had the lowest CTU values (0.89 to 2.4; median 1.9), indicating unenriched to low enrichment conditions, of the three clusters identified by the MDS ordination in the MT-SROCK ecoregion (table 5, fig. 9). Of the four sites included in this cluster, three were in undeveloped (reference) areas and one was associated with a CERCLA site listed as a result of As, Cd, Pb, and Zn contamination (U.S. Environmental Protection Agency, 2010). Given the relatively low CTU value for bed sediment collected from the site associated with the CERCLA area, it is apparent that mine tailings near the site have not contaminated the bed sediment at this site to the extent that they have contaminated bed sediment collected from another site associated with the same CERCLA area (Sample A, fig. 17). The contribution of Pb and Zn to the four bed-sediment samples included in Cluster III ranged from 22 to 50 percent; Cr and Ni accounted for about the same amount, 23 to 57 percent. Trace-element concentrations did not exceed any PEC value (table 2) in any of the four samples included in Cluster III.

The overall richness of macroinvertebrate communities represented in Cluster III (25 taxa) was the same as that for Cluster II (25 taxa); however, Cluster III richness was comprised of a greater proportion of EPT taxa (fig. 18*A*). The most abundant taxa in the macroinvertebrate samples included in Cluster III were mayflies (39 percent), dipterans (28 percent), and caddisflies (16 percent) (fig. 18*B*). Cluster III also contained a substantial number of Elmidae beetles (dominated by *Heterlimnius* sp.), which contributed on average 13 percent (range 7.5 to 14.7 percent) to the overall relative abundance of these communities. Mayflies (*Epeorus sp.* and *Ephemerella sp.*), a caddisfly (*Rhyacophila sp.*), and beetles were the distinguishing taxa for these sites (fig. 17).

Macroinvertebrate communities in the MT-SROCK ecoregion significantly grouped into three clusters and appear to be influenced by trace-element concentrations and, in some cases, possibly physical-habitat disturbances. The lowest taxa richness was observed for the sample which had the highest CTU value (50.3; unclustered Sample A). The macroinvertebrate community sample collected from this site was dominated by Ephemerellidae mayflies and midges belonging to the family Orthocladiinae. It appears that mayflies within the Ephemerellidae family may be tolerant to trace-element exposure in this stream; however, information in the literature regarding the overall resiliency of this family to chemical stress appears to be conflicting. Cluster I includes macroinvertebrate samples collected from a variety of landscapes including undeveloped (reference), mined, and mixed. On average, individuals belonging to the order Diptera constitute 50 percent and mayflies constitute 24 percent of the relative abundance of organisms in macroinvertebrate communities in Cluster I. Midges are the dominant taxa within the Diptera community in this cluster; about 45 percent of these midges belong to the family Orthocladiinae. Of the EPT taxa in Cluster I, relative abundance was generally dominated by a resilient mayfly, *Baetis* sp., and caddisflies belonging to the family Hydropsychidae (*Arctopsychide* sp. and *Ceratopsyche* sp.). Clusters II and III had similar richness metrics; however, Cluster II had more dipteran taxa (Orthocladiinae midges), and Cluster III had a greater number of EPT taxa and Elmidae beetles.

Ozark Highlands Ecoregion (OH)

Sediment samples from streams in the OH ecoregion were representative of undeveloped (27 percent), agricultural (20 percent), and mined (53 percent) land uses and had CTU values ranging from 1.3 to 24.9. The highest CTU values were from bed sediment collected from streams in mined areas. Using hierarchical agglomerative clustering, three significant clusters (I-III) of macroinvertebrate communities emerged in the OH MDS ordination, each containing from two to six communities (fig. 19). Four macroinvertebrate samples collected from streams in two undeveloped and two mined areas did not significantly group with any of the three clusters.

Cluster I is the most distinct cluster of the OH ecoregion MDS ordination. CTU values for the three bed-sediment samples in this cluster ranged from 1.3 to 1.5 (median 1.4) and were collected from streams in agricultural landscapes. All CTU values lie below the 25th percentile (1.7) for the entire regional dataset (figs. 9 and 19). These results indicate these bed-sediment samples are not enriched in trace elements compared to other sites in this study. The contribution of Pb and Zn to the CTU values for these three samples ranged from 17 to 23 percent; Cr and Ni accounted for 55 to 60 percent. The relative contributions of Cr, Pb, Ni, and Zn were consistent with bed sediment collected from other streams in agricultural areas in this study (fig. 10*B*). Concentrations of trace elements did not exceed PEC values in any of these samples (table 2).

The overall richness of macroinvertebrate communities in Cluster I (36 taxa) was similar to that observed for Cluster II and unclustered Sample B; EPT taxa contributed the most to richness (fig. 20*A*). On average, mayflies (56 percent) and Diptera (16 percent) accounted for most of the relative abundance of individuals collected from macroinvertebrate communities included in Cluster I (fig. 20*B*). Cluster I is characterized by two taxa of midges (*Polypedilum* sp. and *Stempellinella* sp.), two taxa of mayflies (*Tricorythodes* sp. and *Stenacron* sp.), a dragonfly family (Gomphidae), and a caddisfly (*Chimarra* sp.). *Tricorythodes* sp. appears to be relatively common in macroinvertebrate samples collected throughout the

OH ecoregion and was found at about 87 percent of the sites (appendix 2, table 2-1). *Tricorythodes* sp. was a distinguishing taxon for Cluster I and Cluster III (where it was common in a sample from a mined area). Other mayfly taxa that were found at higher relative abundances in the agricultural areas were *Caenis* sp. (0 to 24.1 percent), *Leucrocuta* sp. (0 to 11.1 percent), and *Stenonema* sp. (14.5 percent). Heptageniidae mayflies contributed, on average, 15.7 percent to the overall abundance of individuals collected from macroinvertebrate samples included in Cluster I. Many of the taxa in Cluster I are considered tolerant of fine sediment, low dissolved oxygen, and relatively high temperature conditions (Carlisle and others, 2007). *Tricorythodes* sp. and *Stenacron* sp. have been previously reported as tolerant of the effects of agricultural land-use activities on stream habitat and water chemistry (Griffith and others, 2001; Dance and Hynes, 1980).

Clusters II and III are close to one another in the MDS ordination (fig. 19). Cluster II consists of six macroinvertebrate samples from streams in either undeveloped (reference) or mined landscapes. The CTU values for bed-sediment samples in Cluster II ranged from 1.7 to 3.6 (median 2.6). This range in CTU values represents trace-element concentrations in bed sediment that are unenriched to moderately enriched (table 5, fig. 9). The contribution of Pb and Zn to the CTU values for these four samples ranged from 18 to 54 percent; Cr and Ni accounted for 28 to 56 percent. This pattern in Cr, Pb, Ni, and Zn contributions to CTU values does not follow any particular pattern associated with a given land use (figs. 10*A*–10*E*). Concentrations of Pb and Ni exceeded their PEC values in one bed-sediment sample each (in different samples); concentrations of As, Cd, Cr, Cu, Hg, and Zn did not exceed their respective PEC values (table 2) in any of the bed-sediment samples associated with Cluster II sites.

The overall taxa richness (37 taxa) of the macroinvertebrate community in Cluster II was similar to Cluster I (fig. 20*A*). Mayflies (54 percent) and caddisflies (17 percent) accounted for about 71 percent of the individuals in these samples (fig. 20*B*). Macroinvertebrate communities included in Cluster II were characterized by the mayfly, *Plauditus* sp.; the caddisfly, *Ceratopsyche* sp.; and the riffle beetle, *Optioservus* sp. The close proximity of Clusters II and III and the presence of unclustered samples made it somewhat difficult to identify distinguishing taxa. On average, Cluster II communities had

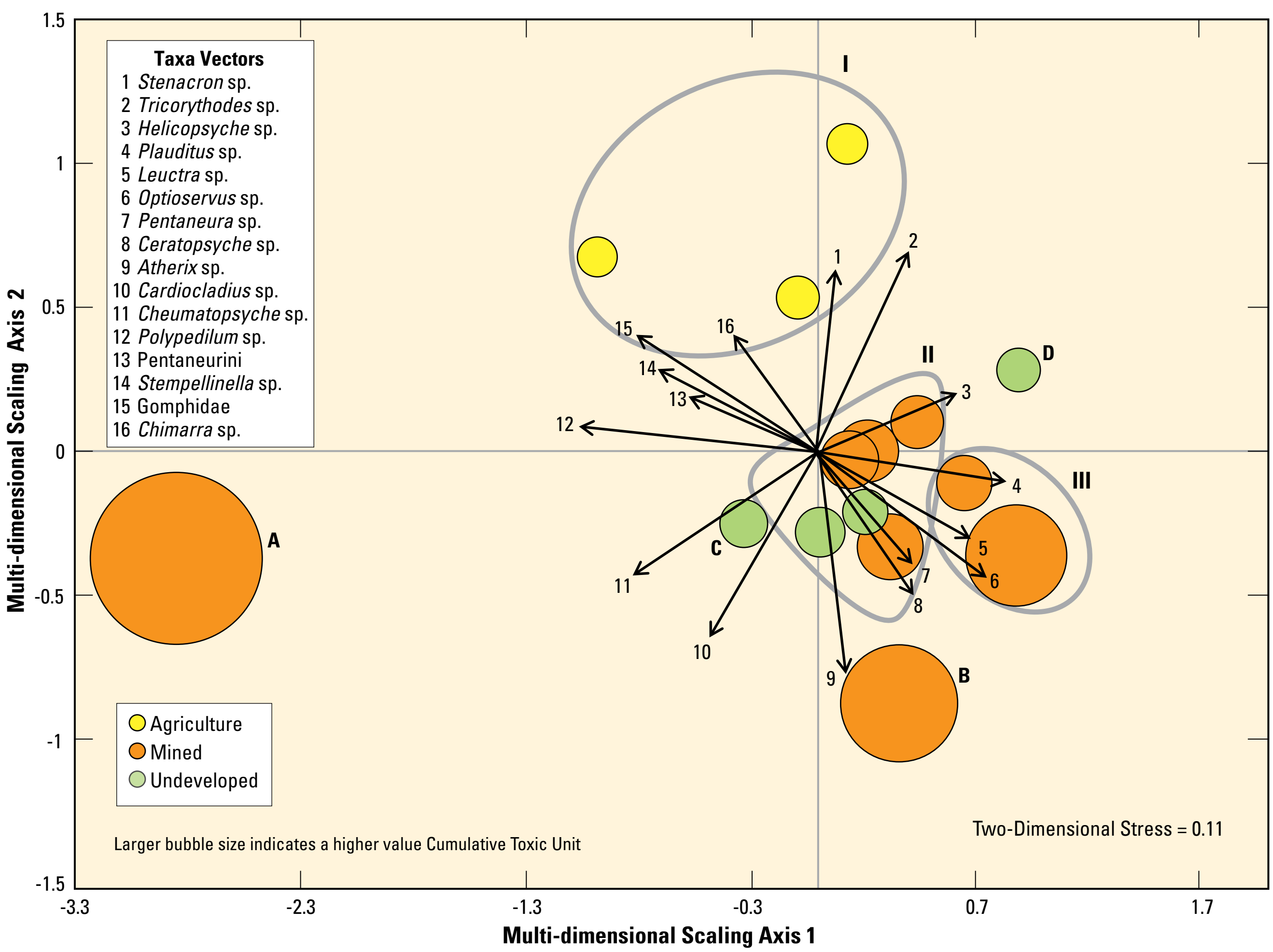


Figure 19. A non-metric multidimensional scaling (MDS) ordination of macroinvertebrate communities in selected streams in the Ozark Highlands (OH) ecoregion and distribution of cumulative toxic units (CTUs), 1992–2000.

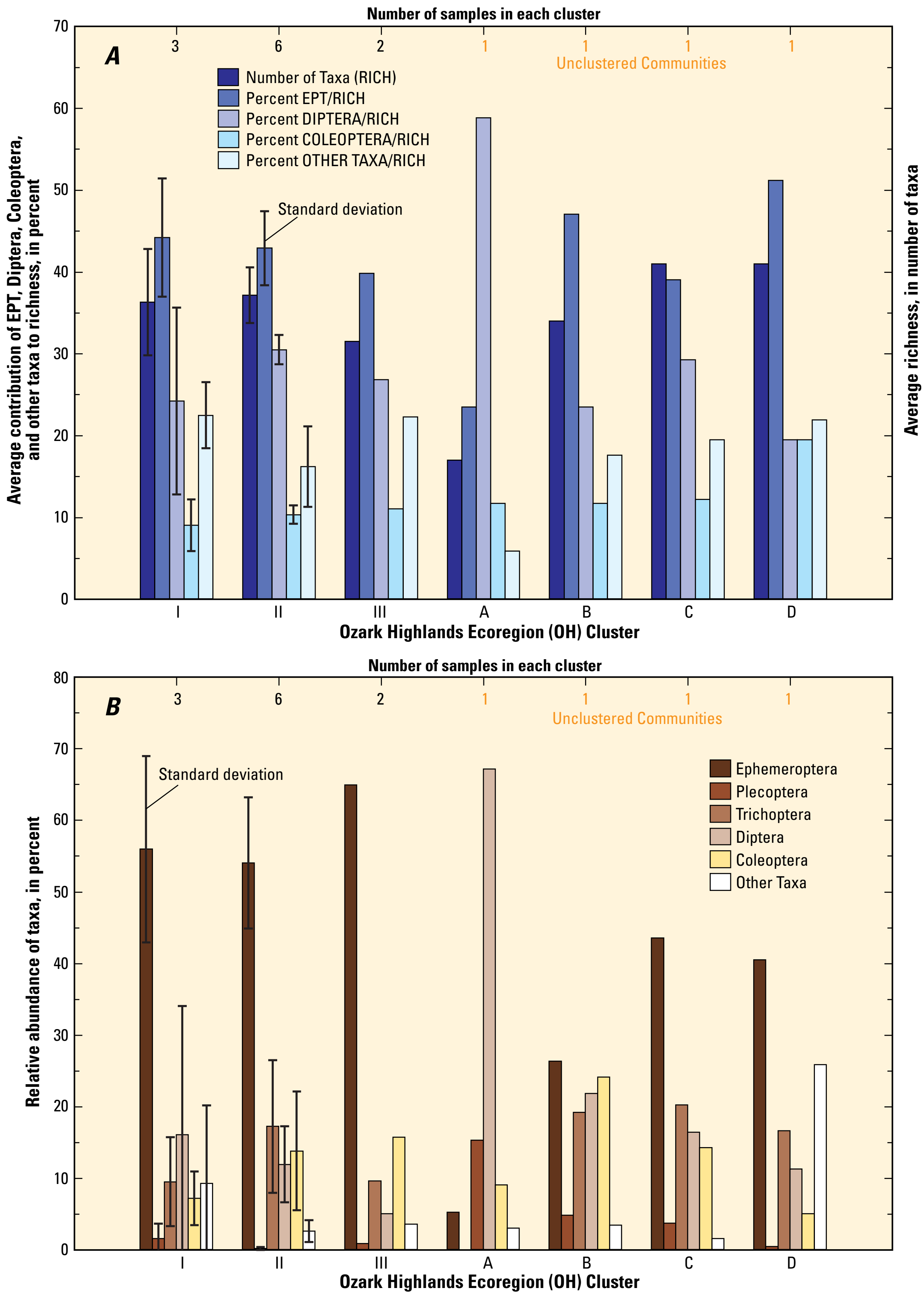


Figure 20. *A*, Average number of taxa (richness) and percent contribution from each of Ephemeroptera, Plecoptera, and Trichoptera (EPT), Diptera, Coleoptera, and other taxa to the overall richness (RICH); and *B*, average relative abundances of Ephemeroptera, Plecoptera, Trichoptera, Diptera, Coleoptera, and other taxa for macroinvertebrate communities in selected streams of the Ozark Highlands (OH) ecoregion, 1992–2000.

higher relative abundances of Heptageniidae mayflies (18 percent) than did Cluster III (6.5 percent).

Cluster III consists of only two sites, both influenced by historic mining-related activities (fig. 19). The CTUs for bed-sediment samples grouped in Cluster III were 2.6 and 8.6 (average 5.6) and ranked from near the 50th percentile to exceeding the 90th percentile for the entire regional study area (fig. 9), indicating the bed-sediment samples ranged from moderately enriched to highly enriched in trace elements (table 5, fig. 9). The average contribution of Pb and Zn to the CTU values for these bed-sediment samples was 44 percent; Cr and Ni averaged 40 percent. Although both sites were influenced by mining-related activities, the pattern in the combined contribution of Pb and Zn relative to the combined contribution of Cr and Ni to the CTU values was not characteristic of the regional mining signature (fig. 10*D*). Concentrations of Pb, Ni, and Zn exceeded their respective PEC values in one bed-sediment sample (the same bed-sediment sample); As, Cd, Cr, Cu, and Hg did not exceed their PEC values (table 2) in either bed-sediment sample in this cluster.

The overall taxa richness (32 taxa) of the macroinvertebrate communities associated with Cluster III was a little lower than that for either Cluster I or II (fig. 20*A*). Mayflies (65 percent) and caddisflies (9.6 percent) accounted for about 75 percent of the individuals in these samples (fig. 20*B*). Both macroinvertebrate samples in Cluster III are dominated by mayflies—55.5 and 74.5 percent, respectively. The mayfly community for the sample with the higher CTU value (8.6) was dominated by *Tricorythodes* sp. (62 percent); the other was dominated by *Isonychia* sp. (26 percent) and was generally more diverse.

Four of the macroinvertebrate samples did not significantly cluster with any of the samples during the cluster analysis (A-D, fig. 19). Two of these samples were collected from streams in mined areas and two from streams with undeveloped (reference) conditions. Bed sediment collected from the two streams in mined areas, Samples A and B, had high CTU values (24.9 and 11.5). CTU values for bed-sediment samples collected from the two reference sites were low (1.6 and 1.9). Both CTU values for the bed-sediment samples collected from the streams in mined areas exceeded the 90th percentile (7.5) for the entire regional dataset (fig. 9), indicating high trace-element enrichment was present at both sites. Unclustered Sample A had the highest CTU value (24.9) for the OH ecoregion and was dominated by Pb and Zn (61 percent); Cr and Ni contributed 4 percent to this CTU. These results show a distinctive pattern in the contribution of these trace elements to the CTU values representative of a regional mining signature (fig. 10*D*). Concentrations of Cd, Pb, and Zn exceeded their respective PEC values (table 2) in this sample. This site (unclustered A) is listed on the 2008 303-d list for impaired water bodies, in part, because of elevated concentrations of Cd, Pb, and Zn in bed sediment resulting from abandoned mine tailings within the watershed (Missouri Department of Natural Resources, 2010). This site had the lowest overall richness (17 taxa) for samples collected from the OH ecoregion. Diptera dominated the taxa richness (59 percent) and relative abundance (67 percent) for this sample (figs. 20*A*–20*B*). The relative abundance of Diptera individuals is the distinguishing characteristic of this macroinvertebrate community (fig. 20*B*). Just about half the relative abundance (55 percent) was comprised of midges belonging to the family Orthocladiinae (*Cardiocladius* sp. and *Chaetocladius* sp.) and tribe Tanytarsini (*Cladotanytarsus* sp.). *Cardiocladius* sp. was more abundant in unclustered Sample A than at any of the other sites in the OH ecoregion.

The CTU value for bed sediment associated with unclustered Sample B (11.5) was primarily composed of Pb and Zn (73 percent), and Cr and Ni contributed 16 percent to this value. These results show a distinctive pattern in the contribution of these trace elements to the CTU values representative of a regional mining signature (fig. 10*D*). Concentrations of Pb, Ni, and Zn exceeded their respective PEC values (table 2) in this sample. The overall richness for unclustered Sample B was similar to the taxa richness for Clusters I and II (fig. 20*A*). EPT taxa contributed most (47 percent) to the taxa richness of the community, and the relative abundance of individuals was fairly well distributed among mayfly (26 percent), caddisfly (19 percent), Diptera (22 percent), and beetle (24 percent) taxa (figs. 20*A*–20*B*). Most of the caddisflies belonged to the family Hydropsychidae (82 percent); most of the Diptera belonged to the family Chironomidae (71 percent) (appendix 3, fig. 3-6). The primary taxon characterizing unclustered Sample B was *Atherix* sp. (fig. 19). This taxon was found at about 33 percent of the samples collected from the OH ecoregion (appendix 2, table 2-1). Although occurring at very low relative abundances (0 to 0.75 percent), *Atherix* sp. was more abundant in this sample than in any other samples from this ecoregion and can be considered a distinguishing taxon of this community (fig. 19). *Optioservus* sp. was the most abundant taxon in unclustered Sample B (13.5 percent).

The CTU values for the bed sediment in samples (C and D) from the reference areas were near the 25th percentile (1.7) for the regional dataset, indicating that trace-element enrichment was not present at these sites. The CTU values for the bed-sediment samples from these reference areas were primarily composed of Cr and Ni (53 and 57 percent); Pb and Zn contributed 20 and 17 percent to these values. The relative contributions of Cr, Pb, Ni, and Zn to these CTU values is consistent with the regional composition patterns for bed-sediment samples collected from streams in undeveloped areas (fig. 10*A*). Concentrations of trace elements did not exceed their respective PEC values (table 2) in either bed-sediment sample collected from streams in these undeveloped landscapes.

The overall taxa richness of unclustered Samples C and D was the same (41 percent); however, unclustered Sample C had a lower number of EPT and a higher number of dipteran taxa (fig. 20*A*). Chironomidae comprised most of the Diptera population (relative abundance of 94 percent) in unclustered Sample C (appendix 3, fig. 3-6). Unclustered Samples C and D were dominated by mayflies (44 and 40 percent, respectively); unclustered Sample D had a higher relative abundance of “other taxa” (26 percent) than did Sample C (1.64 percent, fig. 20*B*). The category, “other taxa”, is comprised of taxa that are not mayflies, stoneflies, caddisflies, beetles (Coleoptera), or true flies (Diptera). Mesogastropoda (*Elimia* sp.) dominated the “other taxa” composition occurring at about 23 percent relative abundance in unclustered Sample D (appendix 2, table 2-2). The caddisfly, *Helicopsyche* sp., was characteristic of Sample D (fig. 19). The relative abundance of this taxon in this community was 9.25 percent, the highest relative abundance of this taxon in streams sampled within the OH ecoregion. The caddisfly population in unclustered Sample D differed from other samples because it was not dominated by taxa belonging to the family Hydropsychidae (appendix 3, fig. 3-6).

Generally, macroinvertebrate distribution patterns in the OH ecoregion, represented by the MDS ordination, included more unclustered communities than the other three ecoregions evaluated for this study. The macroinvertebrate communities in the OH ecoregion that significantly group into three clusters appear to be influenced by trace-element concentrations and possibly land use. The possible influence of land use on macroinvertebrate community structure is suggested by the distinct grouping of the three macroinvertebrate communities, collected from streams in agricultural settings, in Cluster I. For comparison purposes, macroinvertebrate communities sampled from agricultural streams in the XE-EPLAT did not distinctly group into one particular cluster, and the agricultural landscape was uncommon in the mountain ecoregions. The characterizing features of macroinvertebrate communities were the contributions of various mayfly taxa (*Caenis* sp., *Leucrocuta* sp., *Stenacron* sp., and *Stenonema* sp.) to the overall relative abundance at these sites. The lack of trace-element enrichment in the bed sediment at these sites should be noted. Generally, the mayfly communities appear to be relatively diverse compared to samples from the other ecoregions in this study.

The macroinvertebrate communities included in Clusters II and III shared many common taxa, resulting in their close proximity in the MDS ordination. Cluster II included samples collected from undeveloped (reference) and mined areas, whereas Cluster III represented conditions of only mined locations. Concentrations of trace elements in bed-sediment samples were different between these two clusters. Bed sediment in Cluster II was unenriched to enriched in trace elements, whereas Cluster III bed sediment was low to highly enriched. Four taxa distinguished the Cluster II community structure (*Ceratopsyche* sp., *Cheumatopsyche* sp., *Optioservus* sp., and *Plauditus* sp.). *Tricorythodes* sp. dominated the relative abundance (62 percent) of the community in Cluster III with the higher CTU value (8.6).

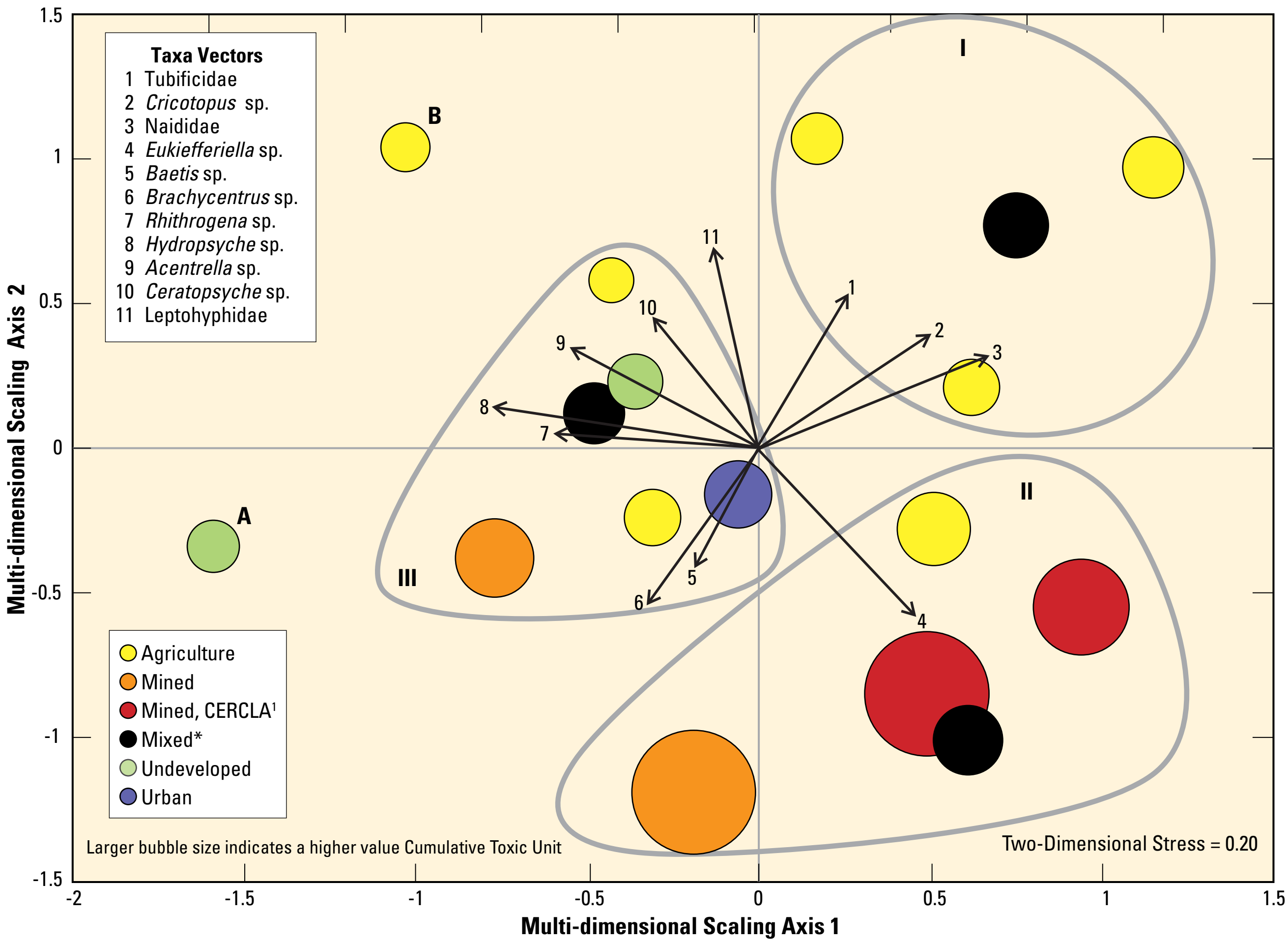
Four macroinvertebrate samples within the OH ecoregion did not group with any of the three clusters. The lowest taxa richness was observed for the unclustered Sample A that had the highest CTU value (24.9). This community was heavily dominated by Diptera, primarily Chironomidae (*Cardiocladius* sp., *Chaetocladius* sp., and C*ladotanytarsus* sp.). This site has been influenced by historic mining practices, and the dominance of Diptera taxa is the defining characteristic of this community. The other unclustered macroinvertebrate community collected from a stream in a mined landscape also had bed sediment enriched in trace elements; however, the community structure was markedly different than that described for unclustered Sample A. Two taxa were characteristic of the unclustered Sample B (*Atherix* sp. and *Optioservus* sp.). Sample B had a fairly balanced representation of relative abundances of mayflies, caddisflies, and Diptera. The taxon *Optioservus* sp. was the most abundant in this sample. The most distinguishing feature of Samples C and D (unclustered communities) from reference sites was the relative contribution of mayflies and “other taxa” to the overall relative abundances observed in these communities. Generally, the taxa within the OH ecoregion were somewhat different than those in the other ecoregions.

Eastern Xeric Plateau Ecoregion (XE-EPLAT)

Samples were collected from streams in the XE-EPLAT ecoregion in undeveloped (12 percent), agricultural (41 percent), urban (6 percent), mined (23 percent), and mixed (18 percent) landscapes. CTU values ranged from 1.0 to 7.5. The MDS ordination and hierarchical agglomerative cluster analysis identified three significant clusters of macroinvertebrate communities (I–III) and two unclustered Samples A and B (fig. 21).

Cluster I consists of four macroinvertebrate communities collected from sites in agricultural and mixed landscapes (fig. 21). CTU values for bed-sediment samples associated with these macroinvertebrate communities included in Cluster I ranged from 1.3 to 2.1; the median CTU value was 1.7, equal to the 25th percentile for the entire regional dataset (fig. 9). These results generally indicate a lack of trace-element enrichment in the bed sediment. The contribution of Pb and Zn to the CTU values in Cluster I ranged from 23 to 69 percent; together, Cr and Ni contributed from 8 to 47 percent (figs. 10*B* and 10*E*). Trace-element concentrations did not exceed PEC values (table 2) in any of the bed-sediment samples collected from these four sites.

The overall richness of macroinvertebrate communities represented in Cluster I (20 taxa) was similar to the richness of the other two clusters and the unclustered Samples A and B (21–24 taxa, fig. 22*A*). This is the only ecoregion examined where this occurred. Although the overall richness values are very similar among clusters, the contributions of the various taxonomic groups differ (fig. 22*B*). Macroinvertebrate community richness in Cluster I primarily consisted of Diptera (48 percent), EPT (26 percent), and “other taxa” (24 percent) (fig. 22*A*). The contribution of Ephemeroptera, Trichoptera, and “other taxa” to the relative abundance in the macroinvertebrate communities in Cluster 1 was highly variable (fig. 22*B*). Taxa characteristic of Cluster I were the Oligochaeta worms (Tubficidae and Naididae) and the Orthocladiinae midge, *Cricotopus* sp. (fig. 21). The contribution of caddisfly taxa to community structure in Cluster I also was highly variable. On



[1] Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA)

*Mixed, sites with agricultural, urban, and (or) mining land uses represented

Figure 21. A non-metric multidimensional scaling (MDS) ordination of macroinvertebrate communities in selected streams in the Xeric Eastern Plateau (XE-EPLAT) ecoregion and distribution of cumulative toxic units (CTUs), 1992–2000.

average, caddisflies contributed about 11 percent to the overall number of individuals in these samples; about 48 percent of the caddisflies were Hydropsychidae (appendix 3, fig. 3-8). The abundance and taxa composition of mayflies in this cluster also were highly variable; however, Leptohyphidae (14 percent) taxa and *Acentrella* sp. (52 percent) did occur at relatively high abundances in two agricultural samples. Given the low CTU values for the bed-sediment samples, other factors are likely influencing the macroinvertebrate community structure in these areas. Streams draining agricultural watersheds are susceptible to nutrient enrichment and deposition of excess fine sediments (Maret and others, 2010; Sidle and Sharma, 1996), and the taxa Tubificidae, Naididae, and *Cricotopus* sp. are documented as having high thresholds to these stresses (Carlisle and others, 2007). Leptohyphidae are present at several of the agricultural sites within the XE-EPLAT ecoregion but particularly at one site included in Cluster I. Even though the information on taxa identification is limited to family, evidence sugggests that these mayflies are likely resilient in ecosystems influenced by agricultural practices (Griffith and others, 2001; Dance and Hynes, 1980).

Five macroinvertebrate samples are included in Cluster II, and the bed sediment at these sites had the highest CTU values (2.3 to 7.5; median 4.5) for the XE-EPLAT ecoregion. Regionally, these bed-sediment samples range from low enrichment to high enrichment conditions (table 5, fig. 9). The streams from which these samples were collected represent a variety of land uses—agriculture, mined, and mixed (fig. 21). The three bed-sediment samples collected from mined areas had the highest CTU values (4.5 to 7.5). Two of the three stream sites included in Cluster II that were influenced by historic mining-related activities were associated with waters listed as CERCLA because of the contamination of bed sediment with a variety of trace elements (aluminum, As, Cd, Cr, cobalt, Cu, fluoride, iron, Pb, manganese, molybdenum, sulfate, and Zn; Colorado Department of Public Health and Environment, 2009; U.S. Environmental Protection Agency, 2010). The contribution of Pb and Zn to the CTU values for these

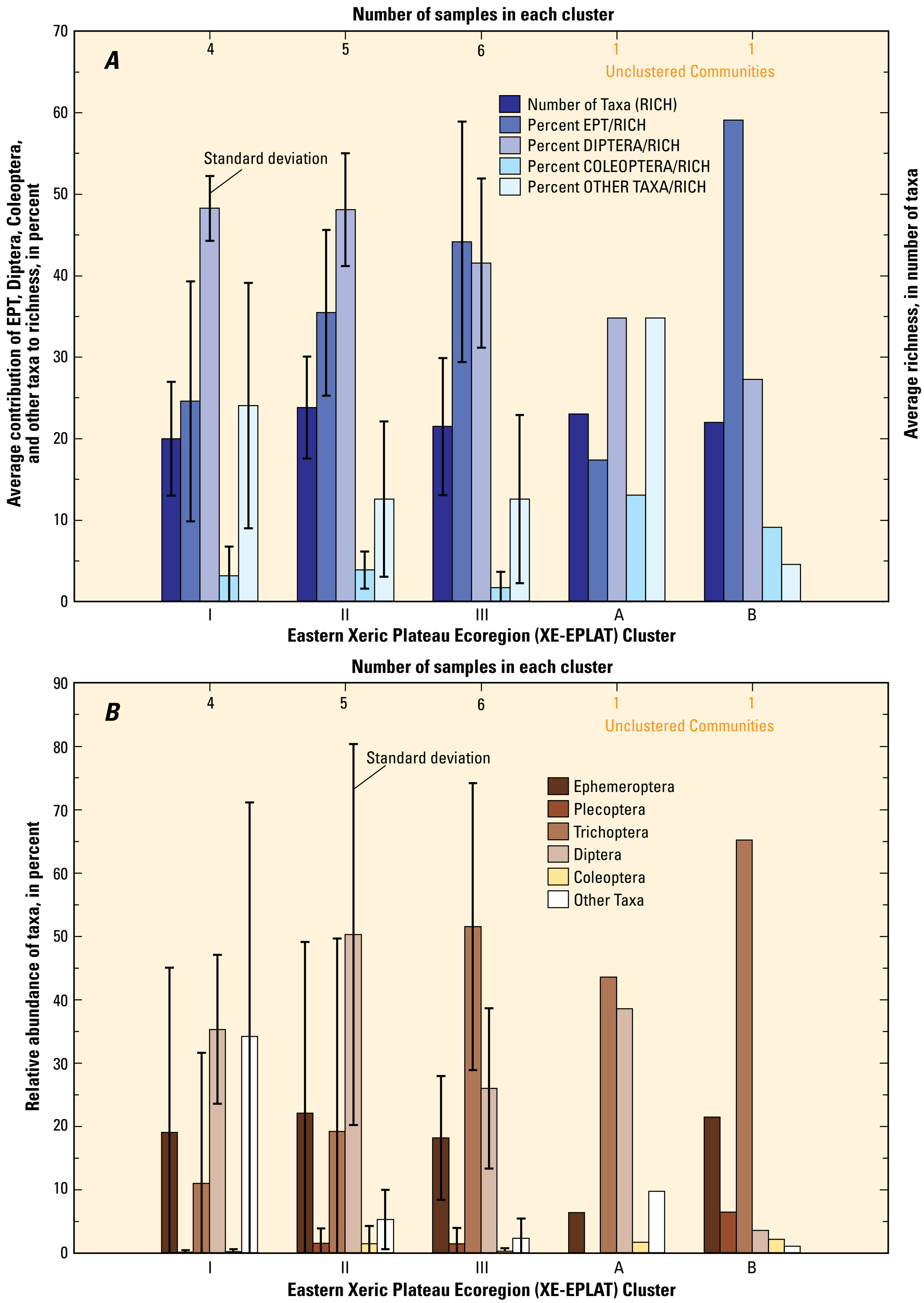


Figure 22. *A*, Average number of taxa (richness) and percent contribution from each of Ephemeroptera, Plecoptera, and Trichoptera (EPT), Diptera, Coleoptera, and other taxa to the overall richness (RICH); and *B*, average relative abundances of Ephemeroptera, Plecoptera, Trichoptera, Diptera, Coleoptera, and other taxa for macroinvertebrate communities in selected streams of the Xeric Eastern Plateau ecoregion, 1992–2000.

bed-sediment samples included in Cluster II ranged from 17 to 48 percent; Cr and Ni contributed from 6 to 45 percent. These results show about the same contributions of both Pb and Zn and Cr and Ni to CTU values, indicating no pattern associated with regional land use (figs. 10*B, 10D,* 10*E*). Concentrations of As, Cu, Ni, and Se each exceeded their respective PEC values, or PEL value in the case of Se, in one bed-sediment sample. However, these exceedences were rarely in the same sample. The PEC value for Pb and Zn (table 2) were exceeded in two bed-sediment samples.

As previously mentioned, the overall taxa richness in Cluster II was similar to the other clusters generated by MDS for this ecoregion. On average, EPT (36 percent) and dipteran (48 percent) taxa contributed the most to overall richness in Cluster II macroinvertebrate communities (fig. 22*A*). Although highly variable, samples included in Cluster II were primarily composed of dipterans, mayflies, and caddisflies (fig. 22*B*). The distinguishing characteristic of macroinvertebrate communities in Cluster II was the presence of the Orthocladiinae midge (*Eukiefferiella* sp.). Some researchers have found that the Orthocladiinae midges such as *Eukiefferiella* sp. are relatively tolerant of trace-element exposure (Gower and others, 1994; Clements and others, 2000; Swansburg and others, 2002). Alternatively, other researchers have found *Eukieferiella* sp. most frequently in streams with relatively low concentrations of trace elements (Pollard and Yuan, 2006).

In Cluster II, the three streams influenced by mining showed some differences in macroinvertebrate community structure. It is likely that the subtle differences in the composition of the CTU values among these sites and possibly site-specific habitat conditions may be contributing to the differences among these macroinvertebrate communities. About half (53 percent) of the relative abundance of individuals comprising the macroinvertebrate community at one of the sites influenced by historic mining activities was composed of the caddisfly, *Brachycentrus* sp. Clements (1994) showed that *Brachycentrus* sp. was positively associated with Zn and elevation, suggesting some degree of tolerance of this taxon to trace elements. *Eukieferiella* sp. was found at all sites included in Cluster II, and the relative abundance of this taxon contributed about 27 percent to the overall abundance of individuals at the two CERCLA sites. The overall stress of the MDS ordination (0.20) suggests that there may be some ambiguity in comparing these macroinvertebrate communities.

The six macroinvertebrate samples included in Cluster III were collected from streams in all five land-use categories—undeveloped, agricultural, urban, mined, and mixed (fig. 21). The CTU values in this cluster range from 1.0 to 3.0 (median 1.7). The highest CTU value (3.0) was for bed sediment collected from the stream influenced by historic mining-related activities indicating moderate enrichment. The remaining CTU values do not exceed the 50th percentile (2.52) of the regional dataset, indicating that trace-element enrichment in these bed-sediment samples was minimal (table 5, fig. 9). The contribution of Pb and Zn to the CTU values in Cluster III ranged from 16 to 33 percent; together, Cr and Ni contributed from 36 to 60 percent. The higher contribution of Cr and Ni to the CTU values suggests a resemblance to regional patterns observed in CTU values representative of undeveloped, agricultural, urban, and mixed land uses (figs. 10*A–10C,* 10*E*). Trace-element concentrations did not exceed their respective PEC values (table 2) in bed-sediment samples collected from these sites. The overall richness of macroinvertebrate communities represented in Cluster III (21 taxa) was similar to the other clusters for this ecoregion (fig. 22*A*). On average, EPT taxa contributed the most (44 percent) to the taxa richness for macroinvertebrate communities included in Cluster III. The contribution to taxa richness was similar for Diptera taxa (42 percent). The macroinvertebrate communities in Cluster III were dominated by caddisfly (52 percent), Diptera (26 percent), and mayfly (18 percent) taxa (fig. 22*B*). Generally, the macroinvertebrate community structure represented in Cluster III is characterized by relatively tolerant EPT taxa. Caddisflies (*Brachycentrus* sp., *Hydropsyche* sp., and *Ceratopsyche* sp.) were characteristic of the macroinvertebrate communities included in Cluster III. In addition to these tolerant caddisfly taxa, *Acentrella* sp., *Baetis* sp., and *Rhithrogena* sp. mayflies were also important in the characterization of these communities; however, these mayfly taxa have a much lower contribution to the total relative abundance than the caddisflies (fig. 22*B*).

Two macroinvertebrate communities did not group with any of the three clusters in the XE-EPLAT ecoregion—one sample from a reference site (undeveloped landscape) and the other an agricultural setting (fig. 21). The CTU values (1.3 and 1.2) for the bed sediment were below the 25th percentile (1.7) for the entire regional dataset, indicating trace-element enrichment is not occurring at these sites (table 5, fig. 9).The CTU values for these two bed-sediment samples were primarily composed of Cr and Ni (47 and 53 percent); Pb and Zn contributed 19 and 24 percent. These results are consistent with the relative contributions of Cr, Pb, Ni, and Zn to CTU compositions for samples collected from streams in undeveloped and agricultural landscapes (figs. 10*A*–10*B*). Trace-element concentrations did not exceed their respective PEC values (table 2) in either bed-sediment sample collected from either stream associated with these unclustered communities.

Overall taxa richness was similar among clusters within this ecoregion; however, the taxa contributing to the overall richness for these two unclustered communities were markedly different from the clustered samples and from each other (fig. 22*A*). On average, Diptera (35 percent) and “other taxa” (35 percent) influenced the richness at unclustered Sample A (undeveloped landscape); however, the relative abundances of Diptera (39 percent) and caddisflies (44 percent) dominated this sample. The relative abundances of the “other taxa” were relatively small (9.8 percent). In Sample A, Diptera were dominated by Ceratopogoninae (6.13 percent) and the midge, *Parametriocnemus* sp. (22.45 percent); the relative abundance of caddisflies was entirely composed of Hydropsyche sp. (43.57 percent). EPT taxa (59 percent) contributed the most to taxa richness in the unclustered Sample B collected at an agricultural site. EPT for Sample B was dominated by caddisflies (relative abundance of 65 percent) of which most belonged to the family Hydropsychidae (*Ceratopsyche* sp. and *Cheumatopsyche* sp.) (appendix 3, fig. 3-8). Mayflies accounted for about 21 percent of the individuals collected from unclustered Sample B, and Leptophlebiidae mayflies (*Choroterpes* sp.) contributed the most (9.3 percent) to the mayfly portion of this community.

The distribution patterns of macroinvertebrate communities within the XE-EPLAT ecoregion reflect exposure to trace elements and possibly land-use activities. The XE-EPLAT ecoregion had the highest level of similarity in richness among clusters of any of the ecoregions included in this study. The relative abundances of mayflies, caddisflies, and ”other taxa” were highly variable for communities in Cluster I. Agricultural settings were represented by two of the three streams included in Cluster I, and bed-sediment samples were not enriched in trace elements. Cluster I was mostly characterized by “other taxa” (Tubificidae and Naididae worms). The midge, *Cricotopus* sp., was also characteristic of the macroinvertebrate community structure of Cluster I. Generally, macroinvertebrate samples collected from streams where historic mining-related activities have bed sediment enriched with trace elements grouped into Cluster II. The relative abundances of mayflies, caddisflies, and “other taxa” were highly variable in Cluster II, which was characterized by the presence of the Chironomidae midge, *Eukieferiella* sp. Cluster III, consisting of sites from a variety of land uses, was characterized primarily by mayflies (*Acentrella* sp., *Baetis* sp., and *Rhithrogena* sp.) and caddisflies (*Brachycentrus* sp*., Ceratopsyche* sp., and *Hydropsyche* sp.). The bed sediment samples collected at the sites included in this cluster were minimally enriched in trace elements. The diverse nature of land uses represented by the streams sampled in the XE-EPLAT ecoregion and the distribution patterns observed in general macroinvertebrate communities suggest that something other than trace-element exposure alone is likely contributing to macroinvertebrate community structure in these samples.

Summary and Conclusions

The objective of this regional-scale study was to characterize trace-element concentrations and occurrence in streambed sediment and to determine the influence of trace elements on aquatic-macroinvertebrate communities. The nine trace elements included in this study—arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), selenium (Se), and zinc (Zn)—were selected on the basis of their ecological significance and the availability of sediment-quality guidelines.

Each of the nine trace elements was first evaluated within each of the five general land-use categories (undeveloped, agricultural, urban, mined, and mixed) for range in concentration and comparison with available sediment-quality guidelines. It was outside the scope of this study to determine a cause and effect relation between land use and trace-element occurrence; however, it was necessary to evaluate the trace elements in the context of land use in order to address ranges in concentrations in bed sediment within each of these landscapes and, if necessary, consider generally accepted influences, other than trace-element contamination, on macroinvertebrate assemblages. Most of the 208 bed-sediment samples (62 to 80 percent) collected from selected streams in the conterminous western United States, Alaska, and Hawaii had trace-element concentrations within natural geochemical concentrations, regardless of land use. Regional baseline and reference-site trace-element concentrations determined in this investigation were similar to baseline concentrations previously reported for bed sediment collected throughout the Nation.

Median concentrations of all nine trace elements were lowest in bed sediment collected from undeveloped (reference) and agricultural landscapes. With the exception of Cr and Ni, median concentrations of the remaining seven trace elements in bed sediment collected from streams in these areas were below the threshold effect concentrations. Median concentrations of Cr and Ni in bed sediment in undeveloped and agricultural areas were between the threshold and probable-effect concentrations for these trace elements. Bed-sediment samples from streams in mined areas were enriched in concentrations of As, Cd, Cu, Pb, Hg, Ni, and Zn. Concentrations of many of these trace elements were elevated in urban (As, Cr, Cu, Hg, Ni, Se, and Zn) and mixed (Cd, Cr, Cu, Pb, Ni, Se, and Zn) landscapes. In most cases, median concentrations of As, Cd, Cr, Cu, Pb, Hg, Ni, Se, and Zn were within the threshold and probable-effect concentrations for these trace elements. A noted exception was observed for Zn in bed-sediment samples collected from streams influenced by mining-related activities, where this trace element was at its probable-effect concentration.

Assessing co-occurrence of trace elements in bed sediment is helpful in identifying areas where toxic effects may be enhanced. Generally, only moderate and strong correlations ($rho \geq 0.5$, $p \leq 0.05$) are discussed herein. Commonly, concentrations of Cr and Ni were strongly correlated in the bed-sediment samples. This was observed only for these two trace elements. In a few instances, concentrations of Cr and Ni were moderately and positively correlated with other trace elements examined in this study. Cr and Ni exceeded probable-effect concentrations in 9 and 18 percent, respectively, of all bed-sediment samples collected throughout the regional study area. Ni exceeded the probable-effect concentration more frequently than Cr; however, in all but one instance, when bed-sediment concentrations of Cr exceeded the probable-effect concentration for this trace element, Ni concentrations also exceeded its probable-effect concentration. In very few samples, when both Cr and Ni exceeded the probable-effect concentrations, the other seven trace elements also exceeded their respective probable-effect concentrations. Although natural or anthropogenic sources of trace elements were not directly evaluated as part of this study, results suggest that in many instances concentrations of Cr and Ni are naturally high in bed sediment in western streams. Concentrations of Zn were moderately correlated with Cd, Cu, Pb, and Hg in bed-sediment samples collected from undeveloped (reference) sites and with Cu in agricultural areas. Bed sediment from streams in undeveloped areas occasionally had concentrations of As, Cr, Pb, and Ni exceeding probable-effect concentrations, indicating that some areas may be naturally enriched in these trace elements above concentrations expected to cause ecosystem impairment.

Concentrations of Cd, Pb, and Zn were moderately correlated in bed sediment collected from urbanized and mined areas. Of the 70 sites evaluated in urban and mined landscapes, concentrations of all three of these trace elements exceeded the probable-effect concentrations in 16 percent of the samples, all of which were from mined areas. As was moderately correlated to Cd, Pb, and Zn in bed sediment from streams in mined areas. Concentrations of As and Cu in these areas commonly exceeded probable-effect concentrations in the same samples where Cd, Pb, and Zn concentrations exceeded probable-effect concentrations. Although Hg concentrations were high in selected bed-sediment samples from areas influenced predominantly by historic mining activities, concentrations exceeding the probable-effect concentration occurred infrequently (3 percent of all samples). All exceedences were in samples collected from areas influenced by mining. With the exception of the moderate correlations between Se and Cu in agricultural bed sediment and with Hg in urban bed sediment, Se was only weakly correlated with the other six trace elements evaluated during this study. The median Se concentration was elevated in urban bed-sediment samples. The only instances where Se concentrations exceeded the probable-effect level were in one sample each from an agricultural and urban stream. These exceedences of the Se probable-effect level did not co-occur with any other exceedences.

Cumulative toxic units (CTUs) were used to evaluate the influence of trace-element mixtures on aquatic-macroinvertebrate community structure. The use of CTUs assumes additive toxicity, which may or may not be the case. Se was not used in the determination of CTU values because this trace element was not analyzed in all bed-sediment samples, rarely exceeded concentrations expected to be harmful to macroinvertebrates, and was seldom correlated to other trace elements included in this study. Gradients in trace-element mixtures were evaluated by examining the range in CTU values. Generally, the lowest CTU values were in bed sediment collected from reference and agricultural areas; the highest CTUs were in bed sediment from mined areas. The median CTU values between undeveloped (reference) and agricultural landscapes and between urban and mixed land-use areas were not statistically different. Median CTU values for bed sediment collected from reference and agricultural areas were below the 50th percentile for the regional dataset indicating minimal enrichment; urban and mixed land-use areas were above the 50th percentile, indicating moderate enrichment in trace elements. Bed sediment collected from streams in mined areas had the widest range in CTU values; the median value was equal to the 90th percentile for the regional dataset indicating conditions of high enrichment. For most landscapes, median contributions of Cr and Ni to CTUs predominated over each contribution of the other six trace elements included in the CTU determinations. In mined areas, CTUs were predominantly composed of Pb and Zn.

Evaluating the results of large-scale regional investigations that rely on macroinvertebrates as indicators is challenging. Regional variability resulting from differences in topography, climate, water quality, and habitat characteristics affects the ability to detect changes in the macroinvertebrate communities in response to an environmental-stressor gradient, especially when multiple environmental stressors are present and biological responses are commonly confounded by additive or dominant effects from one or more stressors. For this investigation, the large-scale MDS ordination of macroinvertebrate community structure was influenced more by regional spatial variability rather than the effects caused by a gradient of trace elements in bed sediment. Even after the analysis was reduced to a sub-regional level, the effects of trace elements in bed sediment on macroinvertebrate community structure were only apparent at ecoregions with the greatest CTUs resulting from past mining activities. Regional patterns in CTU composition determined for general land uses oftentimes did not appear to be reflected in distinguishable macroinvertebrate distribution patterns.

To account for some of the natural variability in distribution patterns of aquatic macroinvertebrates across the study region, ecoregions were used to stratify analyses of macroinvertebrate community structure. Communities within a given ecoregion were expected to be similar enough to distinguish stress-induced changes in structure from other influences on distribution and abundance. Intermediate-scale ecoregions (sub-regional scale) were used to minimize differences in factors such as climate, topography, general geology, and general in-stream habitat characteristics such as water quality and channel substrate. The four intermediate-scale ecoregions used in this investigation were the Mountain Northern Rockies (MT-NROCK), Mountain Southern Rockies (MT-SROCK), Ozark Highlands (OH), and Eastern Xeric Plateau (XE-EPLAT). Each of the four intermediate-scale ecoregions used to evaluate the influence of trace elements on macroinvertebrate community structure had different ranges in CTU values, and the community distribution pattern in each ecoregion appeared to be influenced, at least to some degree, by trace elements. In many cases, macroinvertebrate communities associated with bed-sediment samples with a given trace-element enrichment condition (no enrichment to high enrichment) and collected from a stream in a given landscape showed similar structure.

The four intermediate-scale ecoregions were evaluated using multidimensional scaling (MDS) ordination and cluster analysis with significance testing of the selected clusters. Similar patterns were observed within all four ecoregions. Clusters of sites with the greatest CTUs had greater relative abundances of taxa that are generally considered tolerant to trace-element exposure when compared to clusters with lower CTUs. The common taxa to community composition in macroinvertebrate communities exposed to elevated trace-element concentrations in bed sediment among ecoregions were those belonging to the subfamily of midges, Orthocladiinae. Other taxa resilient to trace-element exposure in bed sediment included the mayflies, *Baetis* sp. and *Tricorythodes* sp.; the Hydropsychidae caddisflies; and the dipteran, *Simulium* sp. In addition, sensitive EPT taxa were generally absent from clusters with higher CTUs.

Given that trace-element concentrations in bed sediment collected throughout the regional study area were predominantly within geochemical baseline concentrations, it is not unexpected that potential trace-element effects on macroinvertebrate communities were observed only at the most-contaminated sites, namely those associated with CERCLA areas. Results of this study are similar to other studies assessing the influence of trace-element exposure to in-stream macroinvertebrate communities in Rocky Mountain streams and in the XE-EPLAT ecoregion. Across the three intermediate-scale ecoregions—MT-NROCK, MT-SROCK, and XE-EPLAT—the mayfly, *Baetis* sp.; the dipteran, *Simulium* sp.; caddisflies in the family Hydropsychiidae; and midges in the family Orthocladiinae were common, demonstrating some tolerance to trace-element exposure. In some cases, the observed results suggest some degree of resilience to possible changes in physical-habitat conditions within stream ecosystems as well. Generally, community composition in samples collected from the OH ecoregion was considerably different. For example, the mayfly community from streams sampled in the OH ecoregion appeared to be relatively more diverse in comparison to the other intermediate-scale ecoregions. One taxon that appeared to indicate possibly impaired conditions by occurring in relatively high relative abundances in the OH ecoregion was the mayfly, *Tricorythodes* sp. The presence of *Tricorythodes* sp. at impaired sites within the OH ecoregion was similar to how *Baetis* sp. dominated the mayfly communities at impaired sites in the MT ecoregions. Given the regional perspective and retrospective nature of this evaluation, site-specific differences in trace-element composition (CTUs) and concentrations and the relative influence on macroinvertebrate communities could not be discussed in greater detail other than by gross generality, and these findings should not be extrapolated outside this context.

In general, the findings of this investigation are consistent with previous studies analyzing the impacts of trace elements on macroinvertebrates. The primary difference between this study and many of the previous investigations is the scale at which patterns in macroinvertebrate community structure were observed. Most of the previous studies have focused on the characterization of macroinvertebrate communities in a single river basin or stream reach. This investigation evaluated macroinvertebrate responses on a larger regional scale. Although many interesting patterns were found, the analyses were limited because of the small number of samples spread across a large ecoregion, with little or no replication at individual sites. The findings of this investigation are useful in that it highlights relations between trace-element exposure and macroinvertebrate response that can be characterized outside local scales. In addition, this study emphasizes the importance of considering the complicated relations between physical and chemical influences that may be imparted on aquatic-macroinvertebrate communities. Natural-resource managers are required to implement best-management strategies intended to balance and maintain necessary resources of adequate quality to maintain healthy ecological systems while simultaneously allowing for continued human development in areas within which these ecosystems reside.

Natural systems are inherently complex and dynamic, and an evaluation of physical and (or) contaminant stress(es) on fauna necessarily will involve an assessment of many components important to the ecosystem being investigated. As described earlier, many studies have investigated isolated components of aquatic systems. When elucidating potential impairments to natural systems, it is important to consider the complicated, integrated, and oftentimes highly variable components of the system in order to place possible impairments into proper perspective.

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Appendix 1-1.

Trace element, land use, and cumulative toxic unit data used to assess the influence of trace elements on macroinvertebrate community structure in selected streams in the conterminous western United States, Hawaii, and Alaska, 1992–2000.

Appendix 1-2.

Average specific conductance, dissolved solids, calcium, magnesium, hardness, pH, and suspended-sediment concentration for each sampling site used to assess the difference in general water-quality characteristics among the five large-scale ecoregions in the conterminous western United States, Hawaii, and Alaska, 1992–2000.

Appendix 2-1.

Relative abundance of taxa occurring at a minimum of 30 percent of the sampling sites within each ecoregion included in this investigation within the conterminous western United States and Alaska, 1992–2000.

Apppendix 2-2.

Phylogony and relative abundance of taxa in macroinvertebrate samples collected as part of this study from selected streams in the western United States, Alaska, and Hawaii, 1992–2000.

Appendix 3.

Community-structure metrics used to evaluate characteristics for macroinvertebrate samples collected from selected streams in four intermediate-scale ecoregions: Mountain Northern Rockies (MT-NROCK), Mountain Southern Rockies (MT-SROCK), Ozark Highlands (OH), and Eastern Xeric Plateau (XE-EPLAT) evaluated in this investigation.

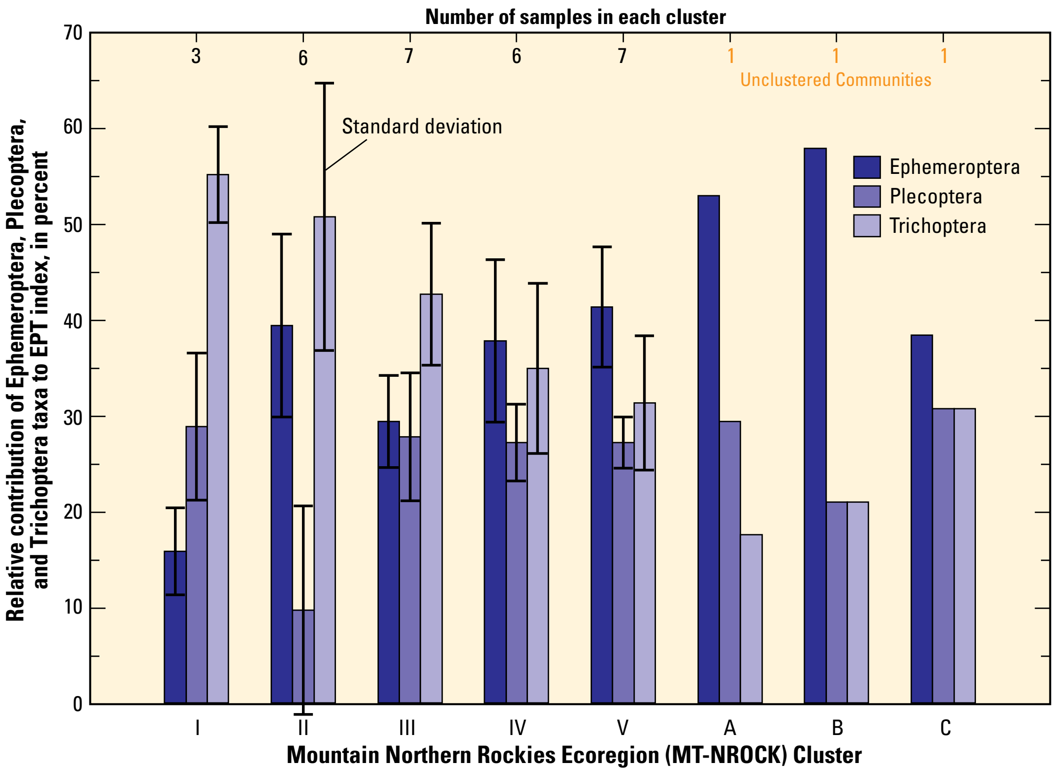

Figure 3-1. Average contribution of each Order, Ephemeroptera (E), Plecotpera (P), and Trichoptera (T), to EPT richness for macroinvertebrate communities in selected streams of the Mountain Northern Rockies (MT-NROCK) ecoregion, 1992–2000. Taxa represented in this figure include rare and common taxa occurring at sites within these MT-NROCK ecoregion streams. Clusters correspond to clusters of macroinvertebrate assemblages within the multidimensional scaling (MDS) ordination for this ecoregion (fig. 15). Clusters identified as "Unclustered Communities" did not group significantly during the cluster analysis.

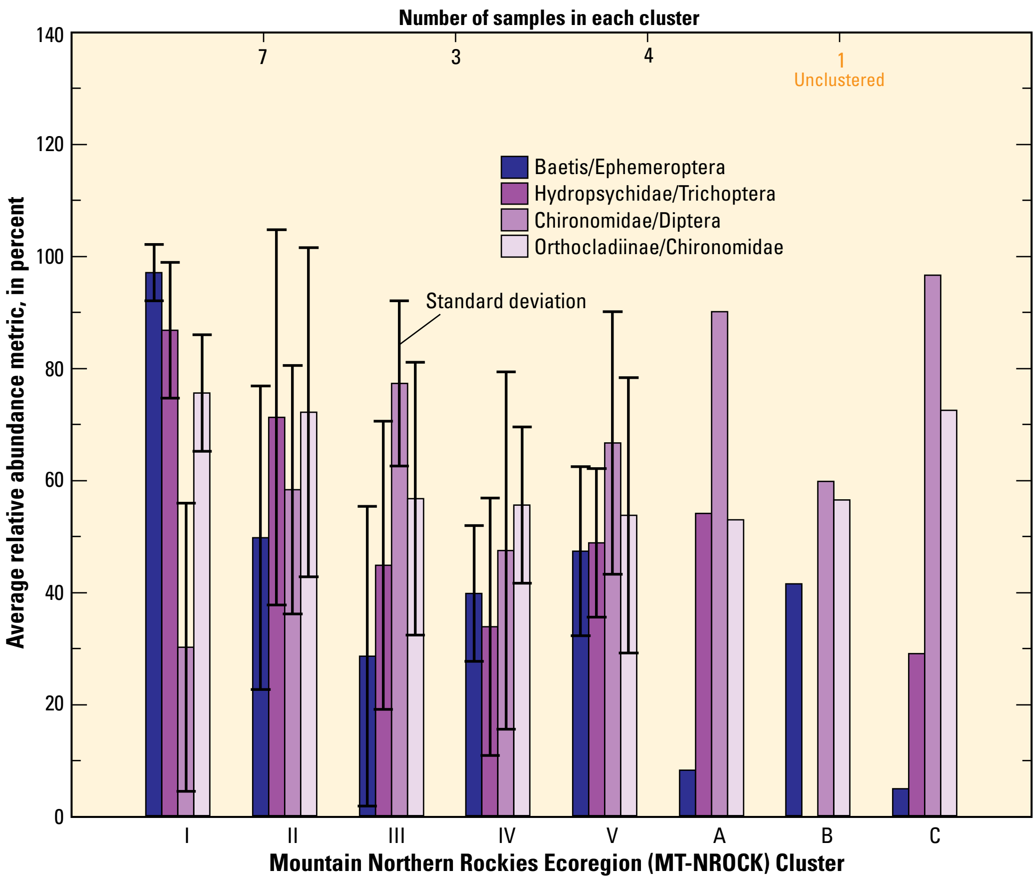

Figure 3-2. Average relative abundance of selected taxa to Ephemeroptera, Trichoptera, Diptera, and Chironomidae relative abundances in macroinvertebrate communities in selected streams of the Mountain Northern Rockies (MT-NROCK) ecoregion, 1992–2000. Taxa represented in this figure include rare and common taxa occurring at sites within these MT-NROCK ecoregion streams. Clusters identified as I-UNCLUSTERED correspond to clusters of macroinvertebrate assemblages within the MDS ordination for this ecoregion (fig. 15). Clusters identified as "Unclustered Communities" did not group significantly during the cluster analysis.

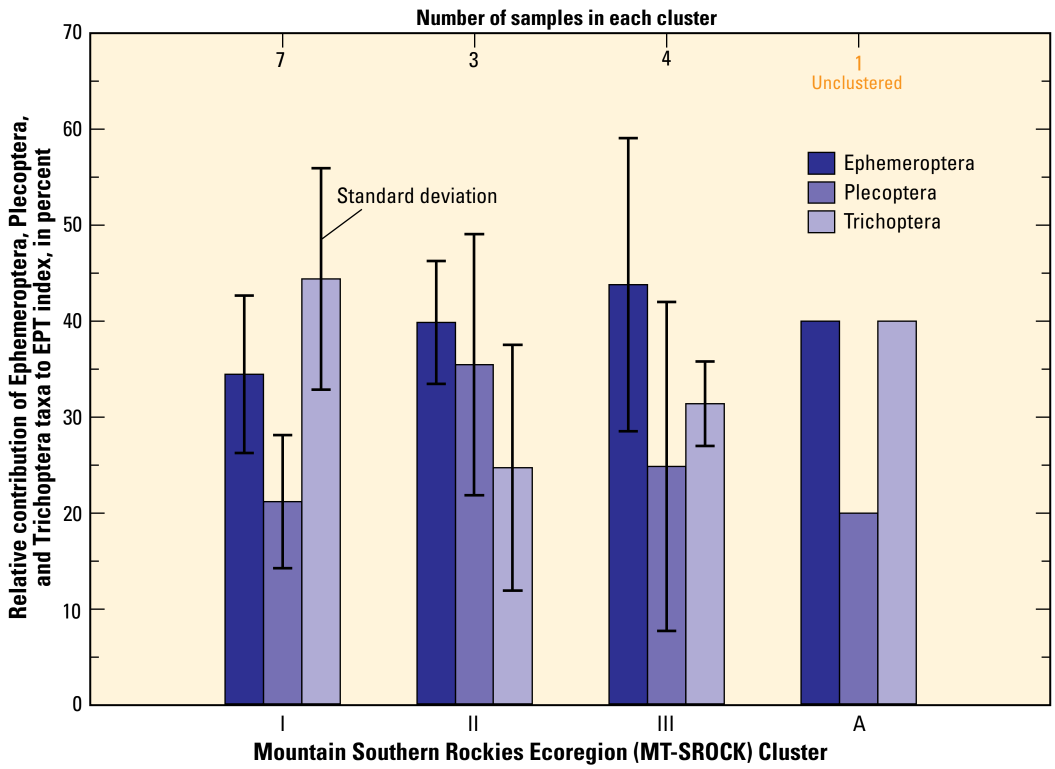

Figure 3-3. Average contribution of each Order, Ephemeroptera (E), Plecotpera (P), and Trichoptera (T), to EPT richness for macroinvertebrate communities in selected streams of the Mountain Southern Rockies (MT-SROCK) ecoregion, 1992–2000. Taxa represented in this figure include rare and common taxa occurring at sites within these MT-SROCK ecoregion streams. Clusters correspond to clusters of macroinvertebrate assemblages within the multidimensional scaling (MDS) ordination for this ecoregion (fig. 17). Clusters identified as “Unclustered Communities” did not group significantly during the cluster analysis.

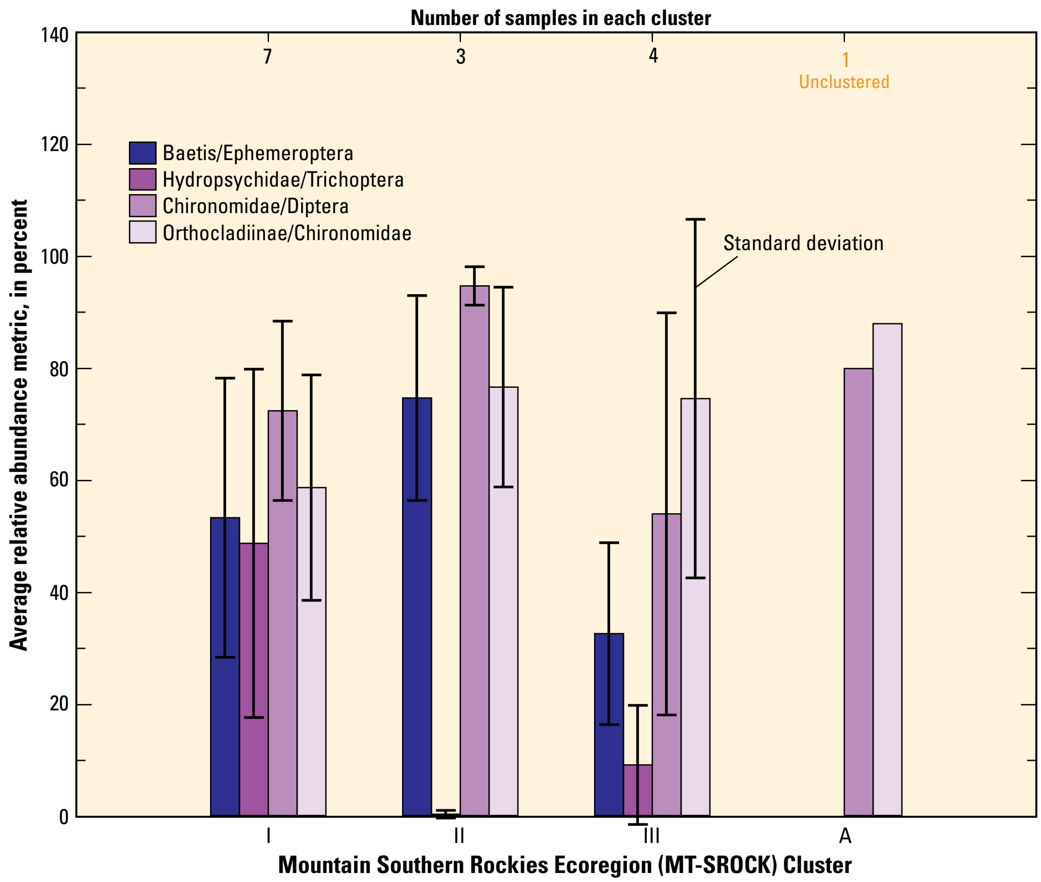

Figure 3-4. Average relative abundance of selected taxa to Ephemeroptera, Trichoptera, Diptera, and Chironomidae relative abundances in macroinvertebrate communities in selected streams of the Mountain Southern Rockies (MT-SROCK) ecoregion, 1992–2000. Taxa represented in this figure include rare and common taxa occurring at sites within these MT-SROCK ecoregion streams. Clusters identified as I-UNCLUSTERED correspond to clusters of macroinvertebrate assemblages within the MDS ordination for this ecoregion (fig. 17). Clusters identified as "Unclustered Communities" did not group significantly during the cluster analysis.

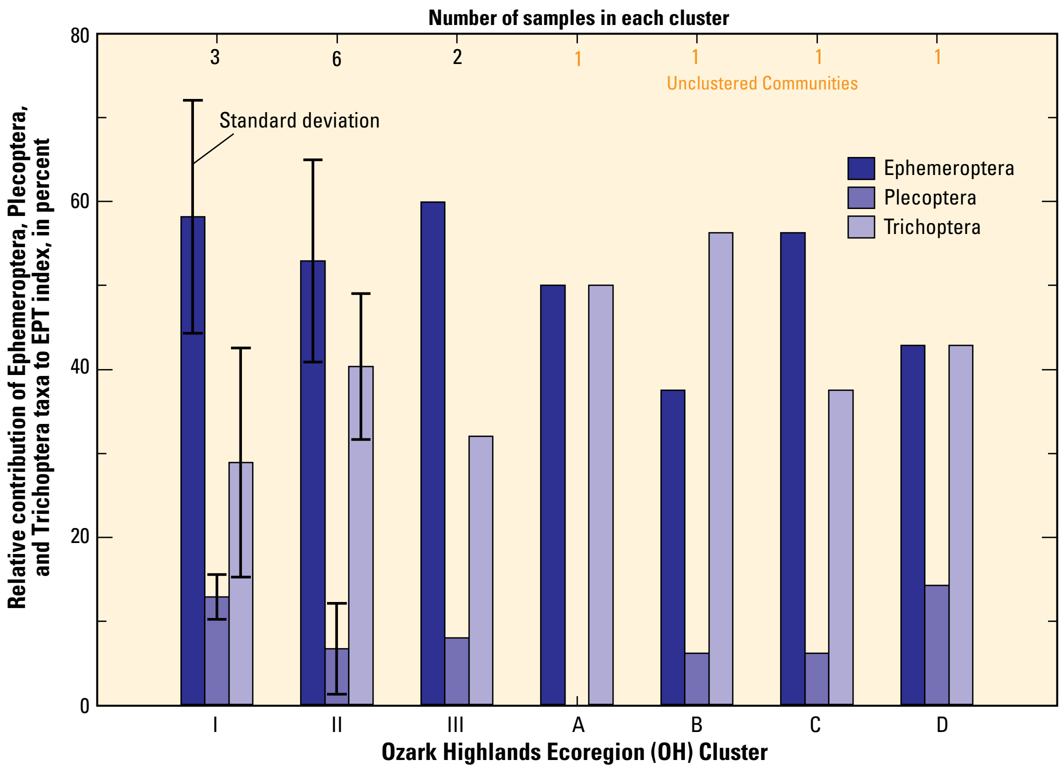

Figure 3-5. Average contribution of each Order, Ephemeroptera (E), Plecotpera (P), and Trichoptera (T), to EPT richness for macroinvertebrate communities in selected streams of the Ozark Highlands (OH) ecoregion, 1992–2000. Taxa represented in this figure include rare and common taxa occurring at sites within these OH ecoregion streams. Clusters correspond to clusters of macroinvertebrate assemblages within the multidimensional scaling (MDS) ordination for this ecoregion (fig. 19). Clusters identified as "Unclustered Communities" did not group significantly during the cluster analysis.

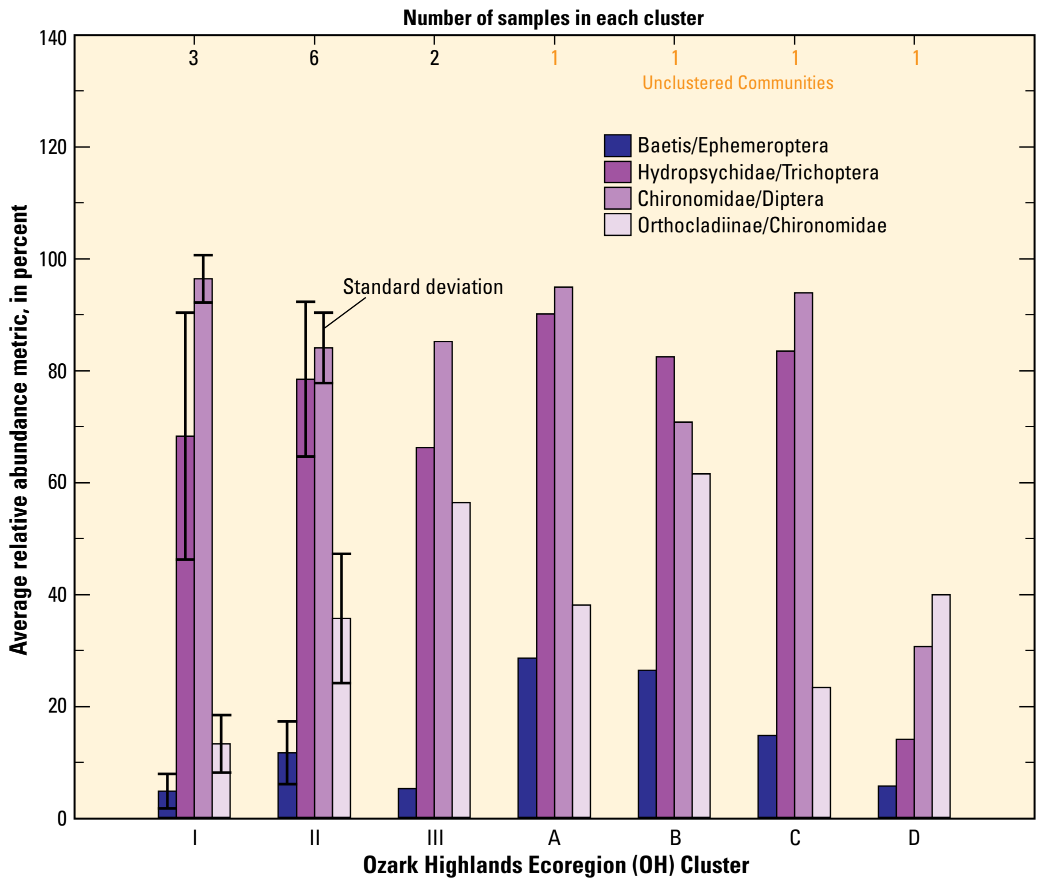

Figure 3-6. Average relative abundance of selected taxa to Ephemeroptera, Trichoptera, Diptera, and Chironomidae relative abundances in macroinvertebrate communities in selected streams of the Ozark Highlands (OH) ecoregion, 1992–2000. Taxa represented in this figure include rare and common taxa occurring at sites within these OH ecoregion streams. Clusters identified as I-UNCLUSTERED correspond to clusters of macroinvertebrate assemblages within the MDS ordination for this ecoregion (fig. 19). Clusters identified as "Unclustered Communities" did not group significantly during the cluster analysis.

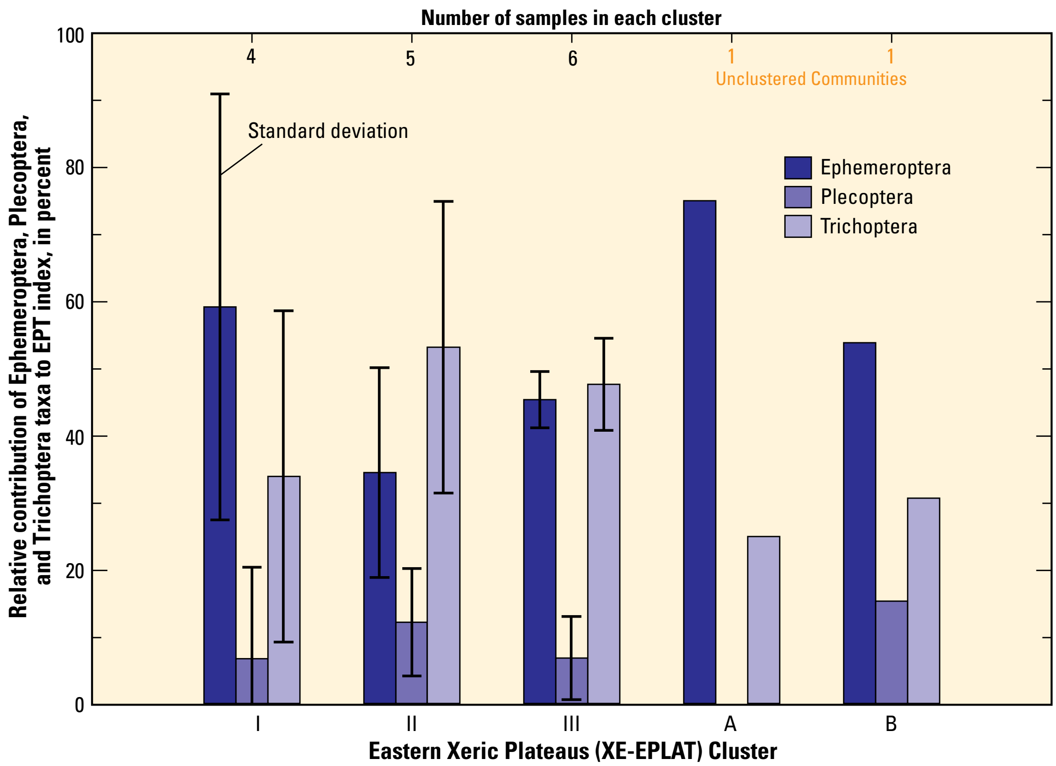

Figure 3-7. Average contribution of each Order, Ephemeroptera (E), Plecotpera (P), and Trichoptera (T), to EPT richness for macroinvertebrate communities in selected streams of the Eastern Xeric Plateau (XE-EPLAT) ecoregion, 1992–2000. Taxa represented in this figure include rare and common taxa occurring at sites within these XE-EPLAT ecoregion streams. Clusters correspond to clusters of macroinvertebrate assemblages within the multidimensional scaling (MDS) ordination for this ecoregion (fig. 21). Clusters identified as “Unclustered Communities” did not group significantly during the cluster analysis.

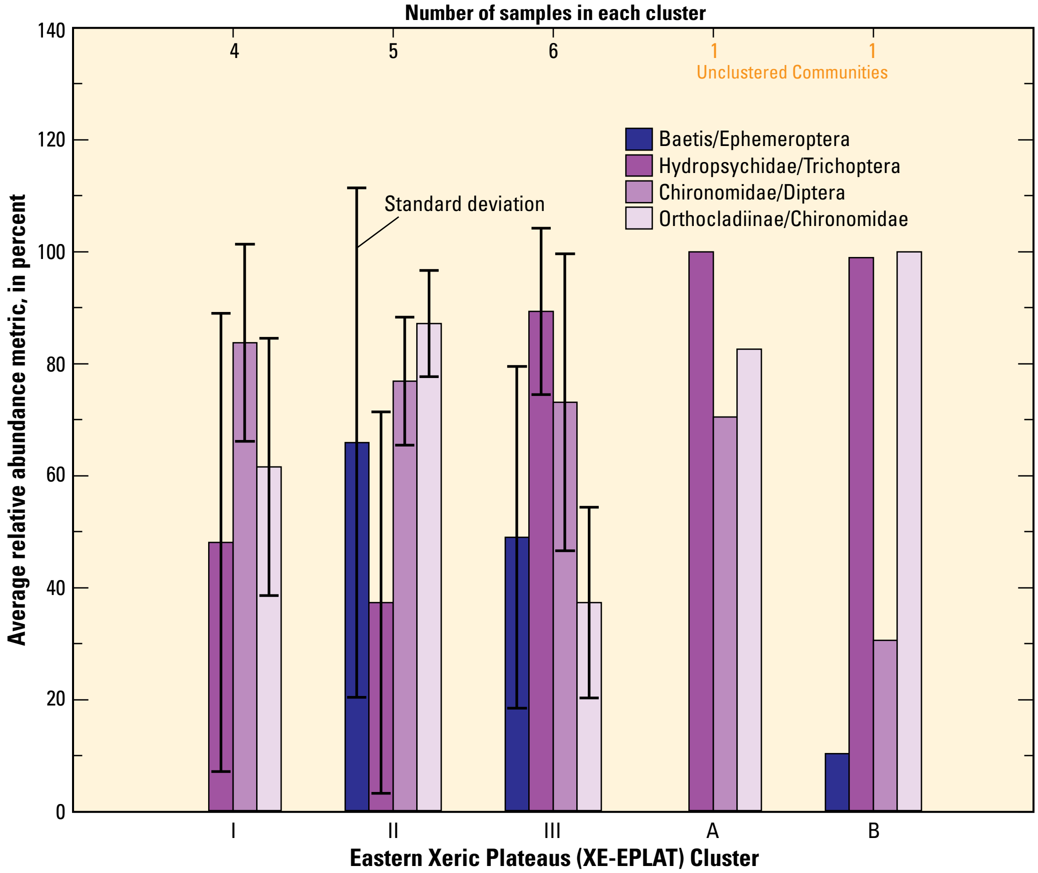

Figure 3-8. Average relative abundance of selected taxa to Ephemeroptera, Trichoptera, Diptera, and Chironomidae relative abundances in macroinvertebrate communities in selected streams of the Eastern Xeric Plateau (XE-EPLAT) ecoregion, 1992–2000. Taxa represented in this figure include rare and common taxa occurring at sites within these XE-EPLAT ecoregion streams. Clusters identified as I-UNCLUSTERED correspond to clusters of macroinvertebrate assemblages within the MDS ordination for this ecoregion (fig. 21). Clusters identified as "Unclustered Communities" did not group significantly during the cluster analysis.

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