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The effects of mine waste contamination at multiple levels of biological organization

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Abstract

This study shows that metal-contaminated sediments cause adverse biological effects at all levels of biological organization, from cellular to ecosystem-level responses, even where the corresponding surface water meets water-quality-based criteria. We studied the effects of contamination from the abandoned Alder Mine, Alder Mill, and Red Shirt Mill located near the town of Twisp on the eastern slopes of the north Cascade Mountains in Okanogan County, Washington (U.S.A.) on fish and wildlife habitat in the Methow River. Ore deposits in the area were mined for gold, silver, copper and zinc until the early 1950s. An up-gradient and down-gradient approach was used to compare impacted sites to control sites. Although the dissolved metal concentrations in the Methow River were below the limits of detection, eight elements were identified as contaminants of potential environmental concern (COPECs) in sediments. Results revealed contamination impacts at ecosystem, community, population, individual, cellular, subcellular, and molecular levels. Metal contaminants in forest soils around the mines were present at concentrations toxic to soil bacteria suggesting that functional properties related to nutrient cycling and energy flow have been effected. Exposed trout in the Methow River showed reduced growth compared to controls. Histopathological evidence is consistent with copper-induced metabolic disease. Glycogen bodies were present in trout hepatocyte cytosol and nuclei and the presence of glycogen inclusions was pathognomic of Type IV glycogen storage disease (GSD IV). This condition suggests food is being converted into glycogen and stored in the liver and that the glycogen is not being converted back normally into glucose for distribution to other tissues in the body, which is a likely cause for the poor growth and development observed in fish and macroinvertebrates. Glycogen storage disease is caused by either a deficiency or inactivation of the glycogen branching enzyme, which results in the synthesis of an abnormal glycogen molecule that is insoluble due to a decreased number of branch points and increased chain length. Further examination of hepatocytes by transmission electron microscopy also revealed the accumulation of electron-dense metal-granules in the mitochondrial matrix.
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1. Introduction

The effects of contamination from abandoned mine waste occur at all levels of biological organization and

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there are potential indicators at each level (Hodson, 1990; Clements, 2000; Clements and Kiffney, 1994). 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 knowledge of the mechanisms of toxicity is less complete at higher levels of organization (Hodson, 1990). Although indicators of toxicity such as morphological changes at the tissue level, ultrastructural changes at the cellular level, and biochemical changes at the molecular level reveal cause-and-effect relationships, impact on ecologically relevant processes are not easy to recognize.

When ecological studies focus on the comparison of groups rather than individuals, direct links between cause-and-effect are often tenuous (Clements, 2000) and susceptible to biases that pertain to the lack of individual data on exposure, outcomes, and confounding variables that contribute to the measured effect. These biases, referred to as the ecological fallacy by Selvin (1958), have been discussed by Morgenstern (1995) and Hopenhayn-Rich et al. (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). 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 indicators depends, therefore, on the examination of indicators at multiple levels of biological organization.

At the subcellular level, the specificity and usefulness of electron microscopy is evident based on the ability to diagnose toxicological and metabolic disorders even when effects at higher levels are not evident (Phillips et al., 1987). In white perch (*Morone americana*), hepatic copper storage (Wilson's) disease is characterized by the progressive accumulation of copper in hepatic lysosomes bound to cytoprotective metallothioneins (Bunton and Frazier, 1994). Saturation of the liver storage capacity results in the distribution of copper to extrahepatic tissues with multiple organ dysfunction. It was found that the diagnosis of Wilson's disease can be made based on the presence of glycogen nuclei, glycogen bodies, copper storage in lysosomes, and especially mitochondrial changes including changes in electron density (Phillips et al., 1987).

Electron microscopy was also used to observe the effect of divalent cations on in vitro cell cultures bathed in media containing calcium, strontium, lead, manganese, barium, and magnesium. Peachy (1964) and Walton (1973) showed that divalent cations accumulate as spherical electron-dense granules in the matrix of mitochondria. The lighter elements (e.g., calcium) produced less dense granules and the heavier elements (e.g., lead and barium) 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 et al., 1996). The effective concentration to cause a 50% decrease in the measured response (i.e., EC_{50}) 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.

This study, conducted along the middle reaches of the Methow River, extending from the confluence of the Chewuch River at Winthrop (River Kilometer 80.6 or River Mile 50.1) to the town of Carlton (Kilometer 43.3 26.8 or River Mile River), attempted to link responses to trace element contamination at multiple levels of organization. The results of this study will help describe the kinds of ecological changes needed to improve the survival and productivity of fish and wildlife populations.

Chinook salmon (*Oncorhynchus tshawytscha*), steelhead/rainbow trout (*Salmo gairdneri*) and bull trout (*Salvelinus confluentus*) migration, spawning and rearing occur in this portion of the Methow River watershed. A survey was conducted by direct underwater observation (snorkeling) on 4 September 1998 to identify salmonids in Alder Creek (Peplow, 1998). The species identified were native steelhead/rainbow trout

(*S. gairdneri*) and Chinook salmon (*O. tshawytscha*). Two redds (gravel nests of salmon eggs) in the Methow River at the Red Shirt Mill were identified on 10 and 23 October 2000. Two coho (*O. kisutch*), parr (i.e., life stage between fry and smolt stages, generally reached by the end of the first summer) were observed on 27 January 2001 in the last pond on Alder Creek after ice melt and before water levels and runoff were sufficiently high to provide surface flow and an outlet from the pond to the Methow River. Upper Columbia River summer steelhead (*O. mykiss*), including the Methow River run, were listed under the Endangered Species Act (ESA) as "endangered" on 18 August 1997. Upper Columbia River spring Chinook salmon (*O. tshawytscha*), including the Methow River run, were listed under the ESA as "Endangered" on 16 March 1999. Bull trout in the Methow River were listed under the ESA as "threatened" on 10 June 1998. Although not an ESA listed species, summer Chinook (*O. tshawytscha*) 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. While it is clear that tributaries to the Methow River have been impaired by heavy metals from abandoned mine waste, the impact of metals from abandoned mines on salmonid habitat in the Methow River has not been determined.

The study estimated the risk and measured the actual impacts on biological endpoints from trace element contamination in soil, surface water, groundwater, and sediments at various levels of biological organization. The objectives of this study were (1) at the ecosystem level, estimate the risk of metal contamination on soil nutrient cycling and energy flow by comparing contaminant levels to benchmarks for ecological risk assessment, (2) at the community level, estimate the effects of dissolved and sediment metal contamination on aquatic macroinvertebrate diversity and abundance, (3) at the population level, use the accumulation of arsenic in bear hair and copper in Aspen leaves and the concentration of copper in the bodies of aspen leaf miner larvae as indicators of exposure to potentially toxic levels of contaminants, (4) at the tissue level, use changes in stained sections of fish liver tissue to detect the occurrence of specific diseases, and (5) at the cellular level, use ultrastructural changes in hepatocytes as indicators of disease.

2. Methods

2.1. Site description

The study site is located near the town of Twisp in Okanogan County, Washington (Fig. 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 4662 km^2, the Methow River flows southward through western Okanogan County and empties into the Columbia River at River Kilometer 843 (River Mile 523.9) near the town of Pateros. The Methow watershed extends approximately 140 km (87 mile) from the confluence with the Columbia River to its headwaters located along the Cascade Crest and the Canadian border.

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. 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 ore deposits that were mined for gold, silver, copper and zinc 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 million years (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 contain high concentrations of bicarbonate and are thus hardwater in nature. 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 climate in the Methow Valley is dominated by Cascade Mountain rain shadow. Mean annual precip-

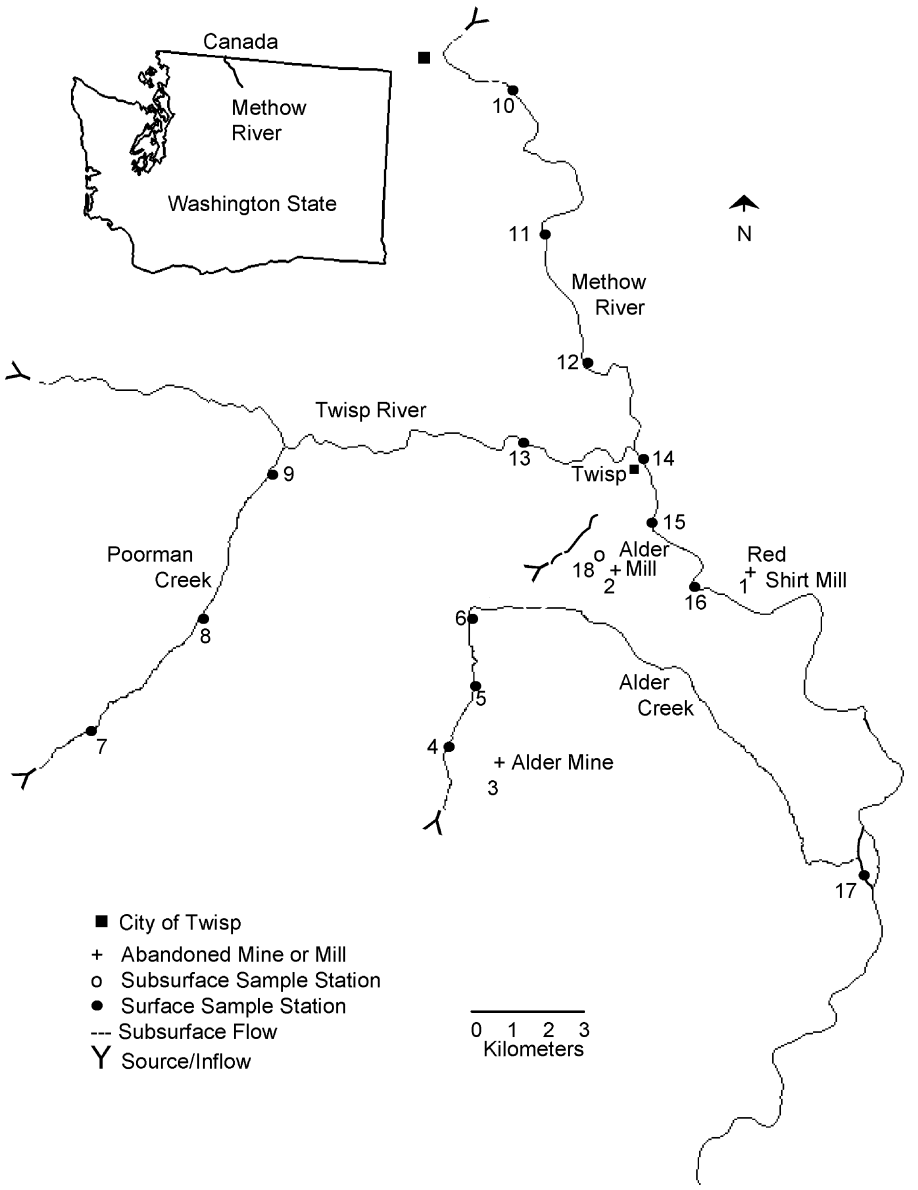

Fig. 1. Methow River, Twisp River and Alder Creek near the town of Twisp in Okanogan County Washington (U.S.A.) with sample stations identified. Contaminant sources are at the Alder Mine, Alder Mill, and Red Shirt Mill.

itation ranges from 25 to 38 cm and the mean annual temperature is approximately 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 snowmelt.

Since streamflow in the basin is primarily driven by runoff from melting snow, flows exhibit a strong peak during spring and early summer with roughly

60% of the mean annual discharge occurring during May and June (Milhous et al., 1976). Streamflow remains relatively high during July, but decreases substantially from August to October in response to a reduced snowpack, low precipitation, and decreased soil moisture. Streamflow in the Methow River reaches an annual low during late September and early October, with some sections going subsurface during dry years. Conversely, during wetter years, autumn precipitation may cause a slight increase in surface stream flows. Winter flows typically remain low in response to low autumn precipitation and freezing winter temperatures. Freezing temperatures retain moisture in the snow pack and freeze soil moisture. Brief warming trends in the winter months can melt snow or cause precipitation to fall as rain, slightly increasing stream flows. Runoff between years is highly variable and maximum and minimum flows for the Methow River at Twisp was 1155 $m^3 s^{-1}$ (May 1948) and 4 $m^3 s^{-1}$ (September 1926).

2.2. Mine and Mill site descriptions

The locations of Alder Mine, Alder Mill, and Red Shirt Mill are shown in Fig. 1. Alder Mine is located approximately 4.8 km southwest of Twisp (Fig. 1). The site consists of two adits (i.e., tunnels open at one end), an adit-discharge 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 to 80%. Estimates from aerial photographs indicate that waste rock covers over 15 ha. The flow rate of drainage from the north adit ranges from 0.14 to 0.42 $m^3 s^{-1}$ (5–15 L min^{-1}). South adit flow is seasonal and ranges from 0 to less than 0.14 $m^3 s^{-1}$ (5 L min^{-1}).

The Alder Mill is located approximately 1 km south of Twisp, Washington, and approximately 500 m west of the Methow River at River Kilometer 63 km (River Mile 39) from the Columbia River. The Mill consists of two buildings, two tailings impoundments, and scattered waste rock and ore piles. The tailings impoundments are estimated to cover approximately 4 ha of surface area. Inputs and springs supplied by Alder Creek feed the upper impoundment creating a contaminated wetlands environment. The phreatic surface in the upper tailings impoundment varies spatially and temporally between 0 and 4 m below the surface depending on time of year and snow melt.

The Red Shirt Mill is also located approximately 1 km south of Twisp at River Kilometer 63 km (River Mile 39) from the Columbia River but approximately 100 m east of the Methow River. The mill consists of a single building and a tailings pile. The tailings pile, which extends to the east bank of the Methow River, is estimated to cover 1 ha of surface area.

2.3. 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 (Fig. 2, numbers 1–10), and one reference well that was isolated from mine impacts (Fig. 2, number 11) was sampled between October 1999 and June 2001. Samples of water from private domestic wells were collected from well casings using disposable Teflon bailers and stored in pre-cleaned 50-mL polypropylene centrifuge tubes.

All other surface water and groundwater samples were collected in pre-cleaned Teflon bottles. Sub-

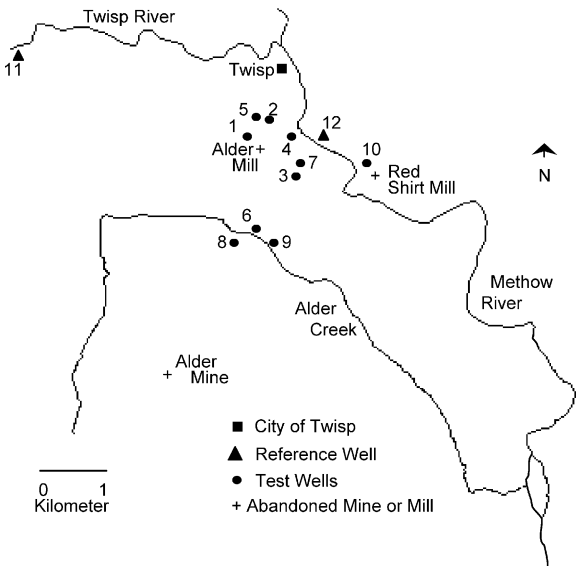

Fig. 2. Location of private drinking water wells and locations where groundwater samples were collected in the vicinity of the contaminant sources at Alder Mine, Alder Mill and Red Shirt Mill. Reference wells number 11 and 12 are also identified.

samples were filtered (Gellman, Inc. 0.45 μm, 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 μm 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 inductively coupled plasma atomic emission spectrophotometry (ICP-AES; Thermo Jarrell Ash® ICAP 61E). Samples were analyzed for arsenic 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 deionized water.

2.4. Contaminants of potential ecological concern (COPECs)

Fig. 1 shows the location of sampling stations for tailings, acid mine drainage, and Methow River water and sediments. A total of 34 grab samples were collected from the waste piles at the three mine sites. Sediments were sampled at each sample station along the mainstem of the Methow and Twisp Rivers. Four replicates of water and sediments were collected at each sample station (10–12) upstream from Twisp and the abandoned mine sites. Ten replicates of water and sediments were collected from each sample station (14–16) downstream from Twisp and the abandoned mine sites. Seven replicates of acid mine drainage were collected at station 3.

Contaminants of potential ecological concern (COPECs) were identified by comparing the concentrations of metals in tailings, AMD, groundwater, and sediments to ecotoxicological benchmarks, which were derived from primary literature (Suter and Mabrey, 1994) for the exposure of aquatic life to chemicals in water; Hull and Suter (1994) for the exposure of benthic organisms to sediments; Will and Suter (1994) for the exposure of soil invertebrates and microbial communities; and by Opresko et al. (1994) for the exposure of wildlife to food, water, and soil.

2.5. Ecosystem level response to contaminants

It was assumed there was an ecosystem-level risk when a contaminant exceeded a toxicity benchmark value for the exposure of soil invertebrates and microbial communities (Will and Suter, 1994) and if the functional property of a community or population is of interest and not the community or population itself (e.g., soil bacteria and nutrient cycling).

Respiration was also measured to provide evidence of ecosystem impacts from trace metal contamination. These measurements were taken along a transect with three sites in a 1-year old clear-cut 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 was weighed into four jars 7 cm in diameter (47% of the respirometer). The open jars of soda-lime were dried 8 h 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 clear-cut 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 h 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 h 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 $g\,m^{-2}$ CO_2 day^{-1}.

2.6. Community level response to contaminants

Surface water, sediment, and benthic macroinvertebrate samples were collected at stations along Alder and Poorman Creeks (Fig. 1). Sample stations included three stations (4–6) on Alder Creek and Poorman Creek

(7–9). Station 4 was located directly below the mine outfall. Two stations (5 and 6) were spaced approximately 0.5 km apart below the mine. All chemical analyses for heavy metals were performed by ICP atomic emission spectrophotometry (Thermo Jarrell Ash® ICAP 61E).

Biological assessments were accomplished using a 0.09-m^2 Surber sampler (15 meshes cm^{-1}, 0.66 mm $mesh^{-1}$), and samples at the six sites (4–9) were collected in triplicate twice, once near high-flow and again near low-flow conditions (June and September 1998). Benthic invertebrate samples were collected from riffles in the same general vicinity as the water and sediment samples. At each of the sample sites, Surber samples were taken and sorted following standard procedures for the analysis of benthic macroinvertebrate community structure. Taxonomic identifications were made primarily using Merritt and Cummins (1996). Organism genera were identified using a 7-65X stereo microscope, 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.

2.7. Population level response to contaminants

Five populations were evaluated for exposure to mine waste contamination. First, health risks to humans (*Homo sapiens*) in the vicinity of the abandoned mine and mill sites were determined based on their exposure to arsenic in contaminated groundwater from drinking water wells. Second, exposure to potentially toxic levels of arsenic was also determined in black bears (*Ursus americanus*) based on the accumulation of arsenic in the hair of these animals. Copper accumulation in aspen leaves (*Populus tremuloides*) and its concentration in aspen leaf miner larvae (*Phyllocnistis populiella* Cham. (Lepidoptera: Gracillaridae)) were measured as third and fourth populations. Finally, in the Methow River, the impacts of sediment metal contamination on the caddisfly larva (*Ecclesomyia* spp.) was determined based on differences in size, weight, and instar (i.e., the individual between insect molting events) development.

In this study, the risks of toxicity and carcinogenicity to humans from arsenic in groundwater were calculated based on the concentration in the water and on an estimate of water consumption rates. This study used the default drinking water intake rate of 2 L day^{-1} for adults (70 kg body mass) (EPA, 1980). To estimate the average daily dose, 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 was expressed in terms of both the noncarcinogenic toxicity and carcinogenic risks. Noncarcinogenic toxicity risk (hazard quotient). Noncarcinogenic hazard quotient was calculated by dividing the average daily dose (μg kg-body-$weight^{-1}$ day^{-1}) by the chronic reference dose (RfD; 0.3 μg As kg-body-$weight^{-1}$ day^{-1}) (Calow, 1998; IRIS, 2001). Carcinogenic risks associated with arsenic were statements of probability and were calculated by multiplying the average daily dose by a cancer slope factor (1/1500 $\mu g\,kg^{-1}$ day^{-1}) (Calow, 1998; IRIS, 2001).

The exposure of resident bears (*U. americanus*) to arsenic was determined using a bear-hair capture technique and a non-consumable liquid lure. Scent attractant was placed on a log enclosed by a strand of barbed wire stretched approximately 50-cm above the ground to snag hair. The use of scent to attract bears ensured there was no possibility of food reward. Six samples of hair from a single station in the vicinity of the Alder Mine were collected for analysis over a 2-year period between 2000 and 2001. Arsenic concentrations in hair were measured by hydride generated atomic fluorescence spectrophotometry and compared to concentrations in reference samples from one male and one female bear, each 10-years-old, that had been raised in captivity (Northwest Trek, Eatonville, Washington) and fed controlled diets in an environment assumed to be free of arsenic.

Samples of leaves from six aspen (*Populus tremuloides*) trees growing on the waste rock at the Alder Mine (Fig. 1, site 3) and samples of leaves from four aspen (*Populus tremuloides*) trees growing on the undisturbed slope on the opposite side of the watershed (Fig. 1, west of site 4) were collected. Aspen leaf miner larvae (*Phyllocnistis populiella* Cham. [Lepidoptera: Gracillaridae]) from aspen (*Populus tremuloides*) 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 inductively coupled plasma atomic adsorption spectrophotometry. Larvae

were rinsed in deionized water, dried, ground, and analyzed for metals by inductively coupled plasma mass spectrophotometry. The Student's *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 significance was computed using SigmaPlot 2001, version 7.0 for Windows. Bioconcentration factors (BCFs) were calculated for the elements in which the body concentrations were significantly different from leaf metal concentrations according to the equation:

$$\mathrm{BCF} = \frac{[\mathrm{metal}]_{\text{Leaf miner larvae}}}{[\mathrm{metal}]_{\text{Aspen leaf}}} \quad (1)$$

where BCF is the bioconcentration factor (dimensionless), $[\mathrm{metal}]_{\text{Leaf miner larvae}}$ the total metal concentration in the worm ($\mu g\,kg^{-1}$ dry weight), and $[\mathrm{metal}]_{\text{Aspen leaf}}$ the total metal concentration in aspen leaves ($\mu g\,kg^{-1}$ dry weight).

Two hundred larvae from each of three sample sites on the Methow River (sites 14–16, Fig. 1) were collected and compared to one hundred larvae from each of four sample sites (10–13) upstream from the abandoned mine sites. Within 1 h following collection, larvae were transported to the laboratory, removed from cases, blotted dry using Whatman #40 filter paper to remove surface water, and weighed. After weighing, the larva were preserved in 70% ethyl alcohol. Head capsule widths were measured using a slide micrometer and a dissecting microscope. 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.

2.8. Individual level response to contaminants

Eighty-four hatchery-raised triploid trout (*O. mykiss*, <35 g) were transferred from a nearby hatchery (Trout Lodge, Quincy, Washington) and equally divided into two pens approximately 1 m on a side. Fish pens were constructed from aquaculture netting on a polyvinyl chloride pipe frame. One pen was located in a Methow River side channel downstream from the abandoned mine site (station 17, Fig. 1) and the other pen was located upstream from the abandoned mine sites (station 13). 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 (07:00–08:00) at 4% of their body weight day^{-1}. Visual examination during feeding revealed that the fish 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 the final body weights measured.

Temperature, dissolved oxygen, and alkalinity were also measured daily at each site. Temperature was measured using Hobo model H8 temperature data loggers. A YSI model 85 meter was employed for the measurement of dissolved oxygen and total dissolved solids. 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 dissolved oxygen were standardized daily before and after use. Current velocity was measured following the method described by Hauer and Lamberti (1996). The Student's *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 SigmaPlot 2001, version 7.0 for Windows.

2.9. Tissue and cell level response to contaminants

Liver samples were excised from five fish per pen at the end of the exposure period. Samples less than 2 mm in diameter were fixed for 12 h at 4 °C in Karnovsky's fixative (5% glutaraldehyde and 4% formaldehyde in 0.1 M cacodylate buffer). Materials were then transferred to 0.1 M sodium cacodylate buffer and stored at 5 °C until transfer to the histopathology laboratory at the University of Washington, Department of Health Sciences. Tissue samples were then dehydrated in a graded concentration series of ethanol and embedded in eponate. Ultrathin sections obtained with a Reichert/Jung Ultra-cut E microtome® were collected on copper grids, contrasted with uranyl acetate and lead citrate. Duplicate samples not contrasted with uranyl

acetate and lead citrate were also prepared to control for the effects of staining on the presence of electron-dense granules in mitochondria. Specimens were observed with a Phillips CM 100® transmission electron microscope. The diagnosis of specific hepatic diseases was determined based on the criteria outlined by Phillips et al. (1987).

Tissue concentrations of metals were measured in resident trout from a farm pond fed by water from Alder Creek (station 6). Four-year-old triploid trout (*O. mykiss*) were euthanized by an overdose of MS-222 (0.1%, pH 7.0) and liver samples were collected from three fish. Samples were assayed by ICP-AES for metals and results were compared to metal concentrations in tissue samples from three fish in the Twisp River (station 13) that had not been exposed to mine waste.

3. Results

3.1. Trace element contaminants of potential ecological concern

Table 1 lists the eight trace elements that are contaminants of potential ecological concern (COPEC) based on the comparison of metal concentrations to toxicological benchmarks. Trace elements in tailings and acid mine drainage, 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 elements on plants, soil heterotrophic processes, wildlife, human health, and aquatic biota.

Table 1
Concentrations of trace elements that are contaminants of potential ecological concern (COPEC) in tailings (Alder Mine, Red Shirt Mill, Alder Mill), acid mine drainage (AMD) (Alder Mine at station 3), groundwater, and Methow River sediments (stations 14–16)

COPEC	Tailings ($mg\,kg^{-1}$)	AMD ($\mu g\,L^{-1}$)	Sediment ($mg\,kg^{-1}$)
As	446 (100)		9 (8)
Cd	33 (20)	3138 (49)	11 (1)
Cr	14 (10)		
Cu	851 (100)		199 (34)
Pb		6470 (271)	49 (47)
Ni			45 (21)
Se	2303 (100)	1267 (20)	
Zn	580 (100)	232654 (859)	

Concentrations are expressed as the 95% upper control limit and benchmark concentrations are given in parentheses.

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 eight trace elements that are contaminants of potential ecological concern. Six trace elements that were contaminants of potential ecological concern (COPEC) exceeded benchmarks for soil heterotrophic processes. Only four minor elements in acid mine drainage exceeded wildlife benchmarks. In the Methow River, dissolved metal concentrations were less than the limits of detection by inductively coupled plasma atomic adsorption spectrophotometry (i.e., ICP-AES) but in the sediments, five elements (i.e., As, Cd, Cu, Pb, and Ni) exceeded toxicity benchmarks for aquatic biota.

3.2. Ecosystem level response to contaminants

Table 1 lists concentrations of the six inorganic elements in tailings that were identified as contaminants of potential ecological concern at the ecosystem level. Since the concentrations of trace elements of potential ecological concern will be relatively constant over time in tailings and because the communities effected by contaminants that exceed benchmark values are immobile (i.e., plants, microbes), no averaging over time was necessary.

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 (Fig. 3). 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

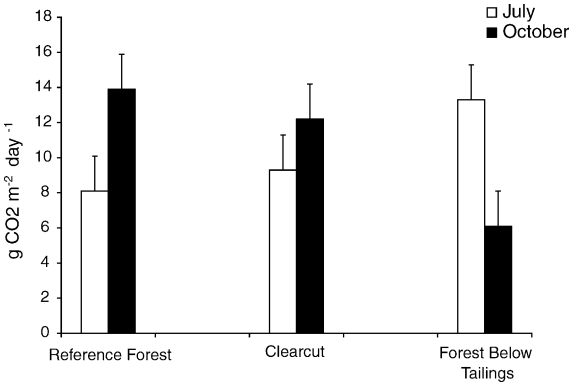

Fig. 3. Average respiration rates for forest soils in July and October 1999. The reference forest soil was adjacent and 100 m north of the Alder Mine and the clear-cut was at the same altitude on the opposing slope of the Alder Creek valley. Error bars indicate S.D.

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.

3.3. Community level response to contaminants

The taxa composition in contaminated Alder Creek was distinctly different from reference stations along Poorman Creek and the 34 most abundant taxa are shown in Fig. 4. The number of taxa and abundance of macroinvertebrates was less in Alder Creek stations 4–6 than in Poorman Creek reference stations 7–9. Abundances in Alder Creek at station 4 were similar to those downstream in Poorman Creek at station 6 ($n = 6$, $r = 0.95$, $p < 0.05$). Taxa richness in Alder Creek revealed an increase with distance downstream from mine outfall ($n = 6$, $r = 0.95$, $p < 0.05$).

Baetis, *Cinygmula*, Chloroperlidae, *Heterlimnius*, 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 Poor-

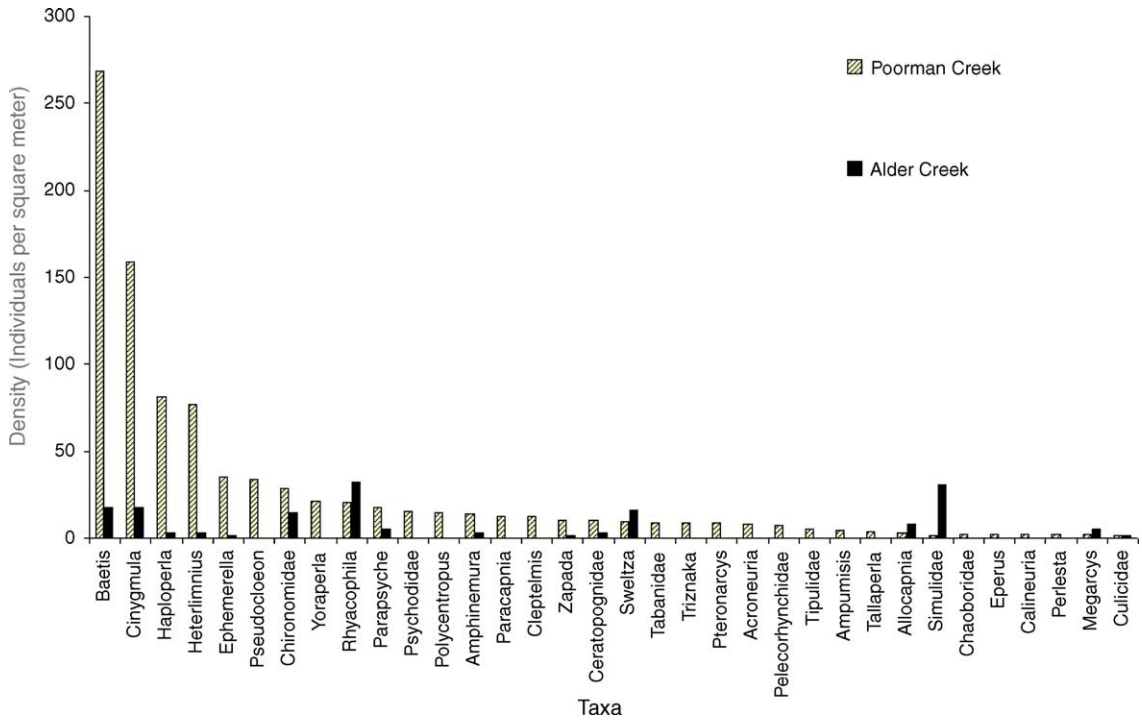

Fig. 4. Abundance of benthic macroinvertebrate taxa in the Poorman Creek at stations 7–9 (Fig. 1) and in the stream below Alder mine at stations 4–6 (Fig. 1). Samples were collected in June and September 1999.

man 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 7–9 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*.

3.4. Population level response to contaminants

The average arsenic concentration in water samples taken between October 1999 and June 2001 from 10 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 to 298 $\mu g\,L^{-1}$. The calculated average daily dose for arsenic ranged from <0.029 to 8.5 $\mu g\,kg^{-1}\,day^{-1}$. The noncarcinogenic hazard quotient for drinking water wells 1–10 ranged from 1.7 to 28.3, which are greater than thresholds of concern for adverse health effects. Carcinogenic risk estimates were also high. On average, one excess death from cancer per 909 adults (1.1E−03) is expected to develop among people drinking water from the wells tested. Carcinogenic risk from drinking water from well 1 was 1-in-77 for adults. No arsenic was detected in the ten samples from reference well 11.

Table 2
Arsenic concentration ($\mu g\,kg^{-1}$) in hair of bears in vicinity of Alder Mine at station 5

Bear number	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	0.89	0.05

Hair was collected using a bear-hair capture technique and a non-consumable liquid lure.

Arsenic was found accumulating in the hair collected from black bears (*U. americanus*) in the vicinity of the Alder Mine (Table 2). Two out of the six samples analyzed from the exposure area exceeded 1 $\mu g\,kg^{-1}$ and the maximum concentration was 1.7 $\mu g\,kg^{-1}$. Remaining hair samples from exposure area were 0.37, 0.48, 0.74, and 0.78 $\mu g\,kg^{-1}$ compared to reference hair samples that were 0.04 and 0.05 $\mu g\,kg^{-1}$.

Table 3 shows that copper and zinc accumulated in aspen leaves from sites contaminated with tailings and that these elements became magnified in aspen leaf

Table 3
The trace elements copper and zinc are chemicals of potential ecological concern (COPEC) because they accumulate in aspen leaves and aspen leaf miner larvae

	COPEC	Aspen leafs	Samples		Aspen leaf miner	BCF
			n	*P*	Larvae (pooled)	
Cu	Unexposed	10 ± 1	4		26	3
	Exposed	15 ± 7	6	0.05	261	17
Zn	Unexposed	150 ± 15	4		255	2
	Exposed	610 ± 126	6	0.00	1050	2

The Student's *t*-test was used to test the difference in metal concentrations in aspen leaves. The bioconcentration factor (BCF) for COPEC transferred from aspen leaves to the Aspen leaf miner larvae based on the formula, $BCF = [COPEC]_{Aspen\ leave\ miner\ larvae}/[COPEC]_{Aspen\ leaves}$. Trace element concentrations in aspen leaves are means plus standard deviation for samples from contaminated and reference sites. Aspen leaf miner larvae were pooled to make 0.5 g dry weight (80–100 larvae). All concentrations are $mg\,g^{-1}$.

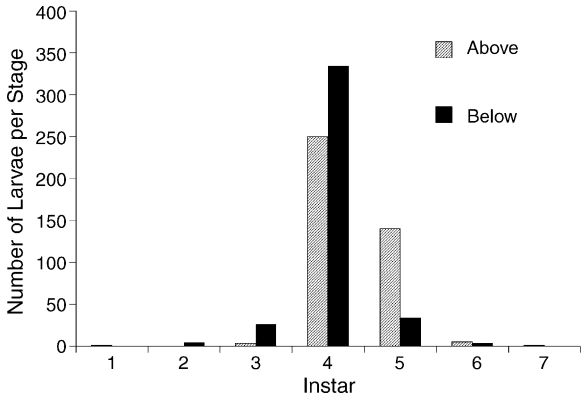

Fig. 5. Number of larvae per instar stage for the caddisfly *Ecclesiomyia* spp. One hundred larvae from sites (14–16, Fig. 1) were collected and compared to one hundred larvae from sites (10–13, Fig. 1) upstream from the abandoned mine sites.

miner larvae (*Phyllocnistis populiella* Cham. (Lepidoptera: Gracillaridae)) that fed on the contaminated leaves.

The mean live body weight of caddisfly larvae (*Ecclesiomyia* spp.) was lower in the Methow River below the mine sites (stations 14–16) than at upstream at stations 10–13 [2.3 ± 0.5 (standard deviations) versus 1.2 ± 0.2 g 100-larvae^{-1}, $P < 0.02$]. Growth patterns were also different between exposed larvae (stations 14–16), for which five larval stages were identified and reference larvae (stations 10–13), for which had seven larval stages were identified. Development of the exposed larvae lagged behind the reference larvae (Fig. 5). It was observed that 84% 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.

3.5. Individual level response to contaminants

The mean body weight of trout (*O. mykiss*) in the exposed group downstream from the abandoned mines (station 17, Fig. 1) was less than the body weights of the upstream control group (station 13, Fig. 1) (65 g $\pm$ 10 (standard deviation) versus 71 ± 9, $P < 0.01$). Mortality among the trout (*O. mykiss*) in the test group downstream from the abandoned mines also exceeded the upstream control group. Three fish died within 96 h following the beginning of exposure compared to no deaths in the control group. Two dead indigenous Coho (*O. kisutch*) parr (life stage between fry and smolt stages, generally reached by the end of the first summer) were also encountered at station 17 during the study period.

With the exception of sediment metals concentrations (Table 1), water quality was good with metals less than the limits of detection. 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 to 8.6. Alkalinity exceeded 190 mg L^{-1} as $CaCO_3$. Dissolved oxygen exceeded 8.3 mg L^{-1} at all stations, which exceeded 8.0 mg L^{-1}, the freshwater criteria for class A (excellent) surface water.

3.6. Tissue level response to contaminants

Examination of liver sections using transmission electron microscopy revealed large glycogen inclusions that were displacing nuclei to the periphery of effected hepatocytes in trout (*O. mykiss*) downstream from mines compared to upstream samples (Fig. 6A and B). Upstream, hepatocytes appeared normal with a lower incidence and less extensive accumulation of glycogen inclusions.

3.7. Cellular level response to contaminants

Examination of liver sections using transmission electron microscopy also revealed glycogen bodies in the nuclei and cytoplasm of hepatocytes in trout (*O. mykiss*) from the pen downstream from the mine sites (Fig. 6C and D). Large, electron-dense granules in the matrix of hepatocyte mitochondria were also observed (Fig. 7).

4. Discussion

Eight contaminants of potential environmental concern occurred in waste associated with mines near the Methow River and the effects were expressed at multiple levels of biological organization. Sources of these contaminants were waste rock deposited on the surface of the three abandoned mine and mill sites, leachate containing contaminants produced by the chemical and biological oxidation of metal sulfide waste and mobilized by infiltrating water from pre-

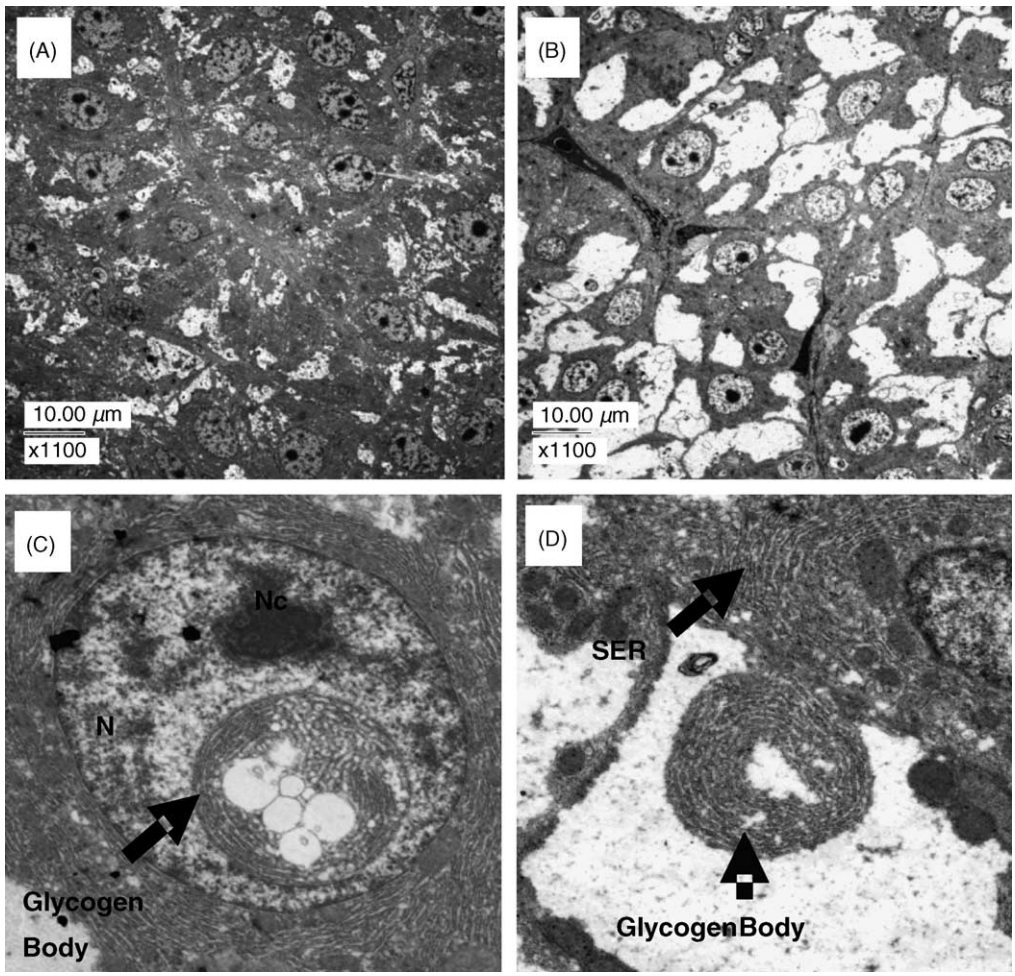

Fig. 6. TEM micrographs of hepatocyte from juvenile triploid trout (*O. mykiss*), maintained 7 weeks in pens and exposed to ambient conditions in a Methow River side channel downstream from the abandoned mine site (station 17), were fixed in Karnovsky's (5% glutaraldehyde and 4% formaldehyde in 0.1 M cacodylate buffer), embedded in Eponate, and contrasted with uranyl acetate and lead citrate. (A) Normal hepatocytes from reference trout maintained 7 weeks in pens upstream from the abandoned mine sites (station 13). (B) Hepatocytes with glycogen inclusions from trout maintained 7 weeks in pens and exposed to ambient conditions in a Methow River side channel downstream from the abandoned mine site (station 17). (C) Glycogen nucleus. (D) Glycogen body in hepatocyte cytoplasm.

cipitation, and contaminated effluent from two mine adits.

Mine waste deposited on forest soils pose a risk to soil microbes, which are also important in regards to nutrient cycling. Soil microbes, which 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 six contaminants exceeded benchmark values for toxicity it is assumed that these contaminants pose ecosystem-level risks.

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 Oc-

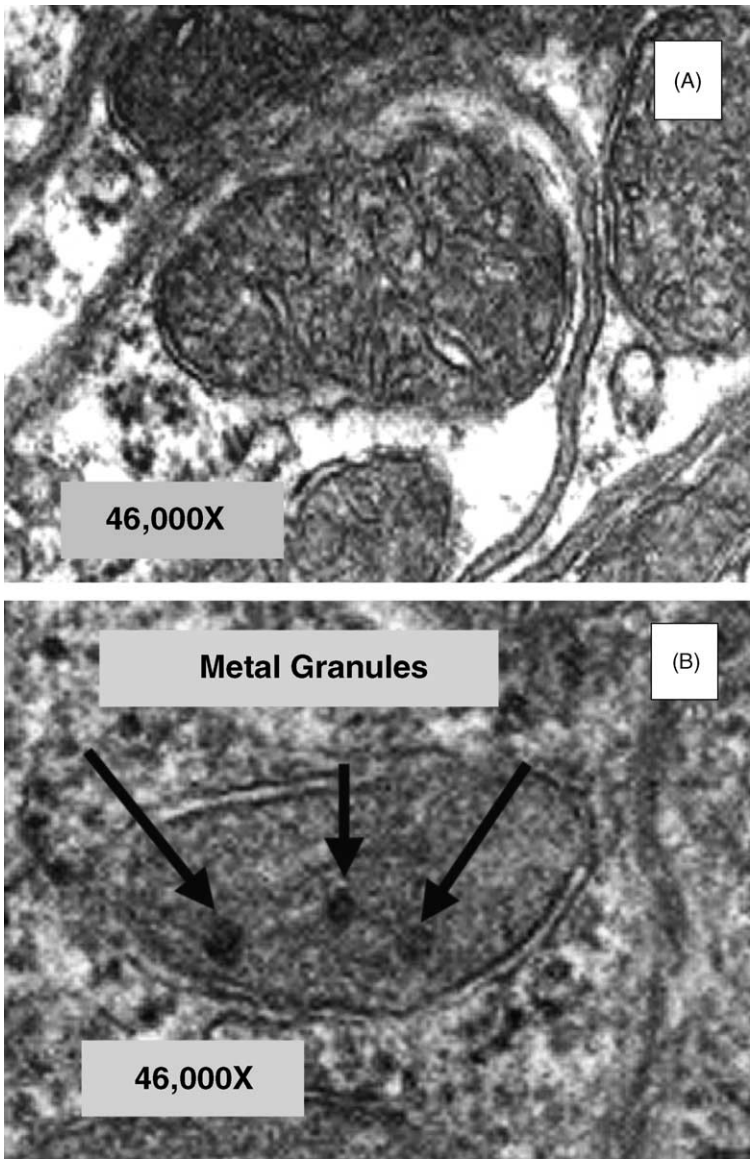

Fig. 7. TEM micrographs of mitochondria in hepatocytes from juvenile triploid trout (*O. mykiss*) fixed in Karnovsky's (5% glutaraldehyde and 4% formaldehyde in 0.1 M cacodylate buffer), embedded in Eponate, and contrasted with uranyl acetate and lead citrate. Duplicate samples not contrasted with uranyl acetate and lead citrate were also prepared to control for the effects of staining on the presence of electron-dense granules in mitochondria. (A) Stained section from trout maintained 7 weeks in pens upstream from the abandoned mine sites (station 13) and (B) stained section from trout maintained 7 weeks in pens downstream from the abandoned mine sites (station 17).

tober 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 is affecting soil microbial respiration and is causing seasonal changes in respiration rates to 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 acidic mine drainage (AMD) flows from station 3 along a subsurface path characterized by extensive faulting and calcite-filled fractures. Carbon dioxide will be released if the calcite fracture fillings are being dissolved by the sulfuric acid in acid mine waters, which are infiltrating from the surface.

Respiration rates increased from 8.0 g CO_2 m^{-2} day^{-1} in July to 13.9 g CO_2 m^{-2} day^{-1} in October in the reference soil adjacent to (but not contaminated by) waste from Alder Mine. Others have noted seasonal fluctuations in soil respiration rates in the clear-cuts in Western Washington were from 9.3 to 12.2 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 15.9 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 13.3 in July to 6.1 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.

The effects of contaminants on plants at the community level are of similar 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. Regression equations by Janssen et al. (1997a,b) that predict BCFs values as a function of soil characteristics suggest that BCFs may be governed by the same factors that determine the equilibrium partitioning coefficients between the soil solid phase and pore water and that the rates of trace element accumulation may be related to the magnitude of ambient trace-element concentration. However, the concentrations of copper, and zinc that were observed in this study in aspen leaves (*Populus tremuloides*) and aspen leaf miner larvae (*Phyllocnistis populiella* Cham. [Lepidoptera: Gracillaridae]) indicate that food-chain transfer and biomagnification from metal-contaminated soils is also possible.

Pathways for the migration of contaminants away from the source at abandoned mine sites involve transport in surface water and shallow groundwater. Runoff waters carry dissolved, colloidal, and suspended contaminants from hillslopes. Likely pathways of transport from source involves leachates that mix with shallow groundwater and emerge as seeps or enter streams through gaining reaches. If rainfall or snowfall exceeds the infiltration capacity then overland flow is produced. However, in the semi-arid study area where soil permeability is high and precipitation is low, subsurface flow is assumed to be the dominant process.

Reduction in the abundance and diversity of benthic invertebrates in Alder Creek below Alder mine show community level effects. In the reference stream community, it is the relatively small set of abundant species that are functionally important because they contribute the most to the biomass of macroinvertebrates and are doing the bulk of nutrient uptake and transfer. 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 (Walker et al., 1999). These minor species provide ecosystems with resiliency by maintaining ecosystem function under changing environmental conditions. When conditions change following contamination, metal-sensitive species are replaced by metal-tolerant species, which reduces diversity and abundance, lowers resiliency and affects the capability of an ecosystem to maintain productivity.

In polluted aquatic ecosystems the transfer of metals through food webs can cause high concentrations in invertebrates and toxicity in fish (Dallinger and Kautzky, 1985). When susceptible invertebrate species are eliminated, metal-tolerant food organisms may become dominant. The tolerance may be based on the capability to accumulate metals, which would lead to increased dietary exposure among fish predators (Timmermans et al., 1989).

Although the concentration of dissolved trace elements in water is important with respect to toxicity, concern over the oral uptake of trace elements contained in contaminated sediments and food is also significant (Spry et al., 1988). It is generally believed that the uptake of adsorbed trace elements is significantly less than the absorption of dissolved forms (Tamaki and Frankenberger, 1992). While the relative importance of the routes of exposure remains unclear, at high concentrations the bioavailability of even a small fraction of

adsorbed trace elements from the diet would be important supporting the proposition that diet is a significant route of exposure (Dallinger and Kautzky, 1985; Hare, 1992).

At the population level, the calculated risk of mortality from cancer in people exposed to As at average concentrations as low as 8 $\mu g\,L^{-1}$ was greater than 1 in 10 000. It has been concluded on the basis of epidemiological studies that arsenic at or above several hundred $\mu g\,L^{-1}$ 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).

Also, the live body weights per 100 caddisfly larvae (*Ecclesiomyia* spp.) downstream from the mine sites were approximately 48% lower than larvae sampled upstream. In both upstream and downstream sample sets, the larvae were comprised mostly of third and forth instar stages. This indicates that *Ecclesiomyia* spp. has a slow-seasonal life-style characterized by distinct changes of larval size with time (Merritt and Cummins, 1996; Irons, 1987). Our observation that larvae in the Methow River downstream from the mines were predominantly stage 4 instars while upriver stage 4 and 5 instars dominated suggesting that development below the mine may be delayed by as much as 1-month using life-history histograms for *Ecclesiomyia* spp. and other slow-seasonal caddisflies (e.g., *Anagapetus bernea*) (Merritt and Cummins, 1996; Irons, 1987). Growth inhibition suggests 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.

Arsenic accumulation in bear (*U. americanus*) hair and cadmium in fish liver suggest a potential for transfer of mine waste contaminants to top predators and indicate that contaminant stress has occurred in both aquatic and terrestrial ecosystems (Dallinger and Kautzky, 1985; Hodson, 1990). Arsenic, which was found to accumulate in the hair of bears (*U. americanus*) 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 (*U. americanus*) equal to 0.89 $mg\,kg^{-1}$, which was 18 times the average for arsenic in the hair of the reference bears (0.05 $mg\,kg^{-1}$). One hair sample from the contaminated site had an arsenic concentration as high as 1.66 $mg\,kg^{-1}$. Since the pattern of arsenic metabolism in bears is unknown and may not be similar to humans, the normal concentration of arsenic in human hair (<1 $mg\,kg^{-1}$) was used as a reference (DHHS, 2000). Although elevated levels of arsenic indicates exposure, the calculation or risk and carcinogenicity among bear (*U. americanus*) or other exposed animals is not possible unless more is known about the timing and duration of exposure and the species specific response to arsenic.

Elevated levels of trace elements in certain animals induce the production of the cytokine-rich protein metallothionein that chelates trace elements, which then accumulates in organs such as the liver (Ow, 1996). A typical host response to metals is a progressive accumulation of the contaminant bound to metallothionein in hepatic lysosomes (Bunton and Frazier, 1994). Reduced growth and increased mortality was noted in individual trout (*O. mykiss*) in pens downstream from the mines compared to upstream control trout (*O. mykiss*). The source of toxic mine waste contaminants may be due either to episodic exposures to contaminants that were not detected during sampling and analysis (Marr et al., 1995; Hanson et al., 2002) or due to dietary uptake of metal-rich sediments (Mount et al., 1994; Dallinger and Kautzky, 1985). While it is not obvious which of these two pathways is responsible for the effects seen, exposure to sediments that are known to be enriched with metals from the abandoned mines is a more likely cause rather than dissolved metals, which were less than the limits of detection during the study period.

Toxins are known to induce biochemical changes in the liver. More drastic systemic dysfunctions occur after the capability of the liver to sequester the toxins ceases (Bunton and Frazier, 1994). Monitoring of the appropriate biochemical parameter in the liver is useful, therefore, for the detection of toxicity at an early stage of exposure. In mitochondria, decreased numbers and increased sizes of matrical granules indicate a toxin-induced change has occurred (Phillips et al., 1987). Divalent cations have been shown to induce the formation of electron dense granules in exposed cells (Peachy, 1964). Metal uptake by mitochondria is by active transport, which causes the deposition of divalent cations on or in pre-existing granules in exchange for calcium. According to Argese et al. (1996), the appear-

ance of electron-dense matrical granules, when compared to in vitro toxicity data from a variety of other bioassays, indicated that the decreased number and increased size of matrical granules is a good general indicator of metal toxicity for several fish and invertebrate species.

Glycogen bodies in the cytosol and nuclei of hepatocytes also are indicators of toxin-induced metabolic disease and are sometimes associated with Wilson's disease, a genetic disorder of copper metabolism in humans (Phillips et al., 1987; Glodblatt and Gunning, 1984; Ostrakhovitch et al., 2002). The accumulation of glycogen inclusions in hepatocytes is pathognomic of type IV glycogen storage disease (GSD IV, Anderson's disease, amylopectinosis) (Sherlock and Dooley, 1997; Ishak and Sharp, 1987). Type IV glycogen storage disease 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, becomes less soluble and glycogenolysis is reduced (Goodman and Ishak, 1999).

Glycogen storage disease, generally an inherited metabolic condition, may also be a part of a toxic process (Goodman and Ishak, 1999). The metals lead, mercury, cadmium, chromium, manganese, molybdenum, nickel, and cobalt are known to cause hepatic glycogenolysis (Goodman and Ishak, 1999; Gill and Pant, 1981). In our study, the occurrence of glycogen inclusions in the liver suggests that metals contaminated sediments may be causing biochemical stress in exposed fish. Elevated concentrations of copper in sediments downstream from the abandoned mines causes a metabolic disorder in which food is first converted into glucose, then to glycogen, and finally stored in the liver. It appears, however, that glycogen is not being converted back into glucose normally for distribution to the tissues.

5. Conclusion

The effects of mine waste contaminants were expressed at all levels of organization, from the cellular to ecosystem level. Linkages that integrate responses across levels of organization were related to the disruption of carbon metabolism at the cellular level. Energy flow from cells, to tissues, organisms, populations, and communities ultimately affect ecosystem function. Toxicity, which begins as a chemical reaction involving contaminants and enzymes related to carbon metabolism, resulted in cellular and tissue level changes that indicate a disease process similar to Type IV glycogen storage disease in fish in the Methow River. Aquatic and terrestrial invertebrate, plant, mammalian populations were also effected. Among aquatic invertebrates, contaminants reduced metal-intolerant species, which were replaced by metal-tolerant taxa and resulted in changes in community structure. Changes at the ecosystem level were inferred when contaminants exceeded benchmark levels that are toxic to soil bacteria, which suggest that their functional properties related to nutrient cycling and energy flow have been affected.

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