Dispersion of Metals from Abandoned Mines and their Effects on Biota in the Methow River

Okanogan County, Washington

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Dispersion of Metals from Abandoned Mines and their Effects on Biota in the Methow River, Okanogan County, Washington

Dan Peplow and Robert Edmonds

Final Report

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Abstract

Dispersion of Metals from Abandoned Mines and their Effects on Biota in the Methow River, Okanogan County, Washington

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A study of mine-waste contamination effects on Methow River habitat on the eastern slopes of the north Cascade Mountains in Washington state, U.S.A., revealed impacts at ecosystem, community, population, individual, tissue, and cellular levels. Ore deposits in the area were mined for gold, silver, copper and zinc until the early 1950's, but the mines are now inactive. An above-and-below-mine approach was used to compare potentially impacted to control sites. The concentrations of eleven trace elements (i.e., Al, As, B, Ba, Cd, Cr, Cu, Mn, Pb, Se, and Zn) in Methow River sediments downstream from the abandoned mine sites were higher than background levels. Exposed trout and caddisfly larvae in the Methow River showed reduced growth compared to controls. Samples of liver from juvenile trout and small intestine from exposed caddisfly larvae were examined for evidence of metal accumulation, cytopathological change, and chemical toxicity. Morphological changes that are characteristic of nuclear apoptosis were observed in caddisfly small intestine columnar epithelial and trout liver nuclei where extensive chromatin condensation and margination was observed. Histopathological studies revealed glycogen

bodies were present in the cytosol and nuclei, which are indicators of Type IV Glycogen Storage Disease (GSD IV). This suggests food is being converted into glycogen and stored in the liver but the glycogen is not being converted back normally into glucose for distribution to other tissues in the body resulting in poor growth. Examination of trout hepatocytes by transmission electron microscopy revealed the accumulation of electron-dense granules in the mitochondrial matrix. Matrix granules contain mixtures of Cd, Cu, Au, Pb, Ni, and Ti. Contaminated sediments caused adverse biological effects at different levels of biological organization, from the cellular to ecosystem-level responses, even where dissolved metal concentrations in the corresponding surface water met water-quality criteria.

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CHAPTER 1

INTRODUCTION

Mining is one of the oldest activities of human civilization dating back to the Stone Age. Even in our modern society demands for mining products continue to grow. However, mining often occurs in environmentally sensitive areas where contamination from operating and abandoned mines can cause severe environmental impact. The apparent disregard for the environmental implications of mining has led the opponents of mining to characterize it as a 'robber economy'. Vernon LaMotte, the mining engineer at Alder Mine from 1939 to 1940 in the Methow Valley in Okanogan County, Washington, described mining as a "liquidation business." In his memoirs, *Stories of the Methow,* LaMotte noted that "mineral deposits are finite resources that, because they are non-renewable, come to an end when the deposit is exhausted." Mining also strongly influences local economies and when mines cease operation communities may die.

People were aware of the environmental effects of mining long before the 1960's when Rachel Carson's *Silent Spring* was published (Carson 1962), before strong environmental laws were put in place in the 1970s, and before the 1980's when analytical methods were developed to determine low concentrations of multiple metals in a single sample. For example, the response of aquatic organisms to toxic water pollution was recognized over 150 years ago (Davis, 1995), and the effect of heavy metals in streams on benthic invertebrates has been noted since the early 1900's (Carpenter 1924). Early research assessing the biological condition of rivers polluted by mine effluent started in 1919 in Wales concurrent with the cessation of lead (Pb) mining in the Aberystwyth district of Cardiganshire. Five years later it was reported that the river Ystwyth was generally barren, except for algae, in comparison to reference streams (Carpenter 1924). Newton (1943) also described the destructive effects of zinc (Zn) pollution from abandoned mines on the same river. Although metalliferous hardrock mines only operate

on average from 5 to 15 years until the minerals are depleted, contamination can occur for hundreds or even thousands of years following the cessation of mining. Contamination of the Rio Tinto estuary, for example, has occurred due to mining of in massive sulfide deposits that began with the Tartessans who developed the first mine over 5000 b.p. (Davis et al. 2000, Palanques et al. 1995).

Mining and ore processing change the topography, hydrogeology, and chemistry of terrestrial and aquatic ecosystems. In metalliferous mining, high volumes of waste are produced because of the low concentrations of metals in the ore. For example, gold (Au), which is commercially viable at less than one-half ounce per ton, creates a huge volume of mine waste when mined. Mine waste historically has been disposed of at the lowest cost by creating heaps of mine spoils on site. Depending on the mineral being mined, the parent rock, and mine spoil heaps can either be inert or contain hazardous constituents.

Pyrite oxidation, a major source of contamination from abandoned mines, is accelerated in mine spoils due to the increased access of air, smaller particle sizes resulting from the mining and milling process, the presence of iron-sulfide oxidizing bacteria, and the frequent saturation of mine spoils by infiltrating meteoric water. Acid mine drainage (AMD) is extremely acidic because pyrite oxidization gives rise to sulfuric acid. AMD accelerates weathering and when surface water infiltrates a mine spoil heap it can leach out soluble salts that contain toxic trace elements at concentrations as high as 0.1 mg L^{-1}.

Many communities of the West had their origins in the mining boom of the late 1880's. Although mining ceased to be a major economic force after the 1930's, the current potential for contamination of groundwater, and fish and wildlife habitats is forcing local residents and State and Federal regulatory officials to identify abandoned mine lands (AML), assess the risk associated with AML, and remediate sites that pose the greatest risk to environmental health. The precise number of AML sites and the scale of the environmental problem is unknown because a complete inventory, which would take years to complete, does not currently exist. In

the absence of a complete inventory, the USGS has implemented an AML initiative to coordinate activities for the cleanup of Federal Lands affected by AMD. The purpose of the AML initiative is to identify watersheds that would be at greatest risk of environmental degradation from AMD.

In Washington State, most AML occurs in environmentally sensitive areas of the Cascade Mountains and Okanogan Highlands of northeastern Washington. Since Hiram Smith, a Washington State legislator, discovered gold near Chopaka Mountain in Okanogan County in 1871, there have been thousands of prospects and mines established in Washington State. Most of the mines were small, unregulated and in operation prior to the 1930's.

Although not included in the AML, the Methow River watershed in the Okanogan County is a watershed at risk of environmental degradation from numerous abandoned mine sites including the Alder Mine, Alder Mill, and Red Shirt Mill, which contain tailings, waste-rock piles, and openings that discharge AMD (Peplow 1998). The contamination of a domestic well by waste water and tailings from the abandoned Alder Mill, which ceased operations in 1952, was confirmed by Spencer (1986), who noted a potential for groundwater contamination. Targets related to groundwater exposure pathways were documented, and a potentially affected human population, estimated to be between 1000 and 1287 residents, was identified within a 6.5-km (4-mile) radius of the Alder Mine and Alder Mill.

As well as the concern about the human population, there is also concern that stream invertebrates and fish could be influenced by AMD from the three sites. We conducted a survey by direct underwater observation (snorkeling) on September 4, 1998 to identify salmonids in the Methow River below the abandoned Alder Mine, Alder Mill and Red Shirt Mill. Spawning sites that occur at the junction of Alder Creek and the Methow River are monitored by the Yakima and Colville tribes and the Chelan County P.U.D. The species identified during the survey were native steelhead/rainbow (*Salmo gairdneri)* and *C*hinook salmon (*Oncorhynchyus tshawytscha)*. Upper Columbia River summer steelhead, including the

Methow river run, were listed under the Endangered Species Act (ESA) as "endangered" on August 18, 1997. Upper Columbia River spring Chinook salmon, including the Methow River run, were listed under the ESA as "Endangered" on March 16, 1999. Bull trout in the Methow River were listed under the ESA as "threatened" on June 10, 1998. Although not an ESA listed species, summer Chinook also spawn in the Methow River and have experienced a severe decline in numbers of returning adults. Summer Chinook are identified as "depressed" by the Washington Department of Fish and Wildlife.

In 1980, the Northwest Power Planning Council was directed by act of Congress to protect, mitigate and enhance fish and wildlife populations that have been affected by the construction and operation of hydroelectric dams. The NWPPC Fish and Wildlife program recognized the potential impact of abandoned mines on fish habitat and linked the Endangered Species Act and the Clean Water Act by describing the kind of ecological change needed to improve the survival and productivity of diverse fish and wildlife populations. To address this issue this study was conducted to determine the environmental effects of contaminants released from Alder Mill, Alder Mine and Red Shirt Mill on the Methow River ecosystem south of Twisp.

My main objectives were to determine: (1) concentrations of macroelements (Ca, K, P, Na, Mg, and S) and trace elements (As, Cd, Cr, Cu, Mn, Mo, Ni, Pb, Ti, and Zn) in ore, tailings, surface water, groundwater, and sediments in and near the Methow River, and if relationships exist between the presence of mining activities and the accumulation of trace elements at concentrations that are potentially harmful to human health and fish and wildlife habitats, (2) the ecological impact at different scales of biological organization (cellular, individual, population and community and ecosystem), (3) the adverse health risks to residents consuming groundwater contaminated with As and trace metals, and the degree of uncertainty associated with the assessed risk, and (4) evidence of trace element toxicity at the cellular level in caddisfly larvae and trout.

This dissertation is presented as a number of chapters. Chapter II contains descriptions of the three sites including locations, soils, vegetation, climate and sampling station locations. Chapter III characterizes the biogeochemistry of ore, tailings, groundwater, surface water and sediments at a number of sites. Data are presented to test the hypothesis that a causal relationship exists between the mining activities and the presence of trace elements at concentrations that are potentially harmful to human health and fish and wildlife habitat in the Methow River valley.

Chapter IV describes the impact of trace elements from abandoned mine waste on organisms at different levels of biological organization; cellular, tissue, individual, population, community, and ecosystem. Emphasis is on invertebrates and fish. The main hypothesis investigated was that the effects of contamination from abandoned mine waste occur at different levels of biological organization and there are potential indicators at each level. The cumulative interpretation of findings from each level was used to suggest a mechanistic linkage that integrates responses across levels of organization.

Chapter V covers an assessment of the risk of adverse health effects from As (arsenic) and trace metal contamination on residents consuming groundwater from wells adjacent to the abandoned mines. The degree of uncertainty associated with the assessed risk is also discussed.

In Chapter VI, We discuss the cytological evidence for trace element toxicity, including apoptosis and the presence of electron dense granules comprised of Pb, copper (Cu), titanium (Ti), Au, nickel (Ni), calcium (Ca) and iron (Fe) in the matrix of mitochondria from trout and caddisfly larvae exposed to contaminated sediments downstream from the abandoned mines .

Chapter VII discusses remediation options and Chapter VIII provides a synthesis of the research and its importance to our understanding of trace element contamination from abandoned mines on the Methow River.

Raw data from the analysis of water, soil, sediments, and biological samples are presented in appendices 1-17. The appendices contain raw data, QA/QC results and endangered fish distribution maps.

CHAPTER 2

STUDY SITES

Location

Study sites were located in the Methow River basin near the town of Twisp in Okanogan County, Washington (Figure 1). The Methow River basin is located in north central Washington east of the Cascade mountains and is bordered by Canada on the north. Draining nearly 4,662 km^2, the Methow River flows southward through western Okanogan County and empties into the Columbia River at km 843 near the town of Pateros (Andonaegui 2000). Three abandoned mine and mill sites are located south of Twisp near the Methow River: Alder Mill, Alder Mine, and Red Shirt Mine (Figures 1 and 2).

Alder Mill

The Alder Mill (48°.21'.13.5"N, 120°.07'.31.6"W, elev. 575 m) is located approximately 1.6 km south of Twisp (Figure 1) and approximately 500 m west of the Methow River at river mile (RM) 39.5 (63.4 km from the confluence of the Methow and Columbia rivers). The Mill consists of several buildings and two tailings impoundments. The impoundments contain approximately 55,845 cubic meters of material (Stewart 1995). Inputs and springs supplied by Alder Creek feed the upper impoundment creating a contaminated wetlands environment. Private residences with groundwater wells are located adjacent to the site (Figure 3).

Alder Mine

The Alder Mine (48°.19'.24.1"N, 120°.09'.38.4"W, elev. 1043 m) is an inactive mine located approximately 4.8 km southwest of Twisp (Figure 1). The site consists of an open adit on the north, an adit retention pond, an open pit, and waste rock dumps. The site is on the north slope of a north-trending ridge. Slopes at the site range from 50-80%. Estimates from aerial

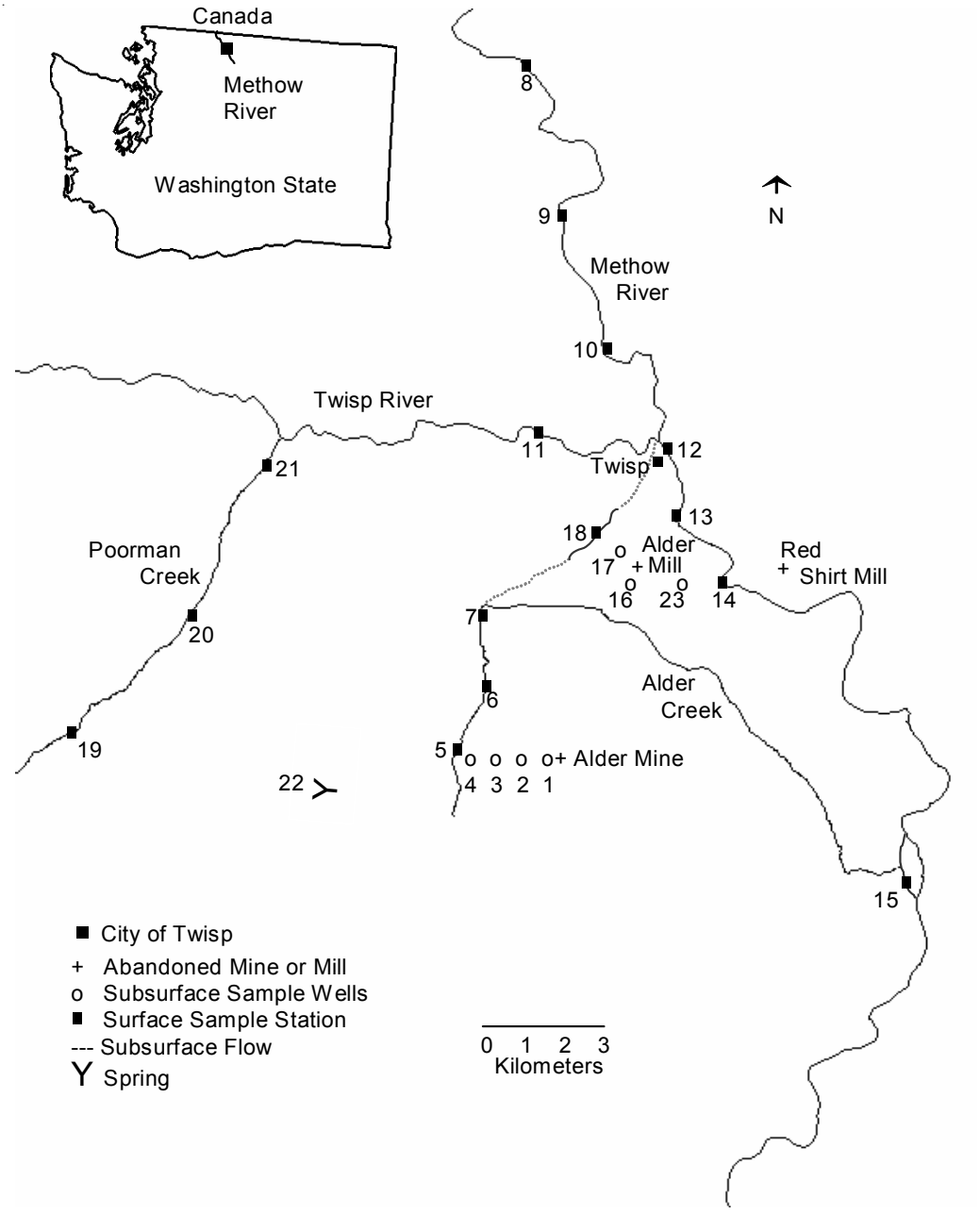


Figure 1. Map showing study area, abandoned mine and mill sites and sample stations.

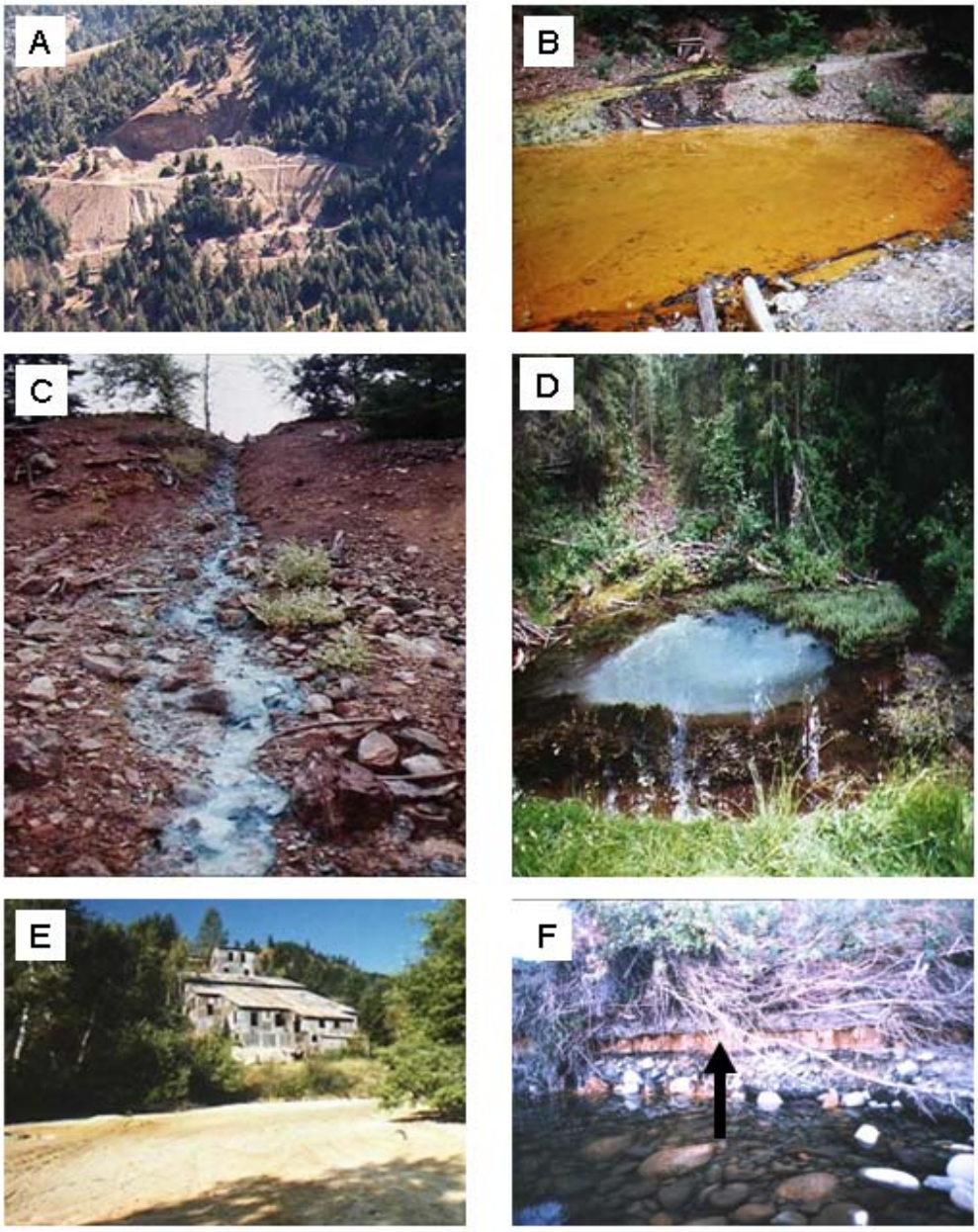


Figure 2. Disturbance at study sites and sources of contamination affecting environmental health. (A) Alder Mine from southeast showing glory hole at top and waste rock covering approximately 16 ha, (B) Acid mine drainage from adit at station 1 (Figure 1), (C) malachite precipitation in channel below waste rock pile, (D) Acid rock drainage entering Alder Creek at upwelling resulting in precipitation of Cu as malachite, (E) Alder Mill with tailings pile in foreground, and (F) Lens of tailings in bank of Methow River at the Red Shirt Mill.

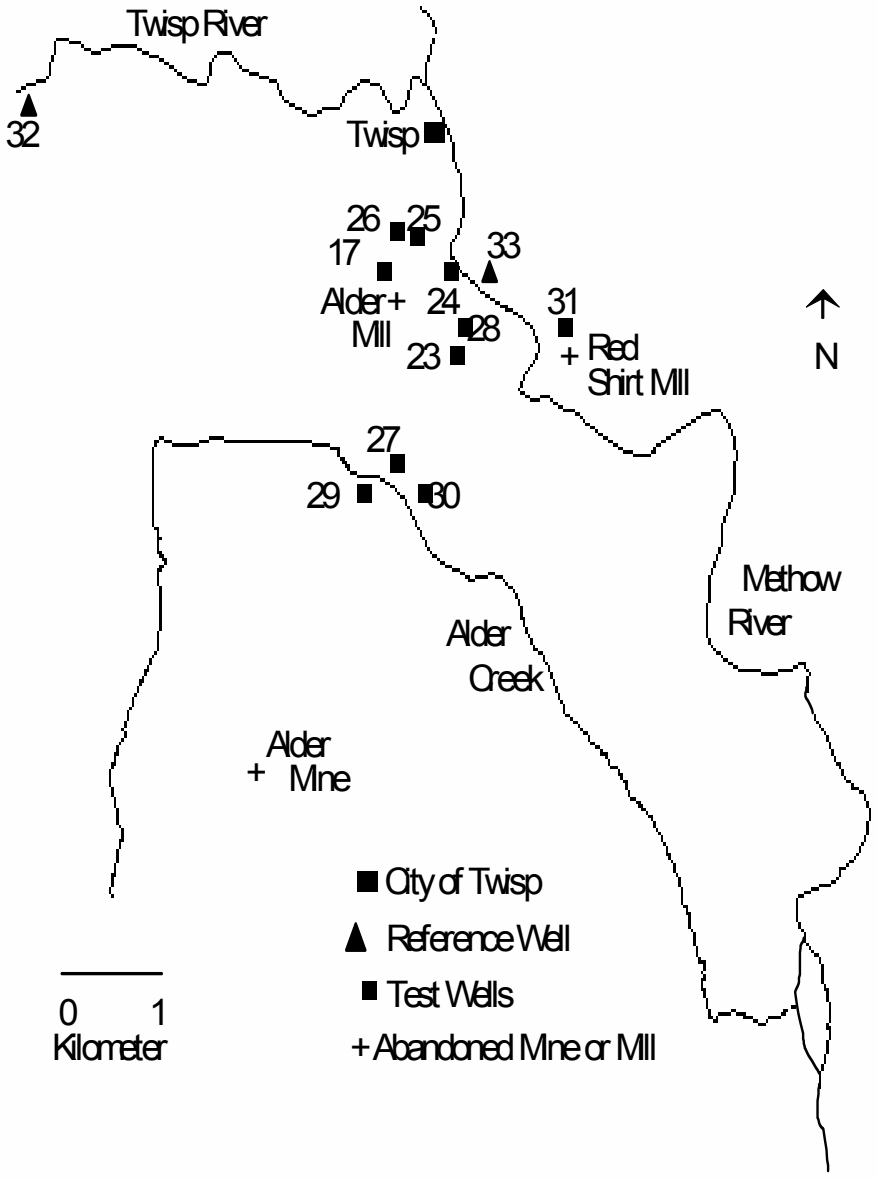


Figure 3. Map of study area showing location of domestic groundwater wells. Sample stations 17 and 23-31 were test wells and sample stations 32 and 33 were reference wells.

photographs indicate that waste rock covers approximately 3.2 ha (USEPA 2000). The flow rate of drainage from the north adit ranges from 5-15 L min^{-1}. The pH of AMD discharged at station 1 was 2.9 ± 0.2 and subsurface samples from stations 2-4 were $< 4.2 \pm 0.1$.

Red Shirt Mill

The Red Shirt Mill (48°.21'.05.0"N, 120°.06'.08.1"W, elev. 487 m) is located approximately 1.6 km southeast of Twisp (Figure 1) and east of the Methow River at RM 39.5 (63.4 km from the confluence of the Methow and Columbia rivers). The mill consists of a single building and a tailings pile. The tailings pile is estimated to cover approximately 4,650 m^2 of surface area and contains less than 30,600 m^2 of material. Approximately 4 m^3 of tailings are recruited annually by the Methow River. The site is located adjacent to the Twisp city limits and residences with private groundwater wells are located on and adjacent to the site.

Topography, Soils, Climate, Hydrology and Vegetation

Topography within the Methow River basin ranges from mountainous terrain along the Cascade Crest to a gently sloping, wide valley found along the middle reaches. Elevation ranges from 2600 m in the headwaters of the basin to approximately 240 m at the confluence of the Methow and Columbia Rivers (Andonaegui 2000). Soils in the valley consist of sandy loams that are underlain by alluvium and glacial outwash with very rapid permeability (Waitt 1972). The major groundwater aquifers of the Methow Valley exist in layers of unconsolidated sediments underlain by bedrock. Groundwater occurrence, movement and availability are primarily related to recharge sources and the configuration of depositional sediments.

The climate in the Methow Valley is within the Cascade Mountain rain shadow. Mean annual precipitation ranges from 25 to 38 cm and the mean annual temperature is below 10°C (USFS 1999). Precipitation is seasonal with roughly two-thirds occurring between October and March. Summers are generally hot and are characterized by extended dry periods. Precipitation

increases in the fall and generally peaks in the winter with most precipitation in the basin occurring as snow between December and February. Since most of the precipitation occurs as snow, the seasonal distribution of runoff is strongly affected by snow storage.

Flows in the Methow River exhibit a strong peak during spring and early summer with roughly 60 percent of the mean annual discharge occurring during May and June (Milhous 1976). Streamflow remains relatively high during July, but decreases substantially from August through October in response to a reduced snowpack, low precipitation, and decreased soil moisture. Streamflow in the Methow River at Pateros generally reaches an annual low during late September and early October, with some sections going subsurface during dry years. Winter flows typically remain low in response to low autumn precipitation and freezing winter temperatures. Runoff between years is also highly variable as reflected in streamflow data from USGS (1996). Maximum and minimum flows for the Methow River at Twisp was 40,800 cfs (May 1948) and 134 cfs (September 1926), respectively.

Vegetation at the site is characterized by Douglas-fir (*Pseudotsuga menziesii*) and ponderosa pine (*Pinus ponderosa)*, which dominate the overstory. Pinegrass (*Calamagrostis rubescens*) dominates the understory to the extent that other species are inconspicuous. Shrubs are normally a minor component of the stand. Soil texture is sandy loam to sand and the parent material is granitic rock. Soils are podzolic. Slope position is mid to lower one-third at approximately 30% with a western aspect.

Aquatic and Terrestrial Vertebrates

The stream and rivers in the area are utilized by resident trout and salmon for habitat and spawning. Beaver ponds and cattail marshes provide nesting sites for waterfowl, game and songbirds. Endangered and threatened species of juvenile salmonids, including bull trout, steelhead, and chinook salmon, use the Methow River and Alder Creek as rearing habitat. Two amphibians, the Pacific Treefrog (*Hyla regilla*) and the Spotted Frog (*Rana pretiosa*) were

observed in the study area. Blackbear (*Ursus americanus*), muledeer (*Odocoileus hemionus*), snowshoe rabbits (*Lepus americanus)* and the bobcat (*Felis americanus*) were also observed in the study area. Unidentified species of bats have also been observed leaving the mine adits.

Geology and Ore Deposits

The massive sulfide ore deposits that were mined for Au, Ag, Cu and Zn are composed largely of chemically precipitated silica in a 4.6-22.9 m wide zone of Cretaceous-Jurassic plutonic (intrusive) igneous stock (granite) in the Newby Group of volcanic rocks (Barksdale 1975). The Newby Group was intruded by the Alder Creek stock, which has been dated at 137 ± 3 m.y. (Burnet 1976, Bunning 1990). Ore minerals were deposited possibly during the emplacement of the Alder stock (Barksdale 1975). Carbonate rocks are found in the drainage basin and the streams and rivers are thus hardwater in nature. There is extensive faulting and calcite-filled fractures in the area where the abandoned mine sites in this study are located (Figure 4). Alkalinity of the Methow River is 103 ± 14 mg L^{-1} and the pH is 7.2 ± 0.5, which is typical of a system dominated by bicarbonate (Stumm and Morgan 1996).

The major component of a tailings sample taken 200 cm below the surface, based on the results from wave dispersive analysis by X-rays (microprobe), showed that plagioclase feldspars were the most common at 26% (Table 1). Minor components were quartz at 14%; chlorite at 12%; and hornblende, magnetite/hematite, muscovite, zircone, and chlinopyroxine at 5-6%. Numerous minerals including ilmenite occurred at levels <1.

The major component of sand and silt fractions of Methow River sediments at RM 40, was plagioclase feldspars at 26% (Table 2). Minor components were quartz at 14%; chlorite at 12%; and hornblende, magnetite/hematite, muscovite, zircone, and chlinopyroxine at 5-6%. Trace minerals include apatite, epidote, and ilmenite.

Figure 4. Calcite-filled fractures in bedrock below Alder Mill and near Methow River site 13.

Table 1. Mineralogy of Alder Mill tailings sample at 200 cm by microprobe analysis.

Mineral	Occurrence (%)
Quartz	80
6% of quartz with Fe coating	
1% of quartz with Fe + Zn or Cu coating	
Barite	4
Pyrite	3
Plagioclase Feldspar	2
Chalcopyrite embedded in quartz	1
Jarosite	1
Apatite	<1
Chlinopyroxine	<1
Chlorite	<1
Epidote	<1
Ilminite	<1
Tephra – Glacier Peak	<1
Mica	<1

Table 2. Methow River sand and silt mineralogy by microprobe analysis.

Mineral	Occurrence (%)
Plagioclase	26
K-Feldspar	6
Quartz	14
Chlorite	12
Hornblende	6
Magnetite/Hematite	6
Muscovite	6
Zircon	5
Chlinopyroxine	5
Apatite	3
Epidote	3
Ilmenite	3
Glass	2
Tephra (Altered Volcanic Fragment)	2
Oxides: Fe, Ti, Rare Earth (La Series)	2

CHAPTER 3

THE CAUSAL RELATIONSHIP BETWEEN MINE WASTE CONTAMINATION AND ENVIRONMENTAL RISK TO AQUATIC AND TERRESTRIAL HABITATS IN THE METHOW RIVER VALLEY

INTRODUCTION

Hardrock mining for metals, such as Au, Ag, Cu, Pb, and Zn, causes considerable environmental damage due to acid mine drainage (AMD) discharged from mine adits and acid rock drainage (ARD) from mine waste and mill tailings piles, even after the cessation of mining activities. Five years after Pb mining activities were ceased near the River Ystwyth in Wales the river was reported to be generally barren, except for algae, in comparison to reference streams (Carpenter 1924). While individual mines are typically in operation only for 5 to 15 years, contamination can occur for hundreds or even thousands of years following the cessation of mining (Davis et al. 2000, Palanques et al. 1995).

Many thousands of small unregulated prospects and mines were established in Washington State starting in the 1870's. Most ore deposits were mined for Au, Ag, Cu, and Zn until the early 1950's. The Methow River watershed in Okanogan County is a watershed at risk of environmental degradation from three sites, the Alder Mine, Alder Mill, and Red Shirt Mill, which contain tailings, waste rock piles, and openings that disperse AMD and ARD that contain heavy metals (Peplow 1998). Acid mine drainage and ARD can accelerate weathering and leach out soluble salts and toxic trace elements at concentrations as high as 0.1 mg L^{-1} (Plumlee et al. 1999). The contamination of a domestic well by waste water and tailings from the abandoned Alder Mill was confirmed by Spencer (1986), who noted a potential for contamination of other drinking water wells in the vicinity. As well as the concern about the human population, there is also concern that stream invertebrates and endangered fish in the Methow River could be influenced by AMD from the three sites.

High levels of trace elements can also occur naturally in groundwater, streamwater and sediments and a number of studies have attempted to separate natural from man-caused loading (Nimick and von Guerard 1998). Isotopes and tracers have been used to try to identify natural sources and loading caused by leaching from unmined ore bodies. However, to date there has been no reliable technique developed to clearly separate them (Dissmeyer 2000).

Thus, epidemiological criteria must be used to determine whether high metal loadings in the vicinity of an abandoned mine cause an observed environmental effect. Susser (1986) and Fox (1991) suggested that the following epidemiological criteria be used to establish a cause-effect relationship: (1) time order [i.e., did cause precede effect in time], (2) strength of association [i.e., what was degree or size of an effect when exposed to a cause], (3) consistency of replication [i.e., has the association been repeatedly observed by different persons, in different places, circumstances, and times], (4) specificity [i.e., does cause lead to only one effect or is effect caused only by one cause], and (5) coherence [i.e., the cause-effect hypothesis should not conflict with generally known facts].

The objectives of this study were to (1) characterize the biogeochemistry of ore, tailings, groundwater, surface water and sediments in the vicinity of the Alder Mine, Alder Mill, and Red Shirt Mill, including the Methow River, and (2) test the hypothesis that a causal relationship exists between the presence of mining activities and the accumulation of trace elements at concentrations that are potentially harmful to fish and wildlife habitats and human health in the Methow River valley.

METHODS

Sampling and Analysis at the Alder Mine:

The location of sampling stations are shown in Figure 1 (Chapter 2). Five rock samples from diverse locations within the Alder Mine waste rock pile were collected for analysis. After the samples were crushed, a piece from the center of each rock was selected and pulverized.

Trace element analysis by both ICP-AES and sequential analysis (Tessier et al. 1979) were performed.

Samples of acid mine drainage (AMD) were collected at station 1 once monthly from May to December 2001. From January to April sample collection at station 1 was limited by poor accessibility due to adverse weather. Samples of acid rock drainage (ARD) were also collected. Zero-tension lysimeters were constructed from 3.5 x 90-cm PVC pipe with 0.625-cm holes drilled in a longitudinal row at the bottom 50-cm of the lysimeter. Three lysimeters were installed prior to ground freeze and snowfall in August 2000 along a vertical transect near the center of the three largest waste piles at Alder Mine, at locations that did not show signs of erosion. The lysimeters were installed using a steel drive-point with a diameter equal to the inside diameter of the PVC. One end of the drive-point had a cap used to drive the lysimeter into the waste rock. The end of the drive point extended 5-cm and was tapered to a point. When completely inserted the drive-point was removed leaving the lysimeter in place with the holes oriented upwards. The lysimeters were also inserted so they sloped out at an angle of 2° and were capped to retain acid rock drainage (ARD). ARD samples were collected as soon as the site was accessible (and while still frozen) in the spring (April 2001), by removing the PVC cap and collecting the leachate in a water sample container.

Sodium chloride (NaCl) injections and geogenic sulfate (SO_4) were used to determine the proportion of AMD entering Alder Creek from the adit as Station 1 (Figure 1) and subsurface sources. In this study, chloride was injected at Station 1 and sulfate, produced geogenically from sulfide mineral oxidation, was used to indicate AMD contamination. Both chloride and sulfate were assumed to be conservative (Stream Solute Workshop 1990, Raforth et al. 2000). Although chloride and sulfate concentrations can decline either by dilution, adsorption or absorption, the assumption that chloride and sulfate are conservative suggests that the sulfate to chloride ratio would remain constant unless additional inputs of contaminated groundwater occurred. Additional inputs of AMD would cause the SO_4 to Cl ratio to increase.

Sulfate concentration was measured at all surface and groundwater stations and gradients from Alder Mine to the Methow River were measured.

Solute was injected into the AMD discharge stream at Station 1. AMD flow was diverted to a 227-L plastic reservoir containing 68 kg NaCl, which was dissolved to a final concentration of 300 g L^{-1} (180 g Cl L^{-1}). Samples were taken from a hand driven 3.8 cm (1.5 inch) PVC well driven to the bedrock at stations 2 and 3 (Figure 1) and from a similar well at the end of the subsurface AMD flow path, 3 m prior to its confluence with Alder Creek (Station 4). Water samples for chloride analysis were stored at ambient temperature for less than four hours in the field then refrigerated in the lab for less than four weeks until analyzed. Conservative solute transport was monitored using chloride and sulfate analysis of water samples by ion chromatography (IC) and in situ electrical conductivity readings.

Alder Creek Water and Sediment Samples

Surface water and sediment samples were collected in triplicate during high- and low-flow conditions between June and September 1998. Sample stations included four stations (5-7 and 15) on Alder Creek and three reference sample stations (19-21) on Poorman Creek (Figure 1). Station 5 was located directly below mine outfall and stations (5, 6 and 7) were spaced approximately 0.5 km apart below the mine. Station 15 was in a side channel of the Methow River below the confluence of Alder Creek . All chemical analyses for trace elements were performed by ICP-AES at the University of Washington, College of Forest Resources laboratory.

Surface water and sediment samples for chemical analysis were collected in triplicate near high- and low-flow conditions between June and September 1998 at each of the four sample stations on Alder Creek and at the Poorman Creek reference stations.

A stream sediment core was taken in an undisturbed depositional pool of Alder Creek at station 22 (Figure 1) following the method described by Church (1993). Cores were taken

using acid-cleaned, 5 cm (2 in) diameter, PVC tubing that was driven into the ground using an eight-pound sledge hammer. The upper end of the core tube was sealed with plastic wrap and a PVC cap. The core was then removed and the base of the core tube sealed as above. The cores were then transported to the laboratory for further processing and sampling. In the laboratory, the cores were extruded using a solid plastic piston. The sediment cores were then sectioned at 2 cm intervals using a plastic knife. The concentrations of Cu and Zn were determined by ICP-AES and normalized to Al concentrations and plotted as a function of depth.

Red Shirt Mill:

Seven samples of tailings material were collected and analyzed for metals by ion coupled plasma - atomic emission spectrophotometry (ICP-AES). Four samples were collected at a depth of approximately 25 cm from sites located approximately 25 m north, south, east and west of a point at the center of the tailings pile. Three samples were also collected from the lens face of the tailings material exposed in the bank of the Methow River.

Sampling and Analysis at the Alder Mill:

The walls of a bore hole in the tailings impoundment at station 16 were sampled at 25 cm intervals and mineralogy was determined using SEM-EDS. A polyvinyl chloride (PVC) monitoring well was installed to the saturated zone of the tailings impoundment at station 16 in October 2000 and water from the saturated zone of the tailings impoundment was sampled monthly between October and December 2000 for metals. Trace metal and As concentrations were determined by ICP-AES and HG-AFS, respectively. The relative proportion of hornblende, as well as the surface etching features, were used to determine the weathering of minerals under acidic soil conditions (Lang 2000).

Methow River Water and Sediment Sampling:

Water and sediments were sampled at each sample station along the mainstem of the Methow and Twisp Rivers (Figure 1). Background levels were estimated based on the average of samples from each of the four stations on the Methow River above Twisp (Figure 1, stations 8 -11). At least four replicates were collected at each sample station. Additional samples were collected in the vicinity of stations 12-15 to provide better resolution of downstream impacts.

Groundwater Sampling

Ten domestic drinking water wells located adjacent to the Alder Mill, near Alder Creek below Alder Mine, and adjacent to the Red Shirt Mill, and two reference wells that were isolated from mine impacts (Figure 2) were sampled monthly between October 1999 and June 2001. Samples of water from private domestic wells were collected from the well casing using disposable Teflon bailers in pre-cleaned 50-mL polypropylene centrifuge tubes.

General Field Methods

Sediment samples were collected using plastic scoops at a shallow depth (<5 cm) and immediately wet sieved in ambient water through a 63 μm sieve. Samples were dried to constant weight at 90°C. All water and sediment sampling equipment was cleaned by washing with Liquinox detergent and sequential rinses with distilled water, dilute nitric acid, and de-ionized water.

Water samples were collected in pre-cleaned Teflon bottles. Subsamples were filtered (Gelman 0.45 mm, disposable 25 mm sterile disposable Acrodisc filter) for determination of dissolved metal concentrations. All water samples for metals analysis were preserved to pH<2 with 0.15% nitric acid and stored at less than 5°C. Samples for As and sulfate analyses were frozen until analyzed. All analyses were performed within 30 days of sample collection. The determination of ferrous iron in water was made using the spectrophotometric reagent Ferrozine

[3-(2-Pyridyl)-5,6-bis(4-phenylsulfonic acid)-1,2,4-triazine Monosodium Salt, Sigma-Aldrich).

General Laboratory Analytical Methods

Samples of water and sediment were analyzed at the University of Washington, College of Forest Resources Analytical Laboratory in Seattle, Washington. Dissolved metal concentrations (0.45mm filtered) were determined by ICP atomic emission spectrophotometry (ICP-AES; Thermo Jarrell Ash ICAP 61E, EPA Method 3050). Ion chromatography (Dionex DX120) was used for the analysis of sulfate. Samples were analyzed for As by Hydride Generated Atomic Fluorescence Spectrophotometry (HG-AFS) (Corns et al., 1993).

The sequential extraction procedure used was a modified version of the Tessier et al. (1979) method. Sequential chemical extractions were performed on 1 g samples using 40 g of the following six solutions and conditions: (1) 0.1 M $Ca(NO_3)_2$ + 0.05 M $AgNO_3$ for 16 h (exchangeable), (2) 1M NaCHCOO at pH 5 for 5 h (carbonates), (3) 0.1 M $NH_2)OH•HCl$ + 0.1M HNO_3 for 30 min (Mn oxides), (4) 0.1M $Na_4P_2O_7$ for 24 h (organics), (5) 0.4M $NH_2OH•HCl$ in 25% v/v CH_3COOH and mix periodically for 6 h in a boiling water-bath (Fe oxides), and (6) concentrated $HNO_3/HClO_4$ using the method for total (residual). Solutions were centrifuged at about 4000 x g for 20 min and decanted. Samples were washed with 40 g of 0.025 M $Ca(NO_3)_2$ for 5 min after extracting fractions 1 to 5. Washings were discarded. Metals that reside mostly in the exchangeable and carbonate fractions are of greatest hazard compared to those that reside primarily in the Mn-oxide, organic, Fe-oxide and residual fractions and were referred to a s available elements.

Data Analyses:

Results were evaluated using five epidemiological criteria that are applicable to ecological studies for the demonstration of causality (Susser 1986 and Fox 1991). These criteria were used to determine whether there is a causal relationship between the presence of

mining activities and the accumulation of trace elements in water and sediments at concentrations that are potentially harmful to human health and fish and wildlife habitat of the Methow River. There are two properties that determine cause and effect. One is time order in which cause precedes effect. The other is direction in which cause must lead to effect. While time order is a criterion onto itself direction is further subdivided into four criterion including strength, consistency, specificity, and coherence. A causal hypothesis (H_a) can be rejected with confidence by only three of the criteria discussed (time order, consistency, and factual incompatibility or incoherence) and strongly affirmed by three criteria (strength of association, consistency, and coherence).

The first criterion is time order. If the causal variable precedes the outcome or nothing can be said about time order, then causality is indeterminate and the other criteria need to be considered. If, however, the effect precedes cause then time order is the most decisive criterion for the rejection of the cause-effect hypothesis (reject H_a).

The second criterion is strength of association, which is the degree to which cause and outcome coincide in their distribution. Chemicals of potential ecological concern (COPEC) were identified by comparing the concentrations of metals to ecotoxicological benchmarks derived from primary literature by Efroymson et al. (1997) and Will and Suter (1994) for the effects of soil contamination on plants and soil microbial processes, Opresko et al. (1994) for the exposure of wildlife to AMD in water, Washington State groundwater criteria (WAC 173-200) for subsurface water (ARD and groundwater), and Hull and Suter (1994) for the exposure of benthic organisms to sediments.

The 90th percentile was selected and used as the default value for background calculations (San Juan 1994). When comparing data sets, the 95% upper confidence limit (UCL) of the contaminated-site data set was compared to the 90th percentile of the background data set. Washington State Department of Ecology's MTCAStat 97 Background Module was used to calculate the 90th percentile values and estimate background trace element

concentrations. The MTCAStat Site Module was used to calculate the 95% Upper Control Limit (UCL).

Statistical significance alone, however, is not a good indication of strength of association because weak associations can be highly significant and large sample numbers can make small differences highly significant. For this reason ratios of relative concentrations that compare minor element concentrations in soil, water or sediments to background or reference levels, referred to as concentration factors (CF), were used as indicators of relative risk. Where estimated background or reference concentrations were zero the elements nominal concentration was used as the CF. Tailings, AMD and ARD were considered sources. Groundwater and river sediments were considered sinks. Strength of association will be evaluated only when the mean concentration of a trace element exceeds benchmark values in both a source and a sink and the corresponding 95% UCL exceeds estimated background concentrations. To evaluate strength of association it is assumed that the larger the CF the greater the relative risk. Also, the stronger the association the more it supports causation (Ha). The weaker the association the more indeterminate it is.

The third criterion is specificity. The ideal is a one-to-one relationship in which a cause has only one known effect and an effect has only one cause. Specificity in the cause of an effect is the most persuasive and the specificity in the effects of a cause is less persuasive. The closer an association is to a one-to-one relationship the more specific it is. Specificity supports Ha, but the absence of specificity is indeterminate and does not support Ho , i.e., if the composition of the AMD is also seen in natural drainage waters then specificity is low and specificity would be considered indeterminate. Specificity in the effects of a cause will be determined by evaluating drainage water, groundwater and stream sediment data from mine-impacted sites in Washington and elsewhere.

Fourth is consistency of results upon replication. The alternative to exact replication is the consistency of a result with other studies that are characterized by a diversity of times,

places, circumstances, people and research designs. In order to minimize inconsistencies among studies used for comparison, only those conducted in areas with similar geologic, climatic, and hydrologic characteristics were considered. Consistency supports causation (Ha), which will be determined by comparing data from studies at similar sites.

The fifth criterion is coherence. The four elements to establish coherence were whether results agreed with general theory, existing facts, biogeochemical principles, and expected concentration gradients that declined with distance away from the source. Results that are in coherence with these factors support causation (Ha). Results that are implausible do not support the null hypothesis (Ho). Instead, it provides grounds to reject the study result.

Quality Assurance/Quality Control

Selection of study sites and sample stations depended on the goals and objectives of the study. Access, location of contaminant sources, mixing zones, and dilution of pollutants were considered. Reference sites were selected that were as similar as possible to the study sites. After obtaining measurements for in situ parameters, samples were collected for water-quality analyses in the order of the parameters' decreasing sensitivity. Deionized water was exposed to the sampling equipment and added to sample containers containing preservative. Field blanks and spikes were prepared in the field under the same conditions as field samples. Samples of water with known amounts of metals were submitted with test samples.

RESULTS

Table 3 lists the eleven minor elements that are Compounds of Potential Ecological Concern (COPEC) based on the comparison of the mean concentrations of the elements to toxicological benchmarks. Minor elements in tailings, AMD and ARD, which are the suspected sources of mine waste contamination, and in Methow River sediment, which is a potential sink for minor element contamination, were compared to benchmarks for toxic effects of minor

Table 3. Mean trace element concentrations in tailings (Alder and Red Shirt Mills), acid mine drainage (Alder Mine at station 1), acid rock drainage (Alder Mine), groundwater and Methow River sediments compared to benchmark values for contaminants of potential concern (COPEC) for plants, invertebrates, wildlife, and soil heterotrophic processes.

	(mg kg^{-1})			(mg L^{-1})	
COPEC	Tailings Mean Concnetration	Plant Benchmark	Microbial Benchmark	AMD Mean Concentration	Wildlife Benchmark
Al	1145	50	600	17	3.8
As	130	10	100	0.2	0.1
B	118	0.5	20	0.3	0.3
Ba	2176	500	3000	0	17
Cd	23	4	20	3	4
Cr	10	1	10	0	4
Cu	2539	100	100	13	65
Mn	192	500	100	9	377
Pb	360	50	900	0.5	4.9
Se	1591	1	198	1.1	0.9
Zn	448	50	100	198	62

(continued next page)

Table 3. (Continued).

	(mg L^{-1})			(mg Kg^{-1})	
COPEC	ARD Mean Concentration	Groundwater Mean Concentration	Groundwater Criteria	Sediment Mean Concentration Stations 12-15	Aquatic Biota Benchmark
Al	815	92	NA	17249	58030
As	33	77	0.05	7	12
B	0	585	NA	5	NA
Ba	43	16	1000	146	NA
Cd	9	3	10	9	1
Cr	10	13	50	35	56
Cu	737	22	1000	146	28
Mn	1078	27	50	580	460
Pb	0	47	50	43	34
Se	0	93	10	429	NA
Zn	2009	136	5000	107	159

elements on plants, soil heterotrophic processes, wildlife, human health and aquatic biota.

In mine tailings, metal concentrations were compared to soil benchmarks that were derived from toxicity studies conducted on plants in the field. This comparison identified 11 COPEC. Eight of the 11 COPEC that exceeded the plant toxicity benchmarks also exceeded benchmarks for soil heterotrophic processes. Only five minor elements (Al, As, Se, Mn, and Zn) in AMD exceeded wildlife benchmarks and three minor elements in ARD exceeded benchmarks. Two COPEC identified in groundwater samples were As and Se, which are both metalloids and typically exist primarily as oxyanions under aerobic conditions. All other COPEC were simple metal cations. In the Methow River, dissolved metal concentrations were less than the limits of detection by ICP-AES but in the sediments, 4 trace elements (i.e., Cd, Cu, Mn, and Pb) exceeded toxicity benchmarks for aquatic biota. While all four of these elements are COPEC in both the tailings (a presumed source) and the sediments (a presumed sink), only Mn is a COPEC in ARD.

The data are presented with respect to the epidemiological criteria related to causality, time order; time order, strength of association, specificity, consistency of replication, and coherence with fact and theory.

Time Order

To determine whether the dissolution of metal sulfides associated with mining preceded the enrichment of stream sediments with trace elements, We measured Zn and Cu concentrations in a sediment core at 2 cm increments. When normalized to Al to correct for variations in organic carbon with depth it was apparent that there were two periods of accumulation. In the earlier period that corresponded to fractions below 14-20 cm, Zn and Cu accumulation appeared lower than in the upper portions of the core that correspond to more recent periods of deposition (Figure 5). These data serve as a record of temporal changes in trace element deposition and show that relatively low background metal accumulation rates

were followed in time by an increase in Cu and Zn contamination.

Strength of Association

Screening minor elements by comparing concentrations measured in different media to benchmarks only identifies elements that are of potential concern. More analysis is needed to determine the strength of association between the measured concentration of trace elements derived from sulfide mineral oxidation and historical mining activities. To determine whether the concentrations of COPEC were significantly different from estimated background concentrations, the 95% UCL of the site data set was compared to the 90th percentile of the background data set and strength of association was determined based on the concentration factor (CF), which is the relative concentrations of trace elements in soil, water and sediments to background or reference levels.

Only three trace elements (i.e., As, Cu and Mn) were detected in both a source (i.e., tailings, AMD or ARD) and a sink (i.e., groundwater or river sediments) at concentrations that exceeded both estimated background concentrations and benchmark values for toxicity. The mean concentration of As in tailings was 130 mg kg^{-1} exceeded both plant and microbial benchmarks for soil. The mean concentration of As in groundwater was 77 μg L^{-1}, which exceeds groundwater criteria by a factor of 98. Copper was a COPEC in tailings with a CF equal to 48 and a mean concentration equal to 2539 mg kg^{-1}. It is also a contaminant in Methow River sediments downstream from the mines with a mean concentration of 146 mg kg^{-1} and a CF equal to 7.

In tailings, the 95% UCL of three of the minor elements that exceeded plant and soil microbial benchmarks did not exceed the estimated background concentrations (Table 4). The remaining eight were all present at concentrations that were greater than background by at least one order of magnitude.

In AMD, three of the five COPECs that were identified exceeded background

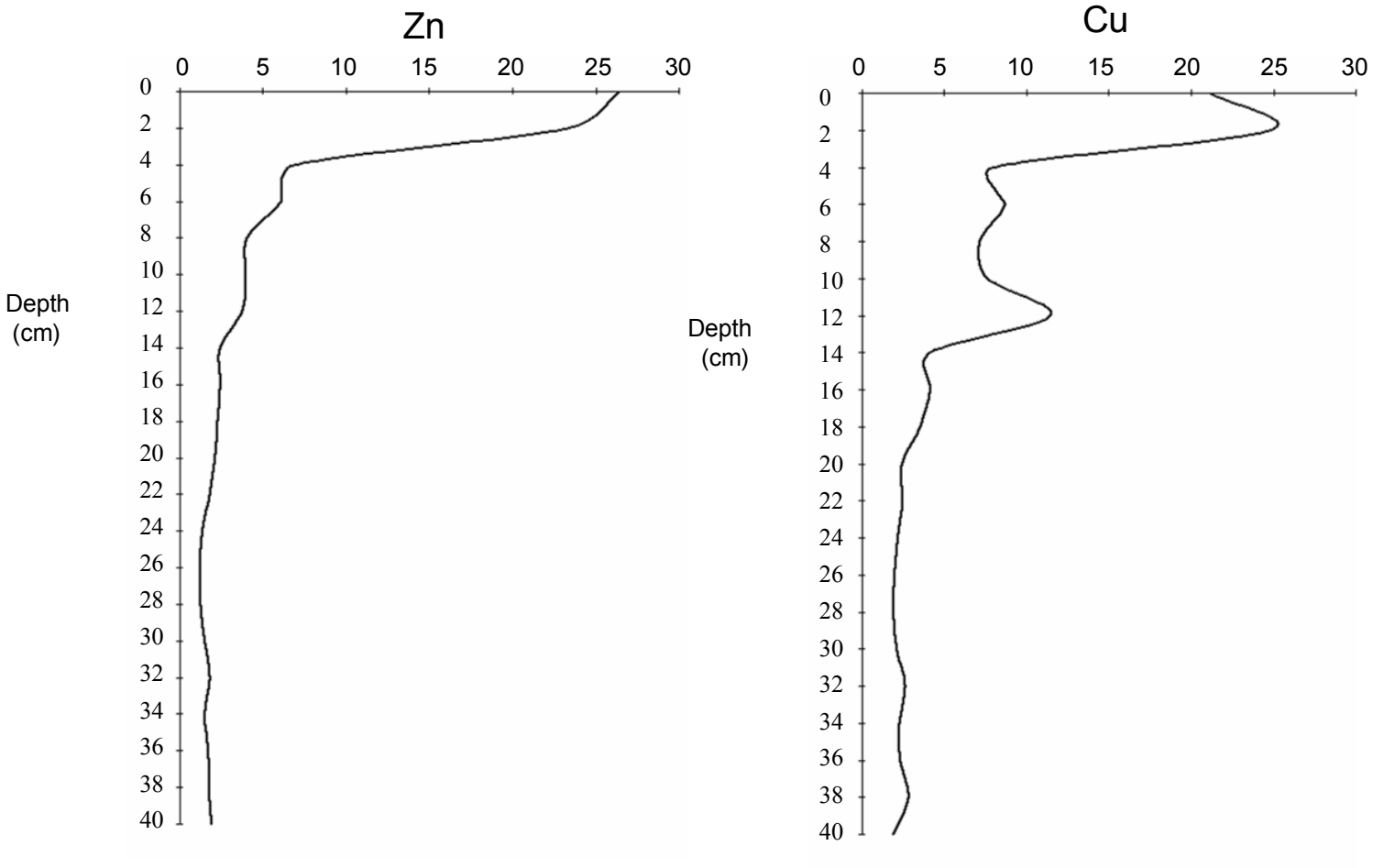


Figure 5. Zinc and Cu concentrations in sediment core from Alder Creek at 2 cm increments. Concentrations were normalized to Al to compensate for changes in organic matter content with depth.

concentrations. The estimated background concentrations for all of the dissolved trace elements were zero in the reference samples and so even Se with a concentration of 1 mg L^{-1} had a nominal CF equal to 1. Aluminum and Zn exceeded background concentrations with concentration factors equal to 20 and 227, respectively. Other compounds that were not identified as COPEC but were present at concentrations significantly higher than background concentrations are Pb, Cd, and Cu with concentration factors equal to 1, 3 and 16 respectively.

In ARD, Al and Mn were COPEC that exceeded background concentrations by factors of 4 and 146 respectively. As in AMD, however, Cu and Zn were not identified as COPEC but were present in higher concentrations relative to background with CF equal to 60 and 2009, respectively. Two COPEC in groundwater (i.e., As and B) exceeded background concentrations but Se did not. The trace elements Al, Cd, Cu, and Pb were also present at concentrations that exceeded estimated background concentrations with CFs equal to 113, 4, 4, and 2, respectively.

Finally, in Methow River sediments, two of three COPEC identified based on concentrations that exceeded benchmarks for aquatic organisms also exceeded estimated background concentrations. The CF was greatest for Cu at 7. Manganese was concentrated over background by a factor of 2. Al (CF = 2), As (CF = 9), and Zn CF = 2) were not identified as COPEC based on sediment concentrations relative to benchmark values for aquatic biota.

Specificity

In Figures 6-8, the median and range for As, Cu and Mn concentrations in AMD, tailings seeps, groundwater, and river sediments are plotted for comparison to data sets from mineralized areas not impacted by mining. Arsenic, Cu, and Mn were skewed to the lower concentrations with the median located near the minimum concentrations and with long tails toward the maximum observed concentrations.

Table 4. Trace elements that are compounds of potential ecological concern (COPEC), their concentrations in tailings, acid mine drainage (AMD), acid rock drainage (ARD), groundwater, and Methow River sediments, and their concentration factors (CF).

	Tailings (mg kg^{-1})			AMD (mg L^{-1})			ARD (mg L^{-1})		
						Alder Cr.			
COPEC	95% UCL (n=28)	90th Percentile E. Wash. Soils (n=80)	CF	90th Percentile 95% UCL (n=7)	Spring Sta. 22 (Fig. 1) (n=7)	ARD CF	Mean Leachate (n=3)	Mean Soil (n=2)	CF
Al	20643	28299		20	0	20	815	219	4
As	446	8	56	0	0		33	46	
B	232			0	0		0	25	
Ba	5619			0	0		43	53	
Cd	56	1	56	3	0	3	9	219	
Cr	22	32		0	0		10	10	
Cu	1343	28	48	16	0	16	737	12	60
Mn	440	836		10	0	10	1078	7	146
Pb	530	13	41	1	0	1	0	0	
Se	2048			1	0	1	0	30	
Zn	995	81	12	227	0	227	2009	1	2009

Table 4. (Continued).

	Groundwater (mg L^{-1})			Methow River Sediments (mg Kg^{-1})		
COPEC	95% UCL (n=108)	90th Percentile Background (n=31)	CF	95% UCL (n=41)	Mean Background Concentration Stations 8-11 (n=18)	CF
Al	113	0	113	19278	12844	2
As	98	0	98	9	0	9
B	711	180	4	56	72	
Ba	19	28		164	114	
Cd	4	0	4	10	11	
Cr	16	11		39	27	
Cu	29	7	4	198	28	7
Mn	35	140		663	371	2
Pb	66	32	2	49	37	
Se	119	461		648	708	
Zn	184	2066		124	70	2

The mean chemical compositions of twelve natural seeps were used as a reference for comparing both AMD and tailings leachate. Eight seeps in the Alamosa River Basin, South Mineral Creek, and Chapmen Gulch in Colorado, and four seeps in an unnamed deposit in Alaska (Plumlee et al. 1999) were selected for comparison. The specificity of the chemical composition of AMD was estimated based on data from 62 adits in eight deposit types in Colorado, California, Montana, Utah, Montana, and Alaska. Data on the chemical composition of AMD from five mines in the vicinity of this study included the Antimony Queen, an unnamed mine in the Gold Creek drainage, the Crescent Mine,an adit at the confluence of North Creek and the Twisp River, and the New Light Mine near Quartz Mountain and the source of the Methow River (Peplow 2000). Tailings water data were from five sites at the Holden Mine in Washington State, eight mines in Alaska, and one in Colorado (Plumlee et al. 1999). Estimates of background chemical concentrations in the Okanogan watershed were based on data from Garrigues and Carey (1999) and Wagner (2003). Data for groundwater at mine sites were from eight sites in British Columbia (Plumlee et al. 1999). The expected concentrations of trace elements in river sediments was based on data for 348 sediment samples from sites throughout the Methow River basin (Grant 1982) plus 18 samples in this study from the Methow River upriver from the mines between Twisp and Winthrop. Expected metals concentrations in sediments of creeks impacted by mining were taken from Raforth et al. (2000).

Arsenic, Cu, and Mn were frequent constituents in both mine site samples and natural analogs of AMD, tailings seep, groundwater and river sediments but the maximum observed concentrations of As and Cu (and Mn in river sediments) were at least one-order of magnitude lower at the natural sites than at the mine site samples. Concentrations in AMD, tailings seeps, groundwater, and river sediments ranged from zero to concentrations as high as 4.8 g L^{-1} Cu in AMD at the Richmond Mine, Iron Mountain, California (Plumlee et al. 1999). The highest maximum copper concentration documented in natural seeps occurred in a sedimentary deposit

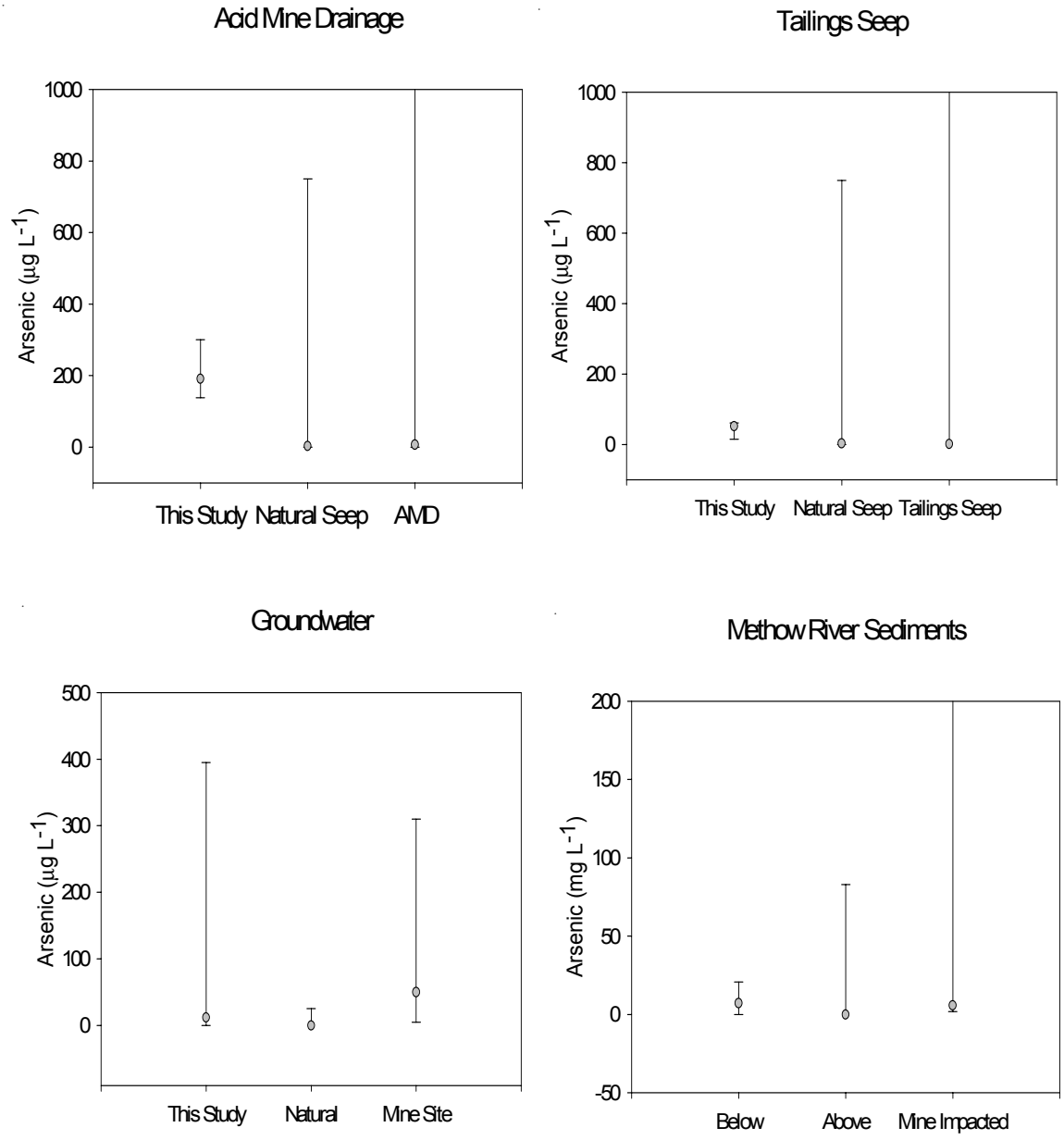


Figure 6. The mean and range of arsenic concentrations in Alder Mine tunnel drai nage(station 1, Figure 1), tailings seep, groundwater and Methow River sediments labeled “This Study” compared to data from Plumlee et al. (1999) and Methow River sediment data above the abandoned mines.

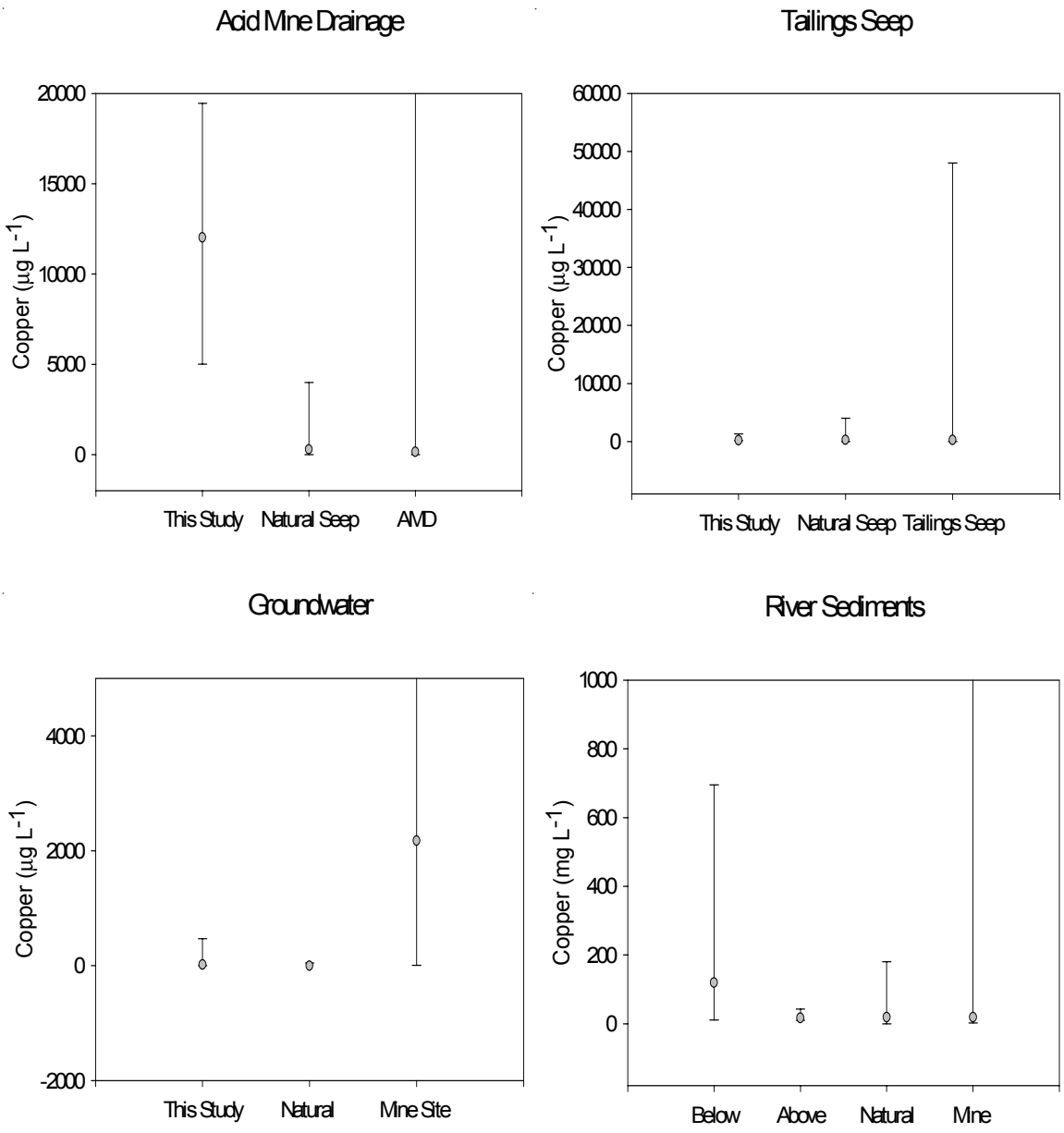


Figure 7. The mean and range of copper concentrations in Alder Mine tunnel drainage (station 1,Figure 1), tailings seep, groundwater and Methow River sediments labeled “This Study” compared to data from Plumlee et al. (1999) and Methow River sediment data above the abandoned mines.

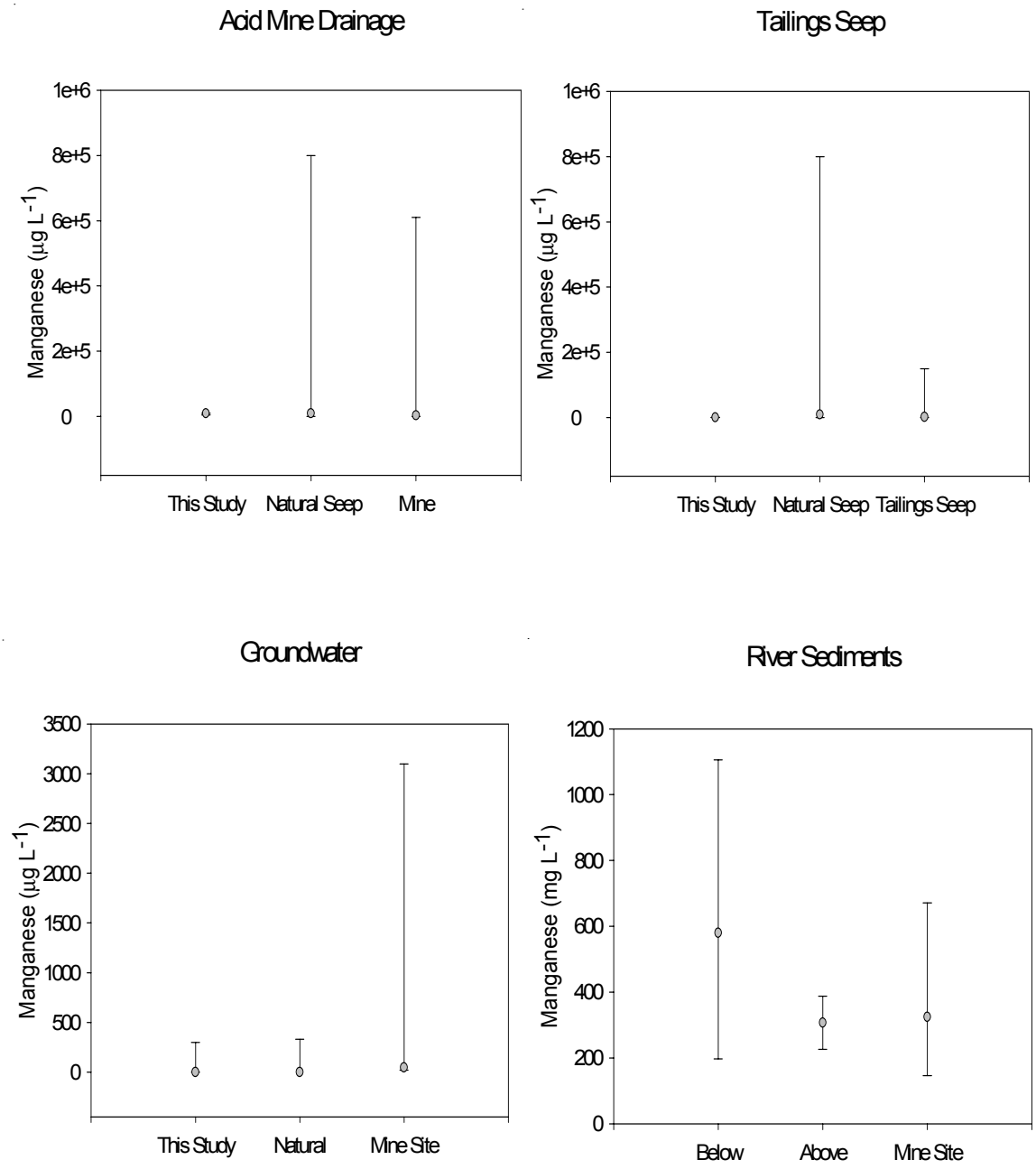


Figure 8. The mean and range of manganese concentrations in mine tunnel drainage,tailings seep, groundwater and Methow River sediments labeled “This Study” compared to data from Plumlee et al. (1999) and Methow River sediment data above the abandoned mines.

at an unnamed site in Alaska that was hosted by shales and cherts with no carbonates where there was more than three-orders of magnitude lower
(4 mg L^{-1}).

Consistency of Replication

Studies used for comparison were those conducted in areas near the study site that had or were assumed to have similar geologic, climatic, and hydrologic characteristics due to their proximity. Data from the Alder mine, Alder Mill and Red Shirt Mill in the Twisp District were compared to data from the Holden Mine. The general geology of both mineralized areas is dominated by igneous and metamorphic bedrock that is host to massive sulfide deposits (Johnson et al., 1997, Raforth et al., 2000). Valley bottoms are overlain with a mixture of unconsolidated glacial, fluvial, coluvial deposits. Precipitation occurs mainly as snow at both locations. Streamflows in the Twisp District are maintained by groundwater whereas in the Holden district are maintained by glaciers in addition to groundwater. Data on the concentrations of Cu and Mn in mine portal drainage from the Holden Mine 43 km (27 miles) south of the Alder Mine (Johnson et al. 1997, Plumlee et al. 1999) were compared to data for Alder Mine portal drainage. Drainage from the portals of four other mines in the Twisp District were also sampled for purposes of comparison. The North Creek and Crescent mines, located approximately 30 km away, and the Antimony Queen and an unnamed mine on the opposite side of Gold Creek, located approximately 8 km from the Alder Mine, were assumed to be comparable in their geology, climate, and hydrology. The concentrations of Cu and Mn in Alder Mine portal drainage exceeded all others (Table 5). The concentrations of Cu and Mn in mine portal drainage water from all sites (Figures 4-6) were within the expected ranges for both natural seeps and AMD (Plumlee 1999). Only dissolved Cu in the Alder Mine portal drainage, which fell within the observed range for AMD, exceeded the maximum concentration for natural seeps (Figures 6-8).

Table 5. Maximum concentrations of Cu and Mn ($\mu g\ L^{-1}$) in mine portal drainage from Alder Mine and five other mines assumed to have similar geology.

	Alder Mine	Holden Mine	North Creek	Antimony Queen	Unnamed Mine	Crescent Gold Cr.	Mine	New Light
Cu	19458	2040	0	25	4	11		2
Mn	10472	114	1335	25	1	2		20

Coherence with Fact and Theory

The association of abandoned mine waste with the occurrence of trace element contamination in mine adit drainage, tailings leachate, groundwater and river sediments at concentrations that are potentially harmful to human health, fish and wildlife must cohere with four preexisting theories: (1) minerals are formed under reducing conditions at high temperatures and pressures and when exposed to atmospheric conditions dissolution is favored according to the laws of thermodynamics and weathering will occur; (2) in the presence of water, reactions occur to establish an equilibrium between a mineral and its surroundings; (3) all minerals are affected by water, which is the transporting medium for minerals, microbes and their products; and (4) reducing conditions in groundwater transport systems favor the transport of trace elements with iron-oxides that are deposited in sediments when they emerge and are exposed to oxidizing conditions at higher pH.

To examine the relationship between mining activities and the accumulation of trace elements, we examined the tailings pile, the hydrological connection between mine waste contaminants and the Methow River, and biogeochemical conditions favorable for the transport and deposition of trace elements in the Methow River.

Operations at the Red Shirt and Alder Mills reduced ore to particles <50μm. To determine whether milling resulted in accelerated weathering rates, we examined tailings particles by SEM analysis and compared them to particles from the interior of ore samples that

had not been subjected to weathering. Pyrite from crushed ore samples showed minimal signs of weathering compared to particles of milled ore in tailings samples that were affected by pitting, rounded corners, and amorphous coatings (Figure 9).

Quartz and pyrite were the most common minerals in tailings samples analyzed by energy dispersive x-ray analysis (microprobe) (Table 1, Chapter 2). Banding and overgrowth of Fe-oxides on quartz and pyrite were extensive indicating that oxidation and reprecipitation had occurred and FeS had been leached out of the pyrite and redeposited as amorphous hydrous iron. Six other minerals were identified as minor based on their frequency of occurrence (i.e., feldspar, mica, hornblende, chalcopyrite, ilmenite, and magnatite). The minerals encountered at trace levels were epidote, chlorite, and tephra. Primary minerals such as sphalerite (ZnS), chalcopyrite ($CuFeS_2$), and galena (PbS) were not detected using SEM-EDS with detection limits that exceed 2%. Framboidal spheres of Si, Al and Fe containing As, Cu, and Zn were identified indicating these elements probably occur in tailings as secondary minerals in colloidal form or as inorganic crystals with amorphous coatings (Figure 10).

At the Alder Mill and Red Shirt Mill sites, increased weathering of tailings particles was also evident based on the distribution of Fe-oxides, which appeared to vary with depth. At depths <25 cm, tailings samples were yellow suggesting the presence of goethite, and at depths between 25-75 cm the color transitions to red indicating an increase in the hematite content, both of which suggest an advanced degree of weathering (Langmuir 1997). Tailings fractions at 75 cm were gray to black indicating a zone of reduced acid sulfate and below 75 cm tailings were gray-green suggesting reducing conditions high in iron and low in sulfate. The occurrence of hornblende, which is easily weathered, was absent down to a depth of 150 cm. From 175-250 cm hornblende increased from 2-20% of the grains analyzed by SEM-EDS (Figure 11). Although weathering appears to decrease with depth it appears to be high in tailings piles to a depth of approximately 250 cm.

Water is the prime transporting vehicle for minerals, which makes the physical

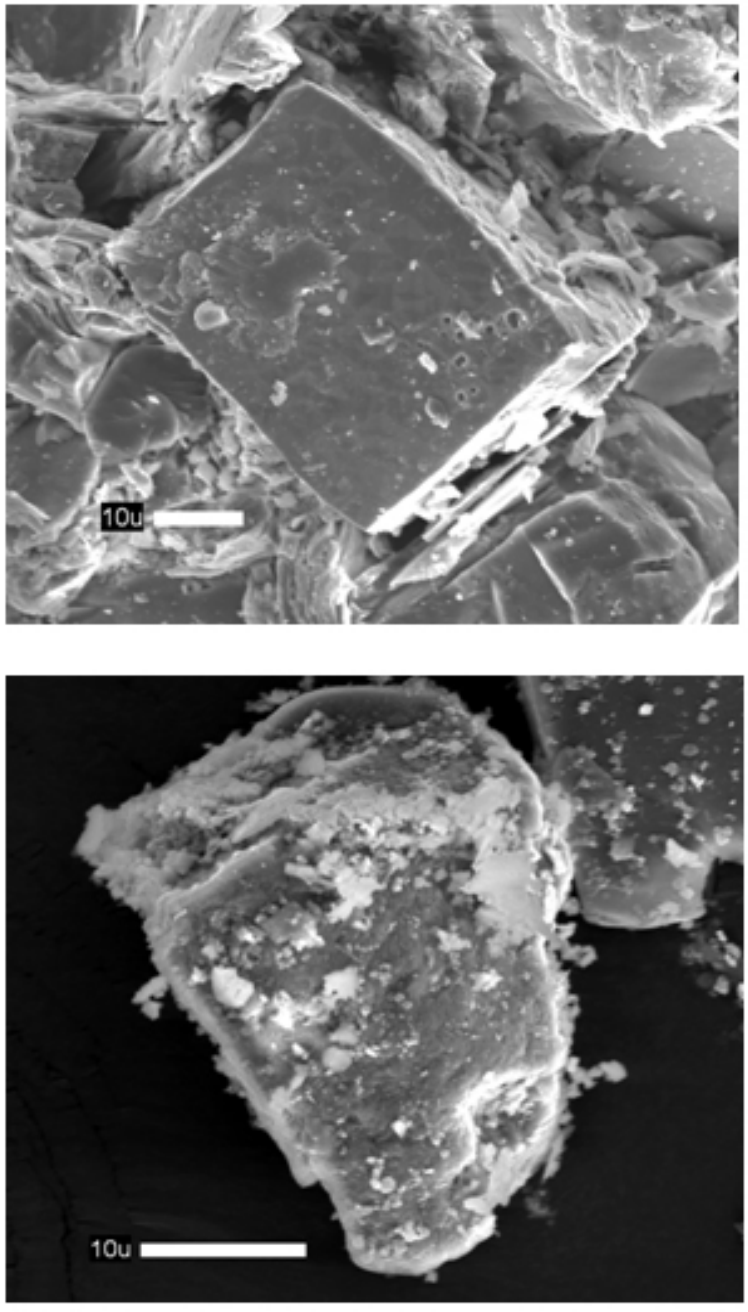


Figure 9. Scanning electron micrographs of pyrite in quartz from center of ore showing little evidence weathering (above) and in tailings from Alder Mill (below).

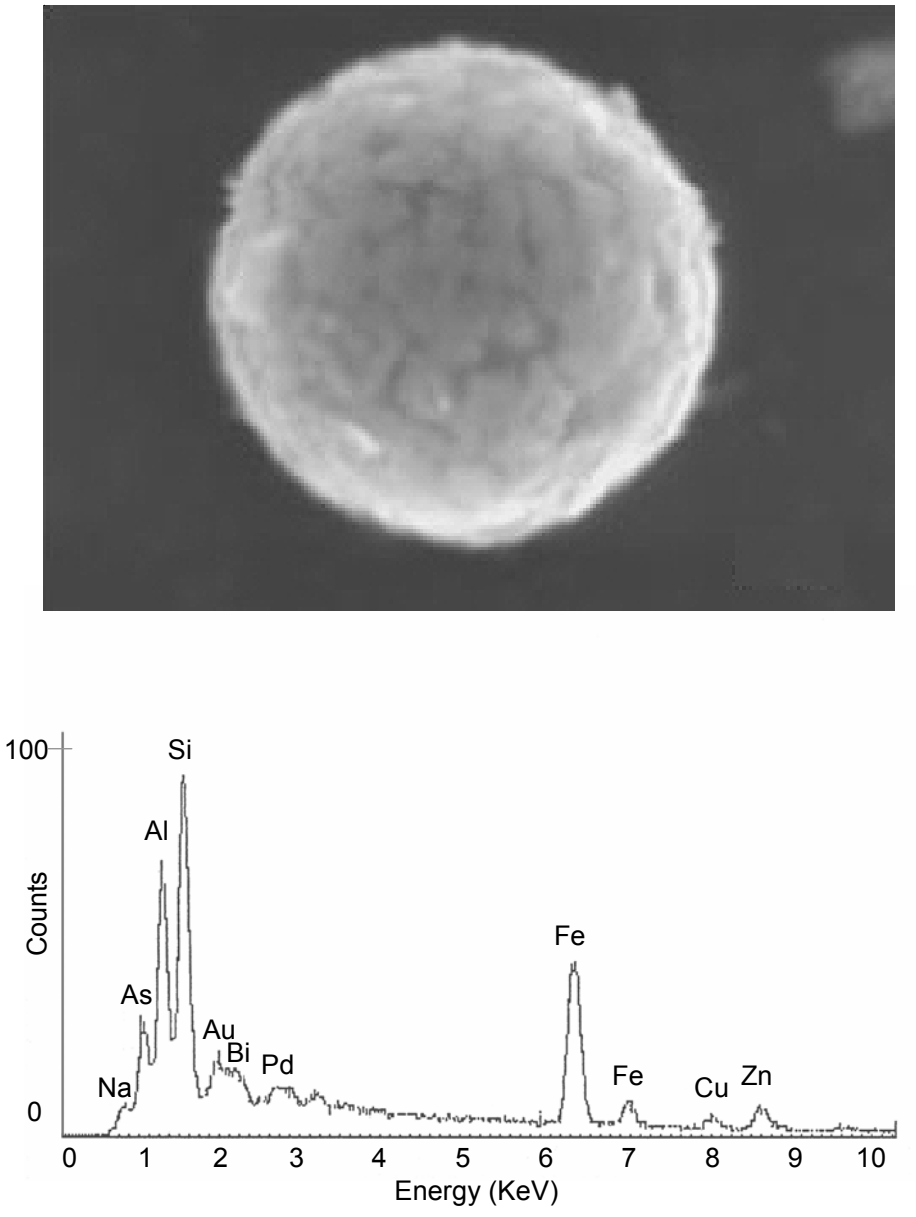


Figure 10. Scanning electron micrograph and EDS spectrum showing results of X-ray analysis of sample of tailings from Alder Mill. The framboidal sphere is approximately 10 mm in diameter, was frrom a sample of tailings from the Alder Mill and is composed of Fe-oxides, and contains Zn, As, and Cu.

processes related to the hydrological cycle largely responsible for their dispersion. Transport of the products of the weathering process promote the continuation of the weathering process because groundwater flow following inputs from precipitation remove the products of mineral dissolution and create conditions that thermodynamically favor additional mineral dissolution.

Was the transport of dissolved metals in AMD from station 1the source of contaminants entering Alder Creek? To answer this question, we used sodium chloride (NaCl) injections and showed there was hydrologic continuity between the AMD at station 1 and Alder Creek water at station 4. At a distance of 322 m between the adit and Alder Creek (slope was approximately 26%), peak Cl and electrical conductivity following Cl injection was detected in Alder Creek 10 hours following tracer injection at the mine adit. At 32 m hr^{-1} the rate of flow appeared to be unrestricted compared to the normal percolation rates for soils that have an inherently high infiltration capacity (10-25 mm hr^{-1}) (Hausenbuiller 1972), and was in the range of that for pipe and overland flow estimated to be > 0.1 m s^{-1} (Selby 1993).

Chloride injection data were also combined with measurements of geogenic sulfate in subsurface water samples (Stations 1-4) to determine whether AMD from the adit at station 1 was supplemented by additional subsurface inputs of AMD from the tunnel or ARD leached from the waste rock. If the discharge from the adit on the surface at station 1 was the sole source of AMD then the concentration of sulfate, assumed to be conservative, should remain constant in the contaminant stream or decline due to dilution or sorption, unless additional inputs of groundwater containing sulfate occur. Although Cl concentrations declined from 612 mg L^{-1} where it was injected into the AMD stream at station 1 to 14 mg L^{-1} at station 4, concentrations of geogenic SO_4 were relatively constant and ranged from 649-743 mg L^{-1} with no detectable trend. The mean SO_4 to Cl ratio increased from 1 to 46. These data suggest there are additional inputs of AMD along the subsurface flow path of AMD between adit station 1 and its confluence with Alder Creek.

Chemical gradients that reveal a causal association were also studied using geogenically

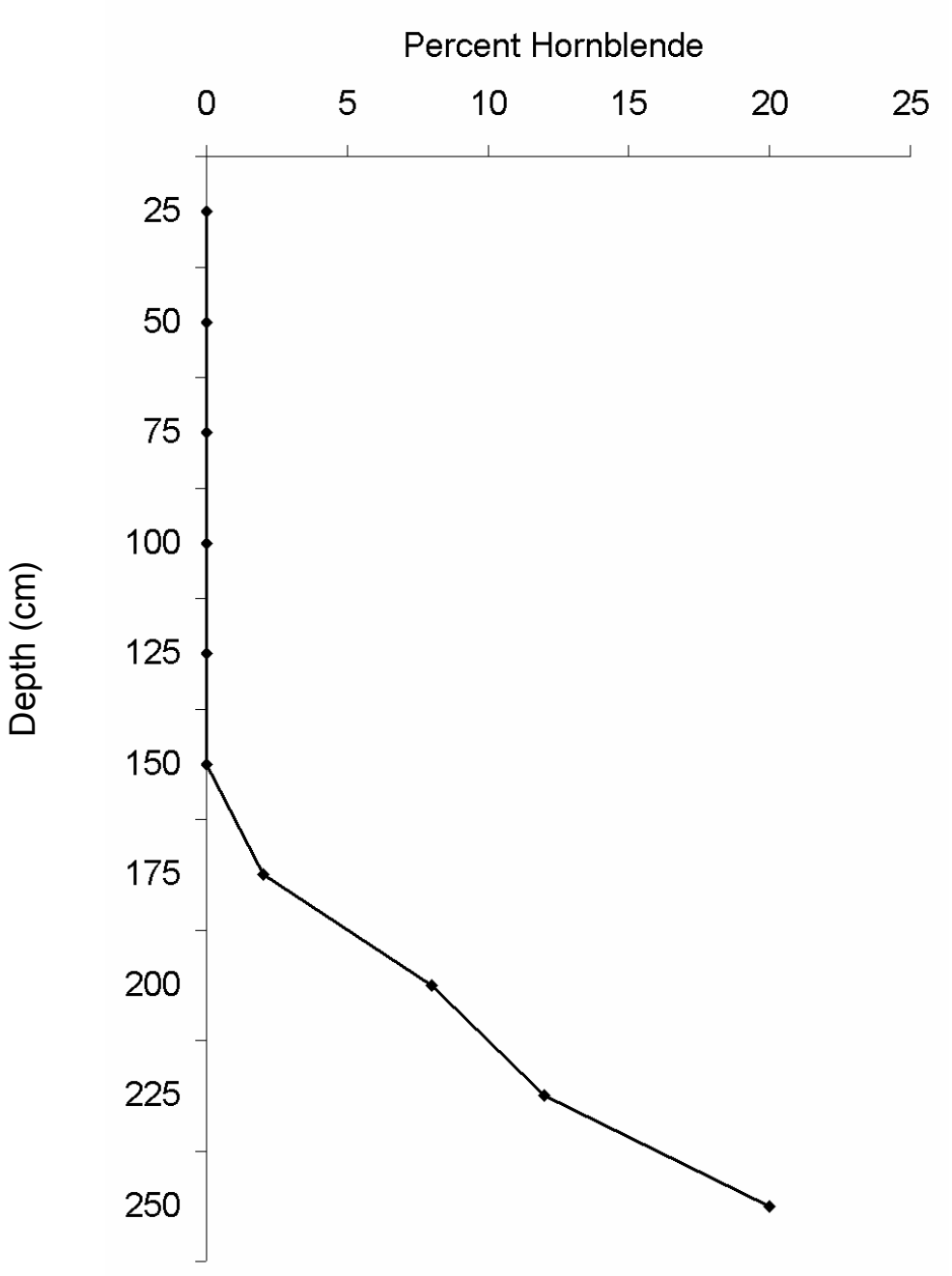


Figure 11. Percent hornblende with depth in core from Alder Mill tailings pile.

produced sulfate as a conservative tracer. The concentration of sulfate was high in water from the saturated zone at Alder Mill station 16 (669 $\pm$ 282 mg L^{-1}) compared to the natural background concentrations of sulfate in surface waters in the Methow Basin, estimated to be 16 mg L^{-1} (n=24), which suggests that sulfide mineral oxidation was occurring at station 16. From stations 16 and 17 northeast to stations 18 and 12, the decline in sulfate concentrations correlated with distance ($r = -0.96$, $p=0.01$, $df=3$). Sulfate concentrations also correlated with distance away from stations 16 (Figure 1) and drinking water well 23 (Figure 2) southeast to station 14 ($r = -0.98$, $p=0.06$, $df=3$).

To determine whether the deposition of red hematite-colored deposits at upwellings in the Methow River below the abandoned mine and mill sites was occurring as a result of abiotic Fe-oxide precipitation or microbial Fe-oxide adsorption and deposition, we analyzed sediments stained red from iron deposition. Red hematite-colored tubules that measure < 1μm in diameter and from a few to over 100 μm in length were observed as mats and attached to sediment particles (Figure 12) near groundwater upwellings at Stations 13 and 14 (Figure 1) in the Methow River. The microtubules appeared to be the sheaths formed by iron-depositing bacteria. SEM-EDS and XRD analysis showed these tubules were similar in composition to the amorphous coatings previously discussed and were predominantly composed of Fe.

Samples of fine sediments in the varve along the margins of the Methow River (Figure 1, stations 12-15) were also analyzed by SEM-EDS. Since it is assumed that the inorganic sediment particles are likely to be soil particles eroded from soils higher up the catchment and because As-, Cu-, and Zn-bearing minerals were not detected, it appears that trace elements exist as contaminants sorbed to coatings on sediment particles. SEM-EDS analysis suggests these coatings are Fe-oxides e.g., siderite ($FeCO_3$) or goethite (FeOOH). The Fe concentration of sediments below Twisp was significantly higher ($p=0.027$, $df=45$) than at the reference sites above by over 72% (32,152 vs. 18,655 mg kg^{-1}, respectively). Figure 13 is a quartz particle and the corresponding EDS spectrum shows Si is the major constituent when the

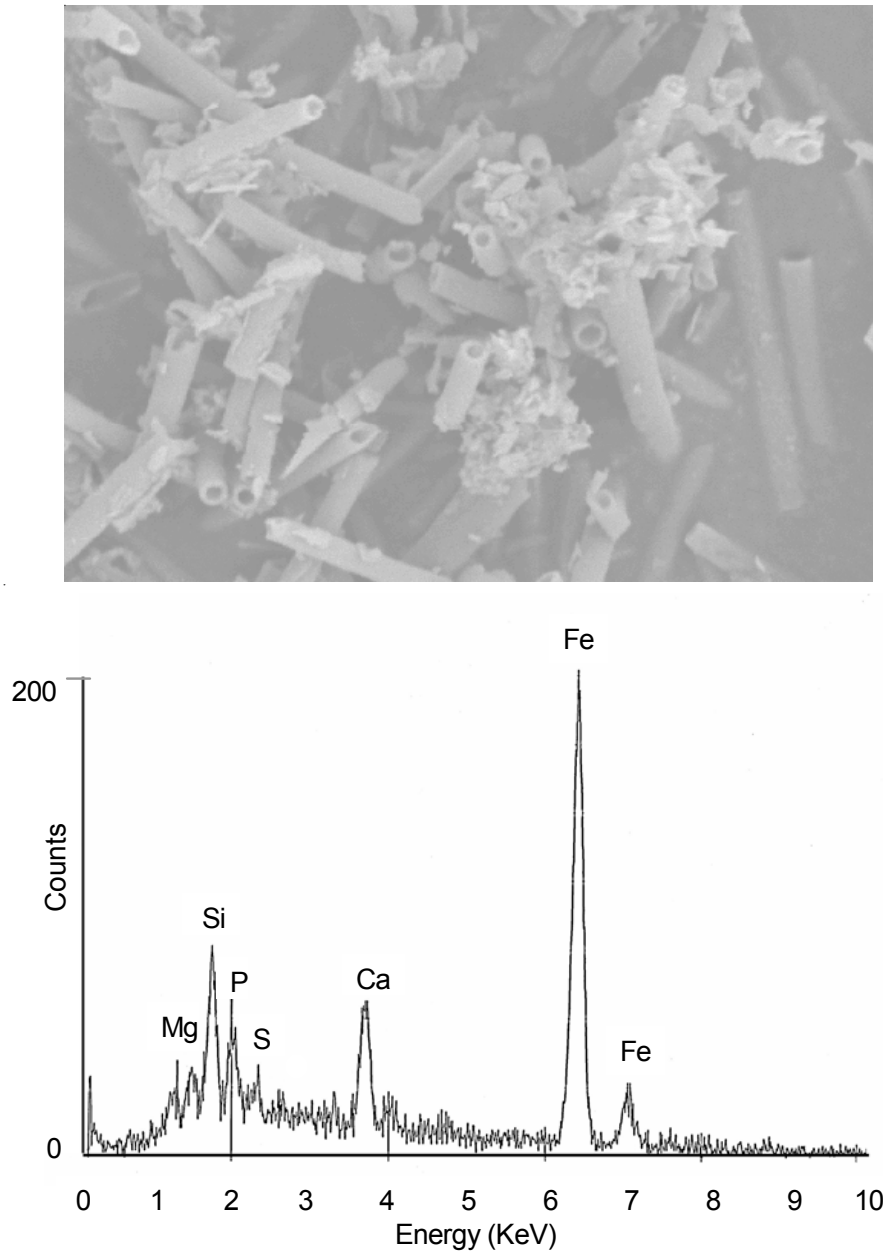


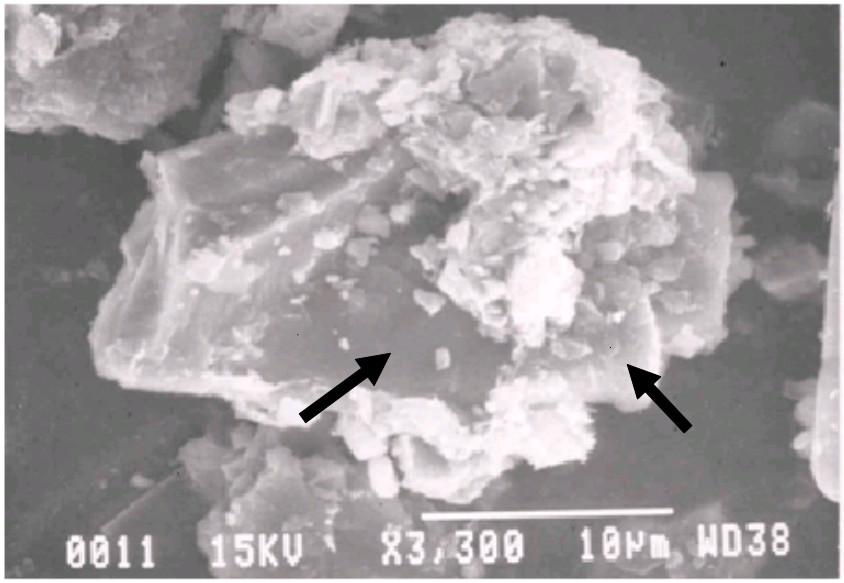
Figure 12. Scanning electron micrographs of Methow River sediments showing abandoned Fe-oxide sheaths from iron-depositing bacteria and EDS spectrum showing composition of mineralized sheaths.

electron beam from the SEM-EDS analysis was directed at an uncoated place on the sediment particle. When the electron beam was focused on an area with an amorphous coating an Fe peak was also observed. The spectrum shows that the coating might also contain Mg, Br, Na, K, Ca, Ti, and K in addition to the dominant Fe peak. The concentration of Ti in Methow River sediments was not determined because it is not detectable by ICP-AES, which was used to perform the quantitative analysis of river sediments.

Sequential extractions and ICP-Analyses were done to determine in greater detail where the different elements were located. The residual fraction of ore, tailings and sediments contained the greatest concentration of total elements (83%), which was comprised mainly of microelements. The trace elements in ore, when considered separately, were found at considerably higher percentages in the available (exchangeable and carbonate) fractions (Figure 14), which suggests that the microelements have the greatest potential for leaching when exposed to low Eh and pH conditions. Of particular interest was Cu at 58% in the available fractions of ore (Figure 12) and at a high total concentration equal to 11,424 mg kg^{-1}. Lead and Zn were also higher in the available fractions (63 and 54%, respectively) but at concentrations that were approximately one order of magnitude lower than copper (~ 1000 mg kg^{-1}). Chromium was high in the available fractions (61%) but the total concentration was low (32 mg kg^{-1}). The percentages of As found in the available fractions was less than 30% but the total concentrations was 261 mg kg^{-1}. Manganese, Ni, Mo, and Cd were found in the available fraction at levels less than 10% and their total concentrations were also low at less than 150 mg kg^{-1}.

DISCUSSION

A causal relationship between the presence of mining activities at the Alder Mine, Alder Mill and Red Shirt Mill sites and the accumulation of trace elements in groundwater



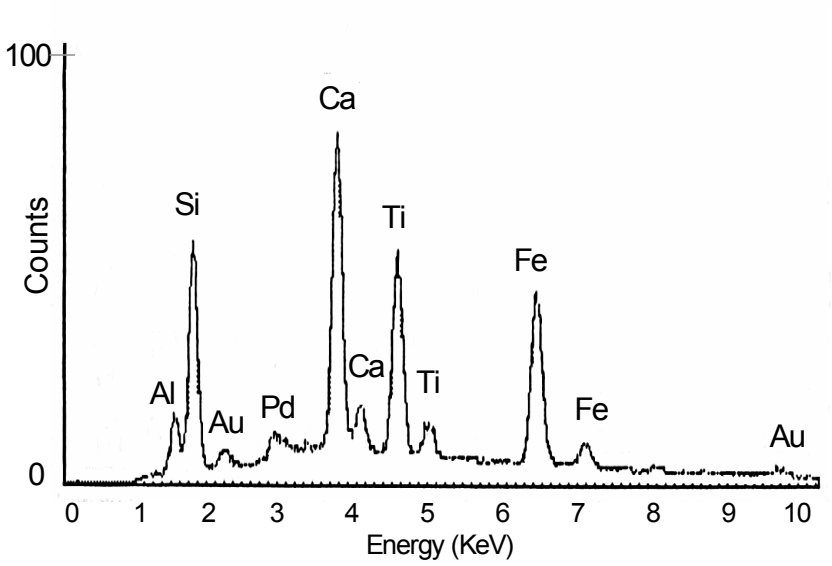


Figure 13. SEM micrograph and EDS spectrum showing quartz particle from Methow River sample and coating that contains Ti.

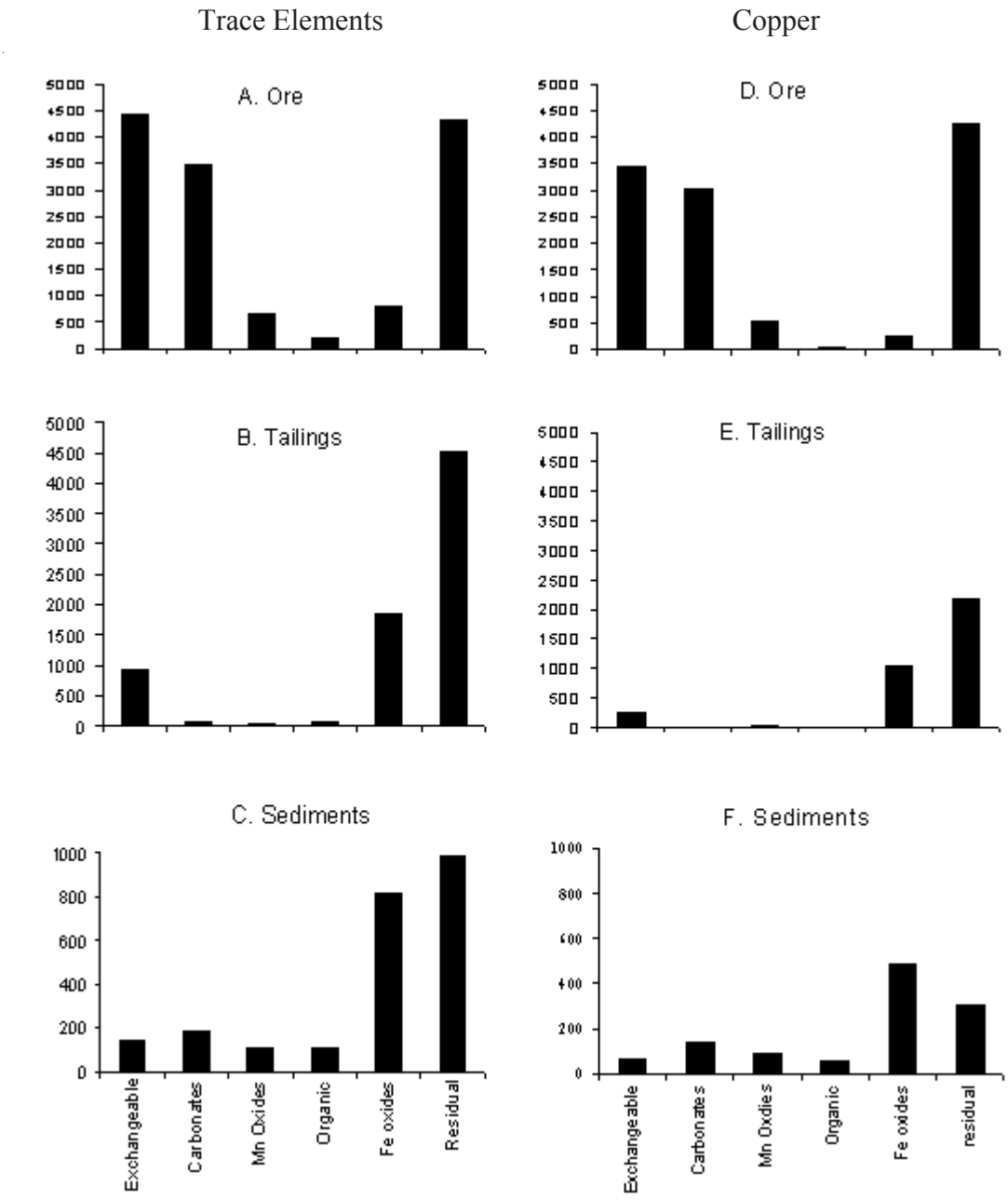


Figure 14. Sequential extractions results for compounds of ecological concern and copper concentrations in ore, tailings and sediments (mg kg^{-1}).

surface water and sediments is apparent when the results in this study were evaluated using the five epidemiological criteria recommended by Sesser (1986) and Fox (1991).

Time Order

Data in this study on the effects of mine waste on aquatic and terrestrial fish and wildlife habitat near the Methow River south of Twist were collected 48 years after the cessation of mining. The combination of metal analyses and radiometric dating would have made the historical reconstruction of contamination possible (Valetta-Silver 1992). Even in the absence of pre-mining background data or radio-isotope data to determine exact age and depositional history of the deposits sampled in the core, the time-order of Cu and Zn accumulation in Alder Creek sediments supports the cause-effect hypothesis (reject H_a).

The sediment profile for Alder Creek suggests historical changes in Cu and Zn accumulation occurred and that there were two periods of accumulation. In the lower fraction, which correlates with the earliest period, Cu and Zn accumulations were lowest. Copper and Zn accumulation increased in the fractions above 20 cm, which correlate with the later period. The cored material came from an area that is assumed to have a relatively fast sedimentation rate and was apparently undisturbed thus supporting its reliability (NOAA 1992).

Strength of Association

When both confidence factors (CF) and confidence in benchmark values are considered, contamination of groundwater with As and Methow River sediments by Cd, Cu, Mn, and Pb appears to be strongly associated with increased weathering of sulfide minerals due to historical mining. There is high confidence in the low benchmark values for Al, which are based on experiments with seedlings and horticultural crops (Efroymson 1997). Trees, however, (especially pines) appear to have the greatest tolerance to Al, which had no effect on

shoot growth rate at 162 mg L^{-1} (Efroymson 1997). A benchmark at this concentration would place the relative risk for pines between B and Cu. Confidence in the benchmarks for As, Pb and Zn is moderate, and for B, Ba, Cr, Cu, Mn, and Se confidence was low due to the small numbers of studies and the lack of supporting data.

In AMD, Zn, Cu, and Se had high CF indicating their concentrations were several orders of magnitude greater than the water quality criteria on which they were based and that they are the elements of greatest concern. In ARD, all of the trace elements (i.e., As, Cu, Cr, Mn, Zn, Pb) had CF that were at least one-order of magnitude greater than the groundwater criteria. When the mean concentrations of trace elements compared to benchmark values for toxicity and 95% UCL compared to estimated background concentrations are both considered, three trace elements (As, Cu, and Mn) are identified as COPEC that appear to be enriched as a result of historic mining activities. Manganese, with a CF of 2 in Methow River sediments, was also concentrated in tailings leachate and exceeded groundwater criteria with a CF equal to 146. Copper had a CF of 7 in Methow River sediments downstream from the mine sites in this study, exceeded plant and microbial toxicity benchmarks and had a CF of 48. Arsenic concentrations in groundwater exceeded criteria and estimated background concentrations by a factor of 98. Arsenic also exceeded soil toxicological benchmarks by a factor of 56. Concentrations of As, Cu and Mn that exceeded toxicological benchmarks at both the source and in the Methow River and had concentration factors that indicated these trace elements exceeded background concentrations by factors ranging from 2 to 146 suggesting the strength of association between the presence of mine waste and the occurrence of As, Cu and Mn contamination in groundwater and Methow River sediments strongly supports causation (Ha).

Specificity

The occurrence of As, Cu and Mn was not specific to either mine impacted or natural sites and the range of concentrations that have been reported suggest that specificity is generally

low in regards to the effects of mining on the chemical composition of AMD, tailings seeps, groundwater, and river sediments. It is clear that the COPEC As, Cu and Mn can occur in natural seeps, groundwater, and sediments that are not impacted by mining activities and not all samples from mine impacted sites are contaminated.

The extremely high maximum concentrations observed in AMD, tailings seeps, groundwater, and river sediments appear to be specific to mine impacted sites. The chemical composition of natural and mine-derived acid rock drainage (ARD) is controlled by deposit geology and biogeochemical processes (Plumlee et al. 1999). Geologic features that control the composition of ARD include the presence of acid-generating iron sulfides, other metal sulfides, and acid-consuming carbonates. Since reactivity of the minerals is a function of grain size as well as the trace-element content of the deposit, mineral processing is likely responsible for the greater range of concentrations observed in mine waters. As it was seen in Figures 4-6 the maximum concentrations for As in groundwater, Cu in AMD, groundwater and river sediments, and Mn in river sediments far exceeded the maximum concentrations observed in the natural analogs. Since these extreme values appear specific to samples from mine impacted sites the causal hypothesis (Ha) is supported.

Consistency of Replication

Opportunities for exact replication to demonstrate consistency and rule out chance are not available. The alternative was to demonstrate the consistency of results from Alder Mine with data from other sites that are characterized by a diversity of times, places, circumstances, people and research designs. In order to minimize inconsistencies among data sets used for comparison, data from areas that were assumed to have similar geologic characteristics were considered. Although the copper and Mn concentrations in the mine portal drainage water from all sites were within the expected ranges for both natural seeps and AMD and the dissolved Cu in the Alder Mine portal drainage, which fell within the observed range for AMD, exceeded the

maximum concentration for natural seeps, there was no apparent consistency of the results from Alder Mine with other studies. It was assumed that the other sites (i.e., Holden Mine, North Creek, Antimony Queen, Gold Creek, Cresent, and New Light) were geologically similar and could be used for comparison. Other differences, due possibly to climate, hydrology, or mineralization, could be responsible for the high concentrations of Cu and Mn in the Alder Mine AMD. Although the results did not demonstrate consistency of results on replication they were consistent with the expected range for AMD reported by Plumlee (1999).

Coherence

Finally, to determine whether the causal hypothesis is plausible the data were evaluated for their theoretical plausibility and factual consistency . Coherence with preexisting facts and theory would support the causal hypothesis (H_a) , while incoherence would detract from the causal hypothesis and may be grounds for accepting the null hypothesis (H_o).

The observation that the subsurface flow of ARD appears to be unrestricted between Alder Mine (station 1) and Alder Creek (station 5) is consistent with the facts related to bedrock morphology. The presence of faulting and calcite-filled fractures in the area where the abandoned mine sites are located (Figure 4) suggests there is a potential that calcite fracture fillings are being dissolved by the acid mine waters infiltrating from the surface. Pyrite oxidation results in acid mine waters where negative pH's occur (Nordstrom et al. 2000). Calcite dissolution would result in modifications of the hydrologic character of the bedrock (Hoch 2000). Once calcite-cemented fractures open the porosity, the reactive character of the rock can potentially be increased resulting in the dissolution, transport, and dispersion of additional trace element contaminants.

The decreased intensity of sulfide oxidation with depth that divides the mine waste dump at Alder Mill into a leached horizon and an accumulation horizon, the banded formation of weathered tailings particles, leachates that are characterized by acid, Fe, sulfate, metals, and

metalloids; and iron oxide coatings on sediment particles that contain sorbed metal and metalloid contaminants indicates there is a temporal variation in oxidation and pore water chemistry that is influenced by seasonal hydraulic flushing (Lin 1996, Plumlee et al. 1999). During dry periods, evaporation of acid waters would be produced by sulfide-mineral oxidation resulting in the precipitation of salts that stores acid and metals until they are flushed during the next period of rain or snowmelt. This theoretical explanation suports the causal hypothesis (H_a).

Furthermore, gradients of sulfate that declined away from the Alder Mill are highly supportive of the causal hypothesis (H_a) (Fox 1991). Sulfide-bearing minerals are formed in reduced conditions out of contact with atmospheric oxygen and are thermodynamically unstable (Plumlee et al. 1999). When sulfide deposits are exposed by natural erosion or by mining to atmospheric oxygen and water, weathering of the sulfides can produce either natural or mining-related acid-rock drainage (ARD). Although climate and the methods used during mining and mineral processing can affect drainage compositions, the compositional range of the leachate is determined by the geologic characteristics of the ore. The mining, milling and tailings disposal process concentrated pyrite sufficiently that the acid generated by sulfide oxidation could easily overwhelm the acid-neutralizing capacity of the tailings and underlying bedrock.

Geochemical processes that regulate the complexation of metals such as Pb, Cu, Zn, Cd, and Ni and metalloids such as As by hydrous ferric oxides (Smith 1999) also support the causal hypothesis. Although dissolved Fe(III) is absent between pH 5 and pH 10, the Fe(III) oxyhydroxides occur in suspended colloidal form in surface- and groundwater within this range (Langmuir 1997). Solids with a diameter between 0.01 and 10 mm are considered colloids (Stumm and Morgan 1996). Coarser particles are considered suspended particles. To be dissolved, therefore, a molecule must have a diameter of < 0.01 mm. If present, some colloidal solids will pass through a 0.45 mm filter membrane used to distinguish dissolved from suspended metals. Colloids can remain suspended indefinitely and it is estimated that 30-70% of the filterable (0.45 μm) ferric iron occurs as colloidal particles (Langmuir 1997). The process of

colloid facilitated transport, described by Kretzschmar et.al. (1999), is a potential mechanism for the translocation of adsorbed metal contaminants. According to the colloid facilitated transport model, metals partition between the surfaces of immobile matrix particles, the aqueous phase, and mobile colloidal particles, which are transported with flowing water. While strongly sorbing contaminants can be highly retarded by immobile matrix particles, colloidal carriers can provide another potential rapid transport mechanism thus providing theoretical suport for the causal hypothesis (Ha).

A potential area of incoherence that, instead of detracting from the causal hypothesis, emphasizes a limit in understanding is that a single chemical potential determines the equilibrium between a mineral and its surroundings (Zajic 1969). In the presence of water, an equilibrium is established between mineral ions in solution and those in the crystalline state. If the reactions in mine waste piles take place at essentially atmospheric conditions (about 25^0 C and one atmosphere), then Eh-pH stability diagrams should characterize the chemical systems and trace elements in AMD should coprecipitate during metal oxide formation. Pyrite oxidation results in acid mine waters that are, however, far from ideal solutions in which dissolved metal concentrations can be as high as 200 g L^{-1}, sulfate concentrations can exceed 760 g L^{-1}, and hydrogen ion activity coefficients can be greater than 1. Under these conditions, ferrous iron and other metallic ions such as Mn^{2+}, Hg^{2+}, and Cu^{2+} form oxides where the Eh potential is positive and they form sulfides, sulfates or chlorides in acidic reducing environments (Kabata-Pendias and Pendias 1992). For transport of trace elements to occur from mine waste sites to groundwater and sediments in the Methow River, therefore, it must be assumed that either flux rates exceed reaction rates or that trace elements are transported as suspended solids.

The observation that trace elements in the sediments from the Methow River were associated with Fe oxides, which would be solubilized under changing Eh or pH conditions and leached easily compared to trace elements in the sulfide or residual phases (Sullivan and Yelton 1988), also supports the causal hypothesis. The extent of sorption of trace elements by Fe-

oxides is a function of pH, the particular metal, the concentrations of aqueous complexing agents that compete for the metals, and the relative concentrations of the various metals competing for sorption sites on the particles. In general, the effectiveness of sorption with increasing pH is As > Pb > Cu > > Zn > Cd, Ni (Sidle et al. 1991, Plumlee et al. 1999).

Adsorption by FeOOH depends on pH and when the pH is less than the Point of Zero Net Proton Charge (PZNPC) of the mineral surface the surface is positively charged, absorbs anions [e.g., $As(III)O_3^{-3}$ and $As(V)O_4^{-3}$] and releases cations (e.g., Cu^{2+}, Zn^{2+} and Pb^{2+}). When pH is approximately equal to the PZNPC (pH 8) the iron oxyhydroxide particles are not repelled and so tend to agglomerate or flocculate and settle out (Langmuir 1999). The exact pH of the PZNPC depends on the species of FeOOH. The exact pH of the PZNPC depends on the species of FeOOH. Studies of diverse mine drainages have shown that the orange to yellow to brown precipitates that commonly line mine-drainage stream beds are poorly crystalline to amorphous iron phases such as ferrihydrate (a hydrous ferric oxide), jarosite (a potassium-iron hydroxysulfate), or schwertmannite (a ferric hydroxysulfate) (Nordstrom and Alpers 1999, Smith 1999). Ultrafiltration studies have shown that 30-70% of the filterable iron occurs as colloidal particles (McKNight et al. 1988).

The microbial production of Fe-oxide sheaths (Figure 10) is significant due to the potential productivity of bacteria and because extensive sheath and ferromanganese deposits have been shown to inhibit algal colonization and growth (Sheldon and Skelly 1990), reduce abundance, diversity and distribution of benthic macroinvertebrates in streams (Wellnitz 1994), and have the potential to scavenge and concentrate trace elements in riverine sediments. Fe-depositing bacteria are commonly scavenge toxic metals from dilute aqueous solutions (Ghiorse 1984, Schultze-Lam 1996) and it is not unusual for bacteria to precipitate an amount of metal equal to or exceeding their cellular weight (Schultze-Lam et al 1996, Beveridge 1989). Iron-depositing bacteria include Gallionella, Sphaerotilus, Clonothrix, and Leptothrix. Leptothrix is common in seeps, pools, and in small patches near the banks of rivers and streams and because

they are ubiquitous in the natural environment they can potentially play a significant role in the development of fine-grain minerals in contaminated sediments.

The findings in this study appear to be consistent with the preexisting knowledge related to the oxidation of sulfide-bearing minerals at abandoned mine sites, the transport of trace-elements in groundwater systems, the accumulation of trace-elements in groundwater and in the bioavailable and potentially toxic exchangeable fraction of aquatic sediments.

CONCLUSION

The weathering of sulfide-bearing minerals exposed to atmospheric oxygen and water by mining has resulted in the contamination of human, fish and wildlife habitat along the Methow River south of Twisp in Okanogan County, Washington:

- Contamination of ground water with As and Methow River sediments by Cd, Cu, Mn and Pb appears to be strongly associated with increased weathering of sulfide minerals at the historic mine sites.

In the absence of reliable methods to separate natural from man-caused loadings of trace elements the use of epidemiological criteria related to time order, strength of association, specificity, consistency of replication, and coherence with fact and theory were used to establish a cause-and-effect relationship among the oxidation of mine waste and the contamination of water, soil and sediments with trace elements (Tables 6 and 7):

- Although no pre-mining background data were available, a vertical profile of trace element concentrations in Alder Creek sediments showed that relatively low background concentrations were followed by an increase in Cu and Zn accumulations after mining began.
- The strength of association between abandoned mine sites and the occurrence of contaminated soil, surface water, groundwater, and sediments was clearly supported by statistical analysis.
- Data from this study generally agree with data from other mine waste contamination

sites supporting the hypothesis that a causal relationship exists between the presence of mining activities and the accumulation of trace elements at concentrations that are potentially harmful to human health and fish and wildlife habitat.

- Biogeochemical conditions and hydrological connections were favorable for the release, transport and deposition of trace elements to soil, water and sediments.

Table 6. Rejection of the hypothesis that a causal relationship exists between the presence of mining activities and the accumulation of trace elements at concentrations that are potentially harmful to human health and fish and wildlife habitat in the Methow River Valley using the criteria strength, consistency, and coherence.

Criteria	Reject Causal Hypothesis (Y/N)
Time Order	N
Consistency	N
Specificity	N

Table 7. Affirm the hypothesis that a causal relationship exists between the presence of mining activities and the accumulation of trace elements at concentrations that are potentially harmful to human health and fish and wildlife habitat in the Methow River valey using the criteria strength of association, consistency, and coherence.

Criteria	Affirm Causal Hypothesis (Y/N)
Time Order	Y
Strength of Association	Y
Consistency	N
Coherence	
Theory	Y
Factual	Y
Biogeochemistry	Y
Chemical Gradients	Y

CHAPTER 4:

CELLULAR TO ECOSYSTEM LEVEL RESPONSES TO TRACE ELEMENT CONTAMINATION

INTRODUCTION

There is considerable concern about the environmental effects of trace element contamination from abandoned mines. These effects can occur at all levels of biological organization. Metal toxicity in soil, water, and sediments following contamination from abandoned mines begins as a reaction between a chemical and an organism at the molecular level. The initial reaction generates secondary and tertiary responses resulting in effects that are observed at all levels of biological organization with potential indicators at each level (Hodson 1990, Clements and Kiffney 1994, Clements 2000). Reduced nutrient cycling and energy flow at the ecosystem level, reduced diversity and abundance at the community level, and reduced growth and increased mortality among individual members of endangered species at the population level are more relevant to resource managers and ecologists than effects at lower levels of biological organization. Higher level effects are particularly relevant when evaluating the impacts of programs, policies or legislation. However, the degree to which cause and effect are related (i.e., specificity) and our knowledge of the mechanisms of toxicity is lowest at higher levels of organization (Hodson 1990). Indicators of toxicity such as morphological changes at the tissue level, ultrastructural changes at the cellular level and biochemical changes at the molecular level better reveal cause and effect relationships. However, their impact on ecologically relevant processes are not easy to recognize.

Studies at the individual level or below are more relevant when exposure varies within the study area, and ecologic studies are more relevant when exposure or effects vary over a larger area. When ecological studies focus on group comparisons rather than on individuals, direct links between cause and effect are often tenuous (Clements 2000) and susceptible to biases that

result from the lack of individual data on exposure, outcomes, and confounders. The effect of these biases on the conclusions drawn has been referred to as the ecological fallacy by Selvin (1958), and has been discussed by Morgenstern (1995) and Hopenhayn-Rich (2000).

At lower levels of organization, the effects of trace element toxicity may be more easily linked to cause, occur more rapidly, and may provide early warnings of toxicological effects on populations (Hodson, 1990, Clements 2000). Despite the greater mechanistic understanding and endpoint-response specificity, effects at lower levels of organization may be limited because the significance of a biochemical response at the ecological level is not obvious. The usefulness of biological assessment depends, therefore, on the examination of indicators at multiple levels of biological organization.

Examination at the cellular, tissue and organ level using electron microscopy has been used to diagnose toxicological and metabolic disorders (Phillips et al. 1987, Roberts 2001). Biological assessment methods, which describe the structure and function of aquatic communities, populations, or the condition of individual organisms, have been used routinely to measure ecosystem health as well as the biological condition of individual sites (Simon 2003). At the ecosystem level trace element toxicity can be measured directly if the property measured is an ecosystem function or indirectly if the effected organisms are valued for their functional properties rather than their community or population properties (Efroymson et al. 1997).

The objective of this study, conducted along the middle reaches of the Methow River in eastern Washington, was to describe biological responses to trace element contamination at different levels of organization. This portion of the river is at risk of degredation because of three abandoned mines sites; the Alder Mine, Alder Mill and Red Shirt Mill. The Methow River is an historic spawning ground for endangered native steelhead/rainbow (*Salmo gairdneri*) and chinook salmon (*Oncorhynchyus tshawytscha*). The overarching hypothesis was that the effects of contamination from abandoned mine waste occur at multiple levels of biological organization and there are potential indicators at each level. If the hypothesis is supported then

the cumulative interpretation of findings from each level can be used to suggest a mechanistic linkage that integrates responses across levels of organization.

METHODS

Cellular Level Responses

The source of data in this part of the study was the electron microscopic observations of hepatocytes from five juvenile triploid trout (*Oncorhynchus mykiss*) from two microcosms, one located at the upstream reference stations 11(Chapter 2) and theother at the downstream station 15 to look for evidence of ultrastructural pathalogy. Eighty-four hatchery-raised triploid trout (*Oncorhynchus mykiss*, <35g), aged 15-weeks, were transferred from a nearby hatchery (Trout Lodge, Quincy, WA) to the study site. Two individuals (time-0 controls) were removed, euthanized (0.1% MS-222, pH 7) weighed and tissue samples were removed following dissection. The remaining 82 individuals were equally divided into two pens.

Fish pens were constructed from aquaculture netting on a PVC pipe frame approximately 1m on a side. One pen was located in a Methow River side channel downstream from the abandoned mine site (station 15, Figure 1) and the other pen was located upstream from the abandoned mine sites (station 11). Fish, maintained in the pens from 7 May 2001 to 11 June 2001, were fed (Rangen 3/32 EXTR 400 Slow Sink food #4974) once daily in the morning (0700-0800) at 4% of their body weight•day^{-1}. Visual examination during feeding revealed that the fish readily ingested the food provided and were satiated daily. Each pen was monitored daily for morbidity and mortality throughout the exposure period. At the end of exposure, fish were euthanized (0.1% MS-222, pH 7) weighed and tissue samples were removed following dissection.

Temperature, dissolved oxygen and alkalinity were measured daily during the exposure period at each site using Hobo model H8 temperature data loggers. A YSI model 85 meter was

employed for the measurement of dissolved-oxygen (DO), total dissolved solids (TDS) and temperature. Alkalinity was measured in the field using the LaMotte Direct Read Titration Kit (Model 221780). A Piccolo Model HI 1295 temperature compensated digital meter was used to measure pH. Conductivity, pH and DO were standardized daily before and after use. Current velocity was measured following the method described by Hauer and Lamberti (1996).

Diagnosis of hepatic liver disease was made using the criteria for identifying toxin induced changes (Phillips 1987). Since hepatocytes produce a limited number of morphological response patterns, an individual toxin may produce several types of ultrastructural changes, and a single ultrastructural change may be associated with a number of toxic agents. Although a detailed analysis of the underlying metabolic disorder is beyond the scope of this study, ultrastructural findings were used to place individuals within groups identified either as normal or within a known group of metabolic disorders. If the findings were pathognomic then specific metabolic diseases were identified and the underlying molecular level mechanisms were inferred from the published literature.

Individual Level Responses

The source of data in this part of the study is the direct observation of 41 individual reference fish from the microcosm at station 11 and exposed fish in the microcosm at station 15. Approximately 100 caddisfly larvae (*Ecclesomyia spp.*) were also collected from each of the four sample stations (Figure 1, Stations 8-11) located in the Methow River upstream and compared to larvae from the four sample sites located downstream from the abandoned mine sites (Figure 1, Stations 12-15). Within one hour following collection, larvae were removed from their cases, blotted dry using Whatman #40 filter paper to remove surface water, and weighed. After weighing, the larva were preserved in 70% ETOH. Head capsule widths were measured using a slide micrometer and a dissecting microscope to compare relative age distribution. Instar groups and corresponding size ranges were identified based on a frequency

distribution histogram of headcapsule width data, which were ranked in ascending order. Head capsule widths that comprised the horizontal portions of graph were assumed to be from the same instar groups and vertical portions of the graph were assumed to be transitions between instar groups. The midpoint of each transition range defined the size range for each instar group.

Since the unit of analysis should be the same for all variables (Morgenstern 1995), trout and caddisfly larvae weights that were collected as individual level observations were expressed as an average for each group. Individual exposure levels, however, could not be measured accurately for large numbers of individuals so average sediment metal concentrations (Chapter. 2, Table 4) were assumed to reflect average group exposures. A second assumption was that the confounding effects due to longitudinal and elevation gradients were negligible because stations 8-10, and 12-15 occur along a 6-km reach near the center of the Methow River basin where the topography is characterized by a gentle slopes and a wide valley floor. Elevation along this reach of the Methow River ranges from approximately 516-m at station 8 to approximately 462-m at station 15, a gradient of less than 10 m km^{-1}. The potential impact of inputs from tributaries on water and sediment chemistry was controlled by including reference station 11 on the Twisp River in the study. Streamwater temperature was monitored throughout the study to control for potential confounding effects.

Population Level Responses in Black Bear

The exposure of resident bears to As was determined using a bear-hair capture technique and a non-consumable liquid lure (Johnson and Kendall 1999). Scent attractant was placed on a log enclosed by a strand of barbed wire stretched approximately 50-cm above the ground to snag hair at station 5. A scent to attract bears to the hair ensured there was no possibility of food reward. The trap was checked bi-weekly from April to September in 2001 and 2002. Since bears exhibit a diminishing response to bait over time as they learn that investigating the liquid scent does not yield food (Johnson and Kendall 1999), it was assumed that hair similar in

color and texture collected closely together in time was from the same individual and hair dissimilar in color and texture and collected over 1-month apart in time was from different individuals. Six samples > 0.5 g each from what was assumed to be separate individuals were collected. Two reference hair samples, 4 g each from 10 year-old male and female black bears held in captivity at Northwest Trek Wildlife Park (Eatonville, WA) and fed controlled diets in an environment assumed to be free of As, were collected in April 2001. Arsenic concentrations in hair were measured by Hydride Generated Atomic Fluorescence Spectrophotometry in the University of Washington Department of Environmental Health .

Arsenic concentrations in the hair from bears in the vicinity of the Alder Mine were also compared to normal levels reported for human hair and differences between test and reference concentrations were interpreted using published regression models associating exposure to accumulation rates in humans. Although toxicity and minimum exposure levels that produce measurable increases in As levels in bear hair have not been defined, regression models in humans show there is a good association between exposure and As accumulation in hair (DSHS 2000) and increases over reference levels would be an indicator of past exposures.

Population Level Response in Aspen Leaf Miner Larvae

Samples of leaves from six aspen (*Populus tremuloides*) trees growing on the waste rock at the Alder Mine (Figure 1, station 1) and samples of leaves from four aspen trees growing on the undisturbed slope on the opposite side of the watershed (Figure 1, west of site 4) were collected. Aspen leaf miner larvae [*Phyllocnistis populiella* Cham. (Lepidoptera: Gracillaridae)] from aspen leaves at the same locations were collected and pooled to provide 0.5 g wet weight (80-100 larvae). Leaves were rinsed in deionized water, dried, ground and analyzed for metals by ICP-AES in the University of Washington College of Forest Resources.

Community

Benthic invertebrate samples were collected in triplicate from riffles in Alder Creek in June and September 1998 in the same general vicinity as the water and sediment samples. Biological assessments were accomplished using a 0.09 m^2 Surber sampler (15 meshes cm^{-1}). Sample stations included three stations (5-7) on Alder Creek and Poorman Creek (19-21). Station 5 was located directly below the Alder Mine outfall. Two stations (6 and 7) were spaced approximately 0.5 km apart below the mine. Benthic macroinvertebrate samples were sorted for identification and the analysis of benthic macroinvertebrate community structure. Taxonomic identifications were made primarily using Merritt and Cummins (1996). Organisms were identified using a 7-65X stereo microscope to genus except for Diptera and Chloroperlidae, which were identified to the family level. Taxa richness and abundance were determined. Triplicate surface water and sediment samples were also collected at the same general locations and time as the Surber samples.

Ecosystem level

The mean element concentrations in forest soils contaminated by mine tailings were compared to ecotoxicological benchmarks derived from primary literature by Will and Suter (1994) for the effects of soil contamination on soil microbial processes. Trace elements that exceeded benchmark values were identified as compounds of potential environmental concern (COPEC) and it is assumed that the contaminant poses an ecosystem-level risk because soil microbial communities are valued for their functional properties rather than their community or population properties. Since this is an inference based on indirect evidence it would be given less weight than the direct measurement of an ecosystem function.

Respiration was also measured to provide direct evidence of ecosystem impacts from

trace metal contamination. These measurements were taken along a transect with three sites in a one-year old clearcut on the west side of Alder Creek across from the mine, three sites in the conifer forest north and adjacent to the mine and three sites in the conifer forest impacted by mine tailings. Respiration was measured three times each in July and October 1999.

Soil respiration was determined using the soda-lime trap method (Edwards 1982). The diameter of each respirometer was 10 cm. Thirty grams of soda-lime were weighed into four jars 7 cm in diameter (47% of the respirometer). The open jars of soda-lime were dried 8-hours at 100° C, capped and the initial dry weight was recorded. In the field, the jars of soda-lime were opened and placed over sample sites located in the forest below the Alder Mine tailings pile, in the forest adjacent to the tailings pile, in a clearcut opposite the tailings pile. The respirometers were installed over the jars. A control using a respirometer capped at both ends was included with each set of samples. The soda-lime was exposed for 24-hours then the jars were capped and returned to the laboratory. Gross weight before drying was recorded, the jars were then opened and dried for 8-hours at 100° C, then the final dry-weight was determined and recorded. Respiration rates of controls were subtracted from total mass and respiration was expressed as g m^{-2} day^{-1}.

Cytology

Portions of the liver < 2mm in diameter were collected from five fish in each microcosm immediately after dissection and portions of the small intestine < 2mm were removed from caddisfly larvae from reference station 9 and test station 13. Tissue samples were rinsed then fixed for 12 hours at 4°C in Karnovsky's fixative (5% gluteraldehyde and 4% formaldehyde in 0.1 M cacodylate buffer). Materials were then transferred to 0.1 M sodium-cacodylate buffer and stored at 4°C until transfer to the histopathology laboratory at the University of Washington, Department of Health Sciences.

Samples of tissue were embedded in paraffin, sectioned at 4-5 μ thickness on dry glass

knives, stained with toluidine blue, and mounted on glass microscope slides. The thick sections were then examined by light microscopy. Tissue samples were also dehydrated in a graded series of alcohol (Hayat 2000), post-fixed for 2 h in 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7.2, and embedded in Eponate. Sections approximately 85 nm thick, obtained with a Reichert/Jung Ultra-cut E microtome, were collected on copper grids, contrasted with uranyl acetate and lead citrate. Specimens were observed with a Phillips CM 100 transmission electron microscope (TEM).

Environmental Sampling and Analysis

All surface- and groundwater samples were collected in pre-cleaned Teflon bottles. Subsamples were filtered (Gellman 0.45 mm, disposable 25 mm sterile disposable Acrodisc filter) and preserved to pH<2 with 0.15% nitric acid and stored at < 5°C. Sediment samples were collected using plastic scoops at a shallow depth (<5 cm) and immediately wet sieved in ambient water through a 63 mm sieve. Samples were dried to constant weight at 90°C.

All analyses were performed within 30 days of sample collection. Samples of water and sediment were analyzed at the University of Washington, College of Forest Resources Analytical Laboratory in Seattle, Washington. The concentrations of metals in water and sediment samples were determined by ion coupled plasma - atomic emission spectrophotometry (ICP-AES; Thermo Jarrell Ash ICAP 61E). Samples were analyzed for As by Hydride Generated Atomic Fluorescence Spectrophotometry (HG - AFS). All water and sediment sampling equipment was cleaned by washing with Liquinox detergent and sequential rinses with distilled water, dilute nitric acid, and de-ionized water.

Data Analysis

The students t-Test was used to compare average weight of fish from the pens upstream and downstream from the abandoned mines. Statistical significance was computed using

Minitab statistical software (version 9). The Wilcoxon paired-sample test (Zar 1996) was used to test the hypothesis that there is no difference in the distribution of instar frequencies between caddisfly larvae populations at stations 8-11 and 12-15.

The students t-Test was used to compare the metal concentrations in leaves and larvae from site 3 to concentrations in the reference samples from trees west of site 4. Statistical. Bioconcentration factors (BCFs) were calculated for the elements in which the body concentrations were significantly different from leaf metal concentrations according to the equation:

$$BCF = \frac{[metal]_{Leaf\ Miner\ Larvae}}{[metal]_{Aspen\ Leaf}}$$

Where BCF = bioconcentration factor (dimensionless), $[metal]_{Leaf\ Miner\ Larvae}$ = total metal concentration in the larvae ($\mu g\ kg^{-1}$ dry weight), and $[metal]_{Aspen\ Leaf}$ = total metal concentration in aspen leaves ($\mu g\ kg^{-1}$ dry weight).

The students t-Test was also used to compare the abundance of invertebrates in Alder Creek and Poorman Creek.

RESULTS

Cellular Level Responses

The livers in trout from both the reference and test microcosms were generally reddish brown with occasional examples that were tan to off white. Dark or black colored livers and evidence of hemorrhaging into areas of necrosis were not observed. TEM microscopic examination of liver sections revealed large glycogen inclusions that were displacing nuclei to the periphery of effected hepatocytes was more extensive in trout downstream from mines compared to upstream samples (Figure 15 A, B). Upstream, hepatocytes appeared normal with a low incidence of glycogen inclusions. In one of five replicates from the downstream

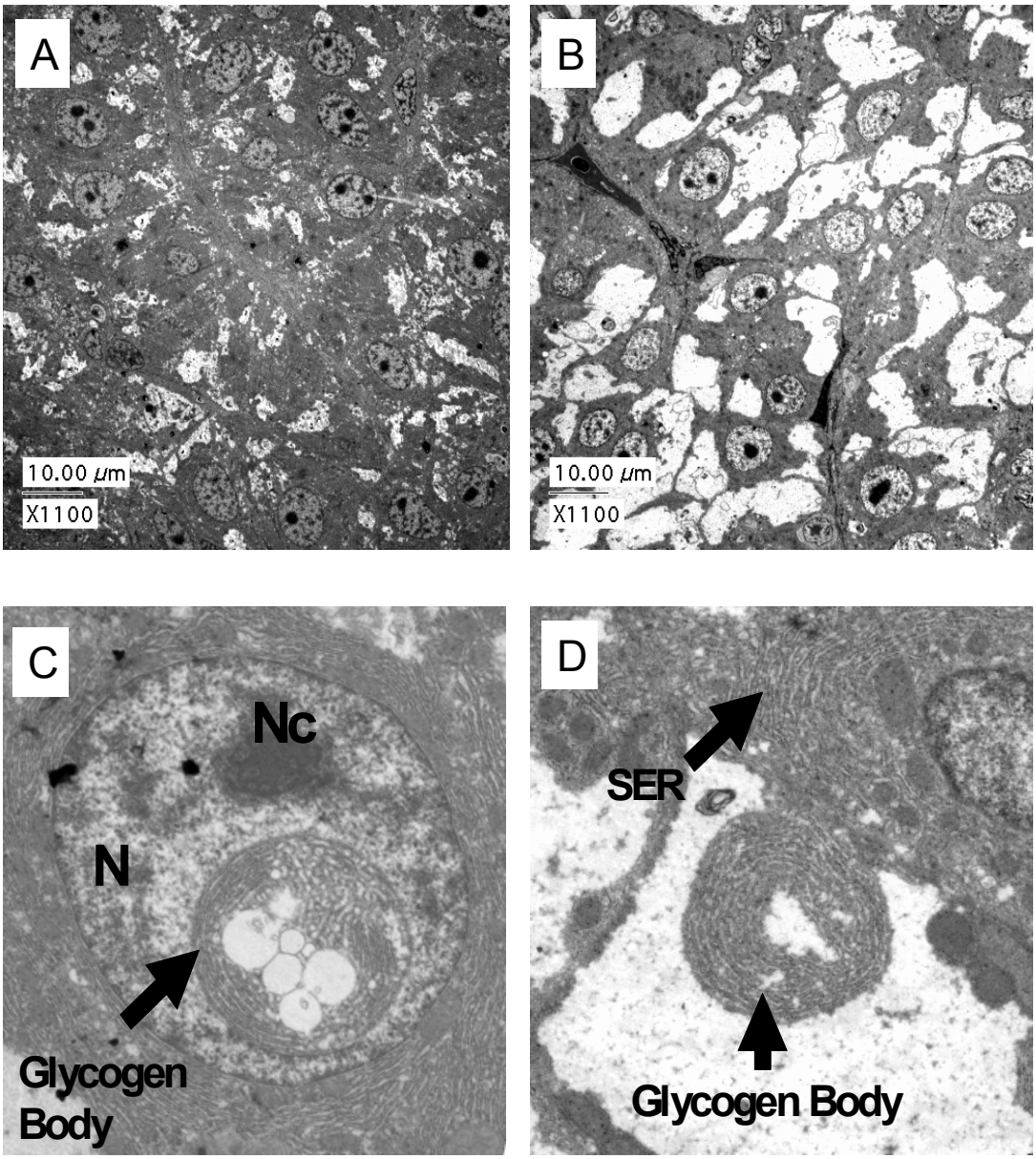


Figure 15. TEM micrographs showing effects of *in situ* exposure to trace element contamination from abandoned mines in the Methow River on juvenile triploid trout (*Oncorhynchus mykiss*) hepatocytes. (A) Section through normal trout liver. x1100. (B) Glycogen accumulation in hepatocytes from exposed trout. (C) Glycogen body in hepatocyte nucleus from exposed trout. x5800. (D) Glycogen body in hepatocyte cytosol from exposed trout.x7900.

microcosm, a proliferation of smooth endoplasmic reticulum, and glycogen bodies in the nucleus and cytosol were observed (Figure 15 C, D).

Individual Level Responses

The mean body weight of trout in the exposed group downstream from the abandoned mines (station 16)was less than the body weights of the upstream control group (station 11) [65g $\pm$ 10 (SD) vs. 71 $\pm$ 9, $P<0.01$]. Mortality among the trout in the test group downstream from the abandoned mines also exceeded the upstream control group. Three fish died within 96 hours following the beginning of exposure compared to no deaths in the control group. Two dead indigenous Coho parr were also encountered at station 15 during the study period.

Four trace elements (i.e., Cd, Cu, Mn, and Pb) exceeded toxicity benchmarks for aquatic biota in sediments at stations 12-15. In the Methow River, dissolved metal concentrations were less than the limits of detection by ICP-AES. Maximum temperatures were less than 16°C, the freshwater criteria for class A (excellent) surface water in the State of Washington (Ecology 1992) and pH ranged from 7.3 - 8.6. Alkalinity exceeded 190 mg L^{-1} as $CaCO_3$. Dissolved oxygen was greater than 8.3 mg L^{-1} at all stations, which exceeded 8.0 mg L^{-1}, the freshwater criteria for class A (excellent) surface water.

The mean live body weight of caddisfly larvae (*Ecclesiomia spp.*) was lower in the Methow River below the mine sites (stations 12-15) than it was upstream at stations 8-11 [2.3 $\pm$ 0.5 g (SD) vs. 1.2 $\pm$ 0.2 g 100-larvae^{-1}, $P<0.02$]. Growth patterns were also different between exposed larvae (stations 12-15), for which five larval stages were identified and reference larvae (stations 8-11) with seven larval stages. Development of the exposed larvae lagged behind the reference larvae as can be seen in Figure 16 which shows that eighty-four percent were mostly 4th instar larvae and only 8% were 5th instar. The reference site had fewer 4th instar (63%) and more 5th instar (35%) larvae.

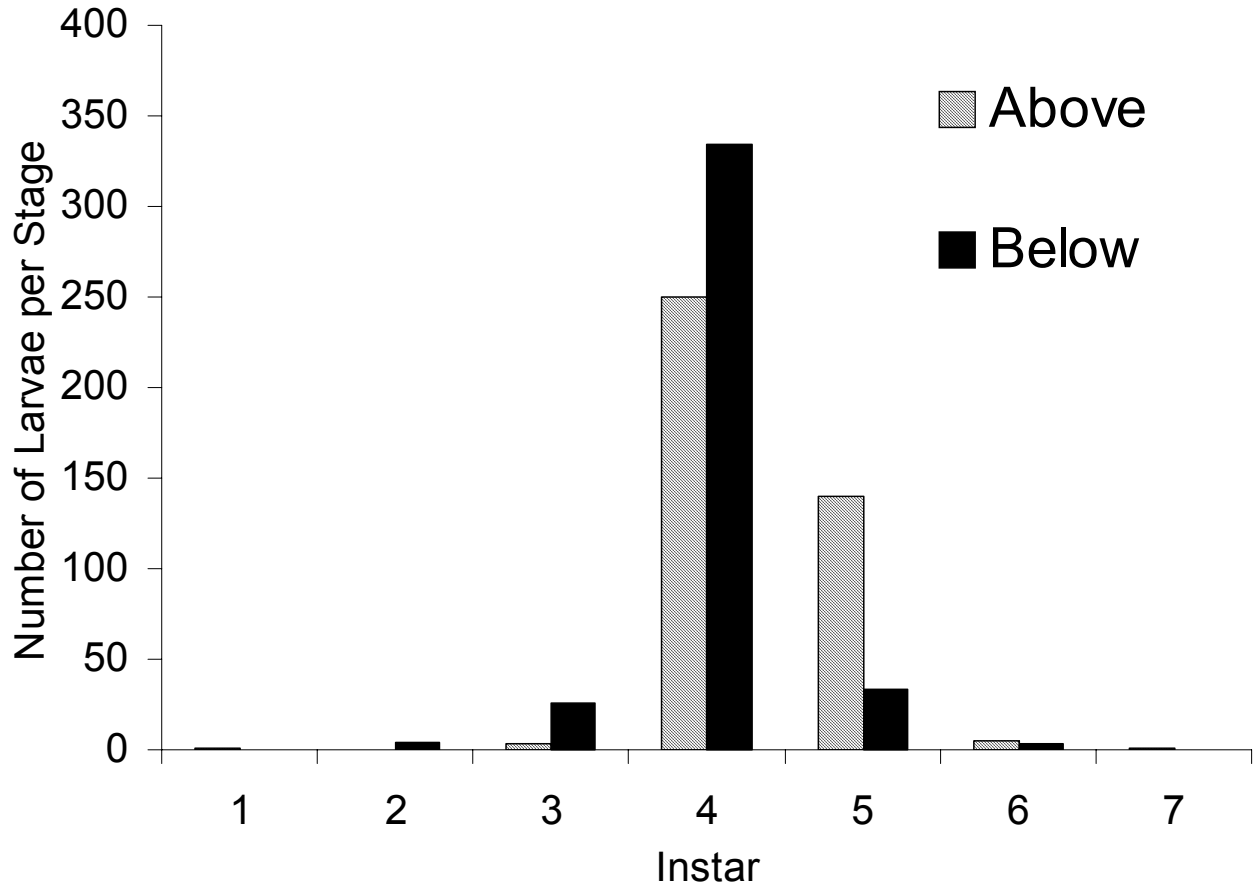


Figure 16. Frequency distribution of indigenous caddisfly larvae (*Ecclesomyia sp*) above and below mines along the Methow River. Development of larvae downstream lagged upstream larvae.

Population Level Responses in Black Bear and Aspen Leaf Miner Larvae

As concentrations were higher in the hair collected from black bears in the vicinity of the Alder Mine than those from bears at Northwest Trek (Table 8). Two out of the six samples analyzed from the exposure area exceeded 1 $\mu g\ kg^{-1}$ and the maximum concentration was 1.7 $\mu g\ kg^{-1}$.

Table 8. Arsenic concentration ($\mu g\ kg^{-1}$) in hair of bears in vicinity of Alder Mine at station 5. Hair was collected using a bear-hair capture technique and a non-consumable liquid lure.

	Alder Mine Bear Hair	Northwest Trek Bear Hair
	1.66	0.05
	1.29	0.04
	0.78	
	0.74	
	0.48	
	0.37	
Average:	0.87	0.05Average

Table 9 shows that eight trace elements accumulated in aspen leaves from sites contaminated with tailings and that 5 of these elements (Al, Cd, Cu, Pb, and Zn) were magnified in aspen leaf miner larvae that fed on the contaminated leaves. The BCFs for Al, Cu, and Pb were greater in leaf miner larva from the contaminated site than they were in the uncontaminated site. The Zn BCF was unchanged and Cd was lower.

Community

The taxa composition in contaminated Alder Creek was distinctly different from reference stations along Poorman Creek. The 34 most abundant taxa are shown in Figure 17. The number of taxa and abundance of macroinvertebrates was less in Alder Creek stations 5-7 than in Poorman Creek reference stations 19-21. *Baetis, Cinygmula,* Chloroperlidae, *Heterlimnius,*

Table 9. Accumulation of COPECs in aspen leaves, aspen leaf miner larvae, in black bear hair and trout liver and gill samples. . The bioconcentration factor (BCF) for COPECs transferred from aspen leaves to the Aspen Leaf Miner larvae based on the formula, BCF = $[COPEC]_{Aspen\ Leav\ Miner\ Larvae}$ / $[COPEC]_{Aspen\ Leaves}$. Trace element concentrations in aspen leaves are means plus standard deviation for samples from contaminated and reference sites. All concentrations are $\mu g\ g^{-1}$.

COPEC		Aspen Leaves	Samples n=	Aspen Leaf Miner Larvae	BCF
Al	Unexposed	14 ± 11	4	522	37
	Exposed	46 ± 15	6	3480	76
As	Unexposed				
	Exposed				
B	Unexposed	33 ± 6	4		0
	Exposed	94 ± 42	6		0
Ba	Unexposed			10.3	
	Exposed			51.8	
Cd	Unexposed	<0.9	4	6.0	6
	Exposed	23 ± 21	6	102	4
Cu	Unexposed	10 ± 1	4	26.0	3
	Exposed	15 ± 7	6	261	17
Mn	Unexposed	78 ± 9	4	22.4	0
	Exposed	104 ± 18	6	57.1	1
Pb	Unexposed	2 ± 1	4	1.2	1
	Exposed	3 ± 3	6	14.4	5
Se	Unexposed	3 ± 0	4		0
	Exposed	12 ± 11	6		0
Zn	Unexposed	150 ± 15 4		255	2
	Exposed	610 ± 2126		1050	2

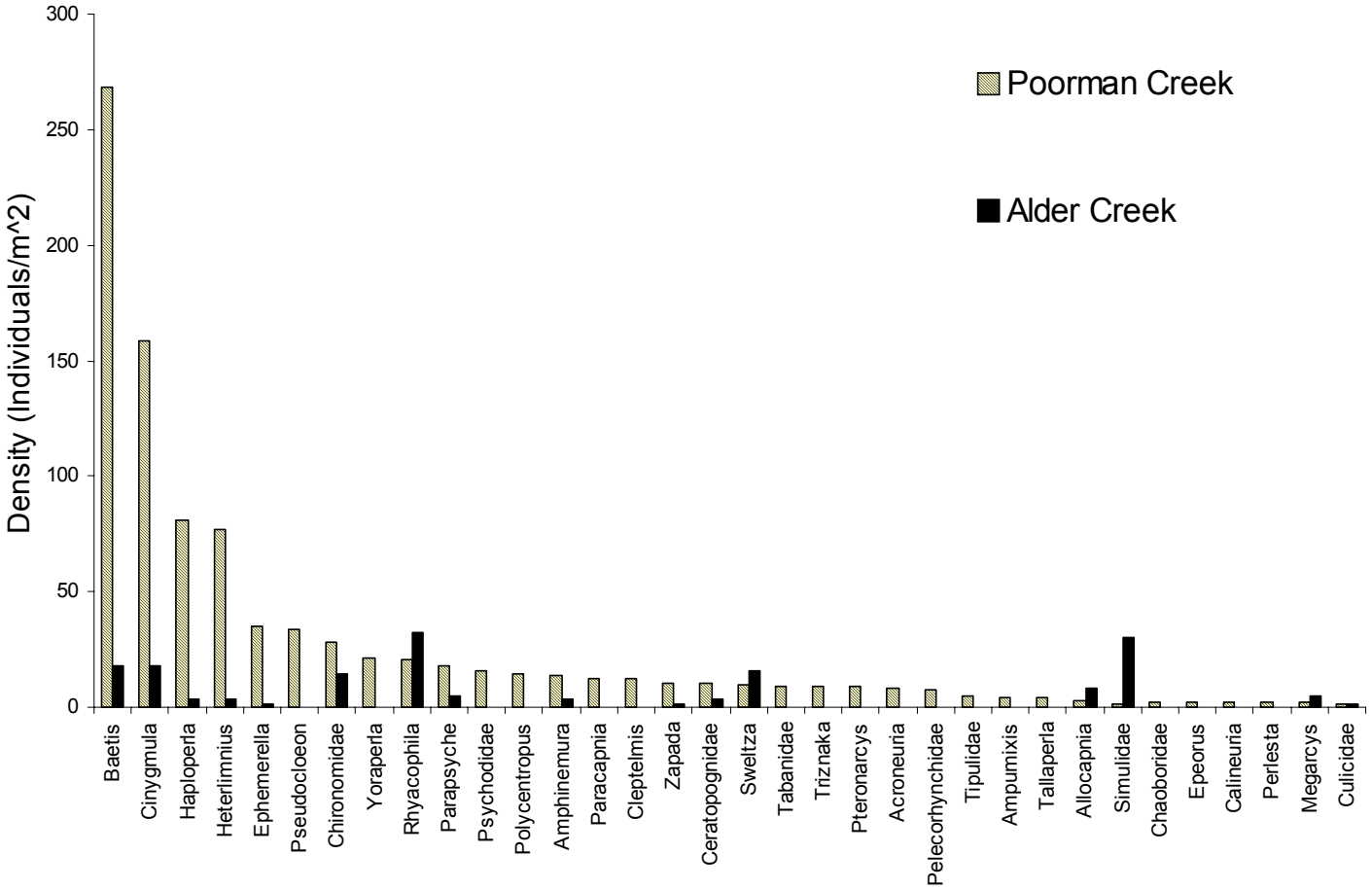


Figure 17. Impacts of trace element contamination on macroinvertebrate community structure. Density of macroinvertebrate taxa in Alder Creek (stations 5-7) are shown in relation to the same taxa in Poorman Creek reference stations 19-21.

and Chironomidae, the dominant taxa based on overall abundance, were found at all sites in the reference stream (Poorman Creek) although not always in the same order. Of the dominant taxa in Poorman Creek, *Baetis,* Chloroperlidae, *Heterlimnius,* and Chironomidae were reduced by at least 50% in Alder Creek. *Cinygmula* was the second most abundant taxa in Poorman Creek and in Alder Creek it was the second least abundant. Of the 48 taxa found in Poorman Creek, 17 taxa (35%) were absent from Alder Creek. Simulidae, which occurred infrequently in Poorman creek, was the dominant taxa in Alder Creek.

For all stations on Alder Creek, 10 taxa (i.e., *Baetis,* Pelecorhynchidae, *Amphinemura, Zapada, Heterlimius,* Simulidae, Chironomidae, Gammaridae, *Polypectropus, Ryacophila*) accounted for 80% of the total individuals, which is similar to reference stations 19-21 in which 11 taxa comprised 80% of the total individuals sampled (i.e., *Baetis, Cinygmula, Haploperla, Heterlimnius, Ephemerella, Pseudocloeon,* Chironomidae*, Yoraperla, Rhyacophila, Parapsyche,* and Psychodidae). At station 4, below the mine outfall, the invertebrate community was dominated by 5 taxa, Simulidae, *Baetis, Zapada, Heterlimnius,* and *Malenka,* which accounted for 80% of the individuals. At station 6, six taxa comprised 80% of total individuals identified including Simulidae, *Heterlimnus, Amphinemura,* Limnocharidae, *Gammarus,* and *Zapada.*

Ecosystem level

In mine tailings, metal concentrations were compared to soil benchmarks that were derived from toxicity studies conducted on plants in the field. This comparison identified 7 COPEC (i.e., Al, As, B, Cd, Cu, Mn, and Zn) that exceeded benchmarks for soil heterotrophic processes (Will and Suter 1994). Benchmarks are values at which contaminants pose a risk to microbial communities that are valued for their functional properties as well as their community or population properties. Concentrations in mine tailings were also compared to estimated background reference levels to calculate the concentration factor (CF), which was used as a

measure of relative risk. The CF of five trace elements suggest Zn (45), Cd (33), Cu (30), As (29), and Zn (7) are elevated relative to background levels.

Carbon dioxide evolution rates were also measured as an index of microbial respiration in the soil contaminated by Alder Mine tailings, a corresponding reference area, and a clear-cut site (Figure 18). In July 1999, the average respiration rates for the tailings contaminated forest soils (13.32 g CO_2 m^{-2} day^{-1}) showed a tendency to be greater than the respiration rate for the reference forest soil (8.10 g CO_2 m^{-2} day^{-1}) and the clear-cut (9.26 g CO_2 m^{-2} day^{-1}). In October, the average respiration rates for the tailings contaminated forest soils (6.08 g CO_2 m^{-2} day^{-1}) showed a tendency to be lower than the respiration rate for the reference forest soil (13.89 g CO_2 m^{-2} day^{-1}) and the clear-cut (12.15 g CO_2 m^{-2} day^{-1}). It is evident from these data that seasonal changes in respiration rates occurred and that respiration increased from July to October in the reference forest and clear-cut samples. In contrast, the respiration rates in the tailings-contaminated forest soil samples decreased from July and October. It should be noted, however, that when the one-way ANOVA was used these means were not significantly different at the 95% probability level.

DISCUSSION

At the cellular- level, the specificity of electron microscopy is evident based on the ability to diagnose specific toxicological and metabolic disorders (Phillips et al. 1987). The glycogen bodies in the cytosol and nuclei and the abnormal deposition of glycogen in hepatocytes of trout from the microcosm at station 15 downstream from the abandoned mine sites suggest the possibility of glycogen storage disease. Glycogen bodies can be an indication of toxin-induced metabolic disease and are sometimes associated with Wilson's Disease, a disorder of Cu metabolism in humans (Phillips 1987, Goldblatt and Cunning 1984, Ostrakovitch 2002). In white perch (*Morone americana*) hepatic copper storage (Wilson's) disease is characterized by the progressive accumulation of Cu in hepatic lysosomes bound to

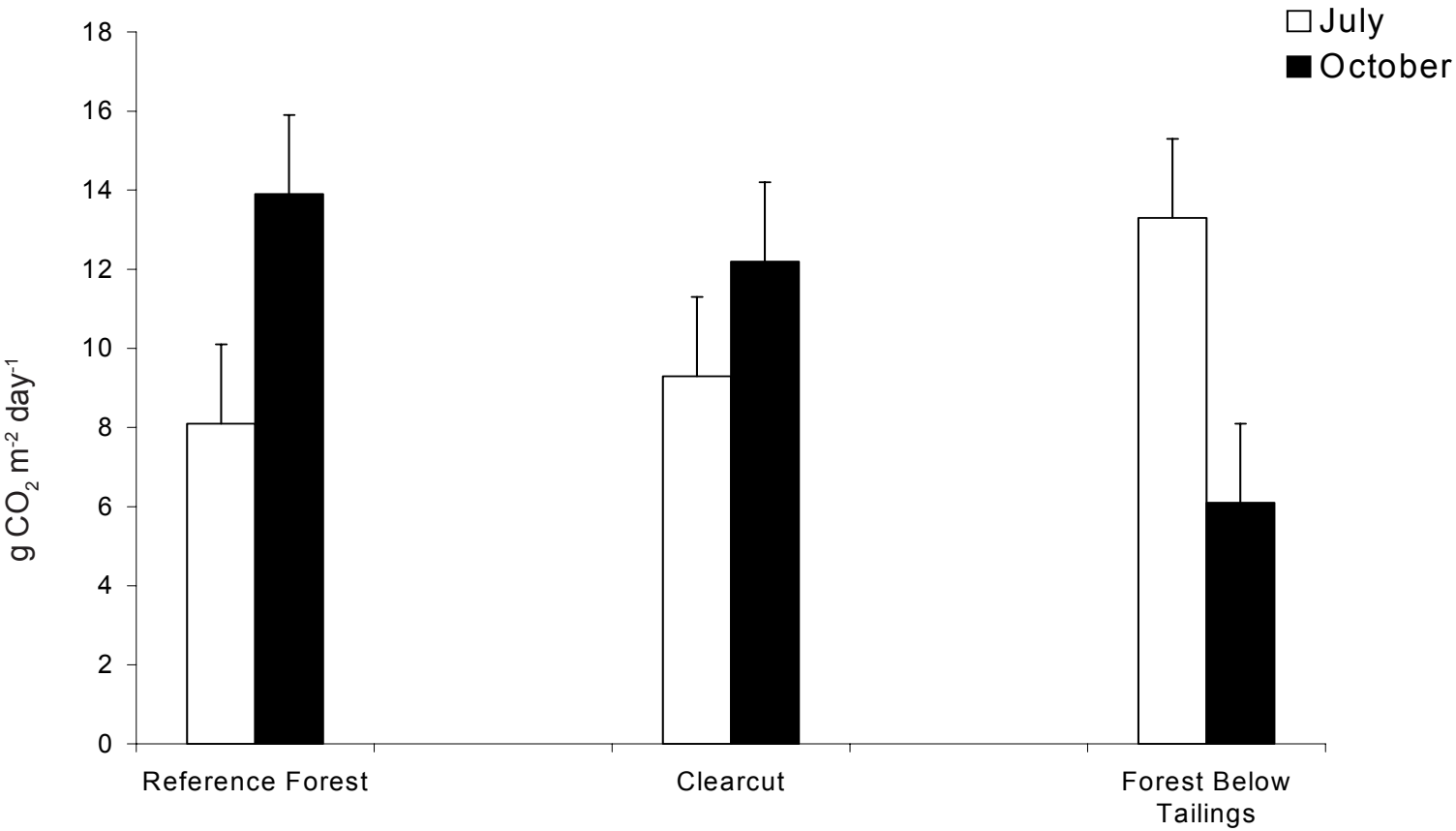


Figure 18. Average respiration rates for forest soils in July and October 1999. The reference forest soil was adjacent and 100-m north of the Alder Mine and the clear-cut was at the same altitude on the opposing slope of the Alder Creek valley. Error bars indicate SD.

cytoprotective metallothionein (Bunton and Frazier 1994). Saturation of the liver storage capacity results in the distribution of Cu to extrahepatic tissues with multiple organ system dysfunction.

Since conventional fixation results in the loss of glycogen, accumulation and deposition of glycogen as inclusion bodies in the cytosol of hepatocytes is visible as clear void spaces within the cytosol. The glycogen inclusions that were observed in the hepatocytes of fish from station 15 is suggestive of type IV glycogen storage disease (GSD IV, Anderson's disease, amylopectinosis) (Sherlock and Dooley 1997, Ishak and Sharp 1979) and can be used to distinguish it from other diseases. GSD IV is caused by a deficiency of the branching enzyme amylo-1,4,1,6 transglucosidase that results in the synthesis of an abnormal glycogen molecule having decreased branch points and increased chain length. Biochemically, the unbranched glycogen, similar to amylopectin, cannot be released and glycogen accumulates (Goodman and Ishak 1999).

While GSD IV may be an inherited metabolic condition it may also be a part of a toxic process (Goodman and Ishak 1999). Lead, Hg, Cd, Cr, Mn, Mo, Ni and Co are known to cause hepatic glycogen storage disease (Goodman and Ishak 1999, Gill and Pant 1981). Alterations in liver glycogen constitute a typical stress response in fish to acute metal salt intoxication (Gill and Pant 1981, Rana et al. 1985). In my study, the occurrence of glycogen inclusions in the liver suggests that metals contaminated sediments may be causing biochemical stress in exposed fish. In addition to elevated concentrations of Cu in sediments downstream from the abandoned mines, Cd, Mn, and Pb were also present at concentrations that exceed toxicity benchmarks for aquatic biota and these trace elements appear to be causing a metabolic disorder in which glycogen is not being converted back into glucose normally for distribution to the tissues. Glycogen storage disease has its most significant impact at the organ level where it serves as the principle intermediary between the dietary sources of energy and the extrahepatic tissues that are the main users of energy. The various forms of glycogen storage disease have

different enzymatic defects and a definitive diagnosis requires demonstration of the specific enzyme defect (Ishak and Sharp 1987, Sherlock and Dooley 1997).

At the individual-level, the lower body weights of microcosm trout and wild caddisfly larvae (*Ecclesiomyia spp*) downstream from the mine sites suggests that glycogen storage disease is causing either a diversion of energy from growth to tissue repair or that mine waste contaminants are influencing the rate of food conversion into usable energy (Ishak and Sharp 1987). My observation that larvae in the Methow River downstream from the mines were predominantly stage-three instars while upriver stage-four instars dominated suggests that development below the mine was delayed by as much as one-month using life-history histograms for *Ecclesiomyia spp* and other slow-seasonal caddisflies (Merrit and Cummins 1996, Irons 1987).

In polluted aquatic ecosystems the transfer of metals through food chains can cause high concentrations in invertebrates and toxicity in fish (Dallinger 1987). When susceptible invertebrate species are eliminated, metal-tolerant food organisms may become dominant. Their tolerance may be based on their ability to accumulate excessive amounts of metals, which would lead to increased dietary exposure among fish predators (Timmermans 1989, Woodward 1995).

The concentrations of Al, B, Cd, Mn, and Zn that we observed in aspen leaves and aspen leaf miner larvae were indicators that metals are being transferred to terrestrial plants and phytophagous invertebrates. These findings show that in addition to aquatic transport the food-chain transfer of metals is another pathway for the exposure of other populations to potentially toxic metals.

Arsenic accumulation in bear hair is also an indicator of exposure. Arsenic, which was found to accumulate in the hair of bears in the vicinity of the Alder Mine, is considered a useful indicator of exposure to arsenic over the preceding 6-12 months (DHHS 2000). We detected an average concentration in the exposed bears equal to 0.89 mg kg^{-1}, which was 18 times the

average for As in the hair of the reference bears (0.05 mg kg^{-1}). Although the physiological response of black bears to As is not known, a 0.1 mg g^{-1} increase in the As concentration in human hair corresponds to a 10-20 mg day^{-1} increase in daily As intake, which increases the risk of death, and systemic, immunological, neurological, reproductive, developmental, genotoxic and carcinogenic effects (DSHS 2000).

Arsenic accumulation in bear hair and Cd in fish liver suggests a potential for transfer of mine waste contaminants to top predators and indicate that environmental degradation has occurred in both aquatic and terrestrial ecosystems (Dallinger 1987, Hodson 1990). While measurable biochemical, physiologic, or other alterations were not observed, these results suggest that multiple pathways are exposing aquatic and terrestrial plants and animals to potentially toxic trace elements.

We hypothesize that when individual fish and caddisfly larvae are exposed to contaminated sediments the diversion of energy causes reduced competition among metals intolerant species and changes in community structure, which are considered a logical result of reduced energy conversion. Reduction in the abundance and diversity of benthic invertebrates in Alder Creek below Alder mine is an example of these community level effects. In the reference stream community, while many rare and infrequently encountered species were observed only a few species made up the bulk of the community. It was this relatively small set of abundant species that were functionally important because they contribute the most to the biomass of macroinvertebrates and are doing the bulk of nutrient uptake and transfer (Walker et al. 1999). The numerous other species that make up a small percentage of the biomass are functionally equivalent to the dominant species but have different environmental requirements and tolerances. It is the minor species that provide ecosystems with resiliency by maintaining ecosystem function under changing environmental conditions. When conditions change following contamination, metals sensitive species are replaced by metals-tolerant species, which reduces diversity and abundance, lowers resiliency and affects an ecosystem's ability to maintain productivity should

there be further changes.

The effects of trace element contamination on forest soil microbial communities also pose ecosystem-level risks because they are important in regards to nutrient cycling. Soil microbes are primary decomposers of soil organic matter, convert nutrients into plant-available forms, and serve as a food source for higher trophic levels. Because this functional property of soil microbial communities is of interest and not the community or population itself and because the concentrations of 11 contaminants exceeded benchmark values for toxicity it is assumed that these contaminants pose ecosystem-level risks. The effects of contaminants on plant communities is of similar ecosystem importance because the production of plant matter influences the cycling of carbon and is a primary source of organic carbon for soils and aquatic ecosystems (Barnes et al. 1998).

The finding that respiration in unimpacted forest and clear-cut soils showed a tendency to be higher in October than in July and that the opposite trend occurred in forest soils contaminated by Alder Mine waste is discussed here even though it is not statistically significant at the 95% level because of the possibility that the difference in respiration trend from July to October in the forest soil below the mine waste pile is biologically significant and that statistical significance would be achieved if sample numbers greater than n=3 were used. The results suggest trace element contamination affects soil microbial respiration. Respiration by soil microbial communities returns C fixed through photosynthesis back to the atmosphere as CO_2 (Barnes et al. 1998). Seasonal changes in respiration rates occur in response to variations in temperature and moisture (Marra 1995). In this study, high temperatures and decreased soil moisture content in the upper portion of the soil profile in July appears to have decreased microbial respiration in the reference forest and clear-cut sites whereas increased soil temperatures in the contaminated forest soils resulted in increased CO_2 evolution in July and reduced CO_2 evolution in October. This would occur if AMD flows from station 1 along a subsurface path characterized by extensive faulting and calcite-filled fractures (Ch. 2,

Coherence) and if the calcite fracture fillings are being dissolved by the sulfuric acid in acid mine waters infiltrating from the surface with the reaction releasing CO_2 .

Respiration rates increased from 8.01 g CO_2 m^{-2} day^{-1} in July to 13.89 g CO_2 m^{-2} day^{-1} in October in the reference soil adjacent, but not contaminated by waste from Alder Mine. Others have noted seasonal fluctuations in soil respiration rates in the clearcuts in Western Washington were from 9.26 to 12.15 g CO_2 m^{-2} day^{-1}, apparently in response to an increase in precipitation (Marra 1995). Gordon et al., (1986) found a higher summer maximum respiration rate of 15.92 g CO_2 m^{-2} day^{-1} in a clear-cut site in a white spruce forest in interior Alaska. Respiration rates in the forest floor below the Alder Mine tailings pile decreased from 13.32 in July to 6.08 g CO_2 m^{-2} day^{-1} in October. It is likely that cooler fall temperatures are reducing the abiotic production of CO_2 that occurs due to the reaction between AMD and $CaCO_3$ in the forest soil and bedrock.

CONCLUSION

Mine waste contamination was detected at different scales of biological organization, from cellular to ecosystem-level. Evidence of metabolic disorders that affect energy conversion in hepatocytes suggested that the mechanistic linkages that integrate responses across levels of organization is related to the disruption of energy exchange in cells, individual organisms, populations, communities and the ecosystem.

- The hepatocytes of trout exposed to elevated trace element concentrations downstream from the abandoned Alder Mine, Alder Mill and Red Shirt Mill exhibited signs of toxin-induced Glycogen-Storage Disease that is sometimes associated with abnormal Cu metabolism.
- Glycogen-Storage Disease at the cellular scale suggests that the lower body weights and retarded development of caddisfly larvae and trout in the Methow River downstream from the abandoned mine sites is causing either a diversion of energy from growth to tissue repair or that mine waste contaminants are influencing the rate of food conversion into usable energy.

- The bioaccumulation of Al, B, Cd, Mn, and Zn in aspen leaves and aspen leaf miner larvae adjacent to Alder mine indicate that food-chain transfer is another pathway for the exposure of other populations to potentially toxic trace elements in addition to aquatic transport.
- High As accumulations in bear hair and Cd in fish liver suggest that mine waste contaminants can be transfered to top predators and indicate that environmental degradation has occurred in both aquatic and terrestrial ecosystems.
- Increased trace element concentrations in Alder Creek resulted in metals-sensitive invertebrate species being replaced by metals-tolerant invertebrate species. Taxa diversity and abundance were reduced and the aquatic community structure was altered.
- The trend for reduced soil microbial respiration in mine waste contamination suggests ecosystem-level functions have been affected.

CHAPTER 5

HEALTH RISKS ASSOCIATED WITH ARSENIC AND TRACE METAL CONTAMINATION OF GROUNDWATER BY ABANDONED MINES NEAR TWISP IN OKANOGAN COUNTY, WASHINGTON

INTRODUCTION

Severe health effects are associated with exposure to inorganic As and metal contaminants in drinking water (DHHS 2000, Oehme 1978). Although the presence of As and trace metals at concentrations exceeding natural background levels is considered an indication that contamination has occurred (EPA 2000a), the risk of adverse health effects depends on their concentration, frequency of contact, and duration of exposure. Ingestion of As in drinking water has been linked with two noncancer health conditions that are a major source of morbidity and mortality (i.e., hypertension and diabetes mellitus), and increased risk of skin, bladder, and lung cancer at very low concentrations (NRC 2001).

Arsenic and trace metal contamination of groundwater and private drinking water wells in the vicinity of the abandoned Alder Mine, Alder Mill and Red Shirt Mill in the Methow River valley near the town of Twisp, in Okanogan County, Washington, U.S.A., was investigated between October 1999 and June 2001. Arsenic and trace metal concentrations that exceed the natural background concentrations for Washington State soils were previously detected by the U.S. Environmental Protection Agency (EPA) in tailings and waste rock samples at the Alder Mill and Alder Mine sites (Spencer 1986, EPA 2000a).

The contamination of a domestic well by waste water and tailings from the abandoned Alder Mill, which ceased operations in 1952, was confirmed by Spencer (1986), who noted a potential for groundwater contamination. Potential targets related to groundwater exposure pathways were documented, and a potentially affected population, estimated to be between 1000 and 1287 residents, was identified within a 6.5-km (4-mile) radius of the Alder Mine and Alder Mill.

The objectives of this study were to (1) measure As and trace element concentrations in groundwater and estimate exposure among residents consuming groundwater from wells adjacent to abandoned mine sites in the Methow River Valley, (2) estimate the risk of adverse health effects from As and trace metal contamination in residents consuming water from private wells, and (3) consider the degree of uncertainty associated with the assessed risk.

METHODS

The study site is located in the Methow River valley near the town of Twisp in Okanogan County, Washington (Figure 1, Chapter 2). Ten domestic drinking water wells located adjacent to the Alder Mill, near Alder Creek below Alder Mine, and adjacent to the Red Shirt Mill, and two reference wells that were isolated from mine impacts (Figure 3, Chapter 2) were sampled monthly between October 1999 and June 2001. Samples of water from private domestic wells were collected from the well casing using disposable Teflon bailers in pre-cleaned 50-mL polypropylene centrifuge tubes. The locations of sample stations were documented using a Trimble (Sunnyvale, CA) GeoExplorer (handheld) GPS unit.

Samples for As analysis were frozen until analyzed. Subaliquotes for metals analysis were also collected and preserved to pH <2 with 0.15% HNO3 and stored at 1 - 5°C. Preserved samples of water were analyzed at the University of Washington in Seattle Washington. Samples were analyzed for As by Hydride Generated Atomic Fluorescence Spectrophotometry in the Environmental Health Laboratory, Department of Environmental Health, School of Public Health and Community Medicine. Dissolved arsenic was speciated by HPLC to distinguish arsenates (AsO_4^{-3}) and arsenites (AsO_2^{-}). Total unfiltered metals (i.e., Ba, Cd, Cr, Cu, Fe, Pb and Se) were determined by ICP-AES (Thermo Jarrell Ash ICAP 61E, EPA Method 3050) in the College of Forest Resources Laboratory.

Estimating Exposure and Risk

In this study, data were summarized using simple descriptive statistics including range, mean and standard deviation. Consumption of contaminants in well water was calculated based on its concentration in the water and on an estimate of water consumption rates. The default drinking water intake rate of 2 L day^{-1} for adults (70 kg body mass) (EPA 1980) was used to estimate the average daily dose (ADD). The daily water intake rate was multiplied by the arithmetic mean concentration of the contaminant in well water, in $\mu g\ L^{-1}$, then divided by 70 kg for adults.

Exposure can be expressed as a noncarcinogenic risk and as a carcinogenic risk. Noncarcinogenic risk was expressed in terms of the Hazard Quotient (HQ). The basic equation for calculating the noncarcinogenic HQ was to divide the ADD (μg kg-body-$weight^{-1}$ day^{-1}) by the chronic reference dose (RfD; 0.3 μg As kg-body-$weight^{-1}$ day^{-1}, 0.5 μg Cd kg^{-1} day^{-1}, 5 μg Se kg^{-1} day^{-1}) (Calow 1998, IRIS 2001). Since there is no Rfd for Pb, a coefficient is given that is based on the ratio of its concentration in water to the action levels for drinking water (i.e., 15 $\mu g\ L^{-1}$, IRIS 2001). Excess risk exists for HQ (or Pb Coefficient) >1. Carcinogenic risks associated with As were statements of probability and were calculated by multiplying the ADD by a Cancer Slope Factor (CSF, 1.5 $\mu g\ kg^{-1}\ day^{-1}$) (Calow 1998, IRIS 2001).

When an RfD value is discussed, the Drinking Water Equivalent Level (DWEL) is also given. DWEL represents the lifetime exposure concentration that is estimated to result in a negligible adverse non-cancer health effect (EPA 2000b). To calculate DWEL, RfD values are multiplied by the typical adult body weight (70 kg) and divided by an assumed daily water consumption rate (2 L).

RESULTS

Arsenic and Trace Metal Contamination

The average As concentration in water samples taken between October 1999 and June 2001 from ten domestic drinking water wells located adjacent to Alder Mill, near Alder Creek below Alder Mine, and adjacent to the Red Shirt Mill ranged from <1 - 298 mg L^{-1} (Table 10). Pb ranged from 0 – 94 mg L^{-1}, Cd 0 - 5 µg L^{-1}, and Se 0 - 390 mg L^{-1} (Table 11). The calculated average daily dose (ADD) for As ranged from <0.029 – 8.5 µg kg^{-1} day^{-1}. Table 12 shows that in wells 22-30, the ADD was equal to or greater than the As Rfd (0.3µg kg^{-1} day^{-1}, IRIS 1999). Two reference wells were also sampled and no As was detected in either of the ten samples from well 32 or the 11 samples from well 33.

Speciation of As in the first of two samples from well 17 revealed As^{3+} was the predominant species at approximately 70% and As^{5+} was present at approximately 30%. In the second sample arsenic was in the oxidized As^{5+} form. No organic or methylated forms of As were detected. Well 17 pH ranged from 5.9-7.4. Dissolved oxygen was present at approximately 125 mM. Iron in well 17 was present in the reduced divalent (Fe^{+2}) form which corresponds at equilibrium to a maximum theoretical Eh of approximately 0 mV at pH 7 at 25ºC (Langmuir 1997).

Lead, Se, and Cd concentrations are given in Table 11. Lead concentrations in wells 17, 23-25, 28, 30, and 31 exceeded 15 µg L^{-1}, the action level for drinking water. The average Cd concentration in well 31 was 5 µg L^{-1}, the action level for drinking water, which was due to a single sample collected in March, 2000, with a concentration of 82 µg L^{-1}. In two wells monitored (17 and 23), Se concentrations averaged 146 and 390 mg L^{-1}, respectively. In contrast to As for which there was very little variation in the monthly concentration, Cd, Pb, and Se concentrations were highest in the spring (April – June) which coincided with peak snow-melt conditions.

Table 10. Average arsenic concentrations (mg L^{-1}) in well water from ten private wells near the Alder Mine, Alder Mill and Red Shirt Mill south of Twisp in Okanogan County, Washington (U.S.A.). Samples were collected at monthly intervals between October 1999 and June 2001. See Figure 3 (Chapter 2) for map showing well numbers and locations.

Well No.	Coordinates (N. Lat./W. Long.)	Number of Samples	Arsenic Concentration Mean ($\mu g\ L^{-1}$) ± SD	
17	48.21.17.3/120.07.40.8	14	298	± 75
24	48.21.30.5/120.07.39.4	6	151	± 59
23	48.20.54.3/120.06.58.6	12	19	± 16
25	48.21.17.1/120.07.10.4	12	12	± 2
26	48.21.32.3/120.07.50.5	4	11	± 1
27	48.20.32.4/120.07.36.8	3	11	± 1
28	48.21.03.4/120.06.58.7	11	9	± 10
29	48.20.36.6/120.08.51.2	1	10	—
30	48.20.27.5/120.07.22.2	13	8	± 4
31	48.21.06.3/120.06.06.8	13	<1	—
32	48.22.02.9/120.10.26.9	10	0	—
33	48.21.13.3/120.06.21.6	11	0	—
—	Okanogan County[1]	62	0	—

Table 11. Average concentrations, average daily dose (ADD) values, and hazard qoutient (HQ) values (noncarcinogenic risk) from lead (Pb), selenium (Se) and cadmium (Cd) exposure based on monthly samples taken between October 1999 - June 2001 from wells near the Alder Mine, Alder Mill and Red Shirt Mill south of Twisp in Okanogan County, Washington (U.S.A.). HQ and carcinogenic risk values enclosed in dashed-line box exceed limits of acceptable risk. Only wells with concentrations greater than zero are listed. Only wells with detectable levels of Pb, Se or Cd are shown.

Metal Contaminant	Well No.	Concentration μg L^{-1}	ADD μg kg-1 d-1	HQ[1]
Pb	31	94	2.7	6.3
	23	64	1.8	4.3
	24	61	1.7	4.1
	30	49	1.4	3.3
	17	35	1.0	2.3
	25	31	0.9	2.1
	28	22	0.6	1.5
	27	10	0.3	0.7
Se	23	390	11.1	2.2
	17	146	4.2	0.8
Cd	31	5	0.1	0.0

[1] HQ = ADD/Reference Dose for Cd (0.5) and Se (5 μg L^{-1}). For Pb, risk coefficient = C/MCL (15 μg L^{-1}).

Arsenic Exposure and Risk Estimates

The estimated ADD, toxic noncarcinogenic HQ and the carcinogenic risk from As at each well is given in Table 12. The noncarcinogenic HQ for wells 17, 24 and 23 ranged from a high of 28.3 to 1.7, which places them above a threshold of concern for adverse health effects. On average, one excess death from cancer per 1601 adults (2.3×10^{-3}) is expected for all wells tested. Carcinogenic risk from drinking water from well 17 was 1-in-77 for adults.

Pb, Se and Cd Exposure and Risk Estimates

The average monthly concentration of Pb in 8 of 10 wells tested ranged from 10 to 94 mg L^{-1} (Table 11). Lead was not detected in two of the wells monitored (wells 26 and 29). The HQ for Pb exceeded 1 in seven wells and ranged from 1.5 to 6.3. For Se, two of the wells monitored (23 and 17) contained Se at 390 and 146 mg L^{-1}, respectively. The HQ for Se was 2.2 in well 23. The average Cd concentration was 5 µg L^{-1} in well 31, due to a single value of 82 µg L^{-1} in a sample collected in March 2000. When averaged over the fourteen-month sampling period, the ADD of Cd was 0.1 µg kg^{-1} day^{-1} and the HQs was less than one.

DISCUSSION

Arsenic and Trace Metal Contamination

Water from private drinking water wells near the Alder Mine, Alder Mill and Red Shirt Mill had elevated concentrations of As relative to drining water standards in nine out of the 10 wells tested. Additional data were available from Garrigues and Carey (1999) who compiled groundwater quality data from 62 wells sampled throughout Okanogan County between 1958 and 1979. These data combined with reference well data from this study suggest that background water quality is good and As, Cd, Pb, and Se are generally below detection limits. The four wells in the Garrigues and Carey study in which detectable concentrations of As were

Table 12. Average daily dose (ADD) values , hazard qoutient (HQ) values (noncarcinogenic risk), and carcinogenic risk from arsenic (As). ADD was calculated assuming 2 L daily water consumption and 70 kg for body weight. HQ and carcinogenic risk values enclosed in dashed-line box exceed limits of acceptable risk.

Well No.	As[1] ($\mu g\ L^{-1}$)	ADD $\mu g\ kg^{-1}\ d^{-1}$	HQ[2]	Carcinogenic Risk[3]	
17	298	8.5	28.3	5.7×10^{-3}	1 in 77
24	151	4.3	14.3	2.9×10^{-3}	1 in 154
23	19	0.5	1.7	3.3×10^{-4}	1 in 1333
25	12	0.3	1.0	2.0×10^{-4}	1 in 2222
26	11	0.3	1.0	2.0×10^{-4}	1 in 2222
27	11	0.3	1.0	2.0×10^{-4}	1 in 2222
28	10	0.3	1.0	2.0×10^{-4}	1 in 2222
29	9	0.3	1.0	2.0×10^{-4}	1 in 2222
30	8	0.2	0.7	1.3×10^{-4}	1 in 3333
31	0	0.0	0.0	0.0×10^{-0}	0

[1] Current Maximum Contaminant Level (MCL) for As is 50 $\mu g\ L^{-1}$ (EPA 2000c)

[2] Chronic Reference Dose (RfD) for As is 0.3 $\mu g\ kg^{-1}\ d^{-1}$ (USEPA 2000b)

[3] Cancer Slope Factor (CSF) for As is 1/1500 μg kg-1 d^{-1} (USEPA 2000b)

found were all located near population centers in the incorporated towns of Twisp, Okanogan, Brewster, and Tonasket suggesting that the As found in these wells might be from anthropogenic sources.

All wells, except one located on the Red Shirt Mill site (well 31), exceeded estimated background levels. Due to the lack of pre-mining groundwater data from the sampled wells, and because water quality can vary over short distances (S&KC PH 2000), it was not possible to define true background values for water quality in the tested wells. The two reference wells (32 and 33) monitored in this study were selected based on both their proximity to the test wells (and an assumed similarity of the groundwater lithology) and their apparent lack of hydrologic continuity with the mine sites.

In aquatic systems, inorganic As occurs primarily in two oxidation states, arsenate (As^{+5}) and arsenite (As^{+3}) (DHHS 2000). Both forms generally exist together although As^{+5} predominates under oxidizing conditions and As^{+3} predominates under reducing conditions. The presence of Fe^{2+} with both As^{3+} and As^{5+} in well 17 reflects a state of disequilibrium in which active oxidation-reduction reactions are occurring as a result of water-mineral interactions and a lack of redox equilibrium (Stumm and Morgan 1996). The pH of well 17 ranged from 5.9 to 7.4 and oxygen was present at 125 μM, indicating an oxidizing environment.

Due to the difficulty in measuring thermodynamically meaningful Eh, the simplified redox classification scheme proposed by Berner (1981) is useful. When oxygen exceeds 30 μM, an oxidizing environment is present and Fe^{2+} is expected to be below the limits of detection at equilibrium. According to the stability relations of As and Fe in As-Fe-O-H-S systems (Vink 1996), the field for ionic arsenate and arsenite species are mostly combined with Fe^{+3} under oxidizing conditions, and under reducing conditions Fe^{+2} would coexist with As^{+1} (realgar, As_2S_2). Evidence from Oscarson et al. (1981) suggests that the kinetics of arsenic oxidation is relatively slow.

The distinction between arsenates (AsO_4^{-3}) and arsenites (AsO_2^-) is often made because a number of studies have noted differences in the relative toxicity of these compounds, with trivalent arsenites tending to be more toxic than pentavalent arsenates (DHHS 2000). These distinctions are sometimes not emphasized because the differences in the relative potency are small (2-3-fold) and may be within the range of uncertainty and because different forms of arsenic may be interconverted both in the environment and in the body.

Arsenic and Trace Element Exposure and Risk Estimates

The average As concentration in all test wells, measured between October 1999 and June 2001, ranged from <1 – 298 μg L^{-1}. In July 2000, the Washington State Department of Ecology sampled 16 wells including wells 17, 23 and 31 described in this study (Roeder 2000). In the WDOE study, Pb, Se, and Cd were not detected in any of the wells tested nor was As in samples from wells 23 and 31. WDOE found As at 360 μg L^{-1} in well 17, which compared to my results (range 159 – 395 μg L^{-1}, mean 298 μg L^{-1}, n=14) and 250 μg L^{-1} in a nearby well not included in our study. In 14 wells sampled by the WDOE, As was reported as 50 μg L^{-1} with the qualifier "U" indicating that As was not detected above the associated value, which is either the sample quantitation limit or the sample detection limit according to EPA methods (EPA 2001).

As concentration in well 23 (298 μg L^{-1}) agree with those reported by WDOE (360 μg-L^{-1}). The WDOE also found 250 μg As L^{-1} in a well that we did not test. One significant difference between my results and those of WDOE is that we measured concentrations over a 14-month period whereas the WDOE collected samples only in July during low run-off conditions. In my study Pb, Se and Cd concentrations varied seasonally.

As Toxicity

Large doses of inorganic arsenic that exceed 60000 μg kg^{-1} body weight, when consumed with food or water, can produce death (DHHS 2000, ATSDR 1989). The US EPA concluded that acute or short-term effects are not seen at 50 μg L^{-1} (US EPA 2000d). Instead, the focus has been on long-term, chronic effects. In 1999, the National Research Council (NRC) concluded that As is associated with both carcinogenic and noncarcinogenic risk (NAS 2001). According to the NRC, however, one limitation of risk assessments based on exposure is that dose is estimated from measuring the concentrations of contaminants in drinking water and assuming an exposure based on drinking water rates.

Noncarcinogenic Risk

The ADD for As, which occurred at concentrations that exceeded 8 μg L^{-1} was greater than the RfD in wells 17, 23, and 31. The lowest exposure level at which there are reports of statistically or biologically significant increases in frequency or severity of noncarcinogenic effects between an exposed population and its appropriate control group (Lowest-Observed Adverse Effect Level, LOAEL) is about 10 – 20 μg As kg^{-1} day^{-1} (DWEL = 350-700 μg L^{-1} water,) (Cebrian et al. 1983, Hindmarsh et al. 1977, Southwick et al. 1981, Tseng 1977, Tseng et al. 1968). In 1999, the National Research Council (NRC 1999) concluded that As might also be associated with an increased risk of high blood pressure and diabetes. The magnitude of the risk, however, is not yet quantifiable (NAS 2001).

There is a range of exposures to As and trace metals from zero to some finite value that is essential to, or at least can be tolerated by humans with essentially no chance of expression of noncarcinogenic effects (Barnes and Dourson 1988). Homeostatic, compensating, and adaptive mechanisms exist that must be overcome before a toxic response occurs. For example, there could be a large number of cells performing the same or similar function whose population must

be significantly depleted before the effect is seen. The As level at which no adverse systemic (noncarcinogenic) effect is expected, (No-Observed Adverse-Effect-Level, NOAEL) based on chronic oral exposure data from studies in humans, is between 0.4 and 0.9 μg As kg^{-1} body weight day^{-1} (DWEL = 14-32 μg L^{-1}) (DHHS 2000). The NOAEL of 0.8 μg As kg^{-1} day^{-1} (DWEL = 28 μg L^{-1}) from the study be Tseng et al. (1968) has been used to derive the arsenic reference dose (RfD = 0.3 μg As kg^{-1} body weight day^{-1}, DWEL = 11 μg L^{-1}). The RfD, which is an estimated threshold exposure that includes an uncertainty factor, is expressed in mg kg-body-$weight^{-1}$ day^{-1} and represents the dose below which toxicity does not occur among humans, including sensitive subgroups, during a lifetime. Tseng et al. (1968) assigned an uncertainty factor of 3, and this value has been adopted by the USEPA (Opresko 1992) because the no-effect group was relatively young thus decreasing the possibility that effects would be detected.

Carcinogenic Risk

In my study, the average calculated risk of mortality from cancer following exposure to As at average concentrations as low as 8 μg L^{-1} was greater than 1 in 1000. Based on epidemiological studies, As at or above several hundred μg L^{-1} causes increased rates of mortality from skin, bladder, and lung cancer (Cebrian et al. 1983; Hindmarsh et al. 1977; Southwick et al. 1981; Tseng 1977; Tseng et al. 1968). Other studies have noted an increased risk of liver and kidney cancer (NAS 2001). Two recent studies contribute to the quantitative assessment of risk for urinary-tract cancers in Taiwan (Chiou et al. 2001) and for lung cancer in Chile (Ferreccio et al 2000).

It is assumed that a carcinogenic response occurs without a threshold and that any dose of a carcinogen is associated with some increased risk (Felter et al., 1998). It is assumed that a small number of molecular events can evoke changes in a single cell that can lead to

uncontrolled cellular proliferation, i.e., cancer (Barnes and Dourson 1988). This mechanism for carcinogenesis is referred to as "nonthreshold" since there is theoretically no level of exposure for such a chemical that does not pose a small, but finite, probability of generating a carcinogenic response. A factor that obstructs the detection of carcinogenesis is the mean latency period, which is the period of time between the critical molecular interaction of a carcinogen within a single cell and the first appearance of a malignant cell. For As, latency has been reported to range from 14 – 41 years (NAS 2001).

A cancer slope factor (CSF) was derived using a linear, no-threshold dose-response model (Petts 1998). Numerous studies in humans have reported dermal effects at chronic dose levels ranging from about 0.01 to 0.1 mg As kg^{-1} day^{-1} (DWEL = 35-350 µg As L^{-1}) (DHHS 2000). The data provided by Tseng et al., (1968) and Tseng (1977) on about 40 000 persons exposed to arsenic in drinking water and 7500 relatively unexposed controls were used to develop dose-response curves for As. Tseng et al. (1968) reported skin cancer rates of 260, 1010, and 2140 per 100 000 in Taiwanese populations whose drinking water contained 300, 300-590, and 600 µg L^{-1} As, respectively. No cases of skin cancer were seen in the control population of 7 500 whose water contained 1-17 µg L^{-1} As.

Although bladder and lung cancers are the main sources of concern (NAS 2001) based on mortality data from studies in Taiwan (Tseng 1977, Tseng et al. 1968), skin cancers are more visible and the time of their appearance can be dated with greater accuracy. There is convincing evidence from a large number of epidemiological studies and case reports that ingestion of inorganic arsenic increases the risk of developing skin cancer (DHHS 2000). Lesions commonly observed were squamous cell carcinomas (SCC), which develop from characteristic patterns of skin changes associated with arsenic exposure.

A time- and dose-related formulation of the multistage model (EPA 1988) was used to predict skin cancer prevalence rates associated with the ingestion of inorganic arsenic at low

concentrations (IRIS 2001). The risk of skin cancer for a 70 kg person drinking 2 L of water per day ranged from 1-in-1 000 to 1-in-500 for an arsenic intake of 1 $\mu g\ kg^{-1}\ day^{-1}$ (DWEL = 35 $\mu g\ L^{-1}$). Based on the pooled data and average well concentrations for each village in the Tseng (1977) study, the unit risk (the upper-bound excess cancer risk from lifetime exposure to water containing 1 μg As/L) was 1-in-20 000). Drinking water concentrations at specified risk levels based on the unit risk value are 2 $\mu g\ L^{-1}$ at the 1 in 10,000 risk level, 0.2 $\mu g\ L^{-1}$ at the 1 in 100 000 risk level, and 0.02 $\mu g\ L^{-1}$ at the 1 in 1 000 000 risk level.

The results of my study indicate that the average risk of risk of cancer from exposure to As is approximately two times the risk of squamous cell carcinoma (SCC) among adults engaged in occupations with high sunlight exposure. Gray et al. (1997) reported that in Rochester, MN, between 1984 and 1992, there were 60 additional cases of SCC on the head and neck per 100,000 people (1 in 1,700) that were attributed to excessive exposure to sun. The average carcinogenic risk associated with As exposure, using the model derived from SSC epidemiological data, was equal to 11 per 10 000 persons (1 in 909).

The use of a cancer risk estimate derived from the Tseng et al. (1968) study for a U.S. population has been the source of debate and a number of concerns have been raised including the applicability of Taiwanese data to the U.S., a possible threshold for arsenic carcinogenicity, nonlinearities in the dose-response curve, differences in health and nutrition between Taiwan and the United States that might increase cancer risk in Taiwan, the possibility that As is an essential element at lower doses, and the possibility of significant exposure to As from sources other than well water (DHHS 2000). These factors contribute to uncertainty in the risk assessment. Several epidemiological studies of small populations (20-200 people) performed in the United States have not detected an increased frequency of skin cancer in small populations consuming water containing As at levels of less than 400 μg L-1 (DHHS 2000). These data suggest that arsenic-associated skin cancer is not a problem in this country. However, these studies lacked

sufficient statistical power to detect the small increases in skin cancer incidence that might have occurred at low doses (DHHS 2000).

Sources of Uncertainty

According to the 1999 Subcommittee on Arsenic in Drinking Water, different individuals can contribute to variability and uncertainty in risk assessments (NRC 1999) because susceptibility to inorganic As can vary due to differences in genetics, metabolism, diet, health status, and sex (NRC 1999). RfDs, expressed in micrograms of chemical per kilogram of body weight per day ($\mu g\ kg^{-1}\ day^{-1}$), are based on the no-observed-adverse-effects-level (NOEL) and are estimates of the daily amount of chemical a person, including sensitive individuals, can ingest over a lifetime with little risk of causing adverse health effects. The methods used to develop RfDs yield numbers with inherent uncertainty spanning perhaps an order of magnitude (IRIS 2001). Applying the agency's methodology, strong scientific arguments can be made for various values within a factor of 2 or 3 of the currently recommended RfD value for As, i.e., 0.1 to 0.8 $\mu g\ kg^{-1}\ day^{-1}$. Uncertainty in the toxicological and epidemiological data from which reference doses are derived is accounted for by applying uncertainty factors (UF). The RfD is derived by dividing the NOAEL by the UF. UFs decrease as the amount of toxicity test data increases. The effect of applying UFs is to lower the estimate of the reference dose and increase the hazard quotient for a given exposure.

One very large study based on 17 000 people detected no effects in any person at an average total daily intake from water plus food of 0.8 $\mu g\ \mu g\ kg^{-1}\ day^{-1}$ (Tseng et al. 1968). This value has been used to calculate the RfD for inorganic arsenic at 0.3 $\mu g\ kg^{-1}\ day^{-1}$ (DWEL = 11 $\mu g\ L^{-1}$) by dividing the NOAEL of 0.8 $\mu g\ kg^{-1}\ day^{-1}$ by an uncertainty factor of 3 (for human variability). For As, a UF of 3 accounts for the uncertainty in whether the NOAEL of the critical study accounts for all sensitive individuals (IRIS 2001). Tseng et al. (1968) assigned an

uncertainty factor of 3 because the no-effect group was relatively young, which decreased the possibility that effects would be detected.

Another source of uncertainty is the estimation of carcinogenic risk and chronic effects levels from acute test data. A key step in CSF development is high- to low-dose extrapolation. Depending on the specific model used to fit the data, extrapolations to the low-dose range can vary by several orders of magnitude, reflecting the potential uncertainty associated with CSF derivation (DHHS 2000).

CONCLUSION

This study revealed contamination by As, Pb and Se in private drinking water wells adjacent to the three abandoned mine and mill sites. These contaminants occurred at concentrations that exceeded estimated background levels and drinking water standards. There appear to be carcinogenic health risks associated with the exposure to As from abandoned mine waste in the Methow River Valley. Additional noncarcinogenic risks are associated with exposure to As, Cd, Pb and Se. Drinking water wells in the vicinity of abandoned mines in the Methow Valley should be tested during times of maximum snow-melt to better determine the extent of risk to human health. Resident's drinking water from the tested wells should consider treating their water or finding alternative sources.

CHAPTER 6

CELLULAR EFFECTS OF TRACE METAL CONTAMINATION IN FISH AND CADDISFLY LARVAE ASSOCIATED WITH ABANDONED MINES IN EASTERN WASHINGTON

INTRODUCTION

Trace element induced toxicity is a serious condition that can affect aquatic organisms in environments contaminated by abandoned mine waste. Toxicity is a chemical phenomenon that begins as a reaction between the chemical and an organism at the molecular level. The initial reactions at the molecular level generate secondary and tertiary responses at the cellular and tissue level that ultimately affect organisms at higher levels of biological organization (Hodson 1990).Reduced nutrient cycling and energy flow at the ecosystem level, reduced diversity and abundance at the community level, and reduced growth and increased mortality among individual members of endangered species at the population level are more relevant to resource managers and ecologists than effects at lower levels of biological organization. However, the degree to which cause and effect are related (i.e., specificity) and our knowledge of the mechanisms of toxicity is lowest at higher levels of organization (Hodson 1990, Clements 2000). At lower levels of organization, endpoints may be more easily linked to cause, occur more rapidly, and may provide early warnings of toxicological effects on populations (Hodson, 1990, Clements 2000).

Indicators of toxicity such as morphological changes at the tissue level, ultrastructural changes at the cellular level and biochemical changes at the molecular level better reveal cause and effect relationships. At the cellular level, the specificity and usefulness of electron microscopy is evident based on the ability to diagnose toxicological and metabolic disorders even when evidence at higher levels is not evident (Phillips et al. 1987). Electron microscopy has been used to show that saturation of the liver storage capacity results in the distribution of

Cu to extrahepatic tissues with multiple organ system dysfunction. It was found that the diagnosis of Wilson's disease can be made based on the presence of glycogen nuclei, glycogen bodies, Cu storage in lysosomes and especially mitochondrial changes including changes in electron density.

Electron microscopy was also used to observe the effect of divalent cations on *in vitro* cell cultures bathed in media containing Ca, St, Pb, Mn, Ba and Mg. Peachy (1964) and Walton (1967) showed that divalent cations accumulate as spherical electron-dense granules in the matrix of mitochondria. The lighter elements (e.g., Ca) produced less dense granules and the heavier elements (e.g., Pb, Ba) produced denser granules 200 – 800 nm in diameter, some of these with less dense cores. The presence of submitochondrial granules accumulating heavy metals was also found to coincide with the toxicity data for aquatic organisms (Argese 1996). The EC50 (50% of the effective concentration) data for submitochondrial granules in *in vitro* cultures, compared to *in vitro* toxicity data from a variety of other bioassays, suggested the matrix granules are indicators of metal toxicity for fish and aquatic invertebrate species. It is not known, however, whether these results are relevant to field conditions.

Water quality is considered to be good at the confluence of Alder Creek and the Methow River near the town of Twisp in the North Cascade Mountains in Okanogan County, Washington, (Milton 1995). Spring Chinook, Summer Chinook, Steelhead/Rainbow and Bull Trout have been found to occur in the Methow River between stations 8 and 15 (Figure 1) and at station 15 a survey conducted by direct underwater observation (snorkeling) identified native steelhead/rainbow trout (*Salmo gairdneri)* and Chinook salmon (*Oncorhynchus tshawytscha*). Two redds in the Methow River at the Red Shirt Mill were identified on 10/10/ 00 and 10/23/00. Two parr (presumably coho) were observed on 1/27/01 in the last pond on Alder Creek after ice melt and before water levels were sufficiently high to provide outlet.The concentrations of Cd, Cu, and Pb (Table 2) in fine sediments (<60µm), however, were higher than background concentrations, benchmarks for aquatic biota (Hull and Suter (1994), and

Environment Canada's Threshold Effects Levels (Persaud et al. 1993). The objective of this study was to determine whether (1) the cellular effects of trace element toxicity observed in *in vitro* cell culture systems occurs among aquatic organisms, including nuclear apoptosis (genetically programmed cell death) and (2) the occurrence of electron dense granules in the matrix of trout hepatocyte and caddisfly gut epithelial cell mitochondria also occurs following exposure to contaminated water and sediments in whole live organisms in microcosm and field settings.

METHODS

Batch Microcosm Caddisfly Cytotoxicity Tests

Thirty mostly 4th and 5th instar caddisfly larvae (*Ecclesomyia spp.*) were collected in June 2001 from sample station 4 (Figure 1, Chapter 2) and divided into three groups of ten each. The three groups of larvae were added to trays (10cmW x 15cmL x 6cmD) in a covered east-facing area with no direct sun exposure. Larvae in different trays were exposed to As-contaminated groundwater, trace element contaminated periphyton, and AMD contaminated stream water (Table 13). Tray 1 contained 500 mol of As in contaminated groundwater from Station 17 (Table 1, Chapter 3); Tray 2 contained a 25 x 25 cm terracotta tile incubated one-year at station 5 where it was colonized with trace-element contaminated sediments and periphyton. Tray 2 also contained 500mL Methow River water from Reference Station 10. The water contained no detectable levels of dissolved metals at time-0; and Tray 3 contained 500 mL water from Alder Creek Station 5, which was contaminated by AMD from Station 1 (Table 13). The three trays were maintained in an insulated chest 46cmW x 76cmL x 46 cmD that contained ice approximately 20 cm deep in the bottom.

The trays were supported on the ice by a one-inch thick piece of Styrofoam. The ice chest was covered by Plexiglas approximately 0.5 cm thick. Medical-grade oxygen was fed continuously to the headspace over the trays at a rate of 0.25-0.5 L min^{-1} to promote

oxygenation of the water in the trays. Temperature, pH, dissolved oxygen, and alkalinity were measured in each tray. Determination of dissolved-oxygen was made using the Winkler Titration method (LaMotte Test Kit Model 221788). Alkalinity was measured using the LaMotte Direct Read Titration Kit (Model 221780). A Piccolo Model HI 1295 temperature compensated digital meter was used to measure pH. A Hobo Model H8 One-Channel Temperature Logger was used for the continuous monitoring of temperature. Larvae were monitored for 24 hours. At the end of exposure, three larvae from each test were dissected and samples of small intestine were collected for cytotoxicity studies.

Perfused Microcosm Caddisfly Cytotoxicity Test

One-hundred-fifty caddisfly larvae (*Ecclesomyia spp.*) were collected from sample station 4 (Figure 1) and divided into six groups of twenty-five each, which were added to wire baskets (30cmW x 30cmL x 30cmD) containing terracotta tiles (25 cm x 25 cm x 2 cm) incubated one-year and colonized with periphyton from Station 11. The tiles in Test Baskets 3-6 were seeded with 6.5 g dry iron-oxide precipitate from Station 1. Tiles in Control Baskets 1-3 were not seeded with dry iron-oxide precipitates and the baskets were maintained in plastic trays 36cmW x 46cmL x 15cmD. Each tray was perfused with well water at a rate of 1.1 L min^{-1}. The metal composition of the periphyton in the two sets of baskets are given in Table 13. Larvae were maintained for 14-d. Temperature, pH, dissolved oxygen, and alkalinity were measured in situ at each sample site. At the end of the 14-d exposure period, one larva from station 4 (Control Larva No. 1), one larva from each of trays 1-3 (Control Larvae Nos. 2-4) and two larvae each from trays 4-6 (Test Larvae 1-6) were dissected and samples of small intestine were collected for cytotoxicity studies.

In Situ Microcosm Caddisfly Cytotoxicity Test

Twenty-five caddisfly larvae (*Ecclesomyia spp.*), collected from sample station 4 (Figure

Table 13. Concentration of metals in water, sediments, and periphyton in caddisfly larvae (*Ecclesomyia spp*) and trout (*Oncorhynchus mykiss*).

		Batch Toxicity Test			Microcosm Toxicity Test		*In Situ Microcosm* Toxicity Test	Trout Toxicity Test	
Element	Ground-water ($\mu g\ L^{-1}$)	Alder Creek Periphyton (mg kg^{-1})	Water over Alder Cr. Periphyton (μg L-1)	Alder Creek Water (μg L-1)	Control Periphyton (mg kg^{-1})	FeOOH Seeded Periphyton (mg kg^{-1})	Red Shirt Mill Periphyton (mg kg^{-1})	River at Alder Cr. Sediment (mg kg^{-1})	Methow Reference Station 11 Sediments (mg kg^{-1})
Al	225	8265	181	233	10774	9078	13337	18227	10720
B	1160	38	0	0	48	150	39	56	35
Ba	7	50	11	35	68	87	132	138	49
Ca	40161	9367	18910	83382	5284	4340	6833	6601	3838
Cd	9	529	9	9	7	48	7	8	4
Cr	41	12	10	45	46	64	30	33	26
Cu	25	292	26	28	85	606	294	291	22
Fe	0	11034	0	0	22350	248135	19409	28858	16608
Mg	31103	3542	4212	29044	6568	5038	5702	7383	5374
Mn	14	374	1	1	261	292	465	601	290
Ni	3	26	3	3	39	60	38	39	27
Pb	30	25	30	30	34	171	94	55	29
S	56936	1591	5251	69240	1010	1546	1199	749	210
Se	30	249	0	30	453	5316	422	280	315
Zn	2	178173	129	42	213	773	258	158	56
pH	8.4	- -	8.3	8.1	7.4 - 7.5	7.4 - 7.5	7.2 - 7.5		
Alkalinity (mg $CaCO3\ L^{-1}$)	227	- -	310	120	156	156			
Dissolved Oxygen	> 17 mg L^{-1}	- -	> 17 mg L^{-1}	> 14 mg L^{-1}	>7.3	>7.2	> 9.1		
Temperature	< 8°C	- -	< 8°C	< 8°C	<12.1	<11.8	<15.1		

1) were added to a 30-cm square basket made of 0.5-cm wire mesh. The basket was secured to rebar driven into the side channel of the Methow River at Station 14 below the Red Shirt Mill where water depth was approximately 10 cm. The basket contained a flat stone approximately 30 cm in diameter (4 cm thick) from the side channel covered with periphyton. The metal composition of the periphyton in the basket is given in Table 13**.** Larvae were maintained for 14-d. Temperature, pH, dissolved oxygen, and alkalinity were measured in situ at each sample site. Larvae were monitored daily and at the end of the 14-d exposure period, five larvae from the basket were dissected and samples of small intestine were collected for cytotoxicity studies.

Cytotoxicity of Wild Caddisfly Larvae

Five wild larvae from the side channel where the *In Situ* microcosm caddisfly cytotoxicity test was performed were collected at the end of the exposure period. The larvae were dissected and samples of small intestine were collected for cytotoxicity studies.

In Situ Microcosm Test of Trout Response to Trace Metal Contamination

Samples in this part of the study were taken from the reference population of fish in the microcosm at station 11 and the fish in the microcosm in the Methow River at station 15 below the mines. Eighty-two hatchery-raised triploid trout (*Oncorhynchus mykiss*, <35g), aged 15-weeks, were transferred from a nearby hatchery (Trout Lodge, Quincy, WA) to the study site and equally divided into two pens approximately 1.1 x 1.1-m .

Fish pens were constructed from aquaculture netting on a PVC pipe frame. One pen was located in a Methow River side channel downstream from the abandoned mine site (station 17, Figure 1, Chapt 2) and the other pen was located upstream from the abandoned mine sites (station 13). Both pens had 1.6-cm rebar extending 0.5-m through two parallel bottom sections, which were weighted down with four large stones from the river to secure the pen in place. Fish,

maintained in the pens from 7 May 2001 to 11 June 2001, were fed (Rangen 3/32 EXTR 400 Slow Sink food #4974) once daily in the morning (0700-0800) at 4% of their body weight•day-1. Visual examination during feeding revealed that the fish readily ingested the food provided and were satiated daily. Each pen was monitored daily for morbidity and mortality throughout the exposure period. At the end of exposure, fish were euthanized (0.1% MS-222, pH 7) weighed and tissue samples were removed. At the end of the exposure period five juvenile trout from each pen were dissected and liver samples were collected for cytotoxicity studies.

General Cytology Techniques

Juvenile trout and caddisfly larvae from each cytotoxicity study were dissected. Sections of trout liver and caddisfly larvae small intestine < 2mm in diameter were collected and preserved in the field in 2.5% glutaraldehyde and 0.1M sodium-cacodylate buffer. The tissue samples were then fixed for 1 h in a final concentration of 1% OsO_4. After dehydration in a graded series of ethanol and embedding in Embed 812, the sections were cut into silver-gray or white sections (approximately 85 nm thickness) using a Reichert/Jung Ultra-cut E. After the sections were collected onto Cu grids and stained with 4% aqueous uranyl acetate for 45 min., the sections were examined and pictures taken using a Jeol JEM 1010 transmission electron microscope operating at 80 keV. Tissues were examined at 1100X for evidence of nuclear apoptosis (Zhao 2001). Magnification was then increased to 34-64,000X and the tissues were scanned until 100 mitochondria were observed. Electron-dense spheres >300A (Rouiller 1960, Peachy 1964) were counted and the average number of granules per mitochondria were calculated.

X-ray Analysis of Metals in Intramitochondrial Granules

Tissues for the analysis of metals in the mitochondrial granules were collected on carbon-coated Ni and Au grids. Energy Dispersive Spectroscopy (EDS) analyses were carried out at 100 keV using a Joel 1200EX STEM scanning transmission electron microscope with a spot size of approximately 9 nm in STEM mode. The sample was scanned at low magnification until a group of granules was located. The magnification was then increased to about 50,000x and a 0.4 x 0.4 mm scan window was placed over an individual granule. EDS analysis of the granule was performed for 100 s. The X-ray analysis system used was a ThermoNoran Voyager 4 with a light element X-ray detector mounted horizontally on the TEM column. Background composition was determined by scanning an area not containing a granule.

After it was discovered that an electron-dense intramitochondrial granule in a wild caddisfly larva from station 14 was comprised of Ti an experiment was performed to test the solubility of titanium oxides in dilute sulfuric acid. A single piece of ilmenite ($FeTiO_3$) was placed in 10% sulfuric acid. After 15-d an aliquot was analyzed by ICP-AES for Ti.

Data Analysis

The Dunnet's test (Zar 1996) was used to compare group means for the number of mitochondrial granules to the control mean (four individuals, 100 mitochondria each). The mean square error (MSE), which is used in the Dunnet's test, was calculated using Minitab statistical software (version 9).

RESULTS

Characteristics of Nuclei and Mitochondria in Control Fish and Caddisfly Larvae

The nuclei and mitochondria of control fish hepatocytes and caddisfly small intestine epithelial cells generally appeared to be normal in appearance (Figure 19). Little variation was

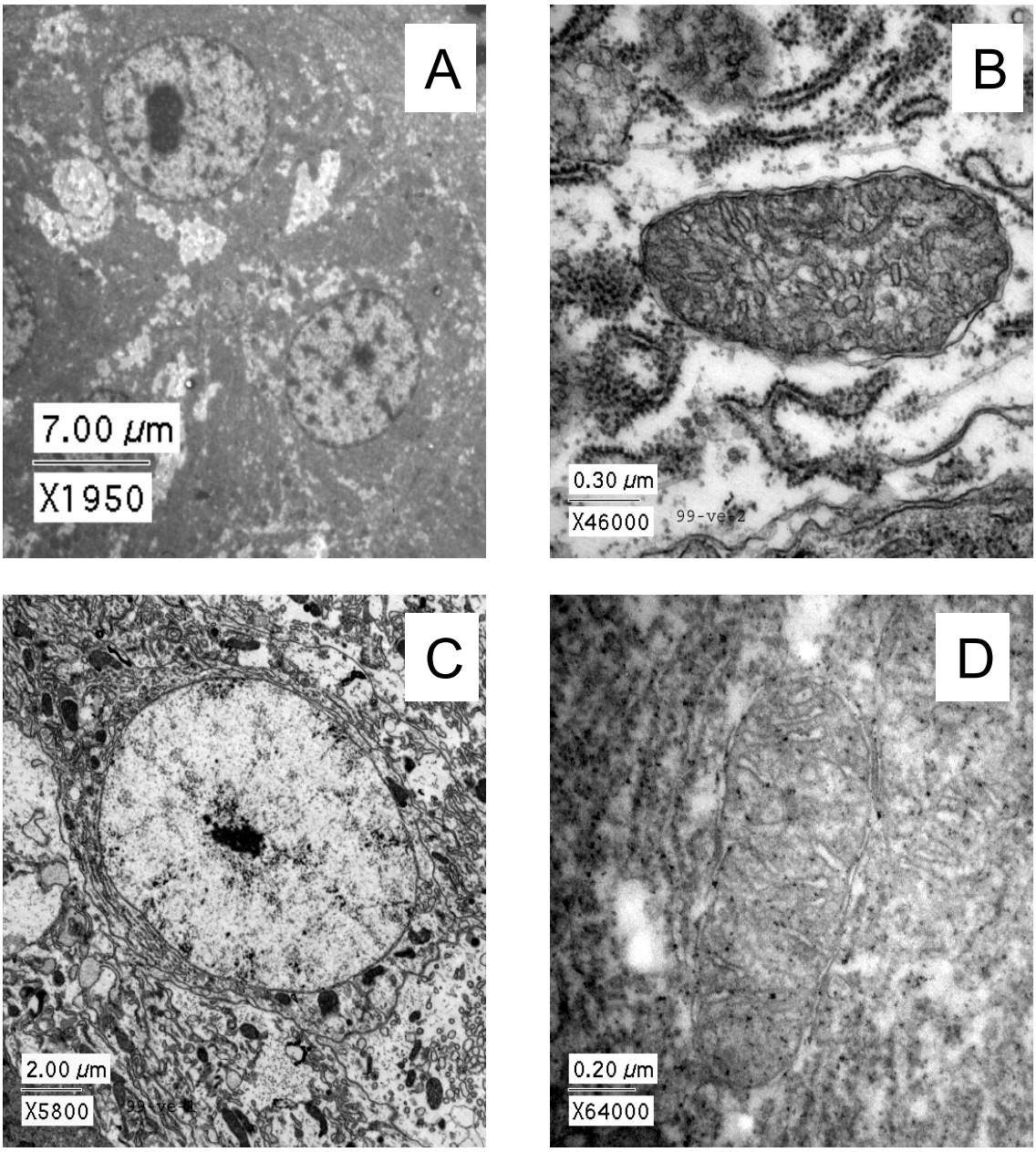


Figure 19. Negative controls. (A) Juvenile triploid trout hepatocyte nucleus from station 11(Figure 1). X1950, (B) Juvenile triploid trout helatocyte mitochondria. X46000, (C) Caddisfly larva normal nucleus. X5800, (D) Caddisfly larva normal mitochondria. X64000.

found in their shape and size. The nuclei were round to oval and measured 5-8 mm in diameter. The nuclear envelope consisted of two visible layers of membrane. The heterochromatin appeared granular or slightly aggregated and sparsely dispersed throughout the nucleus.

Batch Microcosm Caddisfly Cytotoxicity Tests

The mitochondria of caddisfly small intestine epithelial cells from caddisfly larvae exposed to As-contaminated groundwater generally appeared to be normal in appearance (Figure 20A). However, morphological changes that are characteristic of nuclear apoptosis were observed.

Extensive compaction of nuclear chromatin into sharply circumscribed masses that often abut the nuclear envelope and condensation of the cytoplasm was observed in cells from caddisfly larvae exposed to As-contaminated groundwater (Figure 20B). In some nuclei showing chromatin compaction, some chromatin-free nuclear vesicles, which evolved from the nuclear envelope and had no chromatin, were expelled from apoptotic nuclei (Figure 20C). An enlargement of Figure 20C shows the membranes of the apoptotic bodies were also a bilayer derived from the nuclear membrane (Figure 20D). Numerous objects that appeared to be apoptotic bodies containing compacted chromatin and chromatin-free nuclear vesicles were also observed (Figure 20E).

The nuclei of small intestine epithelial cells from caddisfly larvae exposed to trace element contaminated periphyton and AMD contaminated streamwater appeared normal but numerous mitochondria contained dense granules (0.3 – 0.5 μm in diameter) that were scattered randomly among the mitochondria and within the matrix between the cristae (Figure 20F). Table 14 shows the comparison of the mean numbers of electron-dense spheres per mitochondria in small intestine samples from caddisfly larva exposed to trace element contamination in the batch microcosm toxicity test. The means for larvae exposed to water from Station 17 containing high concentrations of arsenic were not greater than the means for reference larvae (Figure 21). The

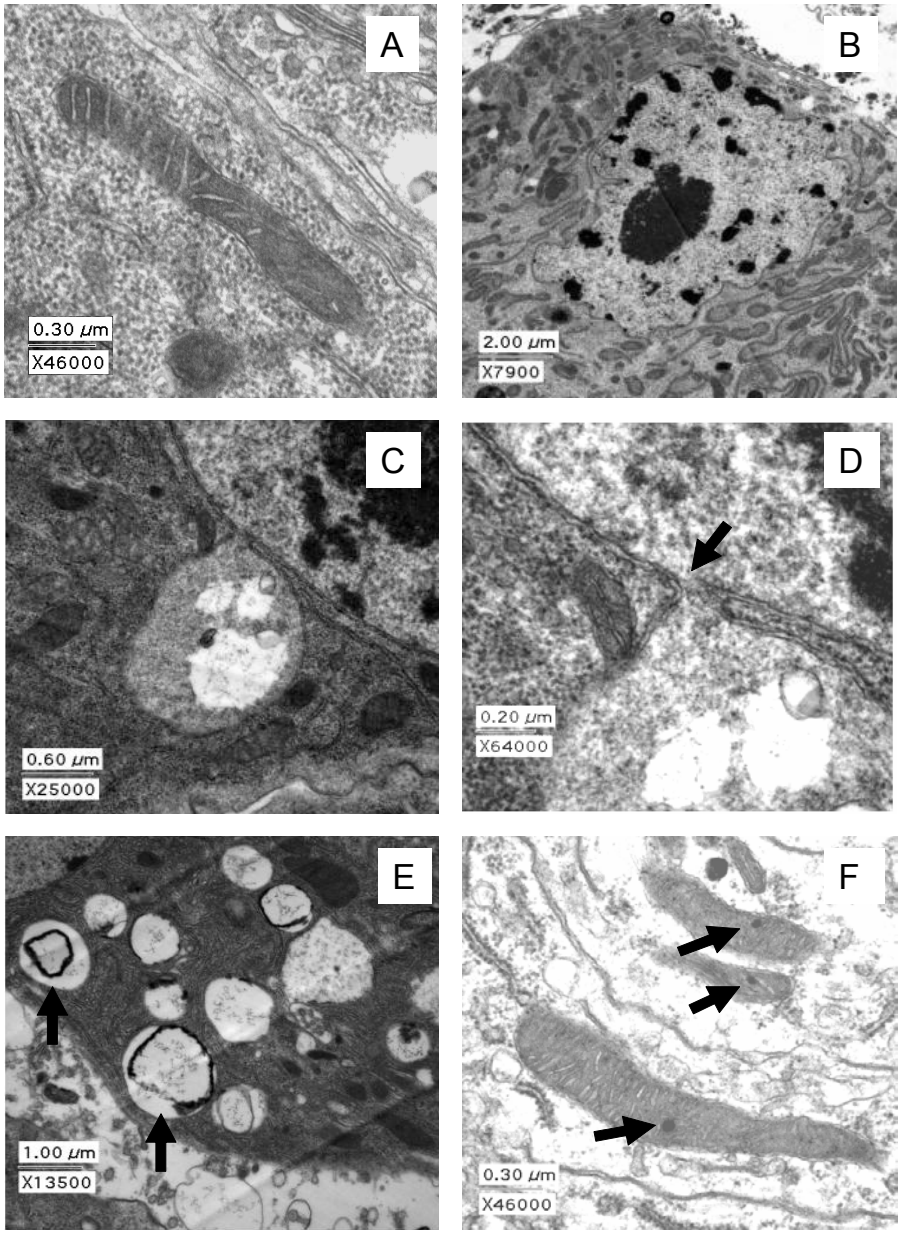


Figure 20. Batch toxicity test. Caddisfly larvae small intestine columnar epithelial cells. (A-E) After exposure to water containing arsenic, (A) Mitochondria with no apparent effects, (B) Chromatin in the nucleus showing condensation and margination, (C) Apoptotic body containing chromatin being expelled from the nucleus, (D) Enlargement of figure C showing bilayer membranes of vesicle, (E) Apoptotic bodies phagocytized by interhepatocytic cell, (F) Electron-dense granules (arrowsa) within the mitochondria of cells from larvae exposed to periphyton containing sediments contaminated with metals.

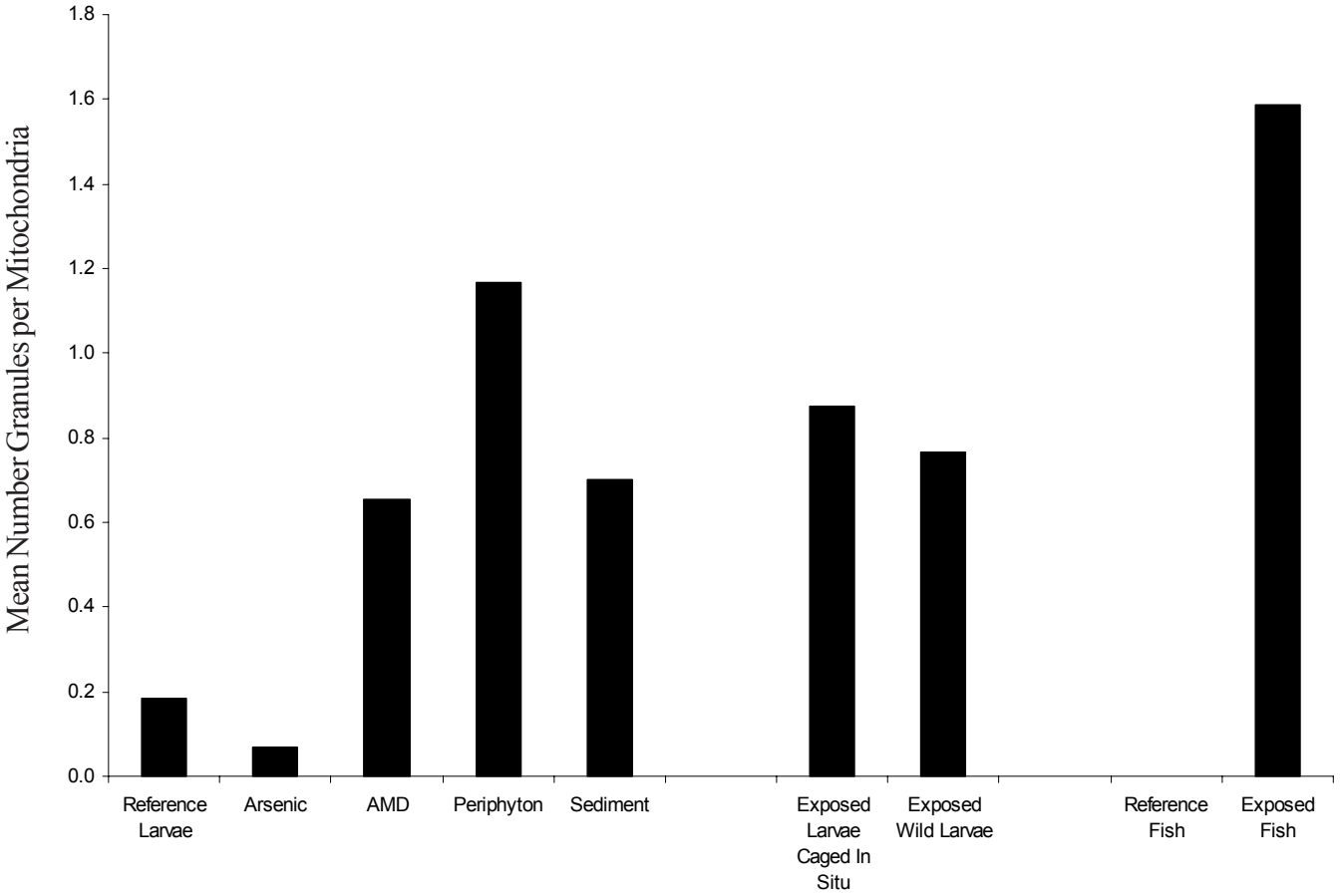


Figure 21. The average number of mitochondrial granules in caddisfly larvae and trout exposed to different levels of metals in their environment including controls, exposure to dissolved As, AMD in streamwater, contaminated sediments in periphyton, periphyton seeded with metal-contaminated precipitates from station 1, and ambient levels of contaminated metals.

Table 14. Incidence of spherical electron-dense granules in mitochondria of caddisfly trout and caddisfly larvae exposed to mine waste contamination. The Dunnet's test was used to compare control means to each other group mean. The data are numbers of spherical electron-dense granules observed in the mitochondria of caddisfly larvae (*Ecclesomyia spp*) small intestine epithelial cells (A-F) and the mitochondria of juvenile triploid trout hepatocytes (G, H). H_o was that $m_{test} \leq m_{control.}$ H_a was that $m_{test} > m_{control.}$

A. Caddisfly Batch Microcosm Toxicity Test: Water from Station 17 Containing High Concentrations of Arsenic ($\alpha = 0.05$, DF = 598, k = 2n mitochondrial: $q_{-0.05(1)} = 1.66$)

		Mean Number Granules/ Mito.	Number of Mito. (n)	MSE	q	Accept/Reject H_o
Control		0.185	400			
Test	1	0.04	100	0.462	Do Not Test	Accept
	2	0.10	100	0.477	Do Not Test	Accept

B. Caddisfly Batch Microcosm Toxity Test: Algae from Alder Creek Station 5 ($\alpha = 0.05$, DF = 598, k = 2: $q_{-0.05(1)} = 1.66$)

		Mean Number Granules/ Mito.	Number of Mito. (n)	MSE	q	Accept/Reject H_o
Control		0.185	400			
Test	1	1.05	100	0.565	10.12	Reject
	2	1.29	100	0.603	12.73	Reject

C. Caddisfly Batch Microcosm Toxicity Test: Streamwater from Alder Creek Station 5 ($\alpha = 0.05$, DF = 598, k = 2: $q_{-0.05(1)} = 1.66$)

		Mean Number Granules/ Mito.	Number of Mito. (n)	MSE	q	Accept/Reject H_o
Control		0.185	400			
Test	1	0.65	100	0.738	6.95	Reject
	2	0.66	100	0.649	5.16	Reject

Table 14 (continued).

D. Caddisfly Perfused Mesocosm Test: Algae from Station 11 Seeded with FeOOH from Station 1 ($\alpha = 0.05$, DF = 512, k = 2: $q_{-\ 0.05(1)} = 1.66$)

		Mean Number Granules/ Mito.	Number of Mito. (n)	MSE	q	Accept/Reject H_o
Control		0.185	400			
Test	1	0.00	19	0.538	Do Not Test	Accept
	2	0.11	19	0.587	Do Not Test	Accept
	3	0.21	19	0.572	0.14	Accept
	4	0.84	19	0.616	6.16	Reject
	5	1.37	19	0.542	0.41	Accept
	6	1.68	19	0.572	7.88	Reject
	Mean Test 1-6	0.70	114	0.672	5.91	Reject

E. Caddisfly In Situ Mesocosm Exposure Test: Larvae from Station 10 in Cages at Station 14 ($\alpha = 0.05$, DF = 493, k = 2: $q_{-\ 0.05(1)} = 1.66$)

		Mean Number Granules/ Mito.	Number of Mito. (n)	MSE	q	Accept/Reject H_o
Control		0.185	400			
Test	1	0.11	19	0.542	Do Not Test	Accept
	2	0.84	19	0.611	3.33	Reject
	3	0.95	19	0.574	4.01	Reject
	4	1.11	19	0.566	4.89	Reject
	5	1.37	19	0.577	6.20	Reject
	Mean Test 1-5	0.88	95	1.03	6.01	Reject

Table 14 (continued).

F. Wild Larvae from Station 14 ($\alpha = 0.05$, DF = 531, k = 2: $q_{-\,0.05(1)} = 1.66$

		Mean Number Granules/ Mito.		Number of Mito. (n)	MSE	q	Accept/Reject H_o
Control		0.185		400			
Test	1	0.16		19	0.27	1.46	Accept
	2	0.21		19	0.23	1.24	Accept
	3	0.37		19	0.183	0.96	Accept
	4	0.95		19	0.763	4.01	Reject
	5	1.11		19	0.923	4.91	Reject
	6	1.21		19	1.023	5.19	Reject
	7	1.37		19	1.183	6.36	Reject
	Mean Test 1-7	0.77	133		0.689	7.05	Reject

G. Trout Positive Controls from Station 7 vs Negative Controls from Station11 ($\alpha = 0.05$, DF = 173, k = 2: $q_{-\,0.05(1)} = 1.66$)

		Mean Number Granules/ Mito.	Number of Mito. (n)	MSE	q	Accept/Reject H_o
Control		0.00	125			
Test	1	0.68	25	0.05	4.35	Reject
	2	0.68	25	0.10	8.55	Reject

H. Trout Exposed Group from Station 15 vs Negative Control Grpoup Station 4 ($\tilde{} = 0.05$, DF = 248, k = 2: $q_{-\,0.05(1)} = 1.66$)

		Mean Number Granules/ Mito.	Number of Mito. (n)	MSE	q	Accept/Reject H_o
Control		0.00	125			
Test	1	1.04	25	0.304	6.57	Reject
	2	1.24	25	0.193	9.83	Reject
	3	1.32	25	0.415	7.13	Reject
	4	1.72	25	0.818	6.62	Reject
	5	2.00	25	1.23	6.28	Reject

hypothesis that the means were equal was rejected for larvae exposed to streamwater from Alder Creek contaminated by AMD at Station 5 and the reference group because the mean differed significantly (Table 14 and Figure 21). For larvae exposed to periphyton from Alder Creek Station 5 the mean number of mitochondrial granules found in cells from the test larvae were greater than the mean for the control larvae and the hypothesis that the means were equal was rejected.

Perfused Microcosm Caddisfly Cytotoxicity Test

In small intestine epithelial cells from caddisfly larvae exposed to periphyton containing iron-oxide precipitates contaminated with trace elements from the adit at station 1, numerous mitochondria contained spherical electron-dense granules compared to the control (Figure 21). Two replicates did not exceed the control. In four replicates, the means for the exposed larvae were greater than the means for the control larvae, but only two were significant based on the Dunnet's test. Overall, when the data for the replicates were pooled (n=114) the mean differed significantly from the control (Table 21).

In Situ Microcosm Caddisfly Cytotoxicity Test

In numerous mitochondria of caddisfly larvae exposed to conditions downriver from the mines at station 14, dense spherical granules were observed (Figure 22A) and the mean number per mitochondria was greater than in the control (Figure 21). In four replicates, the means for the exposed larvae were greater than the means for the control larvae and all were significant based on the Dunnet's test comparing each test to the reference mean. In one replicate, the mean number of matrix granules was less than the control and was not tested using the Dunnet's method. Overall, when the data for the replicates were pooled (n=95) the mean differed significantly from the control (Table 14).

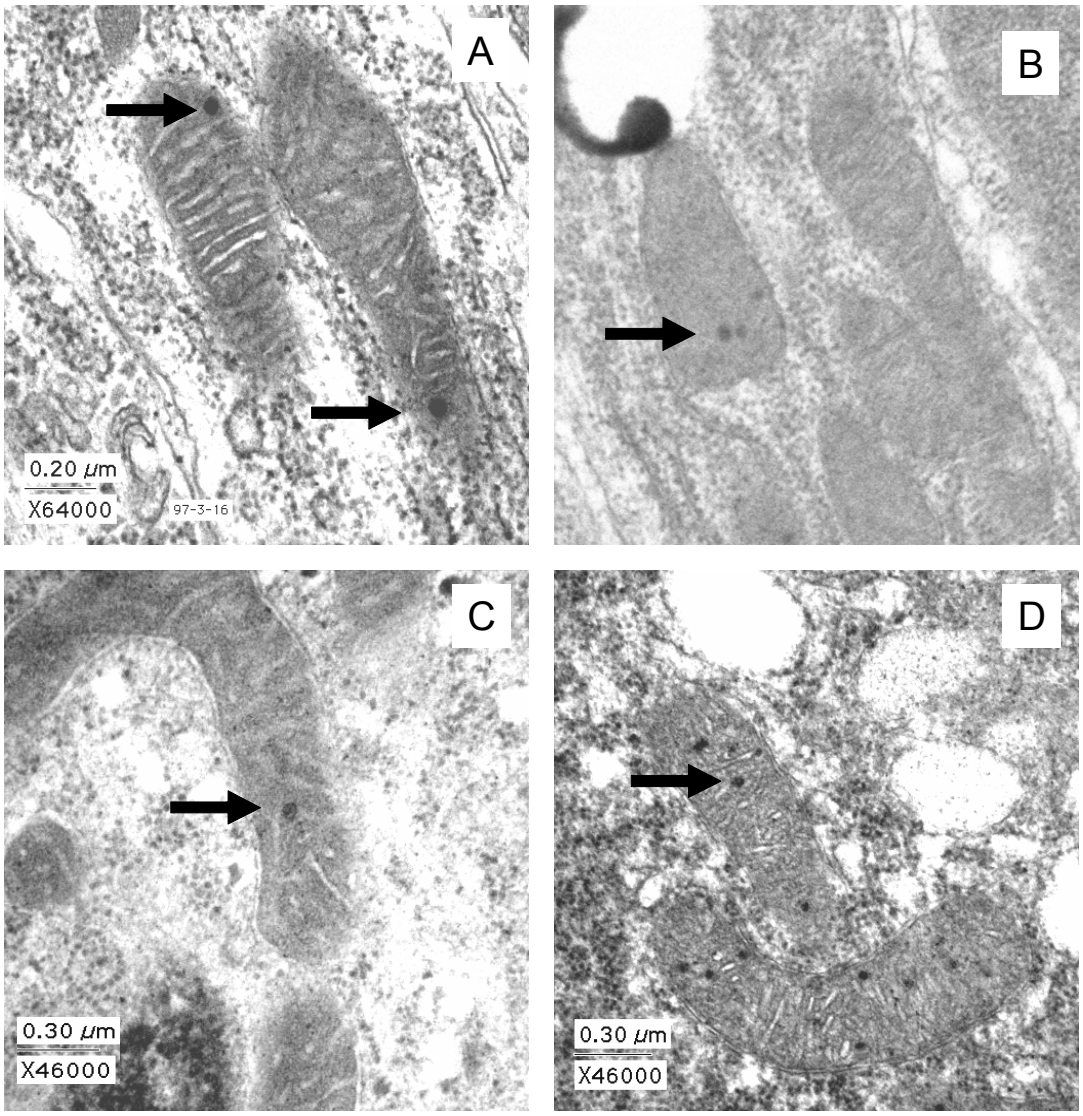


Figure 22. Electron-dense granules in mitochondria (arrows). *(A)* Caged caddisfly larvae at station 14 below the mines, *(B)* Wild larvae from station 14 below the mines, *(C)* Hepatocyte mitochondria from control trout at station 11 above the mines, *(D)* Hepatocyte mitochondria from trout at station 15 below the mines.

Cytotoxicity of Wild Caddisfly Larvae at Station 14

Similar results were observed for wild larvae from Station 14 that were analyzed to see if matrix granules were being formed under natural conditions. The mean number of mitochondrial granules in one replicate was not greater than the control, two replicates were greater but the differences were not significant based on the Dunnet's test, and the means of four other replicates were significantly different than the controls. Overall, when the data for the replicates were pooled (n=133) the mean differed significantly from the control (Table 21).

In Situ Microcosm Test of Trout Response to Trace Metal Contamination

In all five replicates sampled, the mean number of spherical electron-dense granules per mitochondria of trout held exposed to the downriver conditions below the mines at station 15 were greater than the mean number in the control trout and all were significant based on the Dunnet's test comparing each test to the reference mean (Table 14). When the data for the replicates were pooled (n=125) the mean also differed significantly from the control.

X-ray Analysis of Metals in Intramitochondrial Granules

The EDS-spectra for intramitochondrial granules in Figure 23 shows that after the elements found in the system background spectra (Figure 23A) and the grid material are disregarded wild caddisfly larva tissue mounted on Ni grids contained Ti, Ca, and Fe (Fig. 23 B-D). One of at least three granules that were visible in the mitochondria of a second caddisfly larva (Figure 23E) contained Pb (Fig. 23E). A granule in the mitochondria of a hepatocyte from a trout at station 15, mounted on a gold grid contained Cu (Fig. 23F). Figure 23A is a spectrum of the mitochondrial matrix in trout hepatocyte tissue from station 15 where no granules were observed showing peaks for C, Si and O, which are characteristic in background analyses that did not include the grid.

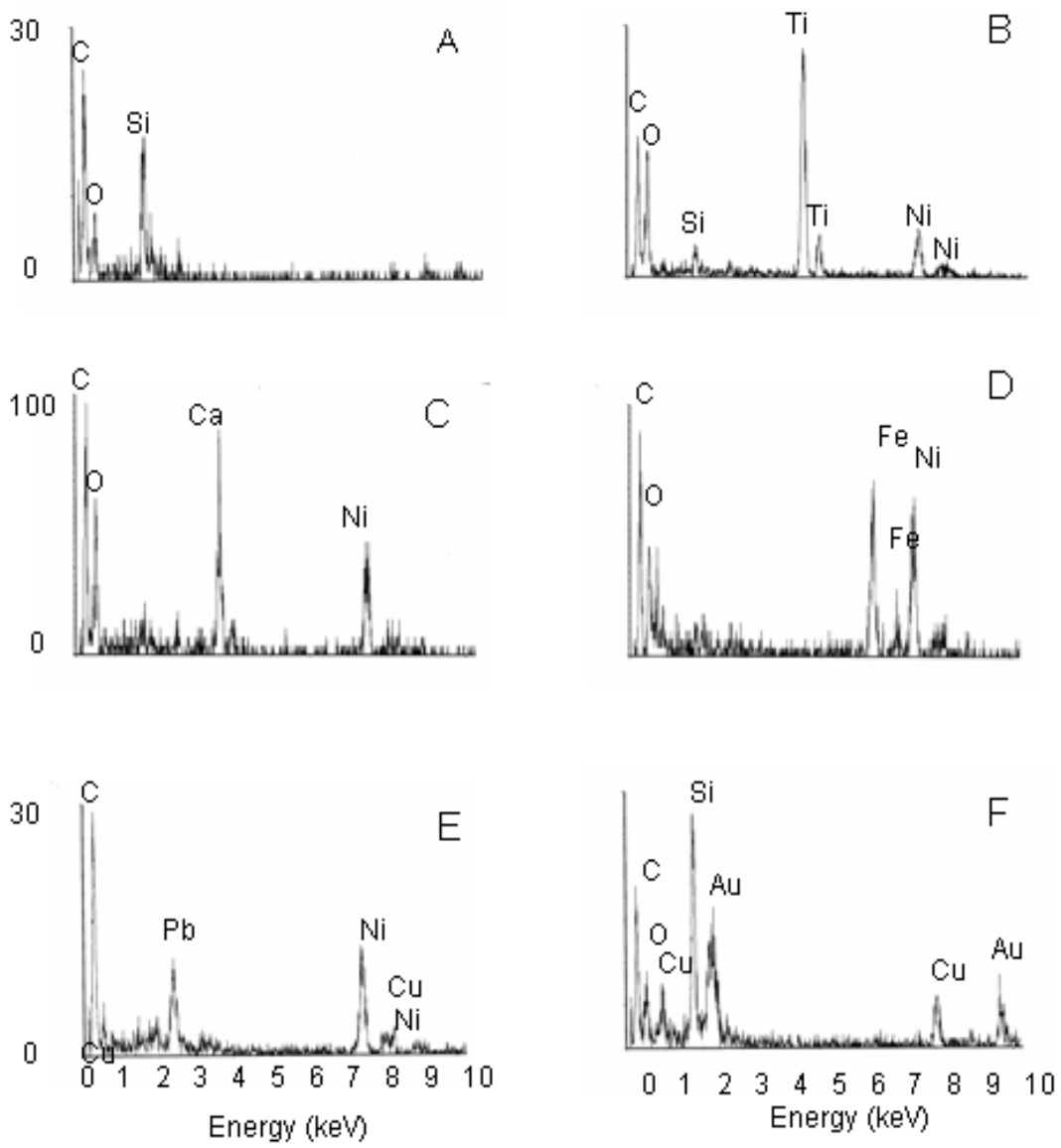


Figure 23. Typical EDX spectra showing composition of electron-dense spheres in matrix of caddisfly and trout mitochondria . *(A)* System background is typical spectra from the analysis of the mitochondrial matrix not including the grid material or matrix granule, *(B-D)* Spectra show Ti, Ca, Fe, and Pb were detected over system background and Ni grid in matrix granules of wild larva in Methow River below mines, *(E)* Spectrum shows Pb was present in matrix of second wild larva, *(F)* Cu was sequestered by matrix granule in hepatocyte mitochondria of trout from Methow River at confluence with Alder Creek .

DISCUSSION

Mitochondria are known to accumulate divalent cations as spherical granules *in vitro* (Rouiller 1960, Peachy 1964). Dense granules were described as numerous in brush border, duodenal and intestinal epithelial cells and hepatocytes and are thought to occur in tissues that transport large quantities of water and ions. Rouiller noted (1960) that increased numbers of granules occurred in the duodenum of animals fed experimental diets high in potassium or sodium. Spherical electron-dense granules in the matrix of mitochondria were produced when *in vitro* cell cultures were bathed in media containing Ca, St, Pb, Mn, Ba and Mg (Peachy 1964, and Walton 1967). In this study, I showed that spherical electron-dense granules occurred in the mitochondria of live caddisfly larvae exposed to trace metals in stream water, sediments, and periphyton. Since mitochondria are implicated in the active transport of Ca across membranes and divalent cations such as Cu and Zn have ionic potentials that are similar to Ca the membranes are thought to be somewhat selective but not reliable sentinels and the granules observed in mitochondria correspond to the available trace metals in the exposure medium. Caddisfly larvae exposed to water containing As did not develop granules in their mitochondria. Arsenic is characterized by the formation of oxyanionic species (e.g., arsenite [$As(III)O_3^{3-}$], arsenate [$As(V)O_3^{3-}$] and would not compete with cations for active transport through membrane channels.

Divalent cations like Cu^{2+} have ionic potentials similar to Ca and they enter the cell and mitochondria by way of active transport mechanisms that regulate transcellular Ca transport (Simkiss 1996). Since cellular and mitochondrial membranes are thought to be selective but not reliable sentinels of Ca transport the presence of metals in mitochondrial granules likely correspond to available metals in the cytosol and medium surrounding the cell.

In caddisfly larvae there are two possible ways that metals may enter the body: the body surface and the alimentary tract (Munger and Hare 2000) and if fish the gills are a third pathway

(Dalllinger et al. 1987). Although it appears that little is known about metal uptake through the skin it is assumed that the skin is impervious to metal absorption (Dalllinger 1987). The gills however, are not only the main organs of gas exchange, they are also important in the uptake of dissolved metal ions in the water. The gills of rainbow trout can show 10-fold increases in Cu accumulation within a few hours of aqueous exposure, and this occurs simultaneously with the appearance of Cu in the blood (Handy 1992, Grosell et al. 1997). In contrast to the gills, the ailimentary tract appears to be the most feasible route for the uptake of metals that are consumed during feeding. Metal concentrations in food are often several orders of magnitude higher and represent a more contaminated source than the soluble forms in water. Although trace metals dissolved in water tend to be more bioavailable than those associated with particles, insect tend to take up the bulk of their metals from food (Hare 1992).

In a study of tissue metal accumulation in rainbow trout exposed to foodborne and waterborne metals (Farag et al. 1994) showed that, in general, metal accumulation in tissues was higher in gill and kidney with waterborne exposures and was higher in stomach and pyloric caeca with dietary exposure. I was not able to discern the exposure pathway for metal uptake in this study. However, in this study where I observed that metal concentrations in the water was, dietary uptake is probably the most important source of metal-loading in the fish and caddisfly larvae that were sampled from the Methow River.

This study also showed that the oral uptake of contaminated periphyton by caddisfly larvae resulted in the development of granules in the mitochondria at numbers significantly greater than in control larvae. Although it is generally believed that benthic invertebrates can accumulate metals from contaminated periphyton, it is assumed that a key to the bioavailability is determined by interstitial water concentrations (DiToro 1990, Jenne 1977, Bissonette 1977 and Ankley 1996).

The ability to induce the formation of electron-dense granules in the mitochondria of caddisfly larvae was further demonstrated by maintaining caddisfly larvae from the reference population in cages and exposing them to ambient levels of contaminants in the Methow River then noting the increase in the average number of granules per mitochondria. Because these results suggest that metals accumulate in mitochondria the appearance of granules in the mitochondria of caddisfly larvae and trout from sites in the Methow River below the mines suggest metals are present at unusual concentrations in the fluid surrounding either the mitochondria or the entire cell of wild caddisfly larvae.

X-ray Analysis of Metals in Intramitochondrial Granules

Since experimental studies suggest that these granules are concerned with the regulation of the internal ionic environment of the mitochondria (Fawcett 1966), the electron-dense particles observed in mitochondria should correspond to metals in the environment that are bioavailable. In this study X-ray microanalytical data indicate that larvae are exposed to unusually high concentrations of Ca, Fe, Pb, and Ti due to their presence in the granules that were analyzed (Figure 23). The accumulation of Ca, Fe, and Pb as spherical granules in caddisfly larvae small intestine epithelial cell mitochondria from station 14 and the accumulation of Cu in the hepatocytes of trout from station 15 suggest that bioavailable forms of these elements are present at high in the environment surrounding the organism, its cells and the mitochondria.

The potential involvement of Ti was unexpected because oxide minerals of Ti (mainly ilmenite and rutile) are very resistant to weathering and they occur practically undecomposed in soils (Kabata-Pendias and Pendias 1992). Titanium, however, dissolves in dilute hydrochloric and sulfuric acids (Ohly 1903) and titanium mobilization might occur in mine waste piles where the oxidation of pulverized ore containing pyrite generates high concentrations of geogenic sulfuric acid . The single piece of ilmenite maintained 14 d in 10% sulfuric acid at room

temperature resulted in a dissolved Ti concentration equal to 2800 mg L^{-1} when the sulfuric acid solution was analyzed by ICP-AES. Pyrite oxidation in mine waste piles results in acid mine waters that are non-ideal solutions in which H_2SO_4 concentration can be as high as 7.6 molal (Nordstrom et al. 2000).

It should be noted that an EDS peak may correspond to more than one element. The two peaks in the spectrum for Ti (Figure 23B) have energies of approximately 4.5 keV for the K_α peak and 5 keV for the K_β peak, which also correspond closely and overlap with the L_α and $L_{\beta1}$ peaks for Ba. Barium, however, would have two additional peaks at 5.3 ($L_{\beta2}$) and 5.6 keV ($L_{\beta1}$), which were not evident on the spectrum in Figure 24. It has long been recognized that titanium-oxide is insoluble in water and all acids except H_2SO_4 (Ohly 1903). More recently, it has been noted that titanite or sphene ($CaTiSiO_5$) and anatase or rutile (TiO_2) are soluble in sulfuric acid (Barksdale 1966, Nordman and Berlin 1986, Dumon and Ernst 1988). Because of TiO_2 solubility in sulfuric acid, titanium mobilization might occur in mine waste piles where pulverized ore is exposed to high concentrations of geogenic surfuric acid.

Mitochondrial Failure and Apoptosis

Mitochondria, in addition to generating ATP, are critical in regulating the complex survival signals that determine whether cells live or die (Kamp 2002). The release of cytochrome c from mitochondria is responsible for mediating apoptosis (Zhao 2001). Normally, the homeostatic regulation of normal tissue mass is effected by the cyclic production of growth factors and death factors, which induce mitosis and apoptoisi, respectively Kerr and Harmon (1991). There is evidence that metal toxicity stimulates apoptotic cell death (Dragan 2001). The precipitation of Ca^{2+} and metals with similar ionic potentials in the matrix of mitochondria, combined with the observation that the mitochondria were often swollen with disrupted cristae, are indicators of mitochondrial failure (Halliwell and Gutteridge2002). Because of a large negative membrane

potential, mitochondria are effective barriers of cycstolic metal transients. Calcium and metals enter the mitochondrial matrix via a transporter, and at low levels stimulates the Kreb's cycle and oxidative phosphorylation. At high levels of cystolic calcium and metals (μM) or when the membrane becomes permeable, loading can result in a catastrophic, irreversible collapse of mitochondrial membrane potential called permiability transition that not only prevents ATP production, but also increases free radical production (Kamp 2002). The lowered ATP availability reduces the ability of the cell to bail calcium and metals out of the cytoplasm into the extracellular volume, which increases cystolic calcium and metal concentrations and free radical production further accentuating mitochondrial instability.

Aside from ATP and free radical problems, mitochondrial collapse releases cytochrome c that activates the caspases that mediate apoptotic cell death (Zhao et al. 2001). It is not clear whether the process that exist in caddisfly larvae and fish are the same but, since the intestinal epithelia in the larvae and hepatocytes in trout are both aerobically poised and energetically demanding, mitochondrial densities are high in both and the loss of mitochondrial function could potentially be injurious due to the loss of ATP, the release of cytochrome c and the initiation of apoptosis.

CONCLUSION

In this study, I showed that spherical electron-dense granules occurred in the mitochondria of live caddisfly larvae and trout exposed to trace metals in stream water, sediments, and periphyton. X-ray analyses suggest that bioavailable forms of Ca, Cu, Fe, Pb and Ti are present at high concentrations in the environment surrounding the organism, its cells and the mitochondria hepatocytes and caddisfly small intestine cells from caddisfly larvae and trout from the Methow River. Chromatin compaction, margination and the observation that large vesicles with bilayer membranes were being expelled from the nuclei of effected cells from caddisfly larvae exposed to As suggest that mitochondrial failure is occurring.

CHAPTER 7

REMEDIATION OF THE ALDER MINE, RED SHIRT MILL AND ALDER MILL SITES

INTRODUCTION

While it is generally agreed that it is necessary to remediate lands disturbed by abandoned mines, there appears to be a large amount of uncertainty as to how it should be done or to what extent. The National Academy of Sciences (1974) defined three categories of remediation: Rehabilitation, reclamation and restoration. Rehabilitation is achieved when the site is returned to a form and level of productivity comparable to the ecological state that existed prior to disturbance. Reclamation is used when the site is hospitable to organisms that approximate the original inhabitants. Restoration is achieved when the site is returned to its original state of ecological integrity, including the full range of variability in biodiversity, ecological processes and structures. Generally, industry favors rehabilitation; regulatory authorities favor reclamation; and ecologists favor restoration (Toy and Daniels 1998).

The goal of remediation also depends on the severity of the disturbance. When communities are at equilibrium and the environmental factors are stable and unchanging, competitive exclusion is the rule and communities become dominated by a few species comprised of the best competitors (McAuliffe 1984). According to the Intermediate Disturbance Hypothesis (Connell 1978, modified for streams by Ward and Stanford 1983) biotic diversity will be greater in communities subjected to intermediate levels of disturbance making reclamation a reasonable goal. However, when disturbances are too severe, and species go extinct because they are not resilient and have low rates of increase, restoration may not be achievable. In habitats exposed to greater than normal disturbance, including in order of increasing severity, erratic flows, hydroelectric dams, organic pollution and finally by acid mine drainage (Ward and Stanford 1983), rehabilitation may be a more reasonable goal.

At the Alder Mine, Alder Mill and Red Shirt Mill, eleven chemicals of potential ecological concern (COPEC) were identified. In mine tailings, metal concentrations were compared to soil benchmarks that were derived from toxicity studies conducted on plants in the field. Based on this comparison Al, As, B, Ba, Cd, Cr, Cu, Mn, Pb, Se, and Zn were identified as COPEC. Eight of the 11 COPEC that exceeded the plant toxicity benchmarks also exceeded benchmarks for soil heterotrophic processes (i.e., Al, As, B, Cd, Cu, Mn, Se, and Zn). Only five minor elements (Al, As, Se, Mn, and Zn) in AMD exceeded wildlife benchmarks and three minor elements in ARD exceeded benchmarks. Two COPEC identified in groundwater samples were As and Se, which are both metalloids and typically exist primarily as oxyanions under aerobic conditions. All other COPEC were simple metal cations. In the Methow River, dissolved metal concentrations were less than the limits of detection by ICP-AES but in the sediments, 4 trace elements (i.e., Cd, Cu, Mn, and Pb) exceeded toxicity benchmarks for aquatic biota. While all four of these elements are COPEC in both the tailings (a presumed source) and the sediments (a presumed sink), only Mn is a COPEC in ARD.

In March 2002, the WDOE requested that EPA assume responsibility for assessing the risk of contamination to the public's health and the environment. The Okanogan Health District, through a grant from the WDOE, has been conducting drinking water well sampling. As a result of the assessments that have been completed these agencies have concluded that conditions at the site have met the criteria for a removal action as stated in the National Contingency Plan (40 CFR Section 300.415) (Sheldrake 2002). Contamination is endangerig the public health and welfare of the environment through exposure to high levels of hazardous contaminants. Arsenic, Cd and Pb were identified as the primary contaminants.

DESCRIPTION OF SITES TO BE REMEDIATED

Alder Mine

The Alder Mine is an inactive mine located approximately 3 miles southwest of Twisp, Washington. The site consists of at least three partially open adits (the north adit, the south adit, and an adit in the open pit), an adit retention pond, an open pit, and waste rock dumps. The site is on the north slope of a north-trending ridge. Slopes at the site range from 50-80%. Estimates from aerial photographs indicate that waste rock covers approximately 3.2 ha (8 ac). The discharge rate of the north adit is approximately 25 lpm and the south adit approximately 3 lpm. It appears that calcite fracture fillings are being dissolved by the acid mine waters infiltrating from the surface and AMD flow between the adit and Alder Creek appears to be unrestricted and in the range of that for pipe flow.

Alder Mill

The Alder Mill consists of several buildings and two tailings impoundments. The impoundments are estimated to contain approximately 55,845 m^3 cubic yards of material (Stewart 1995). Inputs and springs supplied by Alder Creek feed the upper impoundment creating a contaminated wetlands environment. Private residences with groundwater wells are located adjacent to the site.

Red Shirt Mill

The Red Shirt Mill consists of a single building and a tailings pile. The tailings pile is estimated to cover approximately 4,650 m^2 of surface area and contain less than 30,600 m^3 of material. Approximately 4 m^3 of tailings are recruited annually by the Methow River. The site is located adjacent to the Twisp city limits and residences with private groundwater wells are located on and adjacent to the site.

REMEDIATION ALTERNATIVES AND TECHNIQUES

Alder Mine

The remoteness of the location at the Alder Mine, the steep gradients, the complexity of the site, and the apparent alteration of the bedrock affecting the hydrogeology of the site, and most importantly the irreversible production of AMD makes restoration and reclamation unlikely. Rehabilitation may be the only reasonable goal of remediation at this site.

Remedial efforts at the Alder Mine should focus on developing and implementing permanent, cost-effective source control remedies. From the sources of contamination and migration pathways described above we have identified the following as primary remediation goals: 1) mitigate the release of metal contaminants from waste rock piles, 2) mitigate the release of metals from tailings, 3) mitigate dispersion of metals in overland flow paths, and 4) mitigate dispersion of metals in subsurface flow paths.

Waste rock occurs only at the Alder Mine site. The site is characterized by a semiarid climate, sparse native dryland vegetation, a general lack of precipitation (<38 cm), rough topography, poorly developed soils, and cold temperatures. Soils are generally shallow and bedrock frequently exposed. Soils in this area are well-drained stony to sandy loam soils that are made of material weathered from granite, gneiss, and schist. Runoff is rapid and the potential for erosion is high. The waste rock at the Alder Mine is generally unchanged from when it was deposited, at its angle of repose, except where it has been modified by erosion. Currently, runoff from the entire site is concentrated into two channels causing wasting and contamination of the stream with acidic, metal-rich sediments. Remediation activities targeting the waste rock pile at the Alder mine should be designed to minimize erosion, sedimentation, and to reduce surface water runoff velocities in the bare areas with the intent of reducing erosion.

To accomplish this, existing mine-site roads should be used as the template for the creation

of terraces to abate surface runoff and erosion. Five levels of terraces could be constructed that include trench drains to intercept runoff. The terraces could be constructed at a grade equal to approximately 3-5% and these drains would divert flow north and east away from Alder Creek. Runoff will be distributed over a larger area and have a greater opportunity to react with and be neutralized by the calcite detritus present in the forest soil in that area.

In addition to the drains, the terraces will be modified to include regularly spaced holes that will be dug and filled with topsoil to provide a substrate for revegetation. The terraces will offset some of the problems typically associated with dry eastern Washington mine sites by providing shade, shelter from wind, and mechanisms for collecting rain and runoff and retaining soil amendments. Native and fast-growing nitrogen fixing species of trees should be planted to accelerate the natural soil building process and provide an aesthetic cover for the area.

Fences should also be constructed to exclude cattle from the riparian area of Alder Creek and promote the recovery of vegetation. The tributary upstream and to the west should also be fenced-off to protect it as a source of benthic macroinvertebrates for reinoculation of the sites downstream from the mine. Water troughs should be provided to provide an alternative source of livestock water and interpretive signs should be installed to address community questions and concerns.

Alder Mill and Red Shirt Mill

The main contaminants of concern at these sites are toxic trace elements such as As, Pb, Cu, and Zn, that are potentially harmful to human health as well as to terrestrial and aquatic animals and plants. These elements occur naturally in ore and are being released into the environment after the material was crushed, deposited on site and exposed to weathering at the earth's surface. Other contaminants that might have been used in the milling process and released to the environment include Hg and HCN. Complete restoration of these sites is a goal

that is possible assuming that calcite in the fractures in the underlying bedrock have not been dissolved and the hydrogeology irreversibly altered. Nevertheless, because AMD is not being produced on site, if the tailings and contaminated soils that are deposited on site are removed and replaced with clean soil and revegetated with native plants, eventual restoration should be possible regardless of whether or not the bedrock hydrogeology has been altered.

The remedial option that represents the most permanent solution, requires the least ongoing maintenance and would not require riverbank stabilization at the Red Shirt Mill site involves the demolition of mill buildings, excavation of all contaminated soils and tailings, regrading with soil, revegetation, and off-site disposal of excavated soil and tailings (SAIC 2002). Because excavation to pre-disturbance levels is expected to remove materials to a depth of 10 feet in some areas it could increase the potential for flooding. To prevent the alteration of the course of the Methow River and reduce the potential for flooding, the contaminated soils that are removed will have to be replaced with adequate backfill to provide a slope of at least 2% towards the river. Also, pine trees at the site are currently rooted at pre-disturbance levels (Figure 24) making their removal, followed by reforestation at the regraded level, necessary. This represents a permanent remedial action that could restore the Alder and Red Shirt Mills to their full range of variability in biodiversity, ecological processes and structures. It also avoids the need for ongoing maintenance and for riverbank stabilization. Demolition of the mill buildings (Figure 25) and removal of soils (Figure 26) surrounding the buildings began in 2002.

POST-REMEDIATION MONITORING

Under the Model Toxics Control Act (MTCA), monitoring is required for all cleanup actions (WAC 173-340-410). Three categories of compliance monitoring are defined under MTCA (SAIC 2002): (1) Monitoring to confirm that human health and the environment are protected during construction and operation of the cleanup action, (2) Monitoring to confirm

Figure 24. Excavation around pine trees at the Red Shirt Mill site showing elevation prior to deposition of mill tailings (December 2002).

Figure 25. Red Shirt Mill before demolition in June 2001 (left) and after demolition in December 2002 (right).

Figure 26. Excavation of tailings from lower retention pond at the Alder Mill (December 2002).

that the cleanup action has attained cleanup standards or remedial action objectives, (3) Monitoring to confirm the long-term effectiveness of the cleanup action one remedial action objectives have been attained.

CHAPTER 8

SYNTHESIS

Mining often occurs in environmentally sensitive areas where contamination from operating and abandoned mines can cause severe environmental impact. Large volumes of mine waste are often associated with hardrock mining. Acid mine drainage (AMD) leaching from old mines and acid rock drainage (ARD) from mine wastes can contain toxic trace elements at high concentrations. Many abandoned mines exist in eastern Cascade Mountains of Washington, especially in the Methow Valley.

Three such sites are the Alder Mine, Alder Mill and Red Shirt Mill located near Twisp, Washington. These sites contain tailings, waste-rock piles, and openings that discharge AMD and ARD. Although the mine and mills were abandoned in the 1950's, they still have the potential for contaminating private drinking water wells and influencing fish and wildlife habitats in Alder Creek and the Methow River. This study was conducted to determine the environmental effects of contaminants released from the Alder Mine, Alder Mill, and Red Shirt Mill on the Methow River ecosystem.

My main objectives were to determine: (1) concentrations of macroelements (Ca, K, P, Na, Mg, and S) and trace elements (As, Cd, Cr, Cu, Mn, Mo, Ni, Pb, Ti, and Zn) in ore, tailings, surface water, groundwater, and sediments in and near the Methow River, and if relationships exist between the presence of mining activities and the accumulation of trace elements at concentrations that are potentially harmful to human health and fish and wildlife habitats, (2) the ecological impact at different scales of biological organization (cellular, individual, population and community and ecosystem), (3) the adverse health risks to residents consuming groundwater contaminated with As and trace metals, and the degree of uncertainty associated with the assessed risk, and (4) evidence of trace element toxicity at the cellular level in caddisfly larvae and trout.

1. Relationships between the presence of mining activities and the accumulation of trace elements at concentrations that are potentially harmful to human health and fish and wildlife habitats.

We found that elevated levels of trace elements related to AMD and ARD, especially in the Methow River below the Alder Mill, Red Shirt Mill and Alder Mine sites. However, one of the major problems in assessing the effects of AMD and ARD is in separating natural from human-caused loadings. A number of studies have attempted to separate natural from man-caused loading (Nimick and von Guerard 1998) using water-quality data, including isotopes and tracers. However, to date there has been no reliable technique developed to clearly separate natural from human-caused loading (Dissmeyer 2000).

In the absence of empirical methods to partition metal loading between background and mining sources, we used epidemiological criteria, including time-order, strength of association, consistency of replication, specificity of cause-and-effect, and coherence with fact and theory to show there are causal relationships between processes occurring in the vicinity of the abandoned Alder Mine, Alder Mill and Red Shirt Mill sites and the observed environmental effects.

We showed that the weathering of sulfide-bearing minerals exposed to atmospheric oxygen and water by mining has produced AMD and ARD containing elevated concentrations of trace elements that are toxic to fish, wildlife and humans in the Methow valley. Although no pre-mining background data were available, a vertical profile of trace element concentrations in Alder Creek sediments showed that relatively low background concentrations were followed by an increase in Cu and Zn accumulations after mining began.

The strength of association between abandoned mine sites and the occurrence of contaminated soil, surface water, groundwater, and sediments was clearly supported by statistical analysis. Data from my study generally agree with data from other mine waste contamination sites supporting the hypothesis that a causal relationship exists between the

presence of mining activities and the accumulation of trace elements at concentrations that are potentially harmful to human health and fish and wildlife habitat.

2. The ecological impact of trace elements from abandoned mine waste on fish habitat in the Methow River.

We looked for evidence of impact at different scales of biological organization including cellular, tissue, individual, population, community and ecosystem. Liver tissues from juvenile trout and small intestine samples from exposed caddisfly larvae were examined for evidence of metal accumulation, cytopathological change, and chemical toxicity. Morphological changes that are characteristic of nuclear apoptosis were observed in caddisfly small intestine columnar epithelial and trout liver nuclei where extensive chromatin condensation and margination was observed. Metabolic disorders observable at the tissue level indicated the rate of food conversion into usable energy had been reduced. Reduced growth among individual fish and among populations of caddisfly larvae exposed to contaminated sediments appears to be the result of reduced energy conversion. It is hypothesized that the diversion of energy as a result of the physiological response to mine waste contaminants reduces competition among metals intolerant species and results in changes in community structure. It was also hypothesized that when there are changes in community structure and the functional property of that community is altered, then ecosystem function is also affected. At the population level, an increased risk of metal toxicity and cancer from As exposure to humans drinking contaminated well water was also observed.

3. Adverse health effects associated with consumption of contaminated groundwater

Private drinking water wells near the abandoned Alder Mine, Red Shirt Mill and Alder Mill in the vicinity of Twisp, Okanogan County, Washington were contaminated with As, Cd,

Pb and Se. These contaminants occurred at concentrations that exceeded estimated background levels as well as drinking water standards. Using standard risk assessment methods, it was clear there were carcinogenic health risks associated with the exposure to As from abandoned mine waste. Additional noncarcinogenic risks were associated with exposure to As, Cd, Pb and Se.

4. Specific evidence of trace element toxicity at the cellular level in caddisfly larvae and trout.

Trace elements in contaminated sediments downstream from the abandoned mines were toxic to caddisfly larvae and trout. This conclusion was based on cytological evidence, including apoptosis and the presence of electron dense granules comprised of Pb, Cu, Ti, Au, Ni, Ca and Fe, in the matrix of mitochondria in trout and caddisfly larvae.

In conclusion, we showed that, although there are no reliable techniques available to clearly separate natural from human-caused loading, epidemiological methods can be used to determine causal relationships among processes occurring in the vicinity of the abandoned mine and mills and observed environmental effects. We were also able to show that the effects of mine waste contamination are expressed at all scales of biological organization and that there are potential indicators at each scale. The mechanistic linkages that integrate responses across scales of organization appear to be related to the disruption of energy exchange among cells, tissues, organisms, populations, communities and the environment.

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APPENDICES

Appendix 1: Arsenic Methow River Sediments (Above Twisp)

7/1/2001
Atomic Fluoresence

QUANT LIM ->	0.0
DET LIM ->	0.0
	µg/g
Sample Set	As
48.22.25/120.07.13	83.2
48.22.25/120.07.13	0.0
48.22.25/120.07.13	0.0
48.22.25/120.07.13	0.0
48.24.80/120.08.60	76.4
48.24.80/120.08.60	0.0
48.24.80/120.08.60	0.0
48.24.80/120.08.60	0.0
48.26.66/120.09.81	0.0
48.26.66/120.09.81	0.0
48.26.66/120.09.81	0.0
48.26.66/120.09.81	0.0
48.28.46/120.10.75	0.0
48.28.46/120.10.75	0.0
48.28.46/120.10.75	0.0
48.28.46/120.10.75	0.0

Appendix 2: Arsenic Methow River Sediments (Below Twisp)

7/1/2001
Atomic Fluoresence

QUANT LIM ->	0.0
DET LIM ->	0.0
	µg/g
Sample Set	As
48.21.17/120.07.28	1.7
48.21.17/120.07.28	8.6
48.21.17/120.07.28	1.7
48.21.17/120.07.28	6.2
48.21.19/120.06.39	17.5
48.21.19/120.06.39	9.4
48.21.19/120.06.39	5.5
48.21.19/120.06.39	0.0
48.20.87/120.06.43	20.8
48.20.87/120.06.43	17.0
48.20.87/120.06.43	6.9
48.20.87/120.06.43	17.1
48.20.87/120.06.43	1.7
48.20.87/120.06.43	8.0
48.18.26/120.03/99	7.4
48.18.26/120.03/99	0.0
48.18.26/120.03/99	9.4
48.18.26/120.03/99	0.0

7/1/2001
Method: Metals EPA 200.7 (ICP-AES). Arsenic Corns, W.T. and Stockwell, P.B. Journal of Analytical Atomic Spectroscopy. 1993. 8:71-77.

QUANT LIM ->	0.1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	1.3	0.1		
DET LIM ->	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0		
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g		
	Al	**As**	**B**	**Ba**	**Ca**	**Cd**	**Cr**	**Cu**	**Fe**	**K**	**Mg**		
48.22.25/120.07.13	12528.1		83.2	73.7	122.6	5195.0	11.2	24.9	21.4	36348.3		1192.8	4896.0
48.22.25/120.07.13	11512.4		0.0	39.6	67.4	3883.8	4.6	23.5	17.2	18847.5		883.7	5069.8
48.22.25/120.07.13	12039.2		0.0	40.1	72.3	3887.9	4.8	23.7	17.8	19457.0		1054.1	5377.1
48.22.25/120.07.13	9251.7	0.0	30.9	54.8	3047.9	3.7	18.1	13.7	14936.1		853.3	4140.8	
48.24.80/120.08.60	11782.1		76.4	71.2	113.1	4869.8	10.8	23.9	19.3	34939.8		1134.6	4643.0
48.24.80/120.08.60	8581.6	0.0	31.5	53.9	2978.3	3.4	17.5	12.5	15107.6		758.7	3780.7	
48.24.80/120.08.60	10052.1		0.0	33.2	62.0	3357.5	3.8	19.5	15.4	16163.6		869.2	4560.3
48.24.80/120.08.60	10292.1		0.0	33.1	61.8	3362.5	3.9	20.1	15.1	16137.4		956.5	4542.9
48.26.66/120.09.81	11582.5		0.0	35.8	69.0	3730.7	4.4	22.4	16.7	17606.4		1041.1	5271.3
48.26.66/120.09.81	11393.6		0.0	36.2	75.3	4498.4	4.2	21.7	18.1	17285.3		949.6	5001.6
48.26.66/120.09.81	9467.9	0.0	32.8	67.1	4314.2	3.7	19.4	15.3	14620.7		875.6	4012.9	
48.26.66/120.09.81	7331.0	0.0	26.9	45.7	3042.8	0.0	15.4	10.5	12306.7		713.6	3237.9	
48.28.46/120.10.75	9811.5	0.0	35.5	38.8	3073.3	4.3	22.5	14.9	16540.0		630.1	5080.4	
48.28.46/120.10.75	12210.8		0.0	39.1	50.7	3739.1	4.9	25.5	20.6	18818.5		775.7	5721.5
48.28.46/120.10.75	8476.4	0.0	27.7	43.5	3125.9	3.2	17.4	13.1	13129.4		648.6	3791.4	
48.28.46/120.10.75	10483.6		0.0	32.5	44.4	3520.8	4.1	21.3	17.3	15218.3		719.0	4557.0

Appendix 3 (continued)

QUANT LIM ->	0.0	0.0	0.3	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.0
DET LIM ->	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Mn	Mo	Na	Ni	P	Pb	S	Se	Zn	Si	Ag
48.22.25/120.07.13	388.2	28.9	575.0	31.0	884.3	37.3	852.6	734.4	73.2	2300.2	19.1
48.22.25/120.07.13	295.3	19.6	190.4	24.6	672.5	30.7	361.9	429.7	67.5	1479.4	13.0
48.22.25/120.07.13	339.1	20.2	186.0	25.5	665.4	31.0	248.2	442.2	63.7	1633.0	12.9
48.22.25/120.07.13	257.4	15.2	139.9	19.9	522.5	25.8	196.8	346.1	52.1	1042.3	11.2
48.24.80/120.08.60	368.9	25.8	245.5	29.6	860.9	35.0	658.9	704.9	68.0	2259.8	13.3
48.24.80/120.08.60	225.9	13.9	134.7	18.8	557.6	22.9	275.4	337.8	51.1	758.3	12.2
48.24.80/120.08.60	285.1	16.4	145.8	20.9	609.8	36.9	218.7	367.7	54.4	1069.2	11.6
48.24.80/120.08.60	283.2	16.7	155.9	20.9	570.8	27.5	198.9	373.4	53.0	1126.4	12.0
48.26.66/120.09.81	332.2	19.0	176.6	24.1	644.4	29.0	166.4	406.2	61.0	1453.4	12.0
48.26.66/120.09.81	363.4	17.9	178.0	23.2	724.8	28.5	321.9	399.6	69.7	1666.5	11.6
48.26.66/120.09.81	320.2	21.3	181.0	19.7	576.6	28.2	362.0	336.1	56.7	1607.7	0.0
48.26.66/120.09.81	226.6	15.2	129.2	15.7	481.7	20.7	209.2	278.2	43.5	982.5	0.0
48.28.46/120.10.75	247.2	19.4	118.0	24.5	555.6	29.0	226.0	379.7	54.7	1293.3	0.0
48.28.46/120.10.75	334.9	22.3	164.0	27.8	561.9	36.0	166.8	435.8	68.4	1284.7	0.0
48.28.46/120.10.75	241.2	15.4	127.5	18.5	434.1	24.7	205.0	304.1	46.5	595.1	0.0
48.28.46/120.10.75	275.9	18.6	163.6	22.6	482.4	30.5	169.9	354.1	54.0	994.4	0.0

Appendix 4: Metals - Methow River Sediments (Below Twisp)

7/1/2001
Method: Metals EPA 200.7 (ICP-AES). Arsenic Corns, W.T. and Stockwell, P.B. Journal of Analytical Atomic Spectroscopy. 1993. 8:71-77.

QUANT LIM ->	0.1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	1.3	0.1
DET LIM ->	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Al	**As**	**B**	**Ba**	**Ca**	**Cd**	**Cr**	**Cu**	**Fe**	**K**	**Mg**
48.21.17/120.07.28	18051.3	1.7	42.5	120.4	7393.3	12.6	41.5	107.3	23317.8	1423.8	7035.5
48.21.17/120.07.28	14456.1	8.6	50.0	105.9	6694.6	5.8	34.2	70.3	19004.0	1144.2	5754.0
48.21.17/120.07.28	11548.6	1.7	28.7	86.1	6288.8	5.0	27.9	92.9	16399.9	862.7	4784.2
48.21.17/120.07.28	12718.5	6.2	32.9	85.6	5787.5	5.5	31.4	148.5	18275.2	952.4	5599.3
48.21.19/120.06.39	14145.3	17.5	67.1	195.0	6517.9	9.0	34.4	119.7	32683.9	1399.7	5750.4
48.21.19/120.06.39	17410.9	9.4	58.3	159.6	7717.4	8.1	37.8	279.7	27293.4	1617.8	6893.4
48.21.19/120.06.39	13141.4	5.5	43.9	111.2	5820.1	5.3	27.6	154.0	18972.6	1447.1	5020.1
48.21.19/120.06.39	14923.6	0.0	47.9	123.1	5949.4	6.2	32.1	130.5	22200.3	1371.2	6124.0
48.20.87/120.06.43	14914.5	20.8	74.0	154.0	6648.2	9.9	37.2	93.5	35789.3	1431.8	6146.4
48.20.87/120.06.43	15661.4	17.0	72.8	174.7	6882.8	9.6	39.1	98.1	34799.2	1523.5	6395.1
48.20.87/120.06.43	12440.1	6.9	53.2	123.0	5278.4	6.8	30.9	77.9	25588.1	1164.2	5161.4
48.20.87/120.06.43	6814.2	17.1	31.9	73.2	3073.1	4.1	16.8	44.9	15476.0	659.2	2834.6
48.20.87/120.06.43	16138.0	1.7	39.0	117.1	6873.4	6.4	36.5	184.6	21351.7	1215.3	6531.9
48.20.87/120.06.43	20301.7	8.0	48.6	134.8	8911.7	8.0	46.4	72.3	26477.7	1559.0	8469.2
48.18.26/120.03/99	14868.8	7.4	46.2	116.3	8208.7	5.5	34.0	65.0	20426.0	1312.6	6065.6
48.18.26/120.03/99	19477.0	0.0	58.0	128.2	9294.1	7.0	44.5	62.1	25804.2	1938.0	8006.0
48.18.26/120.03/99	17131.7	9.4	53.4	138.3	9996.5	6.4	41.0	77.0	23473.1	1792.1	7065.4
48.18.26/120.03/99	15233.6	0.0	46.9	131.2	8570.8	5.4	34.9	74.0	21185.5	1330.2	6448.6

Appendix 4 (continued)

QUANT LIM ->	0.0	0.0	0.3	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.0
DET LIM ->	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Mn	**Mo**	**Na**	**Ni**	**P**	**Pb**	**S**	**Se**	**Zn**	**Si**	**Ag**
48.21.17/120.07.28	480.4	28.6	329.4	38.6	1349.1	40.4	400.8	523.4	86.1	1186.1	0.0
48.21.17/120.07.28	803.0	23.5	348.8	31.8	1163.6	30.6	326.5	427.8	69.0	908.8	0.0
48.21.17/120.07.28	411.8	19.6	197.5	27.9	1262.3	27.0	281.6	364.1	61.1	677.5	0.0
48.21.17/120.07.28	387.8	23.2	217.0	31.4	1054.8	33.0	407.7	406.7	81.6	1179.9	0.0
48.21.19/120.06.39	424.5	29.4	275.2	35.9	1615.8	44.0	1905.4	676.7	114.9	2813.4	0.0
48.21.19/120.06.39	886.8	31.2	302.6	37.4	1184.2	49.7	1122.2	611.9	134.7	2471.3	0.0
48.21.19/120.06.39	642.1	22.9	250.4	25.5	860.1	41.5	966.3	436.6	99.6	1580.3	0.0
48.21.19/120.06.39	562.0	26.6	223.9	31.9	938.2	46.7	724.8	506.5	102.4	1754.6	0.0
48.20.87/120.06.43	391.7	34.5	272.0	39.0	2211.5	44.8	1363.3	743.6	92.8	2798.4	0.0
48.20.87/120.06.43	394.5	32.2	299.9	39.8	2241.8	45.3	1456.4	722.9	90.2	2340.2	0.0
48.20.87/120.06.43	316.9	24.3	237.7	30.8	1537.4	34.2	992.6	542.7	69.2	1857.9	0.0
48.20.87/120.06.43	196.7	13.8	125.0	17.3	916.0	20.3	641.8	322.6	39.2	865.0	0.0
48.20.87/120.06.43	565.2	26.4	277.9	35.3	1144.6	37.9	493.4	486.1	102.6	1354.6	0.0
48.20.87/120.06.43	607.8	32.6	362.3	44.9	1315.1	45.6	618.9	598.0	100.7	1227.9	0.0
48.18.26/120.03/99	399.7	24.1	263.1	31.7	1014.4	40.2	1427.3	477.1	84.4	1972.6	0.0
48.18.26/120.03/99	441.2	31.9	385.9	40.1	1256.2	49.8	1149.9	600.5	97.0	2144.9	0.0
48.18.26/120.03/99	1076.9	28.7	352.5	36.3	1302.3	43.7	1038.3	546.0	86.4	2399.1	0.0
48.18.26/120.03/99	663.2	25.3	282.9	32.6	1162.9	34.4	1068.2	495.7	78.9	2001.1	0.0

Appendix 5: Aspen Leaf Miner Larvae [Phyllocnistis populiella Cham. (Lepidoptera: Gracillaridae)] - Alder Mine and Reference

Aspen Leaf Miner Larvae [Phyllocnistis populiella Cham. (Lepidoptera: Gracillaridae)] - Alder Mine and Reference
7/1/2001
Method: Metals EPA 200.7 (ICP-AES). Arsenic Corns, W.T. and Stockwell, P.B. Journal of Analytical Atomic Spectroscopy. 1993. 8:71-77.

	Li PPM	Be PPM	Na PPM	Mg PPM	Al PPM	P PPM	K PPM	Ca PPM	Sc PPM	Ti PPM
DET LIM ->	< 0.2	< 0.001	< 6	< 0.3	< 8	< 8	< 20	< 20	< 0.3	< 40
48.19.24.1/120.09.38.4	1.8	0.07	6560	1700	3480	9800	5210	1880	3.9	210
48.19.28.3/120.09.53.3	< 0.2	0.12	4410	2080	522	11000	3480	2840	5.5	50

	V PPM	Cr PPM	Mn PPM	Fe PPM	Co PPM	Ni PPM	Cu PPM	Zn PPM	Ga PPM	As PPM
DET LIM ->	< 0.4	< 0.2	< 0.2	< 50	< 0.1	< 1	< 0.5	< 5	< 0.006	< 0.1
48.19.24.1/120.09.38.4	2.7	6.8	57.1	2100	1.2	1.3	261	1050	1.1	0.6
48.19.28.3/120.09.53.3	< 0.4	6.4	22.4	320	0.16	< 1	26.0	255	0.20	< 0.1

	Se PPM	Rb PPM	Sr PPM	Y PPM	Nb PPM	Mo PPM	Ag PPM	Cd PPM	Sb PPM	Cs PPM
DET LIM ->	< 0.2	< 0.01	< 0.05	< 0.3	< 2	< 0.1	< 0.02	< 0.003	< 0.02	< 0.003
48.19.24.1/120.09.38.4	< 0.2	9.1	11.2	2.2	2.1	0.44	0.68	102	0.20	0.06
48.19.28.3/120.09.53.3	< 0.2	1.4	9.8	< 0.3	< 2	0.96	0.40	6.0	0.07	0.03

	Ba PPM	La PPM	Ce PPM	Ta PPM	Tl PPM	Pb PPM	Bi PPM	Th PPM	U PPM
DET LIM ->	< 0.5	< 0.3	< 0.5	< 0.2	< 0.003	< 0.2	< 0.005	< 0.03	< 0.02
48.19.24.1/120.09.38.4	51.8	9.3	28.0	0.43	0.08	14.4	0.04	3.7	1.5
48.19.28.3/120.09.53.3	10.3	1.4	13.6	0.42	0.03	1.2	0.008	1.4	0.03

*Appendix 6: Caddisfly (*Ecclesiomyia spp*) Methow River*

7/1/2001
Method: Metals EPA 200.7 (ICP-AES). Arsenic Corns, W.T. and Stockwell, P.B. Journal of Analytical Atomic Spectroscopy. 1993. 8:71-77.

	Li PPM	Be PPM	Na PPM	Mg PPM	Al PPM	P PPM	K PPM	Ca PPM	Sc PPM	Ti PPM
Det. Limit	< 0.2	< 0.001	< 6	< 0.3	< 8	< 8	< 20	< 20	< 0.3	< 40
48.21.17/120.07.28	10.1	0.29	21400	4590	16300	6500	10400	6930	4.9	1500
48.21.19/120.06.39	6.2	0.33	8620	3050	13300	5200	6800	6160	2.7	760
48.21.05/120.06.22	6.8	0.33	7960	3240	16700	4300	7920	5770	3.3	680
48.21.05/120.06.22	13.8	0.26	12000	2880	10300	7600	7280	6520	2.2	560
48.18.15/120.04.00	5.6	0.13	14900	2140	3110	8600	6200	1910	1.0	230

	V PPM	Cr PPM	Mn PPM	Fe PPM	Co PPM	Ni PPM	Cu PPM	Zn PPM	Ga PPM	As PPM
Det. Limit	< 0.4	< 0.2	< 0.2	< 50	< 0.1	< 1	< 0.5	< 5	< 0.006	< 0.1
48.21.17/120.07.28	33.2	29.2	764	9300	7.2	14.1	26.0	174	3.9	4.2
48.21.19/120.06.39	22.4	12.3	532	7200	3.8	5.4	20.7	234	3.1	3
48.21.05/120.06.22	24.9	14.5	812	8400	4.7	6.2	34.5	226	3.8	3.1
48.21.05/120.06.22	17.2	7.9	3090	7000	3.9	5.9	27.9	248	2.6	9.3
48.18.15/120.04.00	8.3	3.9	461	2800	3.1	2.5	31.4	278	0.86	3.9

	Se PPM	Rb PPM	Sr PPM	Y PPM	Nb PPM	Mo PPM	Ag PPM	Cd PPM	Sb PPM	Cs PPM
Det. Limit	< 0.2	< 0.01	< 0.05	< 0.3	< 2	< 0.1	< 0.02	< 0.003	< 0.02	< 0.003
48.21.17/120.07.28	0.9	12.3	94.2	7.4	3.0	1.2	0.11	0.48	0.26	0.58
48.21.19/120.06.39	0.6	9.9	81.9	10.3	< 2	2.6	0.02	0.62	0.27	0.64
48.21.05/120.06.22	0.6	10.8	96.7	10.3	< 2	2.6	0.04	0.64	0.2	0.73
48.21.05/120.06.22	0.9	7.7	69.9	7.2	< 2	3.2	0.04	0.44	0.31	0.53
48.18.15/120.04.00	0.8	4.8	22.1	6.1	< 2	2.7	0.05	0.71	0.2	0.24

	Ba PPM	La PPM	Ce PPM	Ta PPM	Tl PPM	Pb PPM	Bi PPM	Th PPM	U PPM
Det. Limit	< 0.5	< 0.3	< 0.5	< 0.2	< 0.003	< 0.2	< 0.005	< 0.03	< 0.02
48.21.17/120.07.28	227	6.3	11.5	0.22	0.05	2.5	0.04	0.92	0.98
48.21.19/120.06.39	258	6.7	10.0	< 0.2	0.07	3.8	0.04	1.2	1.9
48.21.05/120.06.22	310	6.8	10.2	< 0.2	0.09	3.6	0.10	1.1	1.6
48.21.05/120.06.22	290	5.1	7.6	< 0.2	0.05	1.9	0.03	0.75	0.84
48.18.15/120.04.00	116	3.5	4.7	< 0.2	0.02	1.4	0.03	0.43	1.3

*Appendix 7: Trout (*Oncorhynchus mykiss*) Liver Metal Concentration - Alder Creek and Twisp River*

Method: Metals EPA 200.7 (ICP-AES). Arsenic Corns, W.T. and Stockwell, P.B. Journal of Analytical Atomic Spectroscopy. 1993. 8:71-77. September-99

QUANT LIM ->	21.529	3.588	11.961	0.478	9.090	2.153	2.392	0.957	2.153	95.684	9.568
DET LIM ->	6.459	1.077	3.589	0.144	2.727	0.646	0.718	0.287	0.646	28.708	2.871
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Al	As	B	Ba	Ca	Cd	Cr	Cu	Fe	K	Mg
48.22.02.9/120.10.26.9	6.5	1.1	0.0	0.1	877.2	0.0	0.0	306.1	877	13757.6	722.5
48.22.02.9/120.10.26.9	6.5	7.8	0.0	0.0	310.9	0.0	0.0	389.7	1074	14868.2	692.9
48.22.02.9/120.10.26.9	6.5	5.4	0.0	0.0	217.5	0.0	0.0	415.8	1017	14407.8	687.1
48.19.59.45/120.09.27.16	6.5	5.4	0.0	0.0	191.1	12.8	0.0	253.9	65.2	16660.5	761.3
48.19.59.45/120.09.27.16	6.5	4.8	0.0	0.0	263.0	9.2	0.0	71.9	60.1	15999.0	750.6
48.19.59.45/120.09.27.16	6.5	4.9	0.0	0.0	182.6	9.9	0.0	287.8	79.9	12668.3	573.4

QUANT LIM ->	0.239	1.674	23.921	0.718	9.568	7.176	7.176	7.176	0.478	4.784	0.478
DET LIM ->	0.072	0.502	7.177	0.215	2.871	2.153	2.153	2.153	0.144	1.435	0.144
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Mn	Mo	Na	Ni	P	Pb	S	Se	Zn	Si	Ag
48.22.02.9/120.10.26.9	5.5	0.0	4344.7	0.0	14182.6	0.0	10241.1	2.2	92.7	62.9	0.000
48.22.02.9/120.10.26.9	6.7	0.0	4079.9	0.0	14757.9	2.2	9580.2	2.2	101.4	73.1	0.000
48.22.02.9/120.10.26.9	5.8	0.0	3097.4	0.0	13786.7	0.0	9372.2	23.5	91.4	71.2	0.144
48.19.59.45/120.09.27.16	8.6	0.0	2851.1	0.0	14565.7	0.0	6988.5	2.2	81.7	26.7	0.000
48.19.59.45/120.09.27.16	8.7	0.0	3618.4	0.0	14722.5	0.0	7152.6	2.2	74.6	33.1	0.000
48.19.59.45/120.09.27.16	3.0	0.0	4245.8	0.0	11270.8	0.0	7804.5	21.4	63.1	29.4	0.000

Appendix 8: Piezometer Leachate - Alder Mill

7/1/2001
Method: Metals EPA 200.7 (ICP-AES). Arsenic Corns, W.T. and Stockwell, P.B. Journal of Analytical Atomic Spectroscopy. 1993. 8:71-77.

QUANT LIM ->	0.300	0.050	0.167	0.007	0.127	0.030	0.033	0.013	0.030	1.333	0.133
DET LIM ->	0.090	0.015	0.050	0.002	0.038	0.009	0.010	0.004	0.009	0.400	0.040
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Al	As	B	Ba	Ca	Cd	Cr	Cu	Fe	K	Mg
48.21.13.5/120.07.31.6	31975.8	0.0	74.5	42.5	31.0	12.3	14.1	386.9	44732.9	134.1	41171.1
48.21.13.5/120.07.31.6	509.9	0.0	128.6	62.4	217.7	43.5	12.9	16397.8	225537.6	198.8	217.4
48.21.13.5/120.07.31.6	3164.5	0.0	407.5	116.3	1009.7	72.4	27.0	2004.3	274567.5	1213.4	1112.4
48.21.13.5/120.07.31.6	13.7	0.1	0.7	0.0	432.0	0.3	0.1	2.5	385.1	11.6	62.4
48.21.13.5/120.07.31.6	40.5	0.0	0.6	0.0	481.3	0.1	0.3	40.6	60.2	5.9	1215.0
48.21.13.5/120.07.31.6	37.0	0.2	0.3	0.0	468.8	0.1	0.2	35.1	57.6	3.8	221.4

QUANT LIM ->	0.003	0.023	0.333	0.010	0.133	0.100	0.100	0.100	0.007	0.067	0.007
DET LIM ->	0.001	0.007	0.100	0.003	0.040	0.030	0.030	0.030	0.002	0.020	0.002
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Mn	Mo	Na	Ni	P	Pb	S	Se	Zn	Si	Ag
48.21.13.5/120.07.31.6	468.8	59.9	0.0	43.1	436.1	258.2	55979.7	1161.8	242.7	1373.2	0.0
48.21.13.5/120.07.31.6	87.2	49.5	35.5	42.0	356.8	65.3	88497.7	4785.0	306.5	536.1	0.0
48.21.13.5/120.07.31.6	147.9	109.7	92.9	113.3	1260.9	609.3	16558.1	4422.1	964.5	1233.5	0.0
48.21.13.5/120.07.31.6	2.1	0.2	18.4	0.3	2.2	0.2	727.2	6.6	16.5	44.8	0.0
48.21.13.5/120.07.31.6	1.6	0.3	75.4	0.6	3.7	0.2	558.5	8.3	12.6	25.1	0.0
48.21.13.5/120.07.31.6	1.7	0.2	21.9	0.3	2.0	0.2	534.3	2.4	13.7	17.9	0.0

Appendix 9: Piezometer Leachate - Alder Mine

7/1/2001
Method: Metals EPA 200.7 (ICP-AES). Arsenic Corns, W.T. and Stockwell, P.B. Journal of Analytical Atomic Spectroscopy. 1993. 8:71-77.

QUANT LIM ->	0.300	0.050	0.167	0.007	0.127	0.030	0.033	0.013	0.030	1.333	0.133
DET LIM ->	0.090	0.015	0.050	0.002	0.038	0.009	0.010	0.004	0.009	0.400	0.040
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Al	As	B	Ba	Ca	Cd	Cr	Cu	Fe	K	Mg
48.19.24.1/120.09.38.4	0.472	0.061	0.050	0.163	61.895	0.009	0.010	0.243	1.163	4.815	10.787
48.19.24.1/120.09.38.4	1.540	0.015	0.000	0.078	26.621	0.009	0.010	1.343	1.569	25.687	5.780
48.19.24.1/120.09.38.4	0.090	0.052	0.000	0.008	22.245	0.009	0.010	0.131	0.102	20.381	4.897

QUANT LIM ->	0.003	0.023	0.333	0.010	0.133	0.100	0.100	0.100	0.007	0.067	0.007
DET LIM ->	0.001	0.007	0.100	0.003	0.040	0.030	0.030	0.030	0.002	0.020	0.002
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Mn	Mo	Na	Ni	P	Pb	S	Se	Zn	Si	Ag
48.19.24.1/120.09.38.4	0.046	0.000	10.400	0.003	0.185	0.000	50.949	0.000	0.594	4.736	0.000
48.19.24.1/120.09.38.4	1.237	0.000	14.088	0.051	11.138	0.000	27.598	0.000	2.685	6.510	0.000
48.19.24.1/120.09.38.4	0.918	0.000	11.653	0.044	8.379	0.000	28.259	0.000	1.333	5.040	0.000

*Appendix 10: Aspen Leaves (*Populus tremuloides*) - Alder Mine and Reference*

7/1/2001
Method: Metals EPA 200.7 (ICP-AES). Arsenic Corns, W.T. and Stockwell, P.B. Journal of Analytical Atomic Spectroscopy. 1993. 8:71-77.

QUANT LIM ->	29.997	5.000	16.665	0.667	12.665	3.000	3.333	1.333	3.000	133.320	13.332
DET LIM ->	9.000	1.500	5.000	0.200	3.800	0.900	1.000	0.400	0.900	40.000	4.000
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Al	As	B	Ba	Ca	Cd	Cr	Cu	Fe	K	Mg
48.19.28.3/120.09.53.3	9.0	8.4	35.5	26.3	14900.5	0.0	0.0	11.0	45.0	12833.2	1565.9
48.19.28.3/120.09.53.3	9.0	8.7	29.6	24.2	10812.4	0.0	0.0	9.3	45.7	12862.6	1356.4
48.19.28.3/120.09.53.3	9.0	9.6	28.2	20.9	11028.7	0.0	0.0	10.1	52.7	14760.0	1714.5
48.19.28.3/120.09.53.3	30.4	7.9	40.6	44.0	14614.9	0.0	0.0	10.4	104.5	11213.4	1917.5
48.19.28.3/120.09.53.3	11.5	6.9	26.8	23.1	10271.3	0.0	0.0	8.2	49.6	10333.8	1310.9
48.19.24.1/120.09.38.4	35.7	9.1	139.5	6.3	12310.1	11.1	0.0	12.3	79.8	15753.8	3413.0
48.19.24.1/120.09.38.4	62.2	1.5	76.1	5.2	7188.4	18.7	0.0	10.8	133.4	22595.5	3022.3
48.19.24.1/120.09.38.4	37.3	9.5	101.6	7.3	9256.1	20.1	0.0	13.2	83.2	21208.3	3418.4
48.19.24.1/120.09.38.4	27.6	7.5	49.6	15.9	12766.1	0.6	0.0	11.0	65.4	15917.4	1853.1
48.19.24.1/120.09.38.4	63.5	10.6	52.0	4.3	13375.9	61.8	0.0	29.0	93.4	10204.3	5071.0
48.19.24.1/120.09.38.4	47.2	9.2	144.4	6.7	9242.0	23.8	0.0	14.0	60.5	24604.1	3275.8

QUANT LIM ->	0.333	2.333	33.330	1.000	13.332	9.999	9.999	9.999	0.667	6.666	0.667
DET LIM ->	0.100	0.700	10.000	0.300	4.000	3.000	3.000	3.000	0.200	2.000	0.200
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Mn	Mo	Na	Ni	P	Pb	S	Se	Zn	Si	Ag
48.19.28.3/120.09.53.3	77.2	0.0	10.0	0.3	3448.9	3.0	1362.4	3.0	168.3	114.1	0.000
48.19.28.3/120.09.53.3	80.0	0.0	0.0	0.3	2923.4	0.0	1341.7	3.0	138.6	63.2	0.000
48.19.28.3/120.09.53.3	67.1	0.0	0.0	0.3	3635.2	3.0	1384.6	3.0	135.9	116.6	0.000
48.19.28.3/120.09.53.3	89.1	0.0	0.0	2.9	3183.1	2.2	1281.0	2.2	155.6	115.1	0.000
48.19.28.3/120.09.53.3	62.7	0.0	2.0	0.8	2638.1	1.6	1073.9	2.2	119.7	81.8	0.0
48.19.24.1/120.09.38.4	79.0	0.0	0.0	0.3	2596.3	3.0	2291.0	3.0	345.0	203.6	0.000
48.19.24.1/120.09.38.4	96.6	0.0	0.0	0.3	2173.5	0.0	1908.1	3.0	595.6	107.4	0.000
48.19.24.1/120.09.38.4	90.7	0.0	10.0	0.3	2342.9	0.0	2159.9	3.0	651.5	89.2	0.000
48.19.24.1/120.09.38.4	122.6	0.5	0.0	4.4	2919.7	2.2	1915.4	2.2	389.1	241.9	0.000
48.19.24.1/120.09.38.4	119.8	0.5	0.0	4.7	1389.6	8.9	3903.0	31.3	846.9	119.6	0.000
48.19.24.1/120.09.38.4	115.3	0.5	0.0	4.0	2369.0	6.1	2926.7	20.6	829.8	236.8	0.000

7/1/2001

Method: Metals EPA 200.7 (ICP-AES). Arsenic Corns, W.T. and Stockwell, P.B. Journal of Analytical Atomic Spectroscopy. 1993. 8:71-77.

QUANT LIM ->	30.5	5.1	16.9	0.7	12.9	3.0	3.4	1.4	3.0	135.5	13.5
DET LIM ->	9.1	1.5	5.1	0.2	3.9	0.9	1.0	0.4	0.9	40.7	4.1
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Al	As	B	Ba	Ca	Cd	Cr	Cu	Fe	K	Mg
48.21.13.5/120.07.31.6	5821.4	111.5	106.1	1108.9	412.5	23.4	13.5	1488.1	57838.3	2652.2	3586.8
48.21.13.5/120.07.31.6	13945.0	0.0	201.1	1967.5	242.0	51.4	0.0	634.7	235947	7591.6	11201.9
48.21.13.5/120.07.31.6	1643.7	44.6	24.7	5943.3	82.9	5.1	5.4	136.8	13568.4	674.7	1078.8
48.21.13.5/120.07.31.6	3572.8	27.7	36.7	6652.2	157.0	6.4	5.4	106.3	19129.9	1292.5	2490.0
48.21.13.5/120.07.31.6	2896.5	30.9	35.6	1989.6	163.7	6.3	5.3	136.7	19545.1	893.7	1989.2
48.21.13.5/120.07.31.6	2017.6	42.3	26.0	2660.5	114.9	5.8	4.2	124.8	15096.2	407.9	1395.8
48.21.13.5/120.07.31.6	2271.9	32.9	26.5	5201.7	135.0	5.2	4.3	90.4	14907.6	447.8	1578.5
48.21.13.5/120.07.31.6	2101.1	32.8	27.1	4511.3	133.9	5.3	4.2	95.4	15580.2	391.4	1494.6
48.21.13.5/120.07.31.6	4178.1	50.6	40.4	6519.1	184.6	8.1	6.7	133.1	23132.2	615.7	3342.1
48.21.13.5/120.07.31.6	8412.5	21.6	56.6	3155.8	637.8	9.0	10.9	358.3	29257.7	1344.7	5362.6
48.21.13.5/120.07.31.6	10496.9	23.4	54.6	3203.9	785.3	9.4	11.6	313.5	28437.4	1636.8	6960.5
48.21.13.5/120.07.31.6	13622.7	22.4	72.7	1837.4	1693.9	10.5	14.8	454.8	31485.2	1688.7	9290.9
48.21.13.5/120.07.31.6	3182.1	31.5	29.4	6622.3	217.2	5.4	5.0	86.6	15548.9	802.5	2402.9
48.21.13.5/120.07.31.6	16438	17	71	1630	1409	11	16	532	37019	2978	11607

QUANT LIM ->	0.3	2.4	33.9	1.0	13.5	10.2	10.2	10.2	0.7	6.8	0.7
DET LIM ->	0.1	0.7	10.2	0.3	4.1	3.0	3.0	3.0	0.2	2.0	0.2
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Mn	Mo	Na	Ni	P	Pb	S	Se	Zn	Si	Ag
48.21.13.5/120.07.31.6	87.5	89.1	504.1	30.5	458.3	3163.7	13682.6	982.3	983.8	1134.7	37.2
48.21.13.5/120.07.31.6	164.5	93.7	199.3	44.1	628.0	2094.3	32683.9	3541.7	850.5	1750.1	27.184
48.21.13.5/120.07.31.6	27.4	25.8	39.6	8.4	154.8	178.7	2548.7	253.2	142.1	334.2	1.0
48.21.13.5/120.07.31.6	58.3	26.9	107.2	12.7	261.6	363.1	4857.3	366.8	213.3	684.1	1.0
48.21.13.5/120.07.31.6	56.4	21.0	106.6	11.4	246.5	84.4	3219.7	365.3	155.9	651.1	0.0
48.21.13.5/120.07.31.6	41.8	16.7	47.6	8.7	232.7	51.8	1695.5	285.1	140.0	570.4	0.0
48.21.13.5/120.07.31.6	50.7	19.1	47.6	9.1	238.3	45.4	1964.4	282.2	186.1	649.7	0.0
48.21.13.5/120.07.31.6	48.2	19.8	47.6	9.4	253.7	43.7	1912.3	289.4	200.6	548.0	0.0
48.21.13.5/120.07.31.6	104.8	28.5	34.2	14.5	374.1	74.4	4964.4	442.7	242.7	533.3	0.0
48.21.13.5/120.07.31.6	115.0	31.8	120.3	19.7	339.8	116.1	7122.2	588.6	251.8	767.3	0.0
48.21.13.5/120.07.31.6	129.3	33.1	135.3	21.1	376.6	131.6	7597.6	581.3	275.5	698.5	0.0
48.21.13.5/120.07.31.6	165.0	37.1	194.8	24.9	479.9	133.6	9660.6	669.1	334.5	751.5	0.0
48.21.13.5/120.07.31.6	59.2	25.0	52.3	10.8	155.1	105.6	3039.2	298.0	138.7	486.0	0.0
48.21.13.5/120.07.31.6	179	44	283	28	513	172	11410	718	379	1447	0

7/1/2000
Method: Metals EPA 200.7 (ICP-AES). Arsenic Corns, W.T. and Stockwell, P.B. Journal of Analytical Atomic Spectroscopy. 1993. 8:71-77.

QUANT LIM ->	30.5	5.1	16.9	0.7	12.9	3.0	3.4	1.4	3.0	135.5	13.5
DET LIM ->	9.1	1.5	5.1	0.2	3.9	0.9	1.0	0.4	0.9	40.7	4.1
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Al	As	B	Ba	Ca	Cd	Cr	Cu	Fe	K	Mg
48.19.24.1/120.09.38.4	12977.6	0.0	162.6	59.8	395.4	54.8	21.1	54700.3	253365.0	3983.0	11563.6
48.19.24.1/120.09.38.4	18473.2	10.8	68.0	1723.2	507.6	11.1	16.0	494.6	35384.6	3551.1	12710.7
48.19.24.1/120.09.38.4	17.629	0.051	0.334	0.032	171.556	0.131	0.081	39.846	174.501	15.823	57.066
48.19.24.1/120.09.38.4	9.822	0.140	0.050	0.014	371.701	0.034	0.075	28.057	15.266	3.655	23.764
48.19.24.1/120.09.38.4	0.000	0.000	0.000	0.012	32.929	0.000	0.000	0.000	0.000	0.400	5.883
48.19.24.1/120.09.38.4	0.000	0.107	0.581	0.002	19.450	0.000	0.010	0.000	0.000	0.856	10.205
48.19.24.1/120.09.38.4	5744.5	156.8	909.6	235.9		144.6	68.6	3557.4	129321.4	680.3	1990.1
48.19.24.1/120.09.38.4	2824.0	0.0	509.4	104.3		66.1	26.9	1864.4	228216.2	971.0	1014.2

QUANT LIM ->	0.3	2.4	33.9	1.0	13.5	10.2	10.2	10.2	0.7	6.8	0.7
DET LIM ->	0.1	0.7	10.2	0.3	4.1	3.0	3.0	3.0	0.2	2.0	0.2
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Mn	Mo	Na	Ni	P	Pb	S	Se	Zn	Si	Ag
48.19.24.1/120.09.38.4	380.6	78.1	120.6	59.3	487.2	72.1	80453.9	5552.2	1096.9	832.5	41.3
48.19.24.1/120.09.38.4	429.6	47.0	143.9	30.0	550.7	433.7	5355.5	759.2	289.6	804.9	0.2
48.19.24.1/120.09.38.4	1.322	0.141	22.056	0.169	1.400	0.081	384.421	3.488	9.281	3.699	0.000
48.19.24.1/120.09.38.4	0.802	0.007	10.813	0.063	0.601	0.030	367.995	0.472	4.061	1.524	0.000
48.19.24.1/120.09.38.4	0.001	0.000	1.939	0.000	0.215	0.000	17.114	0.000	0.002	4.316	0.000
48.19.24.1/120.09.38.4	0.017	0.000	26.628	0.003	0.262	0.030	24.028	0.030	0.000	3.376	0.000
48.19.24.1/120.09.38.4	282.0	199.9		220.2		1060.0	26613.7	9166.9	1751.7	2878.4	
48.19.24.1/120.09.38.4	134.0	105.8		101.3		536.8	15833.7	2996.3	916.1	494.3	

Appendix 13: Red Shirt Mill Waste

7/1/2001
Method: Metals EPA 200.7 (ICP-AES). Arsenic Corns, W.T. and Stockwell, P.B. Journal of Analytical Atomic Spectroscopy. 1993. 8:71-77.

QUANT LIM ->	30.5	5.1	16.9	0.7	12.9	3.0	3.4	1.4	3.0	135.5	13.5
DET LIM ->	9.1	1.5	5.1	0.2	3.9	0.9	1.0	0.4	0.9	40.7	4.1
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Al	As	B	Ba	Ca	Cd	Cr	Cu	Fe	K	Mg
48.21.05.0/120.06.08.1	4577.1	15.3	37.6	374.2	43.8	6.4	5.6	189.9	21572.2	318.8	3197.6
48.21.05.0/120.06.08.1	26194.7	31.1	101.5	732.2	942.3	17.6	0.5	591.1	58756.1	1427.8	16194.9
48.21.05.0/120.06.08.1	47420.3	0.0	163.4	962.5	184.6	51.6	8.7	1355.3	264502.9	2616.8	> HIGH
48.21.05.0/120.06.08.1	41765.5	0.0	149.5	1179.5	1097.8	45.9	5.5	1166.3	231188.0	2175.4	19291.6
48.21.05.0/120.06.08.1	34815.8	0.0	145.7	1096.7	1116.9	47.1	7.3	1149.5	240701.9	1584.2	19831.1
48.21.05.0/120.06.08.1	3758.599	13.822	65.154	330.726	74.159	7.638	0.010	269.322	28650.786	287.937	2509.071
48.21.05.0/120.06.08.1	31657.279	0.000	174.076	1119.192	989.648	40.372	6.103	1005.889	196949.300	1385.328	17648.996

QUANT LIM ->	0.3	2.4	33.9	1.0	13.5	10.2	10.2	10.2	0.7	6.8	0.7
DET LIM ->	0.1	0.7	10.2	0.3	4.1	3.0	3.0	3.0	0.2	2.0	0.2
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Mn	Mo	Na	Ni	P	Pb	S	Se	Zn	Si	Ag
48.21.05.0/120.06.08.1	92.0	27.3	10.2	13.0	173.6	63.3	761.3	401.1	201.5	958.1	0.0
48.21.05.0/120.06.08.1	447.8	72.3	244.0	39.2	556.1	246.4	2464.2	222.3	645.0	1012.8	0.1
48.21.05.0/120.06.08.1	642.7	113.9	68.8	55.3	796.3	197.7	2060.5	4520.1	812.1	482.6	0.1
48.21.05.0/120.06.08.1	532.3	103.0	80.6	50.2	712.8	199.6	1508.0	3834.6	739.4	961.4	0.1
48.21.05.0/120.06.08.1	545.3	98.9	44.5	50.1	718.1	173.8	1249.0	3976.8	725.1	936.0	0.1
48.21.05.0/120.06.08.1	72.774	28.564	496.064	13.909	170.097	66.682	986.765	0.030	229.933	620.976	0.002
48.21.05.0/120.06.08.1	520.653	83.323	742.801	44.528	659.294	268.274	1365.579	3438.323	627.125	866.237	0.002

Appendix 14: Acid Mine Drainage - Alder Mine

7/1/2001

Method: Metals EPA 200.7 (ICP-AES). Arsenic Corns, W.T. and Stockwell, P.B. Journal of Analytical Atomic Spectroscopy. 1993. 8:71-77.

QUANT LIM ->	0.1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	1.3	0.1
DET LIM ->	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Al	As	B	Ba	Ca	Cd	Cr	Cu	Fe	K	Mg
48.19.24.1/120.09.38.4	6.915	0.138	0.242	0.002	153.844	1.386	0.074	5.013	4.063	3.292	80.617
48.19.24.1/120.09.38.4	14.382	0.186	0.436	0.018	182.742	2.700	0.096	10.557	9.045	5.089	162.482
48.19.24.1/120.09.38.4	14.218	0.245	0.395	0.008	177.448	2.647	0.110	10.049	7.861	3.835	157.373
48.19.24.1/120.09.38.4	16.432	0.191	0.407	0.011	179.075	2.783	0.089	12.027	1.026	8.938	166.724
48.19.24.1/120.09.38.4	18.124	0.301	0.050	0.000	203.947	3.104	0.000	13.717	10.354	4.066	173.063
48.19.24.1/120.09.38.4	23.767	0.199	0.435	0.002	174.917	3.267	0.000	19.458	20.164	5.694	175.655
48.19.24.1/120.09.38.4	22.874	0.183	0.409	0.002	170.147	3.135	0.000	18.612	11.053	4.647	170.276

QUANT LIM ->	0.0	0.0	0.3	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.0
DET LIM ->	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
	Mn	Mo	Na	Ni	P	Pb	S	Se	Zn	Si	Ag
48.19.24.1/120.09.38.4	4.779	0.169	5.582	0.117	1.536	0.230	312.172	0.600	99.309	0.390	0.000
48.19.24.1/120.09.38.4	9.554	0.343	9.279	0.205	1.274	0.288	511.805	1.207	195.131	8.738	0.000
48.19.24.1/120.09.38.4	9.289	0.323	8.780	0.214	1.440	0.287	493.631	1.078	192.538	9.281	0.000
48.19.24.1/120.09.38.4	9.774	0.329	14.632	0.203	4.023	0.239	520.713	1.086	203.832	9.427	0.002
48.19.24.1/120.09.38.4	10.159	0.390	9.643	0.236	1.757	0.770	499.894	1.242	221.800	9.643	0.002
48.19.24.1/120.09.38.4	10.472	0.401	8.733	0.225	1.717	0.756	554.000	1.261	242.000	9.263	0.000
48.19.24.1/120.09.38.4	10.156	0.374	8.745	0.207	1.678	0.659	540.000	1.206	231.000	9.076	0.000

Appendix 15: Methow River Surface Water (Above Twisp)

7/1/2001

Method: Metals EPA 200.7 (ICP-AES). Arsenic Corns, W.T. and Stockwell, P.B. Journal of Analytical Atomic Spectroscopy. 1993. 8:71-77.

QUANT LIM ->	0.1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	1.3	0.1
DET LIM ->	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
SAMPLE SET	Al	As	B	Ba	Ca	Cd	Cr	Cu	Fe	K	Mg
48.22.25/120.07.13	0.000	0.015	0.000	0.014	24.232	0.000	0.010	0.000	0.009	0.709	5.252
48.22.25/120.07.13	0.090	0.070	0.000	0.014	24.887	0.000	0.010	0.015	0.009	0.000	5.601
48.24.80/120.08.60	0.000	0.000	0.000	0.015	25.382	0.000	0.000	0.000	0.000	1.346	5.795
48.24.80/120.08.60	0.000	0.015	0.000	0.012	23.992	0.000	0.010	0.004	0.000	0.400	5.438
48.26.66/120.09.81	0.000	0.015	0.000	0.016	22.721	0.000	0.010	0.000	0.009	0.400	3.662
48.26.66/120.09.81	0.090	0.083	0.000	0.017	21.710	0.009	0.010	0.016	0.009	0.000	3.492
48.28.46/120.10.75	0.000	0.000	0.000	0.015	21.935	0.000	0.000	0.000	0.000	0.000	3.502
48.28.46/120.10.75	0.000	0.015	0.000	0.015	21.188	0.000	0.010	0.004	0.000	0.400	3.404

QUANT LIM ->	0.0	0.0	0.3	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.0
DET LIM ->	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
SAMPLE SET	Mn	Mo	Na	Ni	P	Pb	S	Se	Zn	Si	Ag
48.22.25/120.07.13	0.000	0.000	3.262	0.000	0.134	0.000	2.661	0.000	0.000	4.952	0.000
48.22.25/120.07.13	0.000	0.000	3.484	0.003	0.040	0.000	2.801	0.000	0.000	4.572	0.000
48.24.80/120.08.60	0.000	0.000	3.573	0.000	0.000	0.000	3.007	0.030	0.000	7.624	0.000
48.24.80/120.08.60	0.000	0.000	2.565	0.000	0.201	0.000	1.967	0.000	0.000	4.494	0.000
48.26.66/120.09.81	0.000	0.007	3.497	0.000	0.040	0.000	2.002	0.000	0.000	5.969	0.000
48.26.66/120.09.81	0.000	0.000	3.530	0.003	0.040	0.000	1.900	0.000	0.000	5.311	0.000
48.28.46/120.10.75	0.000	0.000	3.402	0.000	0.000	0.000	1.905	0.000	0.000	5.881	0.000
48.28.46/120.10.75	0.000	0.000	2.558	0.000	0.158	0.000	0.970	0.000	0.000	4.849	0.000

Appendix 16: Meals - Methow River Water (Below Twisp)

7/1/2001
Method: Metals EPA 200.7 (ICP-AES). Arsenic Corns, W.T. and Stockwell, P.B. Journal of Analytical Atomic Spectroscopy. 1993. 8:71-77.

QUANT LIM ->	0.1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	1.3	0.1
DET LIM ->	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
SAMPLE SET	Al	As	B	Ba	Ca	Cd	Cr	Cu	Fe	K	Mg
48.21.17/120.07.28	0.090	0.015	0.000	0.014	24.302	0.000	0.000	0.004	0.009	0.930	4.673
48.21.17/120.07.28	0.090	0.015	0.000	0.015	25.046	0.000	0.000	0.015	0.160	0.400	4.792
48.21.17/120.07.28	0.090	0.015	0.000	0.015	24.266	0.000	0.000	0.004	0.009	0.400	4.652
48.21.17/120.07.28	0.090	0.015	0.000	0.014	24.355	0.000	0.000	0.004	0.036	0.400	4.668

QUANT LIM ->	0.0	0.0	0.3	0.0	0.1	0.1	0.1	0.1	0.0	0.1	0.0
DET LIM ->	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
SAMPLE SET	Mn	Mo	Na	Ni	P	Pb	S	Se	Zn	Si	Ag
48.21.17/120.07.28	0.001	0.000	3.093	0.003	0.040	0.000	2.363	0.000	0.000	5.016	0.000
48.21.17/120.07.28	0.001	0.000	3.298	0.000	0.040	0.000	2.405	0.000	0.002	5.220	0.000
48.21.17/120.07.28	0.001	0.000	3.105	0.000	0.040	0.000	2.362	0.000	0.000	5.011	0.000
48.21.17/120.07.28	0.001	0.000	3.117	0.003	0.040	0.000	2.380	0.000	0.000	5.039	0.000

Appendix 16 (continued)

QUANT LIM ->	0.1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	1.3	0.1
DET LIM ->	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
SAMPLE SET	Al	As	B	Ba	Ca	Cd	Cr	Cu	Fe	K	Mg
48.21.19/120.06.39	0.000	0.015	0.000	0.015	28.044	0.000	0.000	0.000	0.009	0.976	4.380
48.21.19/120.06.39	0.090	0.072	0.000	0.010	27.567	0.000	0.010	0.015	0.009	0.400	4.287
48.21.19/120.06.39	0.000	0.000	0.000	0.009	27.122	0.000	0.000	0.000	0.000	0.973	4.303
48.21.19/120.06.39	0.000	0.000	0.000	0.010	29.040	0.000	0.000	0.000	0.000	0.697	4.543
48.21.19/120.06.39	0.000	0.015	0.000	0.013	30.823	0.000	0.010	0.004	0.000	0.000	4.745
48.21.19/120.06.39	0.090	0.000	0.000	0.020	40.809	0.000	0.000	0.000	0.107	1.127	6.206

QUANT LIM ->	0.1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	1.3	0.1
DET LIM ->	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
SAMPLE SET	Al	As	B	Ba	Ca	Cd	Cr	Cu	Fe	K	Mg
48.21.19/120.06.39	0.000	0.007	3.424	0.000	0.258	0.000	3.249	0.000	0.000	6.346	0.000
48.21.19/120.06.39	0.000	0.000	3.238	0.003	0.265	0.000	3.658	0.000	0.000	4.829	0.000
48.21.19/120.06.39	0.000	0.000	3.146	0.000	0.139	0.000	3.223	0.000	0.000	6.067	0.000
48.21.19/120.06.39	0.000	0.000	3.175	0.000	0.000	0.000	3.356	0.000	0.000	6.054	0.000
48.21.19/120.06.39	0.001	0.000	3.026	0.000	0.040	0.000	3.449	0.000	0.000	5.127	0.000
48.21.19/120.06.39	0.232	0.000	3.607	0.000	0.139	0.000	1.116	0.000	0.000	8.723	0.000

Appendix 16 (continued)

QUANT LIM ->	0.1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	1.3	0.1
DET LIM ->	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
SAMPLE SET	Al	As	B	Ba	Ca	Cd	Cr	Cu	Fe	K	Mg
48.20.87/120.06.43	0.000	0.015	0.000	0.008	15.854	0.000	0.000	0.000	0.000	1.085	5.052
48.20.87/120.06.43	0.000	0.015	0.000	0.002	14.657	0.000	0.000	0.000	0.000	0.400	3.477
48.20.87/120.06.43	0.000	0.015	0.000	0.012	14.922	0.000	0.000	0.000	0.000	0.400	3.238
48.20.87/120.06.43	0.000	0.087	0.050	0.028	60.322	0.000	0.010	0.004	0.309	1.194	13.748
48.20.87/120.06.43	0.090	0.124	0.000	0.028	57.801	0.000	0.010	0.017	1.204	0.799	13.229
48.20.87/120.06.43	0.000	0.015	0.000	0.029	57.046	0.000	0.000	0.000	0.311	0.797	13.531
48.20.87/120.06.43	0.000	0.060	0.050	0.010	53.402	0.000	0.010	0.000	0.009	2.563	12.527
48.20.87/120.06.43	0.090	0.076	0.000	0.023	50.059	0.000	0.010	0.004	0.994	0.873	11.376
48.20.87/120.06.43	0.000	0.015	0.000	0.017	48.662	0.000	0.000	0.000	0.260	1.643	11.140
48.20.87/120.06.43	0.000	0.015	0.050	0.029	56.085	0.000	0.000	0.000	0.261	1.923	12.257
48.20.87/120.06.43	0.090	0.015	0.000	0.017	20.943	0.009	0.000	0.018	0.062	1.386	3.127
48.20.87/120.06.43	0.090	0.015	0.000	0.017	21.033	0.000	0.000	0.015	0.044	1.220	3.168
48.20.87/120.06.43	0.090	0.015	0.000	0.017	21.181	0.000	0.000	0.015	0.038	1.406	3.175
48.20.87/120.06.43	0.469	0.015	0.000	0.028	16.358	0.000	0.000	0.018	0.440	0.680	2.407
48.20.87/120.06.43	8.448	0.015	0.050	0.171	1.056	0.000	0.000	0.232	14.602	3.061	1.964
48.20.87/120.06.43	0.090	0.015	0.000	0.021	0.516	0.009	0.000	0.032	0.240	0.400	0.278
48.20.87/120.06.43	6.436	0.015	0.000	0.177	1.114	0.000	0.000	0.192	9.009	0.400	1.322
48.20.87/120.06.43	0.000	0.015	0.000	0.016	24.221	0.000	0.000	0.000	0.009	0.400	4.188
48.20.87/120.06.43	0.090	0.069	0.000	0.014	22.348	0.000	0.010	0.014	0.009	0.400	3.776
48.20.87/120.06.43	0.000	0.000	0.000	0.014	23.601	0.000	0.000	0.000	0.000	0.400	4.015
48.20.87/120.06.43	0.000	0.000	0.000	0.014	23.636	0.000	0.000	0.000	0.000	0.795	4.031
48.20.87/120.06.43	0.090	0.057	0.000	0.020	29.972	0.000	0.010	0.004	0.041	0.400	4.936
48.20.87/120.06.43	0.090	0.015	0.000	0.002	16.077	0.000	0.000	0.004	0.138	0.672	2.319
48.20.87/120.06.43	0.000	0.054	0.000	0.018	23.339	0.000	0.000	0.015	0.000	0.400	3.856

Appendix 16 (continued)

QUANT LIM ->	0.1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	1.3	0.1
DET LIM ->	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
SAMPLE SET	Al	As	B	Ba	Ca	Cd	Cr	Cu	Fe	K	Mg
48.20.87/120.06.43	0.046	0.000	2.779	0.000	0.338	0.030	3.719	0.000	0.000	2.837	0.000
48.20.87/120.06.43	0.020	0.000	2.089	0.000	0.422	0.030	4.553	0.000	0.000	3.938	0.000
48.20.87/120.06.43	0.037	0.000	2.082	0.000	0.040	0.000	3.095	0.000	0.000	3.038	0.000
48.20.87/120.06.43	0.320	0.007	6.316	0.003	0.370	0.000	5.883	0.030	0.000	7.836	0.000
48.20.87/120.06.43	0.396	0.000	6.168	0.003	0.406	0.000	6.837	0.030	0.002	7.527	0.000
48.20.87/120.06.43	0.365	0.000	6.222	0.000	0.242	0.000	7.445	0.030	0.000	8.312	0.000
48.20.87/120.06.43	0.300	0.000	7.872	0.000	1.612	0.000	5.946	0.030	0.000	11.118	0.000
48.20.87/120.06.43	0.362	0.000	5.715	0.003	0.263	0.000	5.588	0.030	0.000	8.099	0.000
48.20.87/120.06.43	0.204	0.000	6.087	0.000	0.146	0.000	6.428	0.030	0.000	8.694	0.000
48.20.87/120.06.43	0.373	0.000	7.498	0.000	0.203	0.000	17.043	0.000	0.000	8.460	0.000
48.20.87/120.06.43	0.014	0.000	3.019	0.003	0.040	0.000	1.591	0.000	0.000	4.598	0.000
48.20.87/120.06.43	0.001	0.000	3.049	0.003	0.137	0.000	1.648	0.000	0.000	4.626	0.000
48.20.87/120.06.43	0.001	0.000	3.044	0.003	0.157	0.000	1.654	0.000	0.000	4.681	0.000
48.20.87/120.06.43	0.027	0.000	2.171	0.000	0.170	0.030	1.093	0.000	0.000	5.467	0.000
48.20.87/120.06.43	0.070	0.000	1.147	0.003	1.250	0.139	0.520	0.000	0.122	8.413	0.000
48.20.87/120.06.43	0.001	0.000	0.000	0.003	0.000	0.134	0.186	0.000	0.002	0.356	0.000
48.20.87/120.06.43	0.047	0.000	0.000	0.000	0.000	0.119	0.353	0.000	0.002	13.508	0.000
48.20.87/120.06.43	0.001	0.007	3.547	0.000	0.226	0.000	2.379	0.000	0.000	7.181	0.000
48.20.87/120.06.43	0.000	0.000	3.373	0.000	0.225	0.000	2.298	0.000	0.000	4.868	0.000
48.20.87/120.06.43	0.000	0.000	3.452	0.000	0.040	0.000	2.229	0.000	0.000	7.576	0.000
48.20.87/120.06.43	0.000	0.000	3.494	0.000	0.000	0.000	2.173	0.000	0.000	6.934	0.000
48.20.87/120.06.43	0.019	0.000	3.748	0.000	0.188	0.000	4.758	0.000	0.000	5.372	0.000
48.20.87/120.06.43	0.001	0.000	2.206	0.000	0.040	0.030	1.106	0.000	0.000	5.117	0.000
48.20.87/120.06.43	0.000	0.000	3.194	0.003	0.000	0.114	1.833	0.000	0.000	5.740	0.000

Appendix 16 (continued)

QUANT LIM ->	0.1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	1.3	0.1
DET LIM ->	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
SAMPLE SET	Al	As	B	Ba	Ca	Cd	Cr		Cu	Fe	K	Mg
48.18.26/120.03/99	0.000	0.060	0.050	0.036	69.477	0.000	0.010	0.000	0.063	1.209	20.289
48.18.26/120.03/99	0.000	0.000	0.000	0.002	7.435	0.000	0.000	0.000	0.000	0.982	1.634
48.18.26/120.03/99	0.000	0.015	0.050	0.002	17.520	0.000	0.000	0.000	0.000	0.400	8.402
48.18.26/120.03/99	0.000	0.000	0.050	0.008	16.303	0.000	0.010	0.000	0.000	0.826	6.050
48.18.26/120.03/99	0.000	0.015	0.050	0.007	20.470	0.000	0.010	0.000	0.000	0.400	11.186
48.18.26/120.03/99	0.000	0.000	0.050	0.008	17.887	0.000	0.010	0.000	0.000	0.851	6.637
48.18.26/120.03/99	0.000	0.015	0.050	0.013	30.799	0.000	0.010	0.000	0.000	0.986	11.551
48.18.26/120.03/99	0.000	0.015	0.000	0.002	16.174	0.000	0.000	0.000	0.000	0.681	5.913
48.18.26/120.03/99	0.000	0.000	0.000	0.002	6.321	0.000	0.000	0.000	0.000	0.400	0.963
48.18.26/120.03/99	0.090	0.077	0.000	0.008	29.701	0.000	0.010	0.004	0.000	0.886	6.206
48.18.26/120.03/99	0.000	0.000	0.000	0.007	16.179	0.000	0.000	0.015	0.000	0.400	2.815
48.18.26/120.03/99	0.000	0.122	0.168	0.029	86.174	0.000	0.010	0.004	0.033	1.765	26.675
48.18.26/120.03/99	0.000	0.089	0.050	0.028	86.484	0.000	0.010	0.004	0.009	1.650	26.823
48.18.26/120.03/99	0.000	0.061	0.050	0.028	86.738	0.000	0.010	0.004	0.009	1.963	26.413
48.18.26/120.03/99	0.000	0.062	0.050	0.011	31.873	0.000	0.010	0.004	0.000	0.400	10.571
48.18.26/120.03/99	ND	TR	TR	TR	37.7	ND	ND	ND	ND	3.3	24.2
48.18.26/120.03/99	0.0	0.1	0.1	0.0	82.4	0.0	0.0	0.0	0.0	1.4	25.2
48.18.26/120.03/99	0.090	0.114	0.000	0.021	86.507	0.000	0.000	0.016	0.053	1.601	25.111
48.18.26/120.03/99	0.090	0.164	0.050	0.030	90.503	0.009	0.000	0.031	0.077	2.600	25.645
48.18.26/120.03/99	0.090	0.136	0.050	0.029	88.268	0.009	0.000	0.030	0.044	2.552	25.251
48.18.26/120.03/99	0.090	0.128	0.050	0.031	89.227	0.009	0.000	0.027	0.082	2.052	25.728
48.18.26/120.03/99	0.090	0.118	0.050	0.037	93.502	0.009	0.000	0.024	0.009	1.579	26.481
48.18.26/120.03/99	0.090	0.015	0.050	0.041	60.831	0.009	0.000	0.021	0.101	1.665	18.002
48.18.26/120.03/99	0.090	0.015	0.050	0.045	60.470	0.000	0.000	0.018	0.148	1.698	17.940
48.18.26/120.03/99	0.090	0.054	0.050	0.035	60.181	0.000	0.000	0.017	0.088	1.975	17.849
48.18.26/120.03/99	0.090	0.059	0.050	0.034	60.251	0.000	0.000	0.017	0.074	1.669	17.889
48.18.26/120.03/99	0.090	0.136	0.050	0.029	88.268	0.009	0.000	0.030	0.044	2.552	25.251
48.18.26/120.03/99	0.090	0.128	0.050	0.031	89.227	0.009	0.000	0.027	0.082	2.052	25.728
48.18.26/120.03/99	0.000	0.055	0.000	0.035	23.561	0.009	0.000	0.016	0.000	0.862	3.891

Appendix 16 (continued)

QUANT LIM ->	0.1	0.0	0.2	0.0	0.1	0.0	0.0	0.0	0.0	1.3	0.1
DET LIM ->	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
SAMPLE SET	Al	As	B	Ba	Ca	Cd	Cr	Cu	Fe	K	Mg
48.18.26/120.03/99	0.016	0.000	10.090	0.003	0.273	0.000	24.368	0.030	0.000	8.201	0.000
48.18.26/120.03/99	0.000	0.000	0.904	0.000	0.192	0.030	2.025	0.000	0.000	1.694	0.000
48.18.26/120.03/99	0.000	0.000	4.140	0.000	0.353	0.000	11.390	0.000	0.000	3.992	0.000
48.18.26/120.03/99	0.001	0.000	3.203	0.000	0.395	0.030	8.780	0.000	0.000	2.561	0.000
48.18.26/120.03/99	0.000	0.000	5.811	0.003	0.520	0.000	15.528	0.000	0.000	4.985	0.000
48.18.26/120.03/99	0.001	0.000	3.430	0.003	0.493	0.000	9.745	0.000	0.000	5.090	0.000
48.18.26/120.03/99	0.000	0.000	5.602	0.003	0.631	0.030	16.230	0.000	0.000	4.991	0.000
48.18.26/120.03/99	0.001	0.000	2.895	0.000	0.155	0.000	7.794	0.000	0.000	2.285	0.000
48.18.26/120.03/99	0.000	0.000	0.948	0.000	0.040	0.000	0.605	0.000	0.000	1.728	0.000
48.18.26/120.03/99	0.000	0.000	3.133	0.003	0.170	0.000	9.371	0.000	0.029	3.449	0.000
48.18.26/120.03/99	0.000	0.000	1.398	0.000	0.000	0.000	6.152	0.000	0.098	1.845	0.000
48.18.26/120.03/99	0.001	0.007	12.761	0.003	0.547	0.000	34.715	0.030	0.000	10.869	0.000
48.18.26/120.03/99	0.001	0.007	12.333	0.003	0.405	0.000	33.693	0.030	0.000	11.026	0.000
48.18.26/120.03/99	0.000	0.000	12.412	0.003	0.198	0.030	32.627	0.030	0.000	12.935	0.000
48.18.26/120.03/99	0.000	0.000	5.283	0.003	0.232	0.000	13.006	0.000	0.000	4.487	0.000
48.18.26/120.03/99	ND	ND	10.7	ND	0.6	ND	27.0	TR	ND	8.0	ND
48.18.26/120.03/99	0.0	0.0	12.0	0.0	0.4	0.2	27.6	0.0	0.0	9.8	0.000
48.18.26/120.03/99	0.001	0.000	12.274	0.003	0.543	0.094	27.382	0.030	0.000	9.715	0.000
48.18.26/120.03/99	0.001	0.007	11.906	0.045	1.206	0.236	29.647	0.030	0.000	9.837	0.002
48.18.26/120.03/99	0.001	0.007	11.459	0.044	0.620	0.221	29.539	0.030	0.000	9.515	0.000
48.18.26/120.03/99	0.016	0.007	12.079	0.040	0.259	0.195	28.216	0.030	0.000	9.571	0.000
48.18.26/120.03/99	0.001	0.000	12.116	0.003	0.183	0.169	29.434	0.030	0.000	10.423	0.000
48.18.26/120.03/99	0.128	0.000	8.243	0.003	0.277	0.000	9.522	0.030	0.000	9.218	0.000
48.18.26/120.03/99	0.127	0.000	8.270	0.003	0.249	0.000	9.525	0.030	0.000	9.207	0.000
48.18.26/120.03/99	0.126	0.000	8.157	0.003	0.281	0.000	9.475	0.030	0.000	9.132	0.000
48.18.26/120.03/99	0.126	0.000	8.207	0.003	0.264	0.000	9.470	0.030	0.000	9.153	0.000
48.18.26/120.03/99	0.001	0.007	11.459	0.044	0.620	0.221	29.539	0.030	0.000	9.515	0.000
48.18.26/120.03/99	0.016	0.007	12.079	0.040	0.259	0.195	28.216	0.030	0.000	9.571	0.000
48.18.26/120.03/99	0.001	0.000	3.367	0.003	0.000	0.120	1.810	0.000	0.000	5.441	0.000

Appendix 17: Private Groundwater Wells

		Atomic Fluoresence	ICP-AES:					
QUANT LIM ->			7	30	33	13	100	10	100
DET LIM ->		1	2	9	10	4	30	3	30
		µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
		As	Ba	Cd	Cr	Cu	Pb	Ni	Se
48.21.06.3/120.06.05.8	Oct-99	0	0	0	0	0	120	0	0
48.21.06.3/120.06.05.8	Feb-00	0	29	0	0	15	105	3	0
48.21.06.3/120.06.05.8	Mar-00	<1	14	82	0	128	1101	122	30
48.21.06.3/120.06.05.8	Apr-00	<1	0	0	0	0	30	0	0
48.21.06.3/120.06.05.8	Jun-00	<1	0	0	0	0	0	3	0
48.21.06.3/120.06.05.8	Aug-00	<1	0	0	0	0	0	0	0
48.21.06.3/120.06.05.8	Oct-00	<1	0	0	0	0	0	0	0
48.21.06.3/120.06.05.8	Dec-00	<1	29	0	10	4	0	3	0
48.21.06.3/120.06.05.8	Apr-00	<1	28	0	0	4	30	0	0
48.21.06.3/120.06.05.8	Feb-01	<1	25	0	0	0	0	0	0
48.21.06.3/120.06.05.8	Mar-01	<1	29	0	9	18	30	3	0
48.21.06.3/120.06.05.8	Apr-01	<1	30	0	10	4	0	0	0
48.21.06.3/120.06.05.8	May-01	1	2	0	0	0	0	0	0
48.21.06.3/120.06.05.8	Jun-01	3	21	0	0	4	0	0	0
48.21.17.3/120.07.40.8	Feb-00	338	12	9	0	19	169	43	242
48.21.17.3/120.07.40.8	Mar-00	309	11	9	0	26	224	53	283
48.21.17.3/120.07.40.8	Apr-00	384	0	9	0	0	151	0	253
48.21.17.3/120.07.40.8	Jun-00	238	0	9	0	0	0	0	247
48.21.17.3/120.07.40.8	Aug-00	342	0	0	0	0	0	0	0
48.21.17.3/120.07.40.8	Oct-00	160	0	0	10	4	0	3	256
48.21.17.3/120.07.40.8	Dec-00	289	11	9	38	16	0	3	250
48.21.17.3/120.07.40.8	Jan-01	335	7	0	10	4	30	3	30
48.21.17.3/120.07.40.8	Feb-01	395	13	0	10	0	30	0	236
48.21.17.3/120.07.40.8	Mar-01	364	10	9	39	16	0	41	30
48.21.17.3/120.07.40.8	Apr-01	323	10	0	10	0	0	3	30
48.21.17.3/120.07.40.8	May-01	352	2	0	10	0	30	3	0
48.21.17.3/120.07.40.8	Jun-01	234	20	0	10	4	0	3	30

Appendix 17 (continued)

QUANT LIM ->			7	30	33	13	100	10	100
DET LIM ->		1	2	9	10	4	30	3	30
		µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
		As	Ba	Cd	Cr	Cu	Pb	Ni	Se
48.20.54.3/120.06.58.6	Mar-00	12	27	9	10	48	360	91	590
48.20.54.3/120.06.58.6	Apr-00	68	0	9	0	0	208	75	498
48.20.54.3/120.06.58.6	Jun-00	12	0	0	10	0	30	81	585
48.20.54.3/120.06.58.6	Aug-00	16	30	0	0	38	0	82	532
48.20.54.3/120.06.58.6	Oct-00	16	0	0	71	0	30	66	454
48.20.54.3/120.06.58.6	Dec-00	15	28	0	84	31	30	74	500
48.20.54.3/120.06.58.6	Jan-01	15	59	0	86	31	30	74	448
48.20.54.3/120.06.58.6	Feb-01	13	24	0	68	19	60	3	469
48.20.54.3/120.06.58.6	Mar-01	14	25	9	87	34	30	71	460
48.20.54.3/120.06.58.6	Apr-01	12	24	0	74	23	30	70	482
48.20.54.3/120.06.58.6	May-01	14	12	0	41	0	0	0	0
48.20.54.3/120.06.58.6	Jun-01	15	17	0	60	20	30	60	48
48.20.27.5/120.07.22.2	Feb-00	6	21	9	0	25	164	3	30
48.20.27.5/120.07.22.2	Mar-00	19	23	9	0	38	277	43	30
48.20.27.5/120.07.22.2	Apr-00	13	9	0	0	0	125	3	30
48.20.27.5/120.07.22.2	Jun-00	7	0	0	0	0	0	3	30
48.20.27.5/120.07.22.2	Aug-00	7	0	0	0	0	0	0	0
48.20.27.5/120.07.22.2	Oct-00	5	18	0	10	4	0	3	30
48.20.27.5/120.07.22.2	Dec-00	7	19	0	35	17	0	3	30
48.20.27.5/120.07.22.2	Jan-01	5	107	9	10	20	30	3	30
48.20.27.5/120.07.22.2	Feb-01	7	19	0	10	4	9	9	30
48.20.27.5/120.07.22.2	Mar-01	8	18	0	42	21	0	3	30
48.20.27.5/120.07.22.2	Apr-01	8	22	0	10	4	0	3	30
48.20.27.5/120.07.22.2	May-01	8	8	0	10	4	0	0	0
48.20.27.5/120.07.22.2	Jun-01	8	16	0	10	4	30	3	30
48.21.17.1/120.07.10.4	Feb-00	10	8	0	0	18	105	0	0
48.21.17.1/120.07.10.4	Mar-00	12	2	9	0	31	233	3	0
48.21.17.1/120.07.10.4	Apr-00	15	0	0	0	0	30	3	0
48.21.17.1/120.07.10.4	Jun-00	6	0	0	0	0	0	3	0
48.21.17.1/120.07.10.4	Aug-00	11	0	0	0	0	0	0	0

Appendix 17 (continued)

SAMPLE SET		As	µg/L Ba	µg/L Cd	µg/L Cr	µg/L Cu	µg/L Pb	µg/L Ni	µg/L Se
48.21.17.1/120.07.10.4	Oct-00	12	0	0	0	0	0	0	0
48.21.17.1/120.07.10.4	Dec-00	13	8	0	10	4	0	3	0
48.21.17.1/120.07.10.4	Jan-01	12	2	0	0	4	0	0	0
48.21.17.1/120.07.10.4	Feb-01	11	2	9	0	4	0	0	0
48.21.17.1/120.07.10.4	Mar-01	13	2	0	10	4	0	0	0
48.21.17.1/120.07.10.4	Apr-01	14	2	0	0	0	0	0	0
48.21.17.1/120.07.10.4	May-01	14	2	0	0	0	0	0	0
48.21.17.1/120.07.10.4	Jun-01	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry
48.21.03.4/120.06.58.7	Feb-00	40	21	0	0	20	30	3	30
48.21.03.4/120.06.58.7	Mar-00	5	13	0	0	26	147	3	30
48.21.03.4/120.06.58.7	Apr-00	10	2	0	0	0	30	3	30
48.21.03.4/120.06.58.7	Jun-00	5	2	0	0	4	0	0	0
48.21.03.4/120.06.58.7	Aug-00	6	0	0	0	0	0	0	0
48.21.03.4/120.06.58.7	Oct-00	11	0	0	10	0	0	0	30
48.21.03.4/120.06.58.7	Dec-00								
48.21.03.4/120.06.58.7	Jan-01								
48.21.03.4/120.06.58.7	Feb-01	13	27	0	10	4	0	0	30
48.21.03.4/120.06.58.7	Mar-01	6	11	0	43	23	30	3	0
48.21.03.4/120.06.58.7	Apr-01	6	18	0	10	4	0	3	30
48.21.03.4/120.06.58.7	May-01	9	0	0	0	0	0	0	0
48.21.03.4/120.06.58.7	Jun-01	5	0	0	0	0	30	0	0
48.21.17.3/120.07.40.8	Jan-01	67	10	9	37	468	30	3	30
48.21.17.3/120.07.40.8	Feb-01	235	9	0	10	55	30	3	30
48.21.17.3/120.07.40.8	Mar-01	144	18	9	50	28	30	3	30
48.21.17.3/120.07.40.8	Apr-01	105	11	0	36	4	30	3	30
48.21.17.3/120.07.40.8	May-01	170	2	0	10	0	246	0	0
48.21.17.3/120.07.40.8	Jun-01	182	13	0	10	15	0	3	0

Appendix 17 (continued)

48.21.32.3/120.07.50.5	Mar-01	12	16	0	53	27	0	3	30
48.21.32.3/120.07.50.5	Apr-01	11	16	0	39	4	0	3	30
48.21.32.3/120.07.50.5	May-01	12	12	0	0	0	0	0	0
48.21.32.3/120.07.50.5	Jun-01	9	8	0	10	17	0	3	30
48.20.32.4/120.07.36.8	Apr-01	10	42	0	10	4	0	3	30
48.20.32.4/120.07.36.8	May-01	12	19	0	10	0	0	0	0
48.20.32.4/120.07.36.8	Jun-01	10	26	0	10	10	30	3	30
48.20.36.6/120.07.51.2	May-01	10	8	0	10	0	0	0	0
48.20.36.6/120.07.51.2	Jun-01	Dry	Dry	Dry	Dry	Dry	Dry	Dry	Dry
48.22.02.9/120.10.26.9	Apr-00		0	10	10	20	0	0	30
48.22.02.9/120.10.26.9	Jun-00		23	9	0	52	170	3	30
48.22.02.9/120.10.26.9	Aug-00	<1	0	0	0	0	100	3	30
48.22.02.9/120.10.26.9	Oct-00	<1	0	0	0	4	0	3	30
48.22.02.9/120.10.26.9	Dec-00	<1	0	0	0	0	0	3	30
48.22.02.9/120.10.26.9	Jan-01	<1	0	0	10	10	0	0	0
48.22.02.9/120.10.26.9	Feb-01	<1	20	0	10	21	0	3	0
48.22.02.9/120.10.26.9	Mar-01	<1	20	0	10	79	0	0	0
48.22.02.9/120.10.26.9	Apr-01	<1	19	0	10	53	0	0	30
48.22.02.9/120.10.26.9	May-01	<1	20	0	10	77	0	3	0
48.21.13.3/120.06.21.6	Apr-00								
48.21.13.3/120.06.21.6	Jun-00	<1	30	0	0	4	30	3	0
48.21.13.3/120.06.21.6	Aug-00								
48.21.13.3/120.06.21.6	Oct-00	<1	0	0	0	0	0	3	0
48.21.13.3/120.06.21.6	Dec-00	<1	0	0	10	60	0	3	0
48.21.13.3/120.06.21.6	Jan-01	<1	16	0	10	155	0	0	0
48.21.13.3/120.06.21.6	Feb-01	<1	18	0	0	243	30	0	0
48.21.13.3/120.06.21.6	Mar-01	<1	17	0	0	92	0	0	0
48.21.13.3/120.06.21.6	Apr-01	<1	0	2140	2110	2030	2000	2090	2060
48.21.13.3/120.06.21.6	May-01	<1	8	0	10	4	0	0	0
48.21.13.3/120.06.21.6	Jun-01	<1	11	0	0	0	0		0

Appendix 18

QUALITY ASSURANCE AND QUALITY CONTROL

QUALITY ASSURANCE

Selection of study sites depended on the goals and objectives of the study. Access, location of contaminant sources, mixing zones, and the dilution of pollutants were considered. Reference sites were as similar as possible to the study site. In order to compare macroinvertebrate data from different sites, sites were identified that were as similar as possible in terms of gradient, depth, substrate size and heterogeneity, and canopy cover. Sampling bedrock or large-boulder dominated riffles is avoided whenever possible. Water and sediment samples were collected from rifles in the same general vicinity as the benthic macroinvertebrate samples. Samples were collected for water-quality analyses in the order of the parameters' decreasing sensitivity. At least three field replicates were collected at each sample site.

QUALITY CONTROL

In regards to biological samples, all macroinvertebrate samples were replicated in the field. Voucher specimens of all taxa identified were retained. In regards to chemical samples, laboratory standards were run with test samples and compared to the certified concentration. In the field, standard samples were handled following the procedure for the collection of field samples and submitted with the test samples to the laboratory for analysis. The measured concentration standard samples must fall within 10% of the theoretical concentration.

Laboratory and field blanks were submitted with the test samples to the laboratory for analysis. Measured concentrations of blanksshould be less than detection limits.

#61: Fe and Mn were detected in field blank. Were both were < 1% over field spike. Lab blank was 0 and lab spike was <1% below theoretical value therefore deviation must be due to handling. All other results were within specifications. Fe and Mn results from these samples were not used. These deviations had no impact on the test results.

#53: Cu and Zn were detected in field blanks. Both were <3% over field spike. Lab blanks were 0 for both. Lab spikes were <3% below theoretical value. Copper result was used in calculating average concentrations in well water but they were less than drinking water standards. Also used in calculating average concentration in Methow R. surface water but were less than background. Deviations had no impact on test results.

#38: Cu was detected in field blank. Cu was 5% below field spike. Lab blank was 0 and no results were reported for the lab spike. Copper result was used in calculating average concentrations in well water but they were less than drinking water standards. Also used in calculating average concentration in Methow R. surface water but were less than background.

#29: Cd was 2.242 mg L-1 and exceded field spike by more than 10% (i.e., 12%). Field blank and laboratory blanks were 0. Cd results were used in calculating average concentrations in well water but they were less than drinking water standards. Also used in calculating average concentration in Methow R. surface water but were less than background.

#20 and 21: Cd, Pb, and Zn exceded field spike by more than 10% (i.e., 11-15%). Cu and Pb was also detected in the field blank for sample group #20. Lab blanks were 0 and lab spikes were <1% below theoretical value. Cd, Pb, and Zn results were used in calculating average concentrations in well water but they were less than drinking water standards. Also used in calculating average concentrations in Methow R. surface water. Although 15% deviation could have affected individual results the effect on average and the deviation did not justify rejection of the results.

Appendix 19

POTENTIALLY CONFOUNDING EFFECTS OF MERCURY

Methow River sediments downstream from the abandoned Alder Mine, Red Shirt Mill and Alder Mill south of Twisp in Okanogan County, Washington, were associated with toxicity in caddisfly larvae and trout at different levels of biological organization. Eleven chemicals of potential ecological concern (COPEC) were identified based on the comparison of the mean concentrations of the elements to toxicological benchmarks. Trace elements in tailings, AMD and ARD, which are the sources of mine waste contamination, and in Methow River sediment, which is a sink for trace elements, were compared to benchmarks for toxic effects of trace elements on plants, soil heterotrophic processes, wildlife, human health and aquatic biota. This comparison identified 11 COPEC (Al, As, B, Ba, Cd, Cr, Cu, Mn, Pb, Se, and Zn), however, little attention was paid to Hg.

While cinnabar, the ore of mercury, is not known to occur in Okanogan County (Cannon 1975), runoff from abandoned placer gold mines has been identified as one source of mercury to Lake Roosevelt only km to the southeast. Miners used mercury to recover gold at both placer (alluvial) and hardrock (lode) mines. Although mercury is no longer being used in the recovery of gold it is possible that contamination from past activities still exists. There is anecdotal evidence that suggests there is contamination of the Josephine Creek and the upper Methow River as a result of the past use of mercury by gold miners and processors in the Barron area (Peplow 2000). Because of this, confounding by toxicity due to Hg contamination is a concern.

Mercury is a metal that is in liquid form at ambient temperatures and solid at -36°C. In addition to the metallic form, mercury occurs in compounds as monovalent mercurous mercury

(Hg^{+}) and divalent mercuric mercury (Hg^{2+}). Mercury also occurs in nature as organic mercury that is covalently bound to carbon to form methylmercury (CH_3Hg). From the toxicological point of view, mercury compounds are divided into inorganic and organic compounds (Berlin 1986). The carbon-mercury bond is chemically stable due to the low affinity of mercury for oxygen. Mercury also has an affinity for sulfur (-S) and sulfhydryl (-SH) groups. Because sulfhydryl groups are ubiquitous in organisms, when mercury binds to the sulfhydryl groups of proteins in membranes and enzymes they can interfere with membrane structure and function and with enzyme activity.

Organic mercury, formed in the bottom sediments in freshwater systems, is enriched in the aquatic food chain with the highest levels occurring in the predatory fishes. From the aquatic environment, methyl-mercury accumulates in species that consume aquatic organisms. Enrichment has not been observed to the same extent in the terrestrial food-chain (Berlin 1986). Methylmercury is readily absorbed from the gastrointestinal tract and from the lungs and stored in the kidneys and as opposed to other heavy metals, mercury is not concentrated in the liver (Mathews 1984). Symptoms of Hg toxicity, on the other hand, are primarily associated with CNS disorders and renal failure (Donaldson 1984, Mailman 1984).

In contrast, the presence of spherical electron-dense granules occurred in the mitochondria of live caddisfly larvae and trout exposed to trace metals in stream water, sediments, and periphyton are present at high concentrations in the environment surrounding the organism, its cells and the mitochondria of hepatocytes and small intestine cells from caddisfly larvae and trout from the Methow River. The coincidental occurrence of apoptosis and evidence in the liver of glycogen storage disease suggests these metals are the cause of metabolic disorders similar to Wilson's disease, a metabolic disorder of Cu metabolism, that affects energy conversion in hepatocytes. It also suggests that the mechanistic linkages that integrate responses across levels

of organization is related to the disruption of energy exchange in cells, individual organisms, populations, communities and the ecosystem. While there is a potential that Hg contamination and toxicity occurred in the organisms in this study, it is unlikely that the results were confounded by Hg contamination due to the specificity of X-ray analyses, which suggested that bioavailable forms of Ca, Cu, Fe, Pb and Ti for the effects observed at the individual, population, community and ecosystem levels of biological organization.

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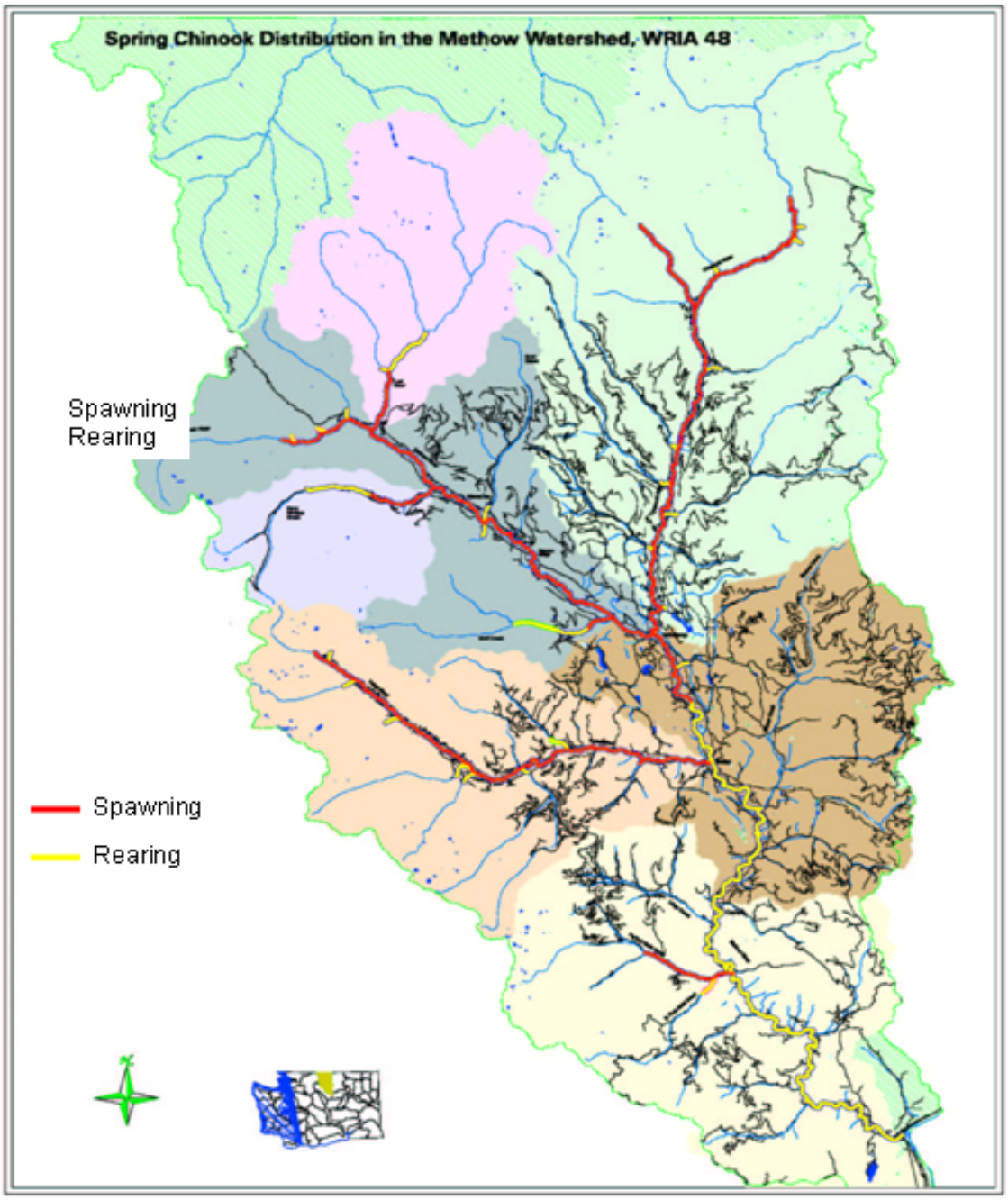
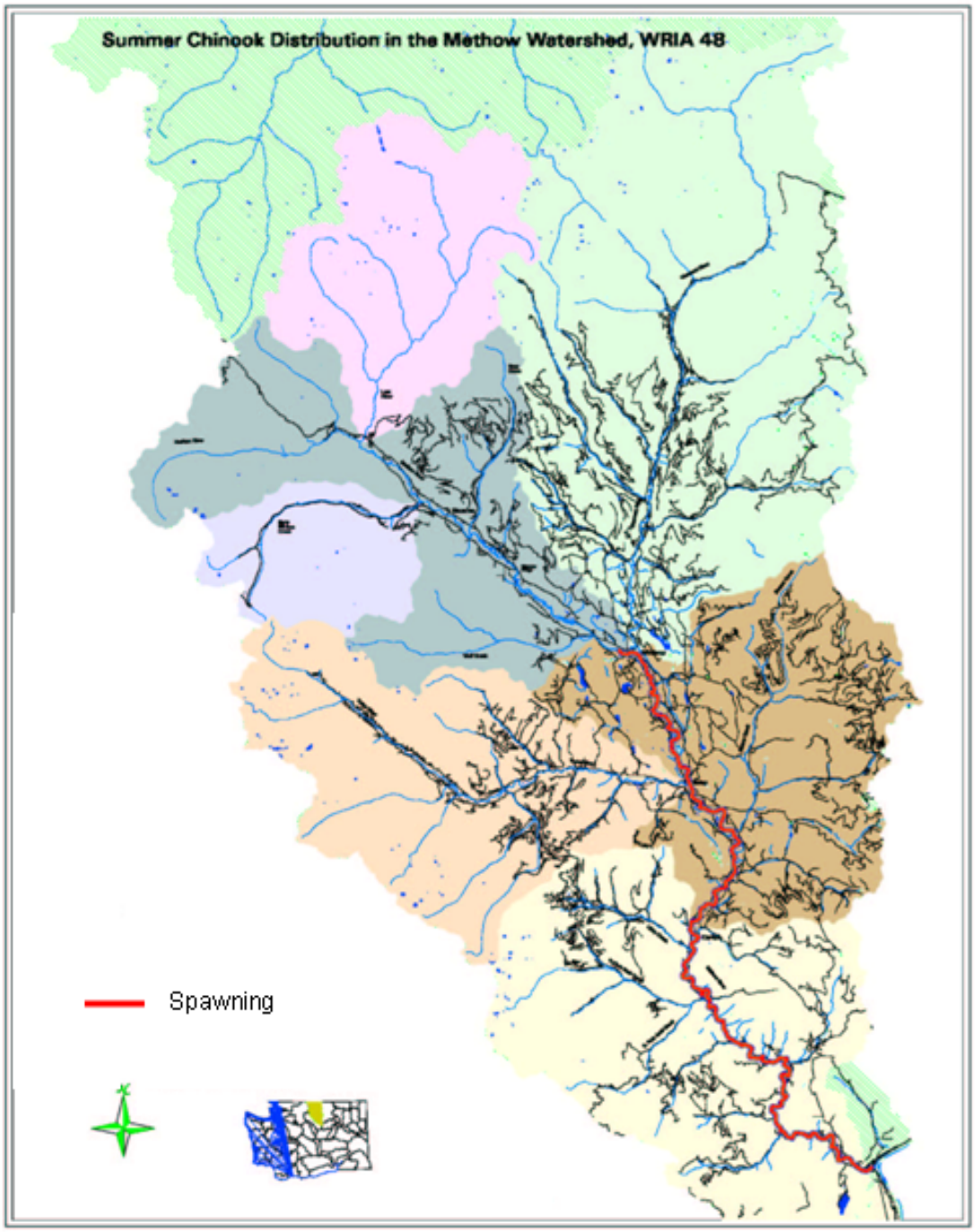
Appendix 20

Map of Spring Chinook Salmon Distribution in the Methow Watershed, WRIA 48 (WSCC 2000).



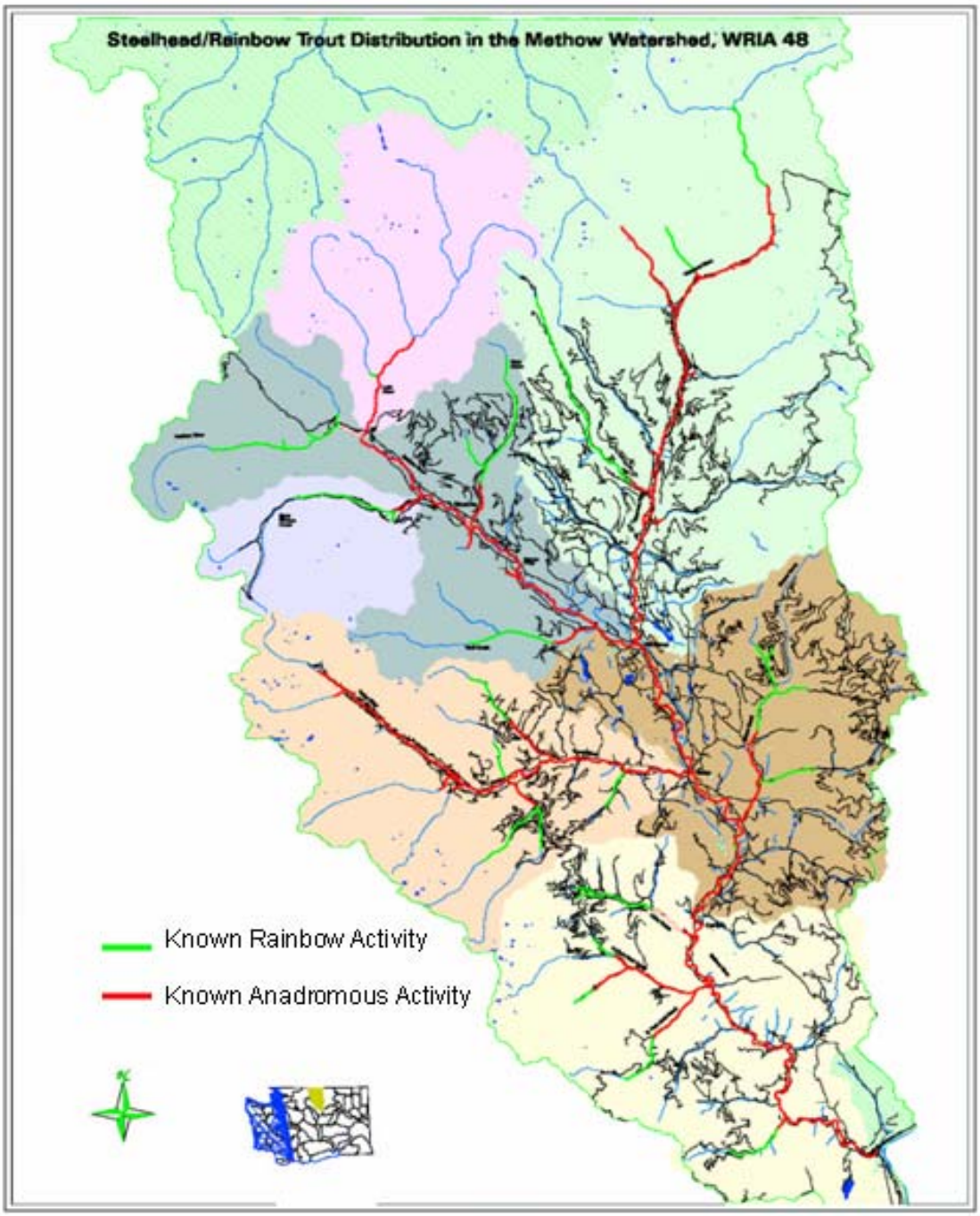
Appendix 21

Map showing summer Chinook salmon distribution in Methow Watershed, WRIA 48 (WSSC 2000).



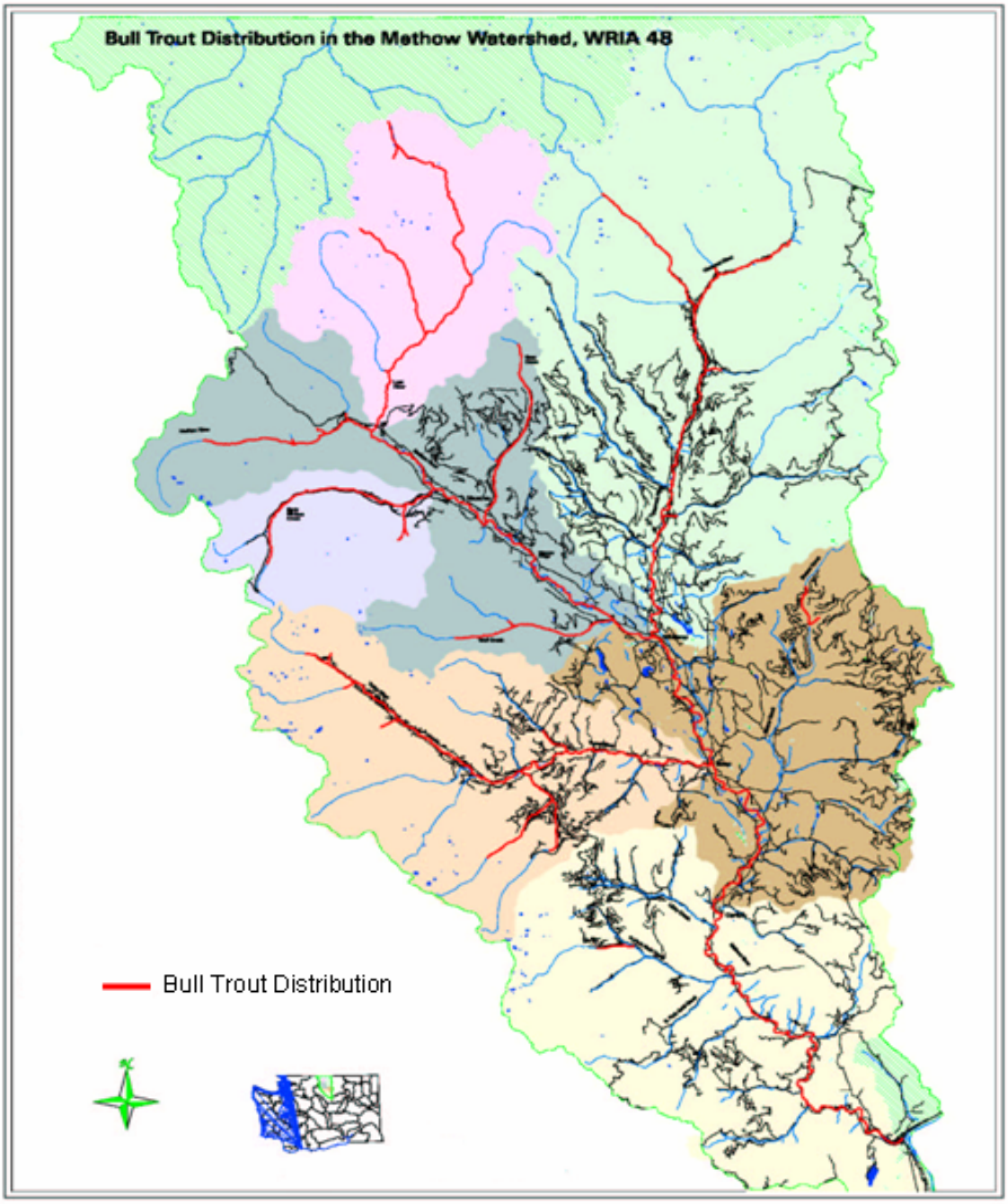
Appendix 22

Map of Steelhead/Rainbow Trout Distribution in the Methow Watershed, WRIA 48 (WSCC 2000).



Appendix 23

Map of Bull Trout Distribution in the Methow Watershed, WRIA 48 (WSCC 2000).



303(d) Data Submittal Cover Sheet

WASHINGTON STATE DEPARTMENT OF ECOLOGY

When you submit your water quality data, please answer the questions below. Completing this form is optional, but would be very helpful to Ecology in assessing your data. If you do not complete this form, please make sure that any cover letter or report you submit with the data provides this same information or that this information can be found in the materials you submit.

*(Note that submission of the **Data Quality Assurance Documentation Form** or equivalent documentation is required, not optional.)*

About the person who is responsible for the data:

Name	Daniel Peplow
Title	Research Assistant
Organization	University of Washington
Address	Box 352100, Seattle, Washington
Phone	(206) 685-8658
Fax	(206) 543-3254
E-mail	dpeplow@u.washington.edu

About the data collection project:

Project and site name (assigned by the collecting party) Dispersion of metals from abandoned mines and their effects on biota in the Methow River, Okanogan County, Washington

Organizations participating in the project

College of Forest Resources, University of Washington, Seattle, WA

About the waterbody that was sampled:

Name of waterbody Methow Watershed

WRIA # 48

About the data:

Is it numeric or narrative data? Numeric (and narrative)

Parameter(s) measured Metals (dissolved and sediment) and biological response

Date(s) collected March 15, 2000 through April 14, 2003

If the data were collected on numerous dates, indicate just the months and years here, and give the precise dates with the data itself.

Frequency of measurement (for example: one-time only, daily, or monthly)

One-time, Daily, Monthly, Annually

Location(s) of data collection Twisp, Okanogan County, Washington

Please provide as much information as you know about the location of where the data was collected, such as the township/range/section, latitude/longitude, river mile, and/or location relative to mapped landmarks, such as bridges and river confluences. If the data were collected in numerous locations, indicate just the general location here, and give the precise locations of each sampling station with the data itself.

About the laboratory performing the analysis, if any:

Name of laboratory College of Forest Resources

Is this laboratory state accredited? Yes

Name of laboratory contact person Dongsen Xue

Phone (206) 543-4691

E-mail dx@u.washington.edu

About the results of the sampling:

What do you consider to be the notable findings, if any, from the data? Contamination of Methow River sediments from abandoned mines near Twisp, Washington can be detected at all levels of biological organization from cellular to ecosystem.

Do you believe the data shows an exceedance of any state Water Quality Standards? If so, which ones?

Yes: WAC 173-201A (also WAC 173-200-010 and WAC 173-340)

Do you believe the data shows that the waterbody should be in a certain assessment category as described in the 303(d) policy? If so, which one?

Unknown

Other information:

Is there a TMDL approved for the measured parameters in this waterbody? If so, which one?

No

Is there a pollution control plan applicable to the measured parameters in this waterbody? If so, please submit a copy of the plan. Do you believe the plan would qualify this waterbody for Category 4, *Has a Pollution Control Plan*?

No

Other comments about the water quality in this waterbody?

With the exception of dissolved and sediment metals, Alder Creek and the Methow River appear to meet Class A (excellent) freshwater criteria.

303(d) Data Quality Assurance Documentation Form

Ecology can use only high quality data for the 303(d) assessment. The person submitting the data must document that a quality assurance plan was followed, and must provide Ecology with a copy of the plan if requested. You can use this form to document that the quality assurance requirements were met, or you can include this statement in a cover letter with the data.

To ask any questions about quality assurance issues or the 303(d) assessment process or for further assistance in submitting data, please contact:

Matthew Green
Department of Ecology
P. O. Box 47600
Olympia, WA 98504-7600
E-mail: 303d@ecy.wa.gov
Phone: (360) 407-6386

THIS DOCUMENTATION IS REQUIRED. Please read the statement below, sign it, and return it to Ecology with your data or to the address above, or please include this statement in a cover letter with the data.

I have read the 303(d) policy regarding data quality assurance (on the second page of this form), and understand that Ecology cannot use the data I am submitting unless quality assurance requirements were met.

The sampling and analysis of this data were conducted under a documented quality assurance project plan or other equivalent quality assurance procedures. If requested by Ecology, I will submit a copy of the quality assurance project plan or procedures for this data.

For a copy of the quality assurance project plan or procedures, I can be contacted at:

Name	Daniel Peplow
Title	Research Assistant
Organization	University of Washington
Address	Box 352100., Seattle., Washington
Phone	(206) 685-8658
Fax	(206) 543-3254
E-mail	dpeplow@u.washington.edu

D Peplow 6/9/03

Signature Date