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Aquatic Insects as Measures of Trace Element Presence:

Cadmium and Molybdenum

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INTRODUCTION

Because of the energy and minerals crises, the tributaries of the major rivers in Colorado are being subjected to large mining operations. Compounding these disturbances are natural background levels of metals, acid-rains from increased fossil fuel burning which will mobilize more elements, and the damming of waters leading to increased evaporation. The resultant levels of metals in water are a serious problem. In flowing streams, metal levels vary from day to day. An indicator is needed to establish maximum levels of toxic metals which were reached in the recent past.

Increasing demands for use and re-use of water emphasize the urgent need for more methods to monitor water quality. Using present methods of standard analysis (American Public Health Association; American Water Works Association; Water Pollution Control Federation (APHA et al.) 1975) a toxic trace element may not be discovered in an aquatic system until it has reached levels which will affect human health. Nehring (1974) used aquatic insects to measure levels of heavy metals in a fish kill. Kormondy (1965) documented the dragonfly, Plathemis lydia, accumulating zinc-65 at high levels. This uptake of heavy metals

by insects, led to the hypothesis that aquatic insects could be used to determine low levels of toxic trace metals in aquatic systems. Results obtained in this manner would be more accurate than results from analysis of a water sample by standard methods. This study investigates the feasibility of using aquatic insects such as mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddis flies (Trichoptera) to measure cadmium (Cd) and molybdenum (Mo) in rivers and streams. An inexpensive, simple, sensitive, and accurate measure of trace elements is examined through the use of insects which spend their larval stage in rivers and streams.

In an effort to explore the use of insects to assay for levels of metals in water, a three part research project was designed. Field research, Part I, sought to define the method and levels at which insects accumulate Cd and Mo and whether all insect species concentrate at the same rate. Part II was a laboratory, flow-through, temporal study to determine the function of time vs concentration in insect uptake and release of Cd. More field work in Part III produced a stream profile of the East River - Upper Gunnison River drainage, Gunnison County, Colorado establishing background levels of Cd and Mo, while putting the results of Part I and II to practical use.

The needs prompting this research will be described in more detail in Chapter I. Chapters II and III will disclose the reasons why Cd and Mo were selected for this study. Field research from the summer of 1979 will be described in

Chapter IV. Chapters V and VI will discuss the controlled laboratory experimentation and the summer field work of 1980, respectively.

I LITERATURE REVIEW

SORPTION BY AQUATIC INSECTS

Nehring (1976) determined that aquatic insects could serve as biological monitors of heavy metal pollution under certain conditions. The concentration of heavy metals by two to three orders of magnitude in the insects made detection of low level metal pollution easier.

Subsequently, Nehring et al. (1979) established through field and laboratory studies that a Field Concentration Factor (FCF) for a genus of aquatic insects could be used to quantify lead levels in water with accuracy:

$$FCF = [e] \text{ in insect} / [e] \text{ in water}$$

FCF = Field Concentration Factor
[e] = Concentration of element

Aquatic insects make possible a more realistic analysis of the total lead content in the stream (i.e., in stream sediments and water) than can be obtained by measuring lead in water samples only.

The FCF appears to be constant for each genus in relation to a specific element (Nehring 1973). Invertebrates reach a state of equilibrium with the concentration of metals in the water at approximately 96 hours, if the concentration of the element in the water is not increasing.

After the insect has equilibrated with the maximum concentration of metals in the water, it will hold the sorbed metals for several days. The insect, therefore, should be a good indicator of the maximum amount of the elements which have been present in the water in the recent past. Also, dried insect samples do not deteriorate with age, thereby eliminating the need for the immediate elemental analysis required for water samples (Nehring et al. 1979).

Nehring et al. (1979) have not clearly defined whether the uptake of metals by aquatic insects is purely a process of adsorption or a combination of adsorption and absorption by a physiological activity, such as assimilation. In other words, is the sorption bioconcentration, "...whereby chemical substances enter aquatic organisms through the gills or epithelial tissue directly from water," or is the sorption bioaccumulation, "...which includes bioconcentration but also uptake of chemical residues from dietary sources" (Macek et al. 1979)? It is clearly not biomagnification, which is a "process by which the tissue concentrations of bioaccumulated chemical residues increases by multiples of whole numbers (for example, X2, X5, X10) as these materials pass up the food chain through two or more trophic levels" (Macek et al. 1979).

Glooschenko (1969) studied live and dead diatoms, and found both forms accumulated mercury at the same rate. Kormondy (1965) reported the uptake of zinc in dragonflies

was a surface adsorption phenomenon and the size (surface/volume) determined the uptake of zinc in these organisms. If the source of contamination in the water is removed, most of the accumulated metal is lost from the insect in one week (Kormondy 1965). Martin (1979) showed that the exoskeletons of moulted copepods (e.g., Undinula vulgaris, Pleuromamma xiphias, Euphasia pacifica) were capable of concentrating trace elements and heavy metals as many as 197,000 times for lead and 6,000 times for Cd.

EXOSKELETON

The exoskeleton provides an outside covering for protection and support which is far more efficient for insects than an endoskeleton. Adult, aerial, aquatic insects are afforded better protection from crashes and shocks (Borror et al. 1976). Likewise, the larval forms are better protected against pressure of water columns, abrasion, and obliteration from effects of current. The degree of hardness varies between and within insect groups.

Structure

The exoskeleton is made up of three layers:

- 1) The body wall or cuticle, containing chitin, protein, waxes and pigments.
- 2) The epidermis layer of cells which secrete the cuticle.
- 3) The non-cellular basement membrane.

The entire outside surface of the insects is covered with these three layers. The trachea and tracheoles are also lined with this material as are the anterior and posterior gut. The tentorii, hardened structures within the head which provide additional support, are also made of this material (Borror et al. 1976).

The outermost cuticle layer or epicuticle is comprised primarily of tightly interwoven globular proteins in varied striations. It is penetrated with wax canals extending from the epidermis and often covered with wax (Andersen 1970; Wigglesworth 1967).

The procuticle, which lies under the epicuticle and above the epidermal cells comprises the bulk of the cuticle. It is made of chitin, protein, pigment and waxes. The chitin is distributed in microfibrils that run perpendicular and/or parallel to the external surface (Andersen 1979; Wigglesworth 1970).

Chitin is primarily a glucose and amine complex. The lipids in the exoskeleton can vary extensively depending upon the insect's diet (Richards 1978). Exoskeletal porosity is provided by the presence of setae, setal sockets, wax gland ducts, striations between protein layers in the epicuticle, and voids between the microfibrils of chitin (Borror et al. 1976; Andersen 1979). Sclerotization or hardening of the chitin, takes place through the cross-linking of cuticle proteins by ortho-quinones. When

sclerotization takes place, a darkening of the exoskeleton occurs (Richards 1978).

Moulting

In order for the insect to grow, it must cast off this chitinous material periodically. Sharp (1970) suggests ecdysis is also required for the insect to relieve itself of waste material such as nitrogenous and carbonaceous matter. To stimulate growth and moulting, the brain produces a steroid hormone called ecdysone; and at the same time, a juvenile hormone, which prevents metamorphosis, is suppressed. Ecdysone stimulates the production of an enzyme which causes histolysis of the upper cuticle layer, separating it from the epidermis which remains, and secretes a new cuticle. Histogenesis also takes place at this time, because the product formed as a result of histolysis provides material for the new cuticle. The entire surface of the foregut, hindgut, trachea, and tentorium are released also. The cast skin is called the exuviae (Richards 1978).

AQUATIC PROBLEMS

Geographically, Colorado sits at the apex of the North American continent. Geologically, Colorado has the potential to provide many of the energy needs of the U.S.A. Colorado also provides water for 14 western states (League of Women Voters (LWV) 1975). These states cover more than

half the land surface of the U.S.A. but receive only 14% of the total water that falls on the U.S.A. Unfortunately, precipitation and stream flow vary enormously in Colorado, as emphasized by excessive spring run-off, drought years, intermittent stream flow, and late summer stream drop-off.

Already, some streams in Colorado are biological moonscapes because of small mining operations and natural background contamination (Goettl and Davies 1971-1979; Woodling 1976; Kaback 1976; United States Environmental Protection Agency (USEPA) 1976b; Colorado Water Conservation Board 1972-1973). Because of the magnitude of some of the present and proposed mining operations in Colorado (United States Department of Agriculture, United States Forest Service (USDA, USFS) 1979a,b; United States Department of Interior, Bureau of Land Management (USDOI, BLM 1977,1978), far reaching effects on water supplies are inevitable. In particular, water pollution from trace elements, including toxic metals, is becoming a major problem (USEPA 1976a).

Compounding the trace-element/heavy-metal-problem is man's proclivity to dam water (Harte and El-Gassier 1978). By damming water for hydroelectric power, recreation, and irrigation, man increases the evaporation factor, and in so doing, increases the concentration of the trace elements and heavy metals in the standing bodies of water.

Technology has developed more and more uses for water, but has not produced new, affordable methods to establish water quality. Today, methods used to test water for metals require refrigeration and immediate analysis, since time and normal temperatures result in deterioration of the sample. Many times, ions adhere to the surface of the water sample container and avoid detection. Often, the cost of analysis prohibits necessary or frequent testing. Data does not exist for many streams. Many streams are analyzed less than once a year. This infrequent testing may not pick up the pulses or surges of trace elements and heavy metals which are prompted by seasonal changes, illegal discharges, rainfall, man-made or natural catastrophes (USEPA 1971-1977). A water sample taken from a river and analyzed today cannot reveal what flowed downstream yesterday. Continuous monitoring and recording of water quality is clearly needed.

The phenomenon of acid-rain is aggravating the metals problem. Normal rainfall, in the presence of carbon dioxide (CO_2), may be slightly acid (USEPA 1979). Oftentimes, in the West, the alkali dust from the deserts will hold the pH at 6.0 or higher (Likens et al. 1979). Any rain under the pH of 5.6 is called acid-rain (USEPA 1979; Likens et al. 1979; Luoma 1980).

As fossil fuels such as coal and gasoline are burned, they produce sulfur dioxide (SO_2) and nitrogen oxides

(NO_x). When these gases come in contact with particulate matter and water, sulfuric acid (H_2SO_4) and nitric acid (HNO_3) are produced.

The acids are deposited in rain and snow, lowering the pH of surface run-off water (Carter 1979; West 1980a,b; Rosenfield 1978). One effect of increased acidity on aquatic ecosystems is the mobilization of trace elements, including toxic metals, from the substrates into solution. Even without rainfall, dryfall, which is very fine particulate matter including salts of sulfur (S) and nitrogen (N) drops to earth. Likens et al. (1979) state that 10 g of S per square meter per year is being deposited in Central Europe. Gravity brings all this solid material to the ground at the whims of the weather and the wind. Regardless if the rain that eventually falls has a pH no lower than 5.6, as it hits the dryfall from the preceding days, an acid solution is formed. This will dissolve large quantities of toxic metals that are found naturally or have precipitated out in the substrates of our rivers and lakes over the years (Glass, N.R. 1979).

Studies are being done on the Boundary Waters Canoe Area (BWCA) lakes of Northern Minnesota to monitor the effects of acid-rain and mercury laden fly-ash on fish which already exhibit high levels of mercury (Glass, G. E. 1979). Aluminum has been identified as one of the problem metals in the acid waters of the eastern U.S.A. As the

aluminum content in the water increases, it interferes with the sodium pump in the gills of the fish and the fish die (Luoma 1980).

It was not until October, 1979 (Likens et al.) and early 1980 (Lewis and Grant) that the presence of acid-rain was documented in the Rockies. John Harte, Ron Hall, and this researcher recorded rains of pH 4.2 at Galena Mountain and Gothic, Colorado during the summer of 1980. The true background level of trace element presence in the streams of Colorado may never be established. It may be too late to measure what has been produced naturally by geochemical weathering. Today's measurements of trace element levels in aquatic systems may already be affected by anthropogenic sources such as mining, ranching, timbering, and acid-rain.

To date nothing has been written about the Rockies being more vulnerable to acid-rain effects than any of the other areas that "are believed to be sensitive to acid deposition" (USEPA 1979) (Fig. 1). The Rockies are more highly mineralized than any other area selected by the EPA as sensitive to acid-rain (Prather 1980). The only other area even remotely comparable to the Rockies is the Eastern Canadian Shield. This area, however, is not quite as mineralized, lies under deep snow a good part of the year, and is not as populated as the Colorado Rockies. Therefore, it may not come under scrutiny for a long time. Acid-rain effects in the mineralized Colorado

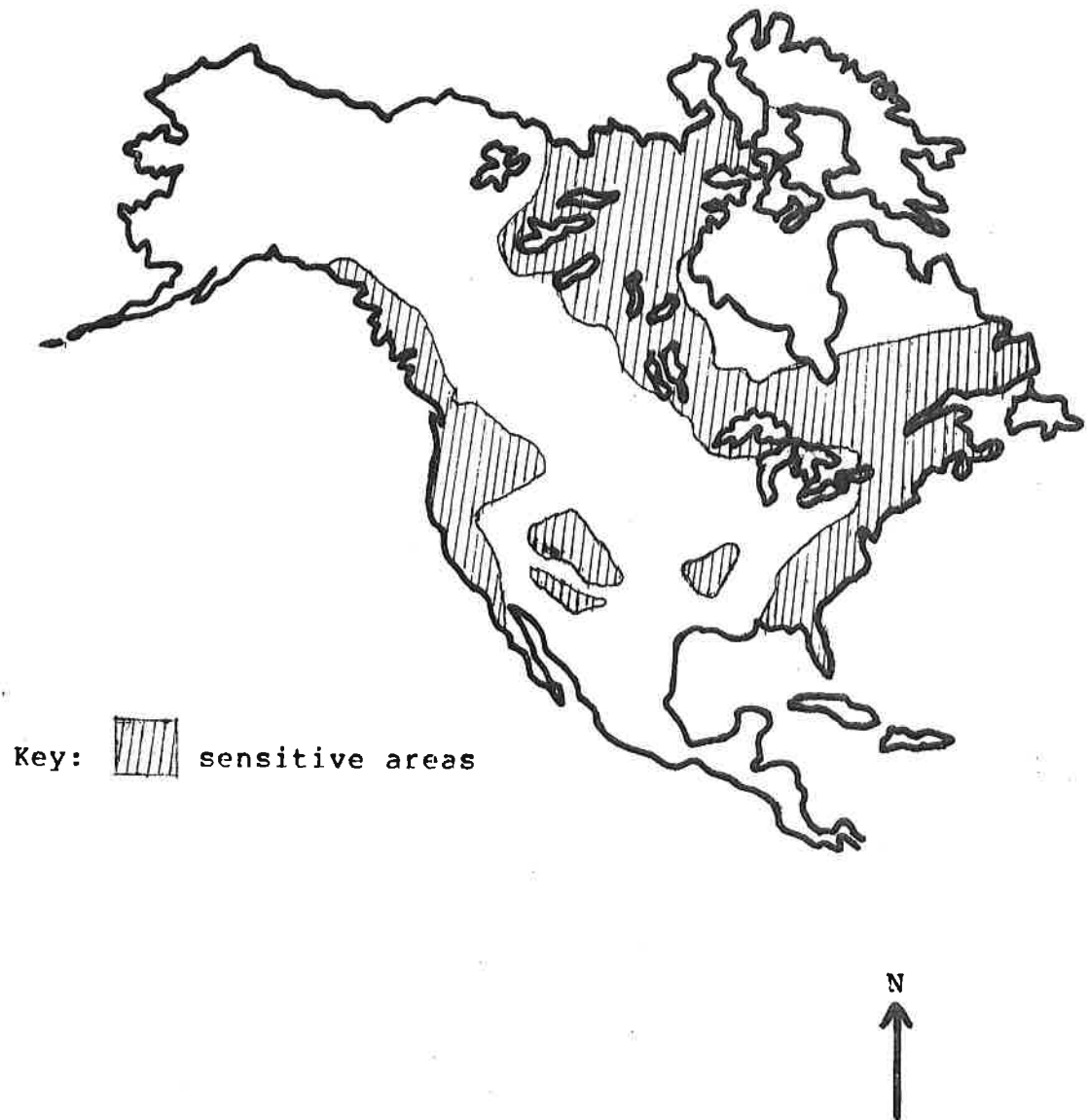


Fig. 1. Areas of the North American Continent sensitive to acid deposition.

Rocky Mountain area, could very well be far more spectacular than in the other areas.

The lack of buffering cations such as calcium (Ca^{++}) and magnesium (Mg^{++}) makes high mountain lakes and streams susceptible to acid contamination. Most low order streams have granite rock for a stream bed and very little allochthonous material. This makes them targets for pH attack as compared to the higher order streams which are loaded with assorted buffering materials, such as silt, organic material, calcareous deposits, and anthropogenic wastes (Hynes 1970).

Federal legislation enabling the use of coal (Council of Environmental Quality (CEQ) 1979) plus the lack of anti-pollutant technology to remove NO_x , assures the increase of acid-rain (Walton 1980; Likens et al. 1979; Luoma 1980). Therefore, methods must be devised to monitor the release of toxic trace elements into the aquatic systems before they reach levels measurable by standard methods of analysis (APHA et al. 1975). By the time the toxic metals reach a measurable level, they will oftentimes be at a level high enough to affect human health. New methods for monitoring increases and measuring low levels of toxic trace elements are needed to deal with the unique problem facing the Rockies.

II LITERATURE REVIEW OF CADMIUM

The need to find a new method for analyzing Cd in riverine systems was established as early as 1974 by Gardiner (1974b). He found that within the first five minutes after a water sample containing Cd is introduced into a container, 10% of the Cd in solution adheres to the inside surface. At the end of 24 hours, 90% or more of the Cd often binds to the receptacle. Water sampling, therefore, is not an efficient method to test for Cd in rivers and streams. Considering the harmful human health effects of Cd, its presence in aquatic systems should not be overlooked.

HUMAN HEALTH EFFECTS

Cd is a toxic substance (USEPA 1976a; LWV 1980). Toxic substances are poisons, often chronic in nature, causing irreversible physiological changes, usually subtle, and may cause harm to future generations (LWV 1980). Cd is teratogenic, mutagenic and carcinogenic (LWV 1980; USEPA 1980). Toxic metals, like Cd, maintain three levels of activity in relation to biological response. Below a certain level there is a no-effect dose range. As the concentration increases, the element evokes biological responses. Some of these responses are reversible and

some are not, while others may be very subtle and go unnoticed. At the third level, the concentration of the metal has a lethal effect (Petering and Petering 1980). A toxic trace element is one that elicits a biological response at less than 1 mg/l (1 ppm).

Long term studies conducted by the USEPA (1980) indicated the average person consumes (via food) or inhales (via cigarettes) Cd at such high levels, that it seemed unreasonable to lower the criterion of 10 ug/l (10 ppb) allowable in drinking water for human consumption. Approximately 6% of the Cd ingested is absorbed (Decker et al. 1958). USEPA (1980) figures show that as much as 25 % and up to 50% of the Cd inhaled, either through smoking or from ambient air concentration, is absorbed. Work done by Decker et al. (1958) confirmed that no absorbed Cd is eliminated. More recently, the USEPA (1980) finds Cd is stored almost entirely in the kidney and liver and may be excreted very slowly. Studies show that people over 50 have less Cd in their tissues than young people. It was thought that after 50, body tissues begin to release Cd. However, USEPA (1980) points out that perhaps the people over 50 carry less Cd because they were not exposed to Cd as heavily as the younger people of today.

Chronic kidney disease (renal tubular dysfunction) occurs in individuals when Cd reaches critical concentrations in kidney tissue (Fleischer et al. 1974). Critical body burden of Cd is estimated to be 200 ug/g wet weight of

renal cortex (USEPA 1980). Cd has been called the "silent killer" because it has been linked with a high incidence of heart attacks and strokes. Because of its affinity for the renal cortex of the kidney, it has been suspected as the cause of high blood pressure (USEPA 1980). A correlation between atmospheric levels of Cd and deaths from arteriosclerotic heart disease and hypertension has been demonstrated. Cd has also been firmly established as a cause of emphysema, from the inhalation of Cd-bearing dust or fumes (USEPA 1980).

There is much disagreement in the literature concerning the effects of Cd on the reproductive systems of both males and females. In vitro studies can produce testicular necrosis, testicular cancer, bladder cancer, and mammary gland and ovarian atrophy (USEPA 1980). The results of population studies correlating Cd with testicular and bladder cancer indicate Cd causing many incidents of both. However, in all the studies done, the subjects were also exposed to high levels of other carcinogenic metals, such as mercury and arsenic (USEPA 1980).

DISTRIBUTION

Cd, like acid-rain, is ubiquitous, and as it becomes more prevalent, more attention and study is being given to it. Its ever-increasing presence in the environment has also been traced to anthropogenic sources. The natural erosion from weathering of rocks and soils is insignificant compared to the release by man.

Cd has received worldwide recognition for the health problems it has created. England and Japan have witnessed serious health problems from environmental Cd (USEPA 1979). Sweden has banned the major uses of Cd and stopped the importing of Cd-containing products (Nilsson 1980). Sweden has the highest percentage of testicular and bladder cancer in the world (USEPA 1980). An International Joint Commission between Canada and the U.S.A. has done extensive research on the problem of Cd in the Great Lakes (Davies 1976).

Fifty percent of the Cd used industrially is for Cd plating of metal products. Twenty percent is used by the pigment industry and 10 percent stabilizes polyvinyl chloride (PVC) (Ranby and Albertsson 1978). The use of PVC pipe for irrigation can increase Cd content of wheat ten times (Ranby and Albertsson 1978). Cd is distributed throughout the environment by means of dry-fall and acid-rain from industrial, utility plant, and automotive discharges. Ambient air and water levels of Cd are so high it is picked up and concentrated by plants and organ tissues of animals leading to very high levels in the diet of humankind.

Eventually, much of the Cd in the environment ends up in sewage sludge (Fleischer et al. 1974). Ideally the sludge should be used as fertilizer, however this facilitates the recycling of Cd through the food chain. The towns of Gunnison and Crested Butte face this problem

with their sludge. In efforts to use the sludge in revegetation projects at mines in the area, very high levels of Cd were found (Ferchau 1980).

Water quality studies done on the East River - Upper Gunnison River drainage show levels of Cd as high as 70 ppb in Coal Creek below the Keystone Mine (Fig. 2). Either through dilution, precipitation, or complexation, by the time the water reaches the Slate River it contains between 10-30 ppb depending on the season of the year (USEPA 1971-1977).

It was this discovery of measurable levels of Cd in the waters of Coal Creek and Slate River that prompted Part I, Field Research, Summer 1979. Not often does one find a perfect situation to do manipulative research. Here was a lotic ecosystem impacted by a toxic trace metal of global importance, with which to compare the sorption of Cd by aquatic insects above and below a mine discharge and document the migration of the Cd after it left the mine site.

CHEMISTRY

Numerous researchers have attempted to explain the binding capabilities of Cd. In discussing the natural removal of Cd from aquatic systems, Gardiner (1974a) points out that Cd will precipitate as cadmium carbonate (CdCO_3), being the least soluble form of Cd in aerobic systems, but, the largest part of Cd is removed by humic materials through

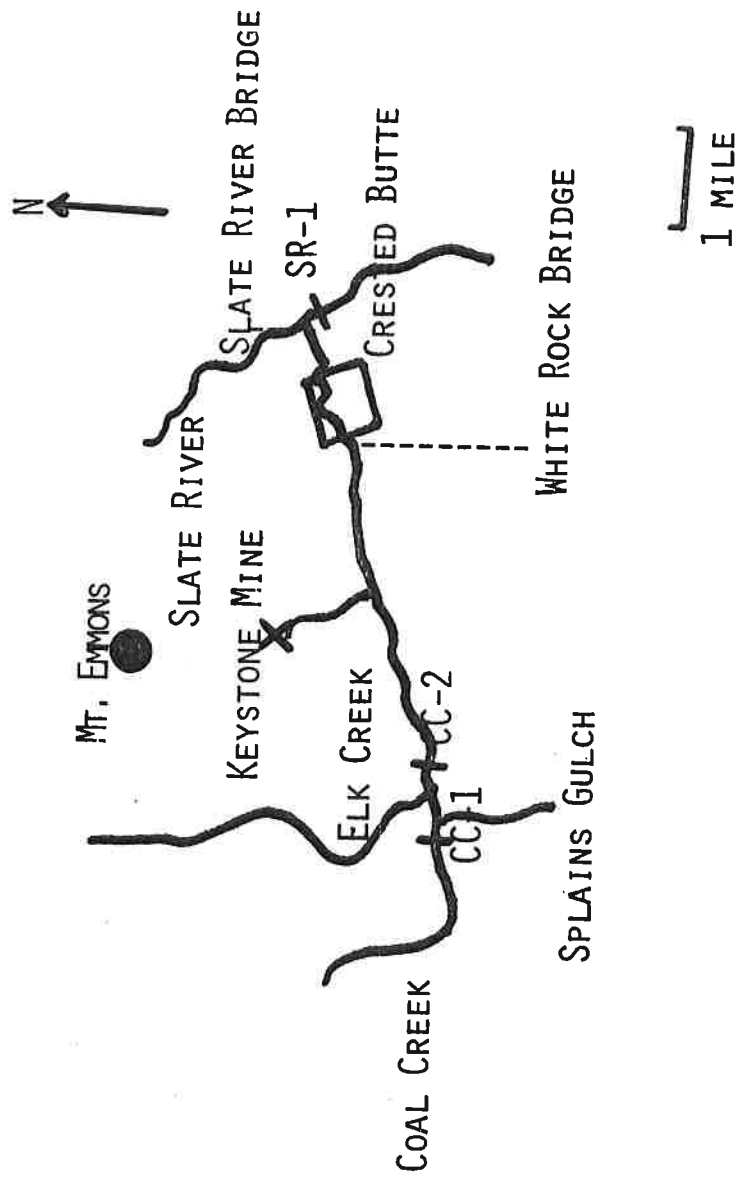


Fig. 2. Coal Creek and Slate River sampling sites, Gunnison County, Colorado.

complexation. The USD0I (1978) states that organic matter in soils fixes Cd through its ion exchange ability and through chelation.

Davies (1976) found that Cd had a "no-effect" toxic level in soft water between 0.7 and 1.5 ppb (dissolved), whereas, in hard water, it was between 13.5 and 21 ppb. He felt it is not because of any complexed Cd bound to humic material that makes the Cd in hard water less toxic, but rather that "some antagonistic reaction inhibiting the toxicity" is involved, such as, other heavy metals in solution.

Cd, in small doses, can induce tolerance by stimulating the production of metallothionine, a protein in the liver. Metallothionine is rich in cysteinyl (sulfur) residues which have an affinity for metals. The Cd binds with the protein and becomes detoxified. The bound form of Cd is inactive. Competition between Cd and other metals such as calcium, selenium, cobalt, mercury, and copper for binding sites with metallothionine has been widely studied (USEPA 1980).

It has been noted that Cd does not build up in the food chain as rapidly as mercury. Cd forms very labile alkyl Cd compounds that do not last very long under natural conditions. Only 6% of the Cd ingested, either as cadmium-protein complexes or Cd ions, is absorbed. However, mercury

forms very stable mercury-carbon compounds. When methylmercury, which has built up in the food chain is ingested, all of it is absorbed.

III LITERATURE REVIEW OF MOLYBDENUM

ECONOMIC DEMAND

Molybdenum (Mo) is a refractory metal exhibiting a high melting point (2610°C), extreme hardness, and high strength at elevated temperatures. Tungsten has the same characteristics but because Mo is half as heavy as tungsten, only one-half the Mo is required to achieve the same effects in metal compounding. This is an advantage in the production of alloys.

The demands for Mo are extensive: in the production of alloys, commercial catalysts, non-toxic constituents of pigments, aqueous corrosive inhibitors, flame retardants, smoke suppressants, solid lubricants, and as a fertilizer for legume seed treatment (Braithwaite 1978). Mo shows little thermal expansion, which makes it suitable for filaments and conductors in the electronics industry (Braithwaite 1978). It is a constituent in stainless steel and such "high-tech, and high-demand" uses as jet engines, utility boilers, and oil pipe lines (Forbes 1980).

DISTRIBUTION

The rationale for selecting Mo for this study was based on the presence of "one of the world's larger molybdenum deposits" in Mt. Emmons, Gunnison County

Colorado (Biddle and Hamlin n.d.) This deposit of molybdenite (MoS_2) at 0.44% could yield an estimated 23-30 million pounds of Mo per year. The Mo deposit lies in a dome 365 m below the surface on the south slope of Mt. Emmons (Fig. 3). The ore will be reduced at a primary crusher near the mine and then will travel down an ore haulage corridor to the mill site, which may be as far as 33 miles from the mine (Biddle and Hamlin n.d.). The mine will pulverize the ore to a very fine sand and remove the Mo by flotation. From each ton of ore, 4 to 5 pounds of Mo will be recovered and the rest of the finely ground material will be placed in a tailing pond which will have the capacity of 100 to 300 million tons (Biddle and Hamlin n.d.). The above provides a perfect situation to explore the possibility of monitoring the release of Mo into the ecosystems of the East and Gunnison Rivers and the Blue Mesa Reservoir as the Mt. Emmons project progresses.

An analogous situation lies to the northeast (Fig. 3) at the world's largest Mo mine, Climax, Colorado. Much research on the distribution of Mo into the ecosystems of the area has already taken place. The mill and mine affect Tenmile Creek, Eagle River, Blue River, and Dillon Reservoir. Although the Climax area is already impacted by Mo and the Mt. Emmons area is not, there is a similarity between the two, in that both mines sit adjacent to tributaries to a major river drainage (Upper Colorado River) and are

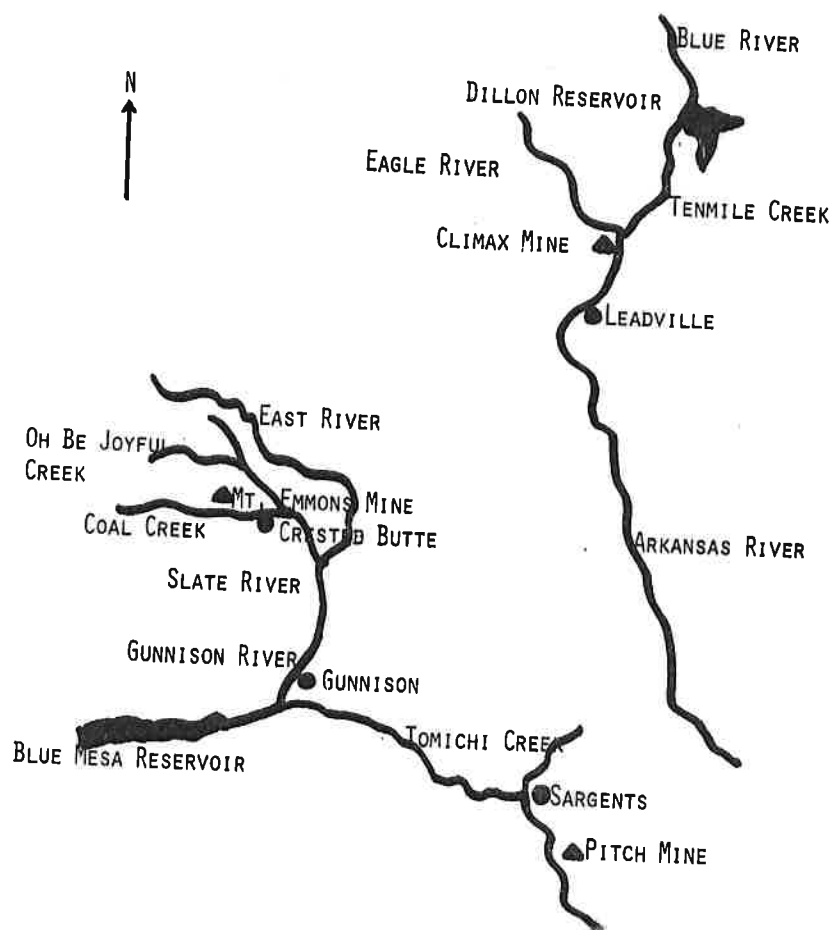


Fig. 3. Locations of two Mo mines and one uranium mine in central Colorado in relation to waters that are, or could be affected by the mining and milling operations.

subtended by large manmade impoundments, Dillon and Blue Mesa, respectively.

Dillon Reservoir serves as a sink for the dissolved metals flowing down Tenmile Creek. Its substrate is comprised of more organic material than the creek, thus providing binding sites for the metals in solution. It serves also as a settling pond for the suspended materials coming down the creek. The oxidized metals that were transported to the lake as a sediment become soluble when finally located in the anaerobic conditions in the bottom of the lake. However, in the case of Mo the pH drop at the bottom of the lake created by anoxic conditions increases its deposition (Jones 1974; Bertine 1972). "The extent of the pollution will be the function of the mass in-flow of pollutants, the buffering capacity of the lake waters and the hydraulic detention time of the lake" (USEPA 1976b). "The ultimate sink for the metals (from mining activity) may be quiescent streams or lakes far downstream from the main section of the polluted stream" (USEPA 1976b). Blue Mesa could very well become the sink for the Mt. Emmons Mine. Levels of Mo as high as 10033 ppb (dissolved) and 32266 ppb (total) have been reported in Tenmile Creek, and studies done on Dillon Reservoir show levels as high as 440 ppb Mo total (USEPA 1971-1977). A simple monitoring procedure using aquatic insects could indicate similar conditions arising in Gunnison County.

Although there are areas where high concentrations of Mo are known to exist, concentrations of Mo in water and the plants surrounding the area are low (Runnells 1975). Areas highly mineralized and disturbed have far more Mo in the environment than similar mineralized areas that are undisturbed (Runnells 1975). Jorden and Meglén (1975) found that "...some 100,000 mi² (of drainage area) is (sic) required to "dilute out" the effects of the point sources" contamination by Mo in Colorado.

Kaback (1976) stated that changes in range management had to be effected along the Blue River because of high Mo levels which caused toxic effects in ruminants (molybdenosis) (Fig. 3). She states that wind blown material from the Climax tailing ponds has caused much of the pollution along with occasional releases from tailing ponds which contain as much as 13400 ppb Mo. All four of the above researchers point out that Mo travels both in solution and suspension. Heavy spring run-off moves large quantities of undissolved Mo as suspended solids downstream from mining disturbances (Le Gendre and Runnells 1975; Runnells 1975).

Historically, the release of Mo into the surrounding ecosystem has been exacerbated by mining; i.e., coal, molybdenum, and uranium (Le Gendre and Runnells 1975). "The median concentration of Mo in streams in the U.S.A. is 0.35 ppb..." and anything over 10 ppb in Colorado is unusual and can be traced to mining activity (Le Gendre

and Runnells 1975). USEPA-Storet Retrieval (1971-1977) data show 55 ppb Mo in the North Fork of the Gunnison River, Gunnison County, Colorado. Le Gendre and Runnells (1975) found Mo "is the principle dissolved metal in the aqueous effluent from many copper, molybdenum, and uranium flotation mills in southwestern U.S.A." because Mo becomes more soluble at the high (alkaline) pH required for the flotation process. At the high pH other metals would have precipitated out as hydroxides. In water from a uranium mill in Southern Colorado the concentration of dissolved Mo was 900000 ppb (Le Gendre and Runnells 1975). A pitch mining company, in its application for a uranium mill on Marshall Pass, estimates 57000 ppb Mo in its tailing pond (Fig. 3) (Homestake Mining Company, 1980). The mill site is upstream from Gunnison County on tributaries serving the Gunnison River and ultimately, Blue Mesa Reservoir.

CHEMISTRY

The element Mo has 7 oxidation states: VI, V, IV, III, II, I, and 0. States VI, V, IV, and III are involved in biological systems. In the natural geological state it occurs as MoS_2 , its anoxic form (Williams 1978). Molybdenite, MoS_2 , is 60% Mo and 40% S. It is found embedded in minute quartz particles in porphyry deposits. Because of this natural physical characteristic the ore has to be ground extremely fine to be milled (Braithwaite 1978).

The fact that Mo has so many valence states, binds covalently in biological systems, and has a strong affinity for S explains the presence of Mo in so many enzyme systems (Williams 1978). Mo is found in the nitrogenase enzyme complex used by bacteria which fix N_2 into NH_4 . Mo, unlike Cd, is an essential trace element in plants (Williams 1978). It is estimated that for every one million atoms of N in a plant there will be found 1 atom of Mo (Thornton 1973). This is a very small proportion of Mo but very important for plant growth and development. Legumes and mustards accumulate more Mo than grasses and shrubs (Thornton 1973). Levels as low as 1.5 ppm Mo in plants have been shown to cause molybdenosis in cattle, sheep and deer (Buck et al. 1976).

In the past, areas with plants and shrubs high in Mo were not identified until ranchers or veterinarians reported cases of molybdenosis. Plant studies were then initiated to confirm the disease (Thornton 1973). Because cattle are moved continuously from area to area for grazing purposes, marginal or subclinical cases of Mo poisoning may be going unnoticed. Wet seasons in areas of high alkalinity can produce plants containing higher amounts of Mo than usual, making Mo exposure a seasonal problem (Thornton 1973). The enhanced solubility of Mo under wet conditions along rivers and streams in the riparian habitat and in swamps often precipitates the disease (Thornton 1973). The Blue River is an example of this phenomenon.

Allaway (1977) speculated that the "probability of molybdenosis (is) consequently greater" in the western U.S.A. because of the predominance of alkaline soils and aquatic systems.

Biologically, there is a species difference in the clinical manifestations, dose tolerance, and cold stress response to Mo. Cattle are more sensitive to Mo than sheep. Horses and deer are more tolerant than sheep. Only cattle exhibit diarrhea at the onset of Mo poisoning. Sheep develop swayback and connective tissue degradation (Underwood 1977).

Mo toxicity is not caused by a Cu deficiency in the animal but the disease will lead to Cu suppression (Thornton 1973). Testicular atrophy and loss of libido in young bulls yields low reproductivity (Underwood 1977). The disease progresses from "emaciation, liquid diarrhea full of gas bubbles, swollen genitalia, anemia, achromotrichia (depigmentation of hair and wool), decrease in milk production, evidence of pain in joints, osteoporosis," and eventually 80% mortality (Buck et al. 1976). Cu is used as an antidote in severe cases and as a deterrent in areas where Mo levels in feed are high. It is thought that the Cu reacts with the active, soluble thiomolybdates in the rumen and precipitates them out as insoluble, inactive copper thiomolybdate (Underwood 1977).

HUMAN HEALTH EFFECTS

"Mo is required for growth, for cellular oxidation, for purine metabolism, and is possibly involved in iron metabolism" (Underwood 1977) and is considered an essential trace element in animals. It is found in xanthine oxidase, an enzyme involved with purine degradation (Williams 1978). Xanthine oxidase converts xanthine to uric acid and could "be involved in the expression of gout and possibly arteriovascular disease" (Solomons and Bekemans 1977). More and more authors are suggesting a relationship to human health effects and high Mo levels in the environment. Nargizyan et al. (1979) correlated production workers' exposure to Mo dust with immediate changes in liver function and high incidence of hypertension, arthritis, myalgia, nervous disorders, and dermal effects in descending frequency. Walravens et al. (1979) surveying workers exposed to Mo dust found elevated blood levels of ceruloplasmin and elevated levels of molybdate in plasma and urine. Because of a high turnover rate of employment, the long-term health effects were not demonstrated.

Agrawal (1980), in seeking a newer more sensitive method to assay Mo in blood, compared the Mo content in blood from 800 normal healthy adults with that of 350 cancer patients. The mean Mo concentration for normal blood was 0.6 ppm; for cancer patients' blood it was 0.98 ppm.

IV PART I: FIELD RESEARCH, SUMMER 1979

Using data collected by AMAX Corporation (1979), the USEPA-Storet Retrieval System (1971-1977), and the Colorado Department of Health, Water Quality Control Division reports on Coal Creek and Slate River (Goettl and Davies 1971-1979), the ideal situation (page 16) was found on which to proceed with field research using aquatic insects as measures of trace element presence. For many years mine waste water has been spilling into Coal Creek. In 1975, a tailing pond broke at the old Keystone Mine above the town of Crested Butte, Gunnison County, Colorado (Fig. 2). Cd levels as high as 70 ppb have been documented in the discharge (USEPA 1971-1977; AMAX n.d.).

METHODS

Research was started in the summer of 1979 on Coal Creek and Slate River (Fig. 2) to determine uptake of Cd and Mo by aquatic insects. In two separate experiments, Run #1 (August 2, 1979) and Run #2 (August 10, 1979) samples of aquatic insects were collected above the Keystone spill in Coal Creek. Run #1 insects were taken from the stream 5.2 road miles above the White Rock Bridge, Crested Butte, Colorado and Kebler Pass Road at the Splains Gulch turnoff. Run #2 insects were collected 4.8 miles above the same

bridge 20 meters below the confluence with Elk Creek. On each run, both live and air-dried insects were transplanted to the Slate River 100 meters downstream from the Slate River Bridge on the Mt. Crested Butte road (Fig. 2).

Insects were individually hand-picked from rocks with teflon coated stainless steel forceps and placed in fiberglass cages. The live transplants were kept in the cages at all times. The air-dried insects were exposed to the atmosphere until dead, and were resubmerged in the Slate River upon transplanting. The larger species common to Coal Creek (e.g., Ephemerella doddsi, E. grandis, Brachycentrus brachycentrus) were collected to accumulate a sample size of 100 mg dried insect. Insects were placed in the Slate River (Fig. 2) in the same fiberglass cages in which they were confined upon collection, and the cages were tied to willows on the edge of the river.

At intervals of approximately 24, 48, and 72 hours, insect samples were cropped from the Slate River and allowed to air dry in their respective fiberglass cages. Upon thorough drying, the samples were placed in plastic bags and stored in a freezer.

At each collection, transplant, and harvest, a water sample was taken. A field procedure for the collection and concentration of dissolved ions was devised (Appendix A). The concentrating of dissolved ions removed the necessity for immediate laboratory analysis. During each sampling period, a liter of water was concentrated 40 times to 25 ml

with the use of an ion-exchange resin column (Biechler 1965; Riley and Taylor 1968). By concentrating the ions in the water, chemical analysis was made easier and the results were not as biased by the adsorption and desorption of the Cd and Mo on the inner surface of the 30 ml sample container.

Run #1 water samples were analyzed at Lawrence Berkeley Laboratory, California by Lockett. All other samples were analyzed at Colorado Analytical Laboratory, Brighton, Colorado. Atomic absorption spectrophotometric (AAS) flame mode analysis was used. In addition, all Mo analyses used APDC-MIBK organic extraction (Davies 1975).

RESULTS AND DISCUSSION

Test Organisms

It was apparent from the results of the first set of chemical analyses that mayflies, Ephemerella doddsi and E. grandis, could not be grouped with the caddis flies, Brachycentrus brachycentrus. The Cd sorption capacity for the two orders were decidedly different (Tables 1-4). For purposes of calculation, all mayflies (except Cinygmula sp.) were of the genus, Ephemerella, and considered as a group.

Cadmium

The Cd concentration of Coal Creek water above the discharge on Run #1 was 2.6 parts per billion (ppb) and on Run #2, 2.9 ppb (Table 5). The Cd level in the Slate River

TABLE 1
INSECT SAMPLE RESULTS COAL CREEK RUN #1

Invertebrate	Exposure Time Hours*	Concentration ppm	
		Cd	Mo
<u>E. doddsi</u> - Reference Ref.) CC-1		1.13	2.06
<u>E. longisimus</u> - Ref. CC-1		1.34	0.78
<u>E. doddsi</u> L**	22	4.14	3.90
<u>E. doddsi</u> D***	22	2.61	0.38
<u>E. doddsi</u> D	28	4.58	8.60
<u>E. doddsi</u> D	28	3.95	0.69
<u>E. doddsi</u> L	28	5.25	0.17
<u>E. doddsi</u> D	43	3.12	0.30
<u>E. doddsi</u> L	43	8.10	0.27
<u>E. doddsi</u> L	70.5	0.91	0.73
<u>E. doddsi</u> L	70.5	6.16	2.40

*Insects were collected at CC-1 and transplanted to SR-1 in the Slate River for the Slate River for the number of hours indicated. Reference samples were collected and dried immediately with no exposure time in the Slate River.

L** = Live insects

D*** = Dead insects

TABLE 1-Continued

Invertebrate		Exposure Time Hours	Concentration ppm	
			Cd	Mo
<u>B. brachycentrus</u>	- Ref. CC-1		1.58	0.87
<u>B. brachycentrus</u>	D	21.5	2.44	1.50
<u>B. brachycentrus</u>	L	28	3.15	0.19
<u>B. brachycentrus</u>	D	28	3.59	0.89
<u>B. brachycentrus</u>	L	43	3.35	0.88
<u>B. brachycentrus</u>	D	49	3.32	0.85
<u>B. brachycentrus</u>	D	70.5	4.12	3.90
<u>B. brachycentrus</u>	L	70.5	3.06	0.69
<u>Cinygmula</u> sp.	- Ref. CC-1		1.72	1.30
<u>Cinygmula</u> sp.	D	70.5	3.93	2.20

TABLE 2
INSECT SAMPLE RESULTS COAL CREEK RUN #2

Invertebrate	Exposure Time Hours*	Concentration ppm	
		Cd	Mo
<u>E. doddsi</u> & <u>grandis</u> - Ref. CC-2		3.55	1.3
<u>E. doddsi</u> & <u>grandis</u> D**	22.75	5.05	0.9
<u>E. doddsi</u> L***	24	4.80	2.6
<u>E. doddsi</u> L	43	9.40	0.4
<u>E. doddsi</u> L	43	13.20	0.1
<u>E. grandis</u> D	43	11.85	1.7
<u>E. doddsi</u> D	43	5.45	1.6
<u>E. doddsi</u> & <u>grandis</u> D	45.25	7.20	1.2
<u>E. doddsi</u> & <u>grandis</u> D	65.25	8.50	2.5
<u>E. grandis</u> L	65.5	12.50	4.5
<u>E. grandis</u> L	65.5	10.10	1.8

*Insects were collected at CC-2 and transplanted to SR-1 in the Slate River for the number of hours indicated. Reference (Ref.) samples were collected and dried immediately with no exposure time in the Slate River.

** = Dead insects

*** = Live insects

TABLE 2-Continued

Invertebrate		Exposure Time Hours	Concentration ppm	
			Cd	Mo
B. <u>brachycentrus</u>	- Ref. CC-2		2.80	2.0
B. <u>brachycentrus</u>	D	22.75	4.25	0.7
B. <u>brachycentrus</u>	L	24	2.80	1.8
B. <u>brachycentrus</u>	L	43	-	-
B. <u>brachycentrus</u>	D	43	3.40	0.7
B. <u>brachycentrus</u>	D	45.25	3.70	1.6
B. <u>brachycentrus</u>	L	65.5	3.65	1.6
B. <u>brachycentrus</u>	D	65.5	9.10	1.5

TABLE 3

EPHEMERELLA: CADMIUM

Run #1

$\bar{X}$ Hours	$\bar{X}$ Cd ppm	$\bar{X}$ Rate	$\bar{X}$ FCF
Reference ₁	1.13		430
25.6 ₅	4.11	0.11	300
43.0 ₂	5.64	0.11	440
70.5 ₂	7.61	0.09	550

FCF ratio = 1.8

Ratio = 9

Run #2

$\bar{X}$ Hours	$\bar{X}$ Cd ppm	$\bar{X}$ Rate	$\bar{X}$ FCF
Reference ₁	3.55		1200
23.38 ₂	4.93	0.06	460
43.45 ₅	9.42	0.14	790
65.08 ₃	10.37	0.11	890

FCF ratio = 1.9

Ratio = 10

$$\text{Ratio} = \frac{\text{FCF day three}}{\text{FCF day one}}$$

Subscripts = N = number of samples

TABLE 4

TRICHOPTERA: CADMIUM

Run #1

$\bar{X}$ Hours	$\bar{X}$ Cd ppm	$\bar{X}$ Rate	$\bar{X}$ FCF
Reference ₁	1.58		600
25.83 ₃	3.06	0.06	230
46.0 ₂	3.34	0.04	250
70.5 ₂	3.59	0.03	255

FCF ratio = 1.1

N = 7

Run #2

$\bar{X}$ Hours	$\bar{X}$ Cd ppm	$\bar{X}$ Rate	$\bar{X}$ FCF
Reference ₁	2.80		970
23.38 ₂	3.58	0.03	330
44.13 ₂	3.55	0.02	280
65.0 ₂	6.38	0.06	550

FCF ratio = 1.7

N = 6

$$\text{Ratio} = \frac{\text{FCF day three}}{\text{FCF day one}}$$

Subscripts = N = number of samples

TABLE 5

LEVELS OF CD FOUND IN COAL CREEK AND THE SLATE RIVER ON RUN #1 and RUN #2

WATER SAMPLES RUN #1

Sample	Date	Cadmium ppb
CC-1	8 2 79	2.6
SR-1	8 2 79	10.0
SR-1	8 3 79	12.8
SR-1	8 4 79	12.8
SR-1	8 5 79	14.0
$\bar{X}$ for Slate River = 12.4		
SD = 1.69 (Standard Deviation)		
s = 2.0 ppb (sensitivity)		

WATER SAMPLES RUN #2

CC-2	8 10 79	2.9
SR-1	8 9 79	9.65
SR-1	8 10 79	10.65
SR-1	8 11 79	13.65
SR-1	8 12 79	11.65
$\bar{X}$ for Slate River = 11.40		
SD = 1.7		
s = 2.0 ppb		

water was 10.0 ppb initially, and increased to 14.0 ppb during the first run. The second run started with Cd at 9.65 ppb, then increased to 13.65 ppb on day three and dropped to 11.65 ppb on day four (Fig. 4). The mean ($\bar{X}$) Cd concentration in the river for the four days of the first run was 12.4 ppb. The $\bar{X}$ concentration for the second run was 11.4 ppb, a difference of 1.0 ppb.

All data concerning insect uptake of metals is reported in parts per million (ppm). The rate of sorption in ppm per hour was calculated by subtracting the amount of Cd in the reference insects from the transplanted insects and dividing by the number of hours the transplanted insects were exposed in the Slate River.

$$\text{Rate of Sorption} = \frac{(\text{Cd concentration transplanted insect}) - (\text{Cd concentration reference insect})}{\text{Number of hours in Slate River}}$$

The $\bar{X}$ rate of Cd sorption for the Ephemerella spp. in Run #1 was identical with Run #2, 0.1 ppm per hour. The $\bar{X}$ rate of sorption for the Trichoptera in Run #1 was 0.04 and 0.03 for Run #2.

The FCF for the reference mayflies on Run #1 was 430 and Run #2, 1200. The reference caddis flies showed a FCF for Run #1 of 600 and Run #2, 970. Tables 1-4 and Figures 5-7 show a comparison of the FCF and the rate of sorption for both runs.

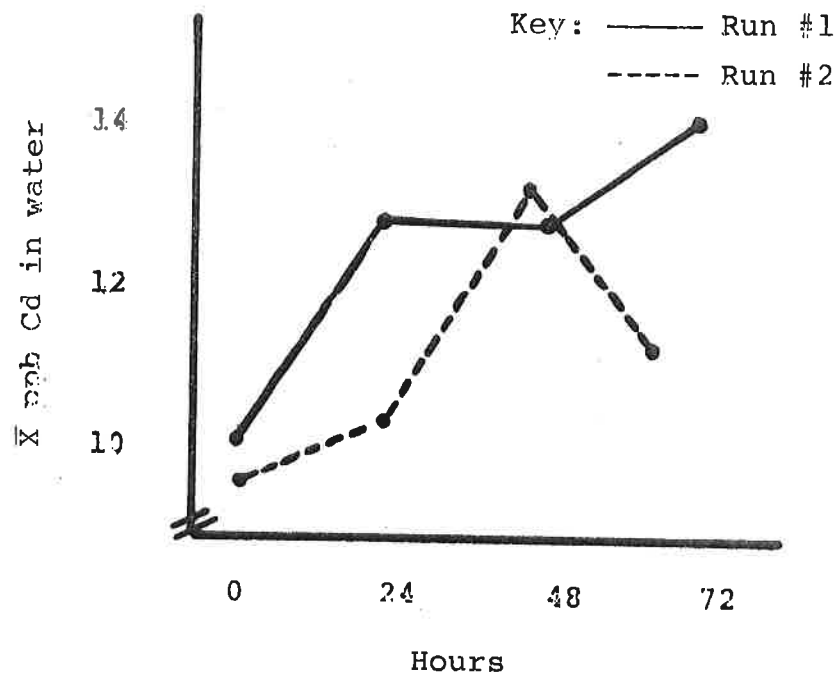


Fig. 4. Levels of Cd in Slate River from zero to 72 hours.

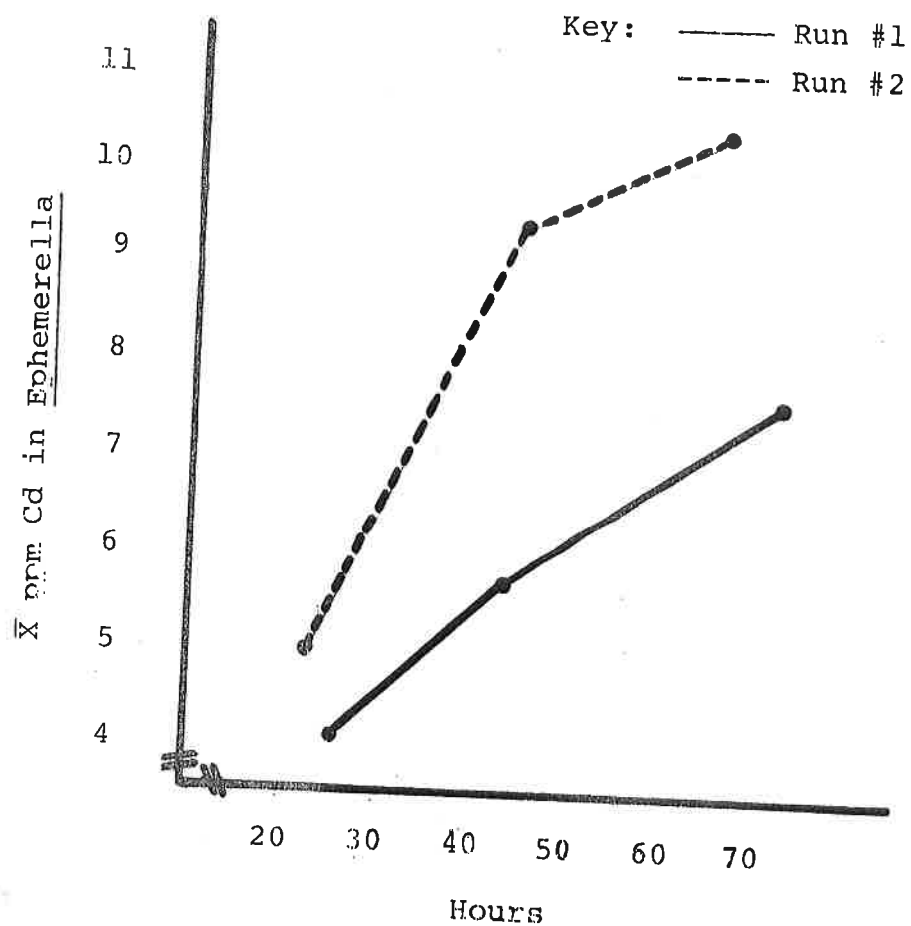


Fig. 5. Levels of Cd in *Ephemerella* from day two to day four after transplant to Slate River.

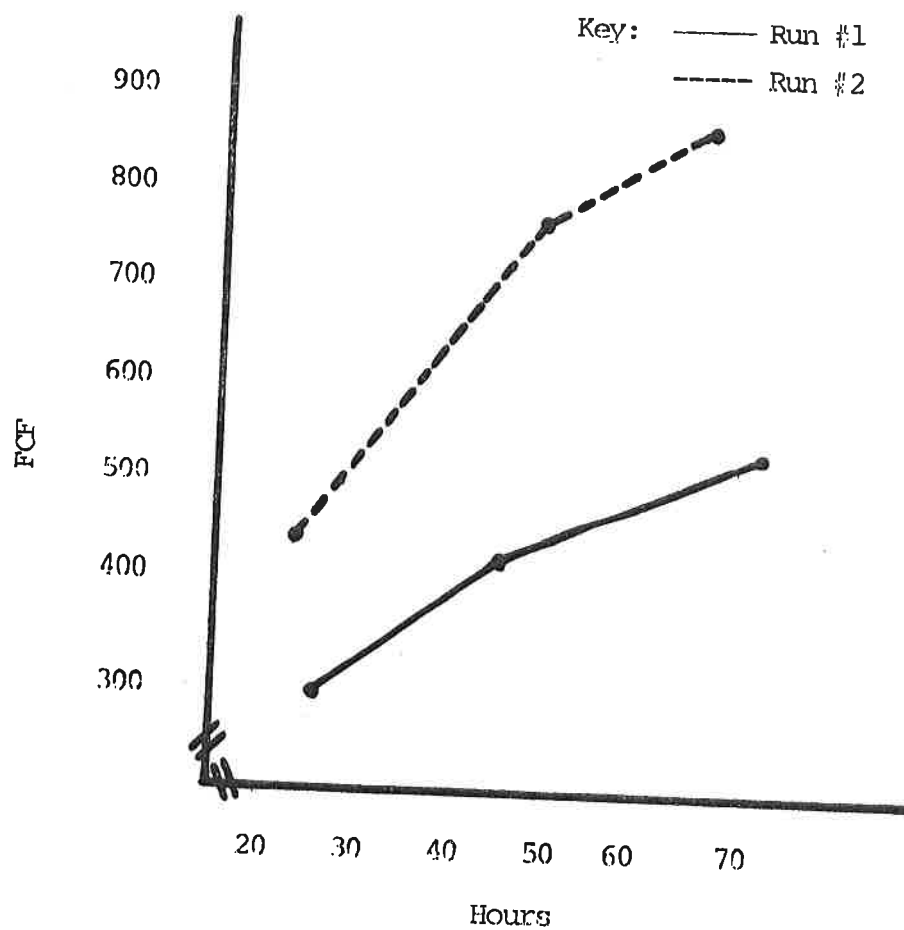


Fig. 6. Field Concentration Factor (FCF) exhibited by Ephemerella from day two to day four after transplant to Slate River.

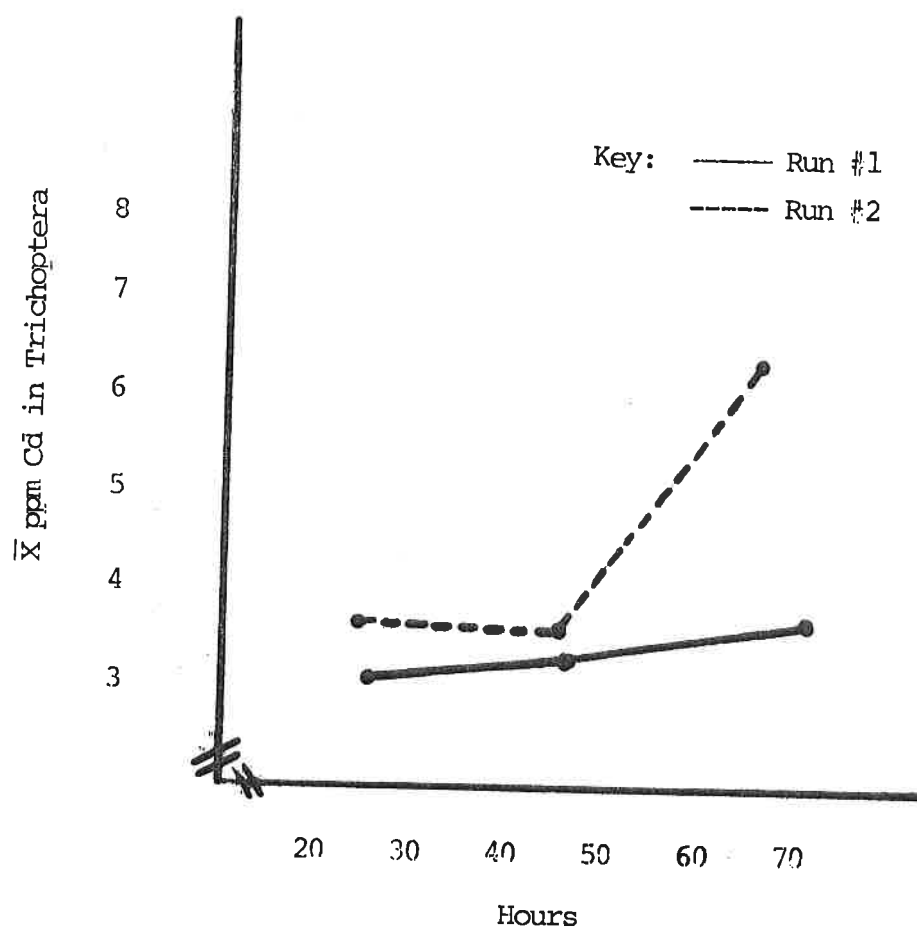


Fig. 7. Levels of Cd in Trichoptera from day two to day four after transplant to Slate River.

A ratio was derived by dividing the FCF after 3 days' exposure in the river by the FCF for one day in the river. A comparison of the live and dead insects (Tables 6, 7) in Run #2 showed that live mayflies sorbed Cd with a $\bar{X}$ FCF for the three days of 760 and exhibited a ratio of 1.9 between day one and three. The dead subjects, with a $\bar{X}$ FCF of 680, had a ratio of 1.9. Run #1 cannot be compared because there were no 72 hour dead Ephemerella samples. The caddis flies in Run #1 had a $\bar{X}$ FCF of 235 for live insects and 250 for dead subjects, with ratios of 0.97 and 1.3 respectively (Tables 8, 9).

Cd levels in the Slate River were similar on both runs (Fig. 4). These results compared favorably with Calgon Laboratory (AMAX 1979) Cd levels for the same stretch of river thus bearing out the presence of Cd in upper Coal Creek where the element had not been reported in the past when using standard methods of analysis.

There were periods of rain storms between the two runs which could account for the lowered level of Cd (9.65 ppb) in the Slate River at the start of Run #2. Storms during the afternoon and night of August 8 in the surrounding mountains could have been the diluting factor on the August 9 Cd level (Fig. 2). There was no precipitation at any of the stations or the Keystone Mine during the time of the experiments. Only the volume of the water in the Slate River was affected.

TABLE 6

A COMPARISON BETWEEN LIVE AND DEAD EPHEMERELLA - RUN #1

LIVE-CADMIUM

$\bar{X}$ hours in Slate River	25.0 ₃	43.0 ₁	70.5 ₂
$\bar{X}$ Cd ppm	4.7	8.1	7.6
$\bar{X}$ FCF	350	650	550
$\bar{X}$ rate ppm/hr	0.14	0.16	0.09

Ratio = 1.6

DEAD-CADMIUM

$\bar{X}$ hours in Slate River	26.0 ₃	43.0 ₁	*
$\bar{X}$ Cd ppm	3.7	3.2	*
$\bar{X}$ FCF	270	250	*
$\bar{X}$ rate ppm/hr	0.10	0.05	*

Ratio = *

$$\text{Ratio} = \frac{\text{FCF day three}}{\text{FCF day one}}$$

Subscript = N = number of samples

* = no sample

TABLE 7

A COMPARISON BETWEEN LIVE AND DEAD EPHEMERELLA - RUN #2

LIVE-CADMIUM

$\bar{X}$ hours in Slate River	24 ₁	43.0 ₂	65.5 ₁
$\bar{X}$ Cd ppm	4.8	11.3	10.1
$\bar{X}$ FCF	450	970	870
$\bar{X}$ rate ppm/hr	0.05	0.18	0.1

Ratio = 1.6

DEAD-CADMIUM

$\bar{X}$ hours in Slate River	22.75 ₁	43.75 ₃	64.88 ₂
$\bar{X}$ Cd ppm	5.05	8.17	10.5
$\bar{X}$ FCF	470	670	900
$\bar{X}$ rate ppm/hr	0.06	0.11	0.11

Ratio = 1.9

$$\text{Ratio} = \frac{\text{FCF day three}}{\text{FCF day one}}$$

Subscript = N = number of samples

TABLE 8

A COMPARISON BETWEEN LIVE AND DEAD TRICHOPTERA - RUN #1

LIVE-CADMIUM

$\bar{X}$ hours in Slate River	28 ₁	43 ₁	70.5 ₁
$\bar{X}$ Cd ppm	3.1	3.35	3.1
$\bar{X}$ FCF	230	260	220
$\bar{X}$ rate ppm/hr	0.06	0.04	0.02

Ratio = 1.0

DEAD-CADMIUM

$\bar{X}$ hours in Slate River	24.75 ₂	49 ₁	70.5 ₁
$\bar{X}$ Cd ppm	3.01	3.32	4.12
$\bar{X}$ FCF	220	240	300
$\bar{X}$ rate ppm/hr	0.06	0.04	0.04

Ratio = 1.4

$$\text{Ratio} = \frac{\text{FCF day three}}{\text{FCF day one}}$$

Subscript = N = number of samples

TABLE 9
A COMPARISON BETWEEN LIVE AND DEAD TRICHOPTERA - RUN #2
LIVE-CADMIUM

$\bar{X}$ hours in Slate River	24 ₁	*	65.5 ₁
$\bar{X}$ Cd ppm	2.8	*	3.65
$\bar{X}$ FCF	260	*	310
$\bar{X}$ rate ppm/hr	0.0	*	0.01

Ratio = 1.2

DEAD-CADMIUM

$\bar{X}$ hours in Slate River	22.75 ₁	44.13 ₂	65.5 ₁
$\bar{X}$ Cd ppm	4.25	3.55	9.1
$\bar{X}$ FCF	400	280	780
$\bar{X}$ rate ppm/hr	0.06	0.02	0.1

Ratio = 2.0

$$\text{Ratio} = \frac{\text{FCF day three}}{\text{FCF day one}}$$

Subscript = N = number of samples

* = no sample

The reference insects from Run #1, which were collected farthest upstream, had the lowest $\bar{X}$ concentrations, 1.13, 1.34, and 1.58 ppm. Run #2, collected 0.4 miles downstream, held $\bar{X}$ concentrations of 3.55 and 2.8 ppm. More sampling needs to be done farther downstream to see if the trend continues.

The insects reflected a decrease in concentration rate at the same time the Cd level in the water decreased (Fig. 4,5). It is not possible to establish a curve or pattern for the rate of sorption from the results of this study. Every set of insects showed a decrease in sorption as the 72nd hour approached, except for the caddis flies in Run #2. In this run, one dead caddis fly sample showed an extremely high sorption level at 65.5 hours.

The Trichoptera did not sorb as readily as the Ephemeroptera. Within the mayfly order, E. grandis was the heaviest concentrator (Tables 1,2). Nehring (1980) stated that he obtained the same results with E. grandis. E. grandis is a crawler that is readily found on the underside of rocks and in the substrate of streams. Its close association with the detritus and sediments in the water could account for the higher level of Cd it exhibited compared to the caddis flies, or other mayflies. E. doddsi, which showed less Cd concentration, is found on large cobble on the upstream areas of riffles.

Molybdenum

No Mo was found in the water on either run. Reference insects from Coal Creek on Run #1 showed 1.2 ppm Mo in the mayflies and 1.3 ppm in the caddis flies (Table 1). In Run #2 the mayflies showed 1.3 ppm and the caddis flies, 2.0 ppm (Table 2). Tables 10 and 11 show breakdowns of Mo levels for the two orders of insects over the three day period.

Although no Mo was found in either set of water samples, Mo was found in measureable amounts in all but one insect sample. This was a 43 hour transplant in Run #2 (Table 10), which coincides with the drop in Mo on the second day on both runs (Fig. 8, 9: Table 10) and then started to increase up to 70 hours. However, with the rest of the insects, as the rate of sorption of Cd peaked, at 43 hours, the Mo content of the insect dropped. As the rate of sorption of the Cd slowed down, the Mo content in the insect began to increase (Fig. 10). This phenomenon needs to be further explored.

It is realized that Mo may be an essential trace element in the insects as it is in man, and the level in the ^{reference} control insects represents an intrinsic form. However, the increase after two days in the river allows one to speculate that there is more Mo in the river than in Coal Creek.

Despite limited sampling, the following conclusions are consistent with the data:

1. Aquatic insects are capable of concentrating

TABLE 10

A COMPARISON OF MOLYBDENUM LEVELS IN TWO INSECT FAMILIES

<u>Ephemera</u>		Trichoptera			
	$\bar{X}$ hours in Slate River	$\bar{X}$ Mo ppm		$\bar{X}$ hours in Slate River	$\bar{X}$ Mo ppm
Run #1	25.6 ₅	2.75		25.8 ₃	0.86
	43.0 ₂	0.57		46.0 ₂	0.87
	70.5 ₂	1.57		70.5 ₂	2.3
		$\bar{X} = 1.63$			$\bar{X} = 1.34$
Run #2	23.4 ₂	1.75		23.4 ₂	1.25
	43.5 ₂	1.0		44.1 ₃	1.15
	65.1 ₃	2.93		65.4 ₂	1.55
		$\bar{X} = 1.89$			$\bar{X} = 1.32$

Subscript = N = number of samples

TABLE 11
A COMPARISON OF MO LEVELS IN INSECTS
LIVE VS DEAD

	<u>EPHEMERELLA</u>		<u>TRICHOPTERA</u>	
	$\bar{X}$ hours in Slate River	$\bar{X}$ Mo ppm	$\bar{X}$ hours in Slate River	$\bar{X}$ Mo ppm
Run #1 Live	25 ₂	2.04	28 ₁	0.19
	43 ₁	0.27	43 ₁	0.88
	70.5 ₂	1.57	70.5 ₁	0.69
	$\bar{X} = 1.29$		$\bar{X} = 0.59$	
Run #1 Dead	26 ₃	3.22	24.75 ₂	1.20
	43 ₁	0.30	49 ₁	0.85
	*	*	70.5 ₁	3.90
	$\bar{X} = 1.76$		$\bar{X} = 1.98$	
Run #2 Live	24 ₁	2.60	24 ₁	1.80
	43 ₂	0.25	*	*
	65.5 ₁	1.80	65.5 ₁	1.60
	$\bar{X} = 1.55$		$\bar{X} = 1.70$	
Run #2 Dead	22.75 ₁	0.90	22.75 ₁	0.70
	43.75 ₃	1.50	44.13 ₂	1.15
	64.9 ₂	3.50	65.25 ₁	1.50
	$\bar{X} = 1.97$		$\bar{X} = 1.12$	

Subscript = N = number of samples
* = no samples

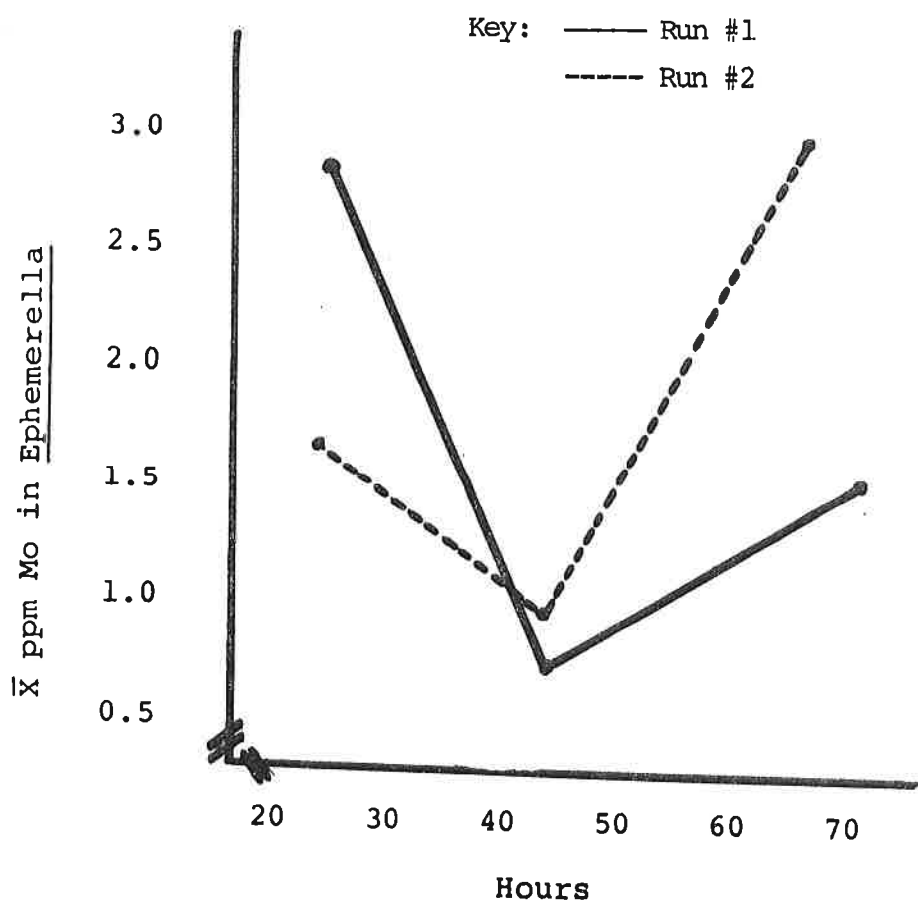


Fig. 8. Levels of Mo in Ephemerella from day two to day four after transplant to Slate River.

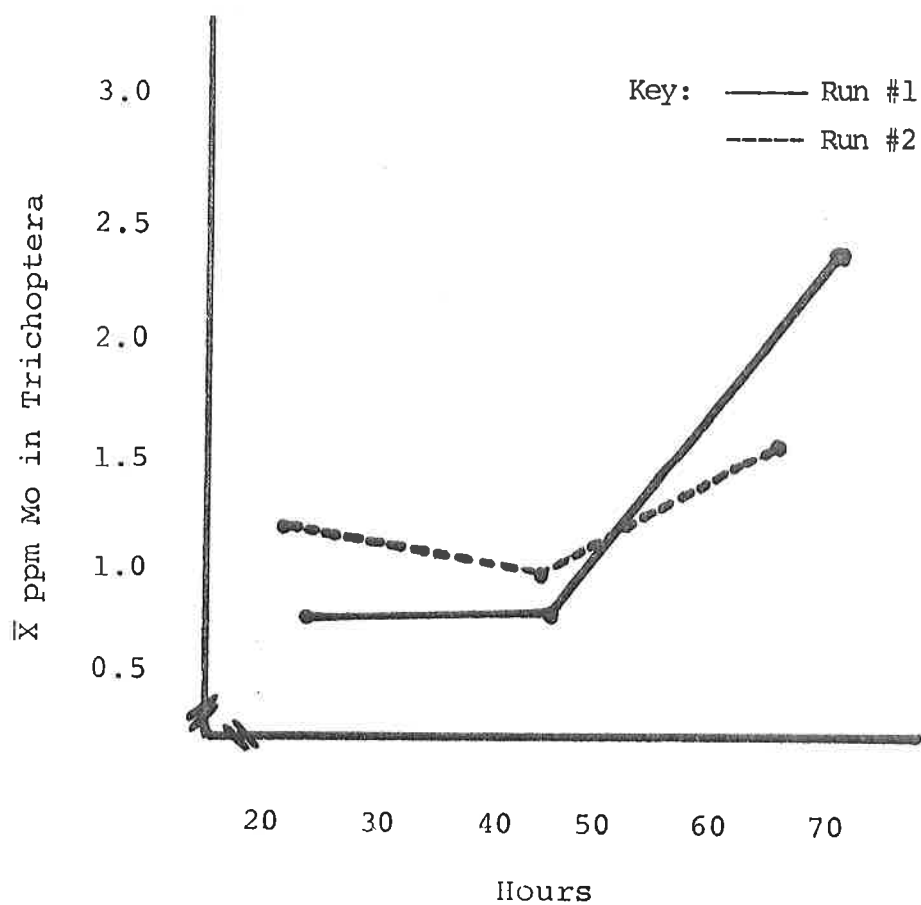


Fig. 9. Levels of Mo in Trichoptera from day two to day four after transplant to Slate River.

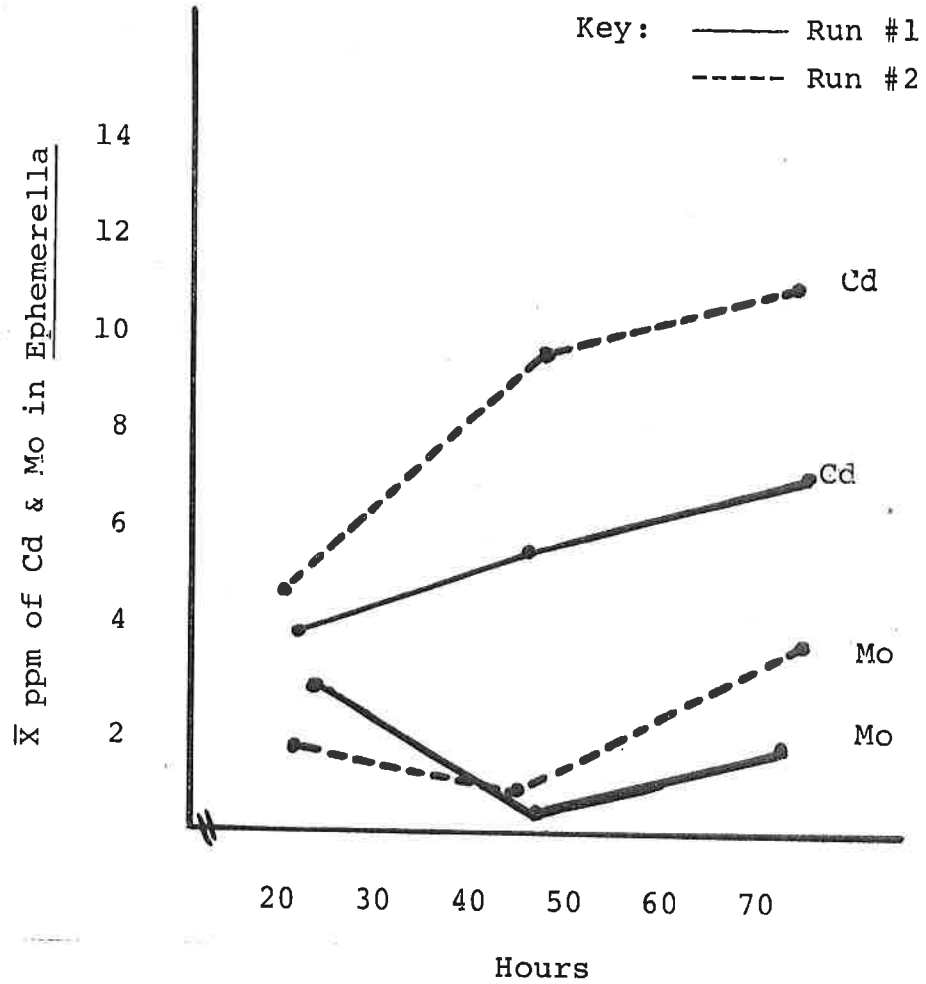


Fig. 10. A comparison of Cd and Mo concentrations in Slate River transplants of mayflies.

Cd and Mo even when the concentration of the metals in the water is very low. For instance, when the level of Mo in the water is below detection after concentrating the ions in the water 40X, the insects still exhibit a measurable uptake of the Mo.

2. The insects appear to sorb the Cd at a predictable rate.

3. Of all the species used in this study, E. grandis exhibits the highest uptake.

4. Mayflies concentrate Cd and Mo from the surrounding water more than caddis flies.

5. Levels of Cd in both live and dead insects are similar enough to suggest that the uptake is not a physiological process.

6. The dynamics of Cd and Mo within the system are different.

7. If equilibration between water and insect concentration of Cd takes place, it is after 72 hours.

8. Although no Mo was found in the water samples (concentrated 40X), the dynamics of Mo within the system are observable in the insect, providing a method for monitoring low levels of elements in aquatic systems.

9. The most important information extracted from this research is the fact that, with only one water sample at each collection, transplant, and harvest, and very few multiple insect samples, a useful relationship was found between insect element level, and water element level.

V PART II: LABORATORY EXPERIMENTATION

The results from Part I (1979) yielded encouragement to pursue further work in establishing the FCF Method for water analysis. It was imperative that more testing be done to establish equilibration and release times, and to prove through geographical sampling that there was a constant relationship between specific species and exposure to specific metals. Consequently, flow-through temporal studies were undertaken to determine insect sorption and desorption rates and thus establish equilibration time. If sorption were too slow, the method would not register the short-term changes in concentration necessary to monitor the pulses and declines of a metal in a stream. Conversely, if depuration were too fast, short-term variations in concentration would obscure important longer-term trends. Since the literature did not contain sufficient information to permit the selection of experimental parameters, a pilot study was first performed to determine approximately the range of values of the independent variables involved: in this case, time and concentration of Cd.

PILOT STUDY

The results in Chapter IV show that the insects equilibrate sometime after 72 hours. Nehring et al. (1976)

mention that equilibration takes place at approximately 96 hours with lead, zinc, copper, and silver. However, from previous data there was little to indicate how long insects hold a metal once they accumulate it. From the results of a pilot study, the discrete collection times for the flow-through study could be fixed. Not only would a pilot run provide insight into release rates, but it could alleviate any problems that might occur during a ten or 12 day flow-through experiment. It was important to know what would be physically possible for an individual researcher to handle on an around-the-clock study; i.e., how many concentrated water samples could be taken and resin columns regenerated in one day; were the tanks efficiently designed to run for ten days consecutively; and what equipment might be needed as back-up in case of breakdown.

To this point, in this research, all insect samples were comprised of multiples of the larger species available in Coal Creek. Nehring (1980) advised using large single insects per sample for in-vitro studies. At least ten individual insects per sampling would be adequate for statistical analysis and to determine the standard deviation between individuals. All research was designed to be tested against an alpha level of 5%. Work done by Eddy et al. (1974) showed that small insects within a genus will often show the highest FCF. Consequently, in

the pilot study, three insects in one sample and one large individual in a second sample were used for comparison.

The pilot run was designed as a partial static study. Insects were placed in a tank treated with one dose of Cd. The only other addition of Cd to the tank than the initial specific quantity was to replace Cd removed when taking a liter of water for analysis. At the end of 72 hours, the insects were removed from the Cd treated water and placed in a tank of fresh water with a flow-through rate of 19 liters per hour.

Methods (Pilot Study)

Insects (Pteronarcella badia) were collected from the Gunnison River at GR-1 (Fig. 11). They were placed in a holding tank with rocks for cover and willow leaves for food. All tanks used in the study held 75 liters of water and were fitted with 2 x 58 cm air wands and three air stones. City of Gunnison charcoal filtered water was used. Five days later, a control sample of three small and one large P. badia were taken for analysis (see method described page 30). At the same time 20 P. badia were placed in a tank with water containing 100 ppb Cd. At the end of 72 hours, a control sample of four insects was taken from the tank containing Cd and the rest of the insects were placed in a fresh water tank with a flow-through rate of 19 liters per hour. After six hours in the fresh water, one large and three small insects were collected for

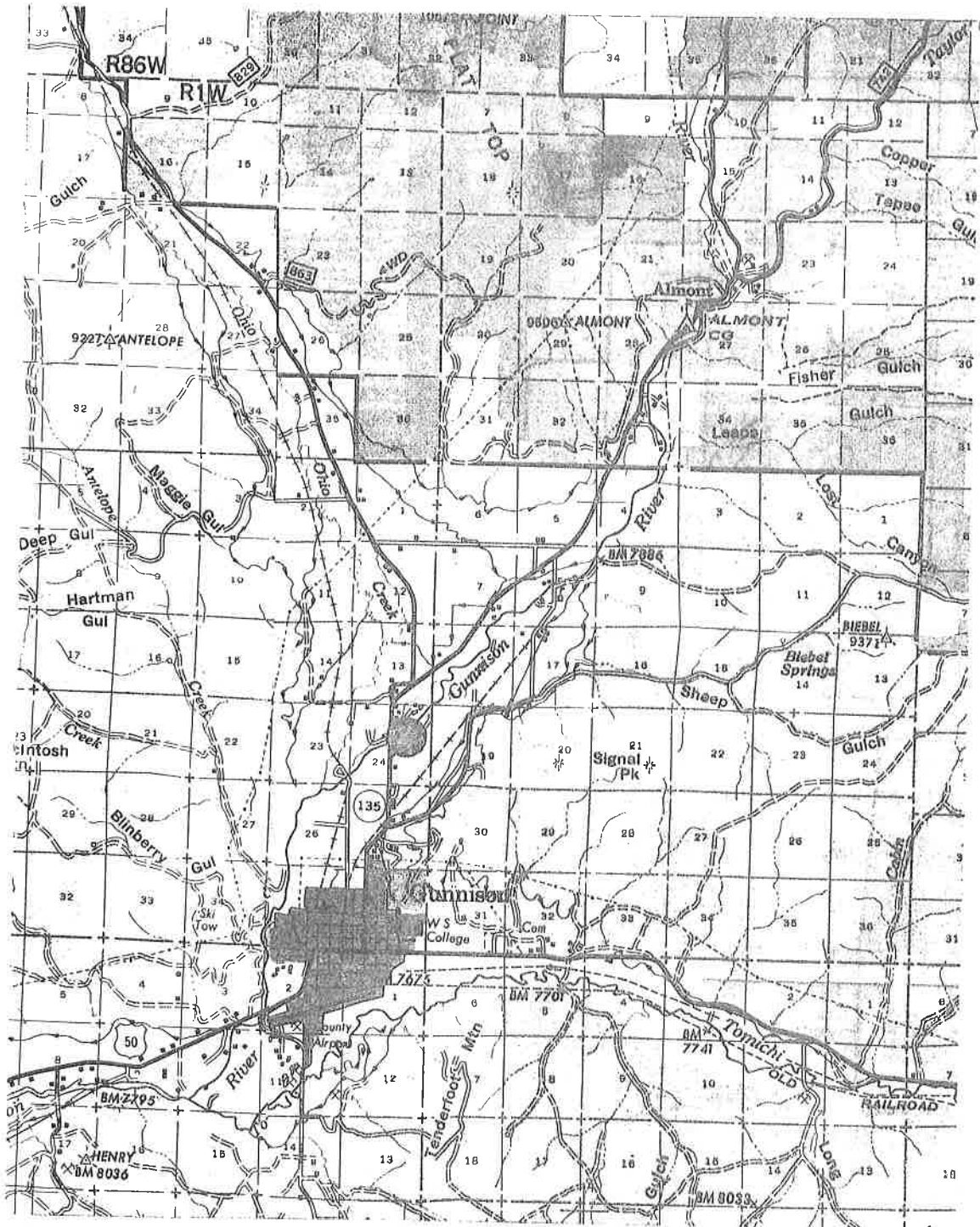


Fig. 11. Location of GR-1 on U.S. Forest Service map, Gunnison National Forest, Gunnison County, Colorado.

analysis. A similar collection was made at 48 hours. At each transplant and cropping, a water sample was collected (Appendix A).

Results (Pilot Study)

The multiple insect samples sorbed more Cd than the single larger insect (Fig. 12). The multiple insect sample released 24% of the Cd in six hours and the single insect lost 28% in the same amount of time. At the end of 48 hours, the multiple sample had lost 62% and the single 53%. Extending the curve in Fig. 12 (dashed lines) one can project a release of the recently sorbed Cd by 72 hours.

Water level of Cd in the exposure tank at the time the insects were introduced was 97.6 ppb (Table 12). After transplant to fresh water, at the end of 72 hours there was no measurable Cd in the fresh water. However, the insects were still exhibiting elevated levels of Cd at that time. This will be discussed at the end of the chapter, since the phenomenon also occurred in the flow-through study.

FLOW-THROUGH STUDY DESIGN

After 48 hours exposure to one dose of Cd at approximately 100 ppb, the P. badia began to act sluggish and were turning slightly white which may have been caused by bacterial decomposition forming a gelatinous film around each one. Since the insects in the flow-through study would be exposed to Cd-water for longer intervals, a

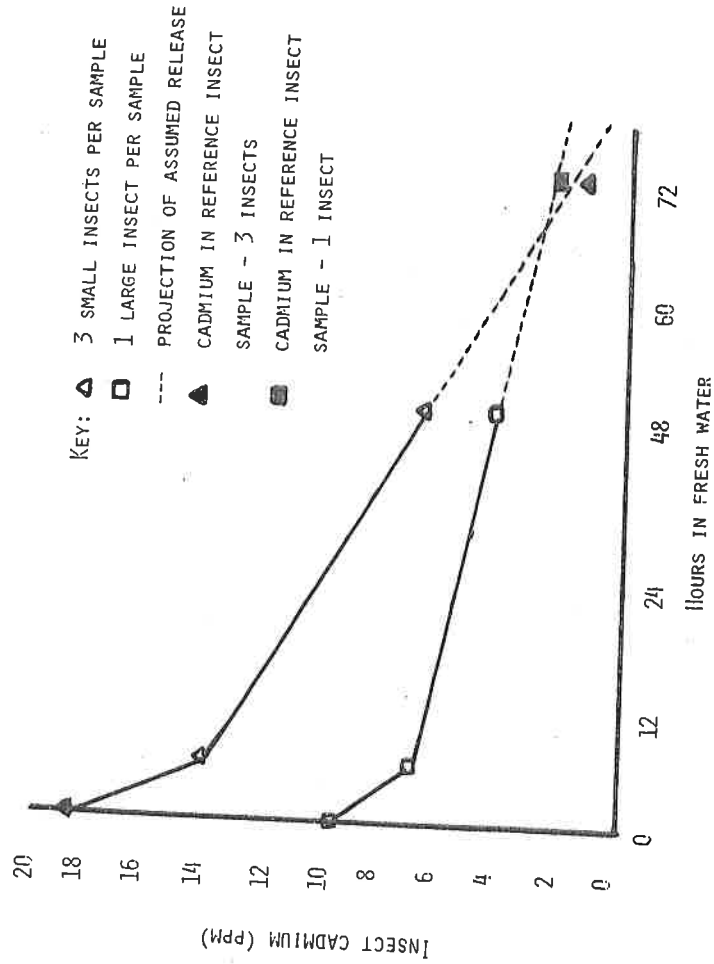


Fig. 12. Levels of Cd in insects after transplant to fresh water in pilot run. Insects had been exposed to one dose of 97.4 ppb Cd for 72 hours previous to transplant.

TABLE 12
LEVELS OF CD IN WATER (PPB) AND INSECTS (PPM) DURING PILOT STUDY

Time	Water Cd	Single Insect Cd	Triple Insect Cd	Mean Insect Cd
Reference Sample	0.0	2.46	1.83	2.15
Zero hour (one dose Cd)	97.4			
72 hours after exposure to one dose Cd	0.3	9.74	18.78	14.36
6 hours after transplant to fresh water	0.0	7.13	14.22	10.68
48 hours after transplant to fresh water	0.1	4.68	7.11	5.90

lower level of 25 ppb Cd was chosen for the flow-through experiment. Cd is often found in the Slate River at this level (USEPA 1971-1977).

The largest single P. badia weighed 82.2 mg after drying to constant weight. A larger dried sample weight of 100 mg or more was desirable to facilitate AAS analysis. Pteronarcys californica, a larger stonefly than P. badia, was used for the flow-through study. To confirm the results from Part I (1979) that both live and dead insects sorb at the same rate, air-dried (dead) insects were introduced in the study.

Methods

Subjects

P. californica were collected on the Colorado River, Parschal, Colorado using a 1/4 inch seine stretched across the river as the substrate upstream was disturbed. Naiads were transplanted in Colorado Division of Wildlife oxygen bombs, charged with 15 pounds of oxygen, and packed in snow. Insects were placed in 75 liter holding tanks with rocks providing natural habitat conditions. Willow leaves and fresh budding willow branches were provided for food. Subjects had a minimum of 30 days to acclimate to laboratory conditions in the holding tanks before being exposed to experimentation. All subjects were female and over 2 cm long.

Apparatus and Procedure

Five glass tanks, 30 x 30 x 76 cm, were maintained with a constant flow of water by means of a siphon system. Charcoal filtered City of Gunnison water was used with an exchange rate of 19 liters per hour. Each tank was equipped with two 58 cm air wands and three standard air stones to maintain a high dissolved oxygen (DO) level. DO varied from 7.0 to 7.2 ppm. Ambient light from a west facing wall of windows provided nearly normal light conditions. Room temperature was maintained at 15.5° C or less with water at 13° C $\pm$ 1. pH was 7.7 $\pm$.3 and the specific conductivity was 460 umhos.

To maintain a constant flow of Cd at 25 ppb through the exposure tank, a manually operated diluting system was designed. Two 75 liter tanks were elevated 15 cm above the exposure tank. Water was allowed to enter one of the two raised tanks. When this was full, stock solution of CdCl₂·2½H₂O was added to the water to achieve 25 ppb. This tank was then siphoned into the exposure tank which was being relieved of water at the same rate by means of siphons. While these two tanks were functioning together, the third tank was being filled with water. When the third tank was full, Cd was added, etc. The procedure was kept in progress around the clock. Each tank was outfitted with the usual air wands to prevent stagnation, effect mixing, simulate stream flow, and maintain a high DO.

Insects were taken from the holding tank and placed in the exposure tank containing Cd (25 ppb). In three daily transplants, they were removed from the Cd water and placed in fresh water, and later harvested from the fresh water. Air-dried (dead) insects were introduced at the beginning and were to be harvested at each time interval along with the live insects. Because of putrefaction, only one sample of dead insects exposed to Cd for 72 hours and a second sample exposed to Cd for 72-hours followed by transplant to fresh water for 24 hours were used. All other dead subjects were removed from the tanks (Fig. 13).

Insects were taken from the tanks with a dip net washed between each use with deionized distilled water. They were placed in a fiberglass screen cage and rinsed with 10 ml deionized distilled water, air dried, and stored in plastic ziplock bags.

All water samples were taken according to the procedure outlined in Appendix A. Additional insects (20% more than number needed) were introduced in the beginning and available throughout testing in case of attrition, i.e.; death, emergence, moulting, and cannibalism. All samples were stored in a freezer until the final sample was collected. At that time they were sent to Colorado Analytical Laboratory for AAS analysis.

Insect Transplant Steps

1. Stoneflies (190) exceeding 2 cm in length were removed from the holding tank. Ten insects were air-dried

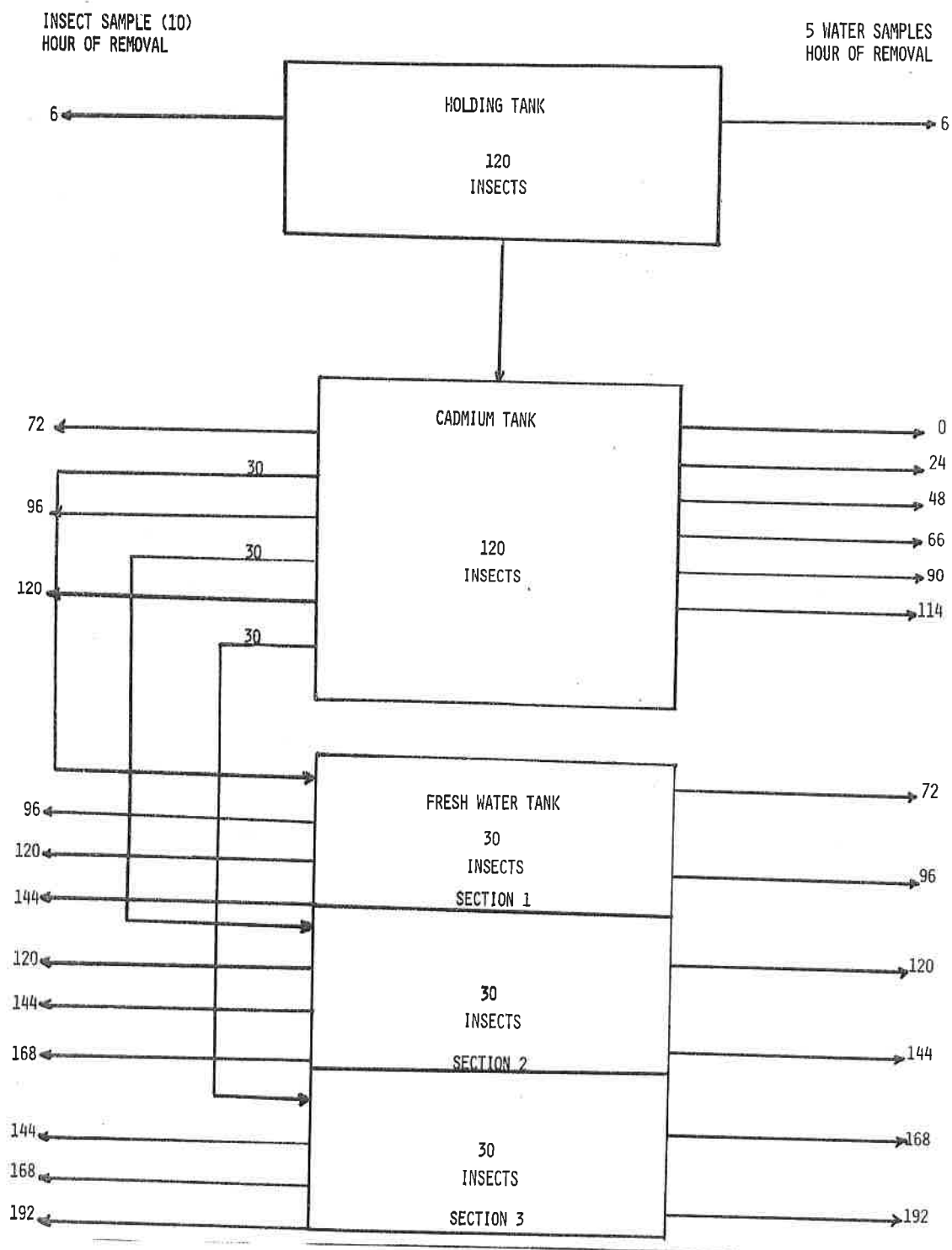


Fig. 13. Simplified flow chart for flow-through temporal experimentation.

and used as a control. At the same time, 120 live insects and 60 air-dried insects were placed in a tank holding water containing 25 ppb of Cd.

2. At the end of 72 hours, 40 insects were removed from the tank containing Cd. Ten insects were air-dried, bagged, and refrigerated. Thirty insects were placed in section 1 of a tank supplied with fresh water. This tank was partitioned into three equal sections with fiberglass screening. Twenty of the dead insects were removed from the Cd tank, ten placed in this tank in section 1 and ten held as a control.

3. At the end of 96 hours, 40 insects were removed from the Cd tank, ten were air-dried and 30 were placed in section 2 of the above mentioned tank.

4. At the end of 120 hours, the last 40 live insects were removed from the Cd tank. Ten were air-dried and 30 were placed in section 3 of the fresh water tank.

To eliminate handling as much as possible, the insects to be used as "dead" subjects were removed from the handling tank and placed in fiberglass screen cages in groups of ten individuals. The insects were air-dried in these cages and then transplanted from tank to tank in the separate cages. From the time they were selected from the holding tank, they were not touched again until they were removed from the cages and individually placed in ziplock plastic bags or discarded because of putrefaction.

Insect Cropping Steps

1. Ten insects in section 1 of the untreated tank were cropped at the end of 24 hours and air-dried and bagged. At this time, ten dead insects were collected and bagged and all other dead subjects were removed from the tanks.

2. Ten insects in section 1 along with ten insects in section 2 were cropped at the end of 48 hours, air-dried, and bagged.

3. The last 10 insects in section 1, along with ten insects in sections 2 and 3, were cropped, air-dried and bagged at the end of 72 hours.

4. The last ten insects in section 2 and ten insects in section 3 were cropped at the end of 96 hours, air-dried and bagged.

5. The last ten live insects in section 3 were cropped at the end of 120 hours, air-dried and bagged.

Water Sample Steps

Five water samples were taken at each of the above steps as follows:

1. A control sample was taken from the holding tank before 190 insects were removed.

2. A Cd tank sample was taken just before the insects were introduced.

3. A Cd tank sample was taken at 24, 48, 72, 96, and 120 hours.

4. A fresh water tank sample was taken just before introduction of the first insects at 72 hours.

5. A fresh water sample was taken at the end of 96, 120, 144, 168, and 192 hours.

Results and Discussion

Moulting

P. californica were brought in from the Colorado River on April 27, 1980. During their period of acclimation, on May 20, 1980, they began to moult and emerge. Up until this time, an ambient air temperature of 10° C was maintained in the laboratory. However, several days before the metamorphosis, temperatures outside the building exceeded 10° C and the room temperature could no longer be held constant. Fraley (1979) noted that increased stream temperatures caused insects to emerge earlier than usual. Since many of the insects continued to moult throughout the experiment, it seemed prudent to collect exuviae and moultees if emergence occurred near a discrete time when other insects were being transplanted or cropped. Two such samples were collected and analyzed for Cd content along with the rest of the subjects. The moultees exhibited low levels of Cd similar to those found in the control insects, whereas the exuviae bore the bulk of the sorbed Cd. The freshly emerged moultees assayed for 2.3 and 3.0 ppm Cd and the control insects had a mean level of 1.9 ppm.

Consequently, when individuals in the sample of ten insects held 3.6 ppm Cd or less, it was assumed they had just moulted and their value was dropped, reducing the N to 9 or less. In each case where this was done, the results are subscripted with the N value (Table 13).

The exuviae held a higher concentration of Cd than was exhibited by the whole insect exposed to the same level of Cd for the same amount of time. The insect retained the majority of her weight as she shed the exuviae, and the exuviae removed the largest part of the Cd upon separation. Using the total amount of Cd in both moult and cast skin and totaling the weights, in each instance, the concentration of Cd was within the $\bar{X}$, plus or minus the SD of the sample insects harvested at the same time (See appendix B).

Equilibration

One of the goals of the laboratory investigation was to establish at what time equilibration is reached between Cd in the water and Cd in the insect. Unfortunately, the sorption rate did not approach zero because the level of Cd in the water of the exposure tank did not stabilize until 72 hours (Fig. 14). It would have been no great problem to keep the insects in the exposure tank until they had been exposed to five consecutive days of Cd at 25 ppb. Cropping and

TABLE 13

CD LEVEL IN INSECTS THROUGHOUT FLOW-THROUGH STUDY

Exposure Hours Insect Sample		Mean Cd ppm	SD
Exposure Cd/Exposure Fresh			
0	Control	1.9	0.5
72		24.8 ₇	6.5
72	Dead	28.3	6.4
72/24		11.7 ₈	4.5
72/24	Dead	46.2	55.7
72/48		14.6	11.8
72/72		28.9 ₉	20.9
96		47.4	26.4
96/24		25.0 ₉	10.7
96/48		13.8	5.8
96/72		33.8	28.2
120		111.0	93.4
120/24		42.4 ₉	32.6
120/48		14.5 ₉	6.4
120/72		9.7	5.6

Subscript = N = number of samples

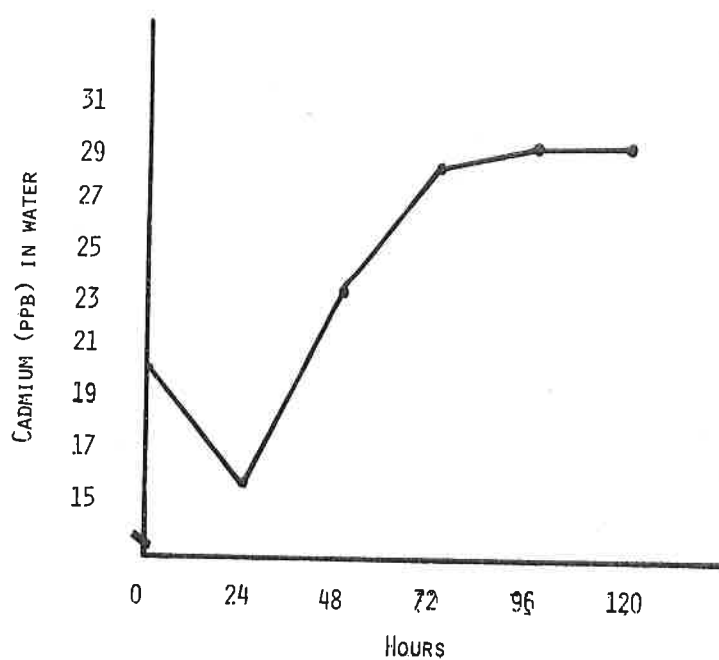


Fig. 14. Levels of Cd in the water in the exposure tank of the flow-through temporal study.

transplanting commenced on day three as planned since the lack of stabilization of Cd was not determined.

The drop in Cd from time zero to 24 hours reflects a competition which could be attributed to the hoses and tanks binding some of the Cd, plus the sorption afforded by the 270 insects that were being exposed in the tank. This is a classic example of what can happen when close monitoring of a system, in this case, access to immediate AAS analysis, is not available. Davies (1976), in an attempt to understand the complexation of Cd with humic substances found a similar situation. He noticed a Cd concentration drop in Cd treated water that he later "... attributed to adsorption of Cd on the walls of the glass cell used in the analysis."

Sorption

Fig. 15 is not a true sorption curve for Cd on P. californica. The dashed line extending from 72 hours is a projection of what might have occurred if water levels of Cd had been maintained at 25 ppb from zero hour. Considering the Cd levels in the exposure tank did not level off until the 72nd hour, this might be a truer curve than the one depicted by the data collected. By running more tests under similar conditions and collecting samples at closer intervals, one should be able to define the exact curve of sorption. The work must be done with an AAS at immediate disposal

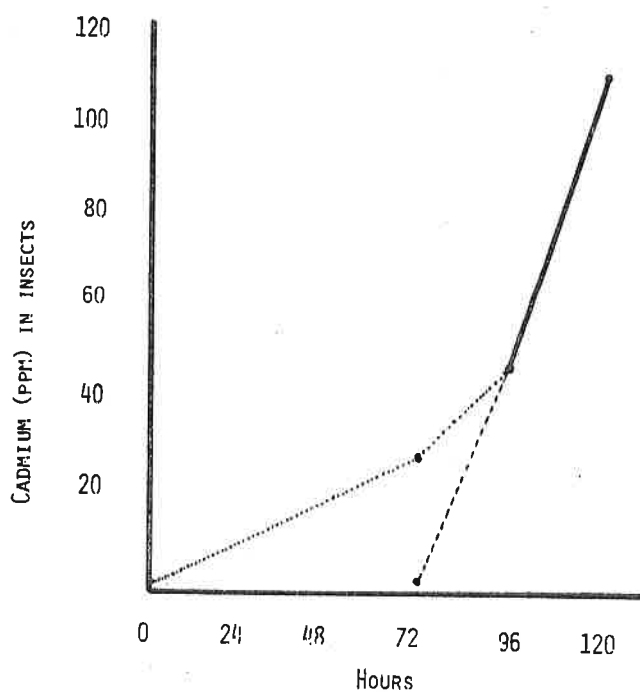


Fig. 15. Levels of Cd in insects exposed to a mean level of 25.07 ppb Cd from zero to 120 hours. Dashed line --- represents what might have occurred if insects had been introduced at 72 hours when Cd water levels stabilized. Dotted line ... indicates the time period when Cd water levels fluctuated.

and with the help of an assistant so that the testing can continue around the clock with samples being collected on an hourly basis until a steady-state or equilibrium is reached.

Desorption

In an effort to economize, three fresh water tanks were condensed into one tank with two water penetrable partitions creating three sections in the one tank. At the time, the design seemed efficient and labor saving. Because of the shifting of Cd between the different insect groups, simple desorption never occurred in the tank (Appendix D). Instead, data shows the insect-Cd-water shifting toward a steady state. A similar result to that of the pilot study was reproduced when the insects assayed for high levels of Cd, yet little or no Cd was found in the water.

The most interesting result indicated by Fig. 16 is the terminally upswinging curve of the 72 and 96 hour transplants after being placed in the fresh water flow-through system. The 72-hour live sample appears to have lost Cd when placed in the fresh water tank, and at that time it reversed its loss and started to take up more Cd. When the 120-hour sample was introduced, the 72-hour sample appeared to take up more Cd, eventually holding more Cd than it held after 72 hours in the exposure tank. The 96-hour transplant followed the same pattern, and appeared to never exceed the level of Cd with which it started. The above data were analyzed using

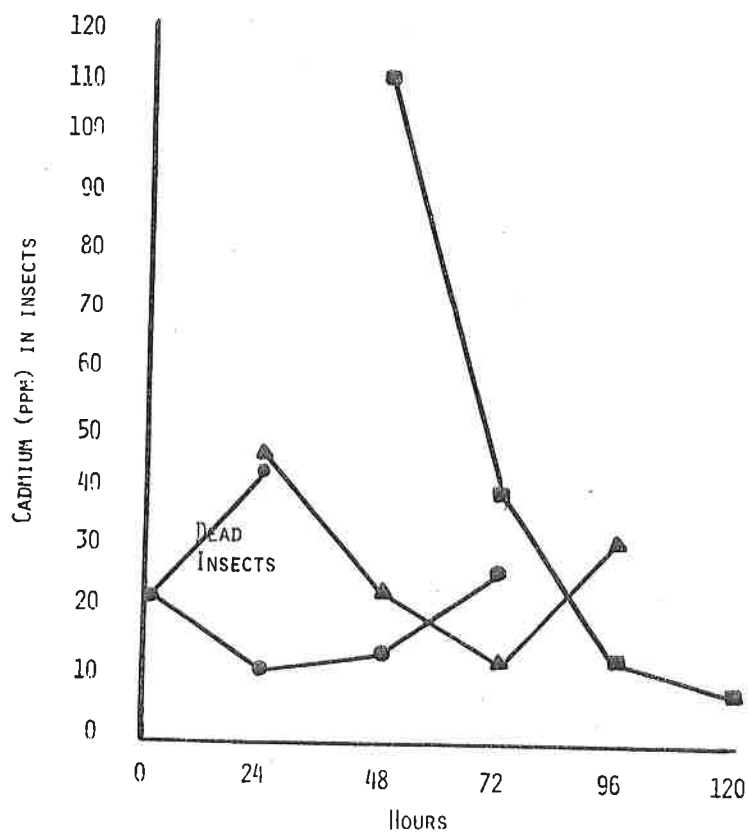


Fig. 16. Levels of Cd in live insects after being transplanted to fresh water. ● = insects exposed to Cd for 72 hours. ▲ = insects exposed to Cd for 96 hours. ■ = insects exposed to Cd for 120 hours.

a two way analysis of variance and these differences were found to be significant. However, with Scheffe's Test, significance was not shown (Appendix D). At the end of the study the 120-hour sample exhibited less Cd at each sample interval, which would be expected because there was nothing added to the tank to provide it with more Cd. It was at this point that significance was borne out by Scheffe's Test.

As soon as a new batch of insects was introduced into the fresh water tank, it appears as though a new exchange process commenced with the Cd ions causing insects holding low levels of Cd to start collecting Cd from insects holding more. This hypothesis assumes that within samples, individual insects were shifting the more loosely bound Cd back and forth between each other. With an exchange of 19 liters per hour of fresh water, Cd was slowly being depleted from the system. However, it is apparent that the exoskeleton of the insect has a much stronger attractive force over Cd than the water.

Gardiner (1974b) noted that the more active binding sites for Cd will be used first, and as the more active sites are filled, the less active sites will be used. Often, differences in complexation and steric hindrances will prevent strong binding. Perhaps, as the unused more active binding sites on the surface of the insects with low Cd concentration become exposed to insects with higher

Cd concentrations, the more loosely bound Cd on the newly introduced insects shifted.

Dynamics of Cd in the System

By plotting the $\bar{X}$ values of Cd in the insects at each sample interval, the Cd loading of the fresh water system was approximated. The fresh water system started with zero ug Cd until 30 live and 30 dead insects were introduced, containing a total of 77.8 ug Cd. At the end of the 24 hours, 29% of the Cd was flushed out of the insects. The live insects lost 34% Cd, while the dead insects lost 16%. At the end of the run, when there was no insect competition between samples, the release was 43% (Fig. 17). The maximum amount of Cd, 335 ug, occurred at 48 hours upon introduction of the 120-hour Cd-exposed insects which brought 294 ug Cd to the system. After 24 hours, larval Cd decreased 56%. This loss exhibited at 72 hours can be attributed to the following dynamics: (1) the constant flow of fresh water was drawing Cd out of the system, which at this time held the most insects, (2) the exoskeletons of the two previously introduced insect samples containing less Cd provided new exchanges which kept Cd moving in the water, (3) and perhaps part of the Cd bound to the heavier-laden 120-hour Cd-exposed exoskeletons was not bound as firmly. The rapid rate of desorption from the heavier Cd-laden insects may reflect the higher rate of sorption as the insects reached the higher concentration at 120

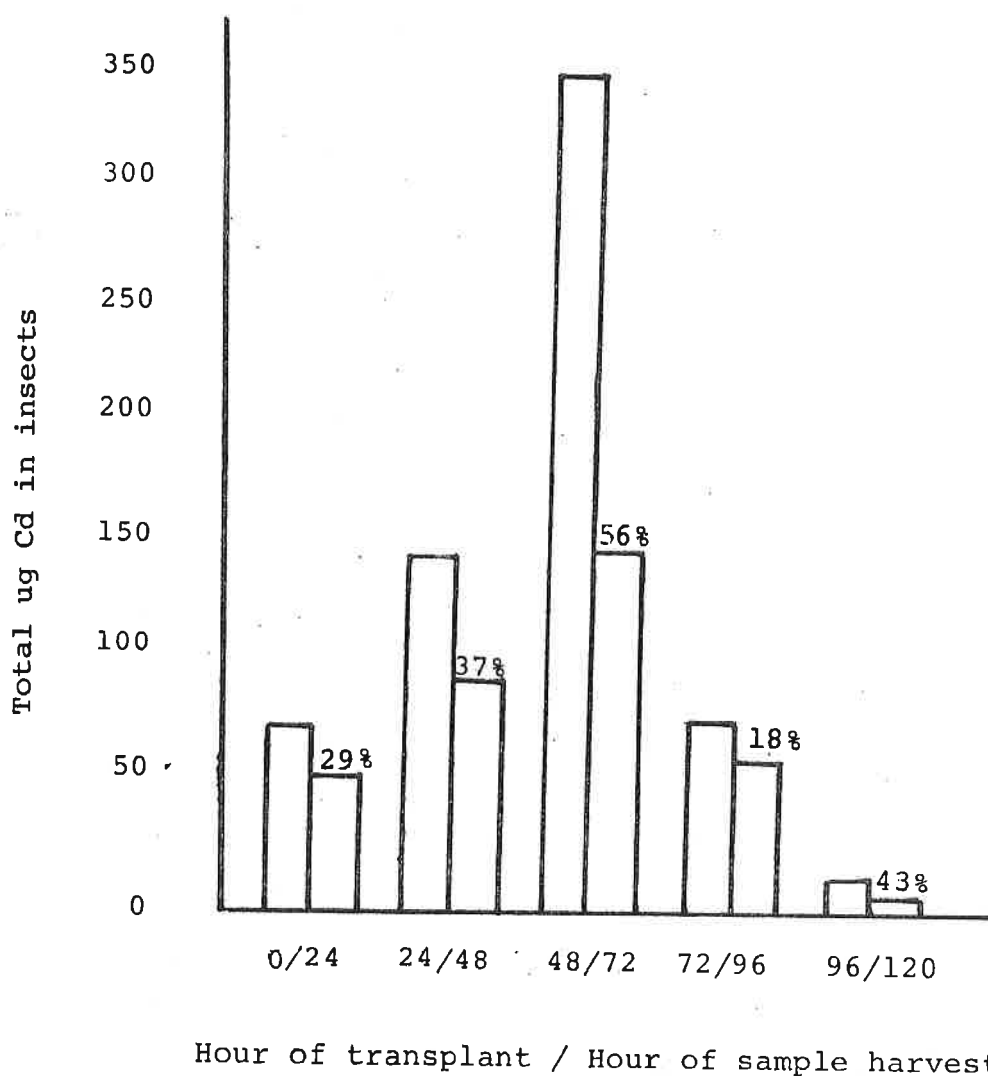


Fig. 17. Total ug Cd in fresh water system as 72, 96 and 120 hour Cd-exposed insects were added and harvested from fresh water tank. Percentages represent total ug Cd lost at each time interval. Insects exposed to Cd 72 hours were introduced at 0/ and cropped at /24, /48 and /72. Insects exposed to Cd 96 hours were introduced at 24/ and cropped at /48, /72 and /96. Insects exposed to Cd 120 hours were introduced at 48/ and cropped at /72, /96 and /120.

hours (Table 13). As the rate of binding becomes more rapid, the binding appears to be less permanent.

There are two kinds of adsorption: physical adsorption and chemisorption (Alberty and Daniels 1979). The concentration of a solute is the force causing physical adsorption. In the case of exoskeletal Cd, physical adsorption can be reversed by lowering the concentration of Cd in the water. By comparison, chemisorption requires the formation of chemical bonds. The bonding can be tight or loose. Chemisorption cannot be reversed readily by exposing the exoskeletal Cd to Cd-free water.

Since the exoskeleton is porous (Borrer et al. 1976; Andersen 1979), a phenomenon called hysteresis may take place when Cd is sorbed. If the pore entrance is narrower than the inside of the pore, it takes a higher concentration of Cd in the water to enter the pore than is required for release of the Cd. Because of the porous nature of the exoskeleton, there must be some entrapment of Cd. From all the data collected thus far, it appears that depuration time is shorter than accumulation time. This suggests that hysteresis does occur during the uptake and release of the Cd. In the case of the exoskeleton, there could be a combination of physical adsorption (ion-exchange) with a small amount of chemisorption (complexation). This is borne out by the pilot study where the greatest loss of Cd was during the first six hours after transplant to fresh water (Fig. 12), and the rates of release as

demonstrated in the flow-through study (Fig. 16) by the 120-hour Cd-exposed insects.

It has been shown that the exoskeleton and exuviae provide a very attractive site for Cd to settle out of solution. If, as Sharp (1970) says, insects moult to remove waste materials, perhaps the insects in the Cd laden stretches of rivers are moulting more often than their counterparts living under less Cd stress.

It is yet to be determined if insects can develop a true Cd tolerance, or instead, moult more often to compensate for the increased exoskeletal Cd burden. Perhaps, a self defeating situation is materializing where too much demand is being placed upon a natural biological system capable of recycling undesirable material. The increased Cd loading from anthropogenic sources may be placing too much stress on the insects with resultant depletions in their populations.

A Comparison of Live and Dead Insects

The level of Cd in live insects exposed 72 hours in water with a mean Cd level of 25 ppb (Table 13) was 24.8 ppm (SD = 6.5) and dead insects exposed the same amount of time held 28.3 ppm (SD = 6.36). Using a t-test for unrelated samples with different N values, there is no significant difference between the sorption of Cd by live and dead insects ($t = 1.104$, $df = 15$, $p > 0.05$). This implies that the accumulation of the Cd by the insect is

through bioconcentration or ion-exchange (Macek et al. 1979). If there is any sorption because of a dietary or physiological process, it is so insignificant, it has no effect on the FCF. This is not a food chain accumulation (biomagnification). It is nothing more than the direct partitioning of the element, Cd, between the insect, the water, and solids in the surrounding ecosystem, i.e., humic acid in the substrate of a stream; or, as in the lab, tank walls and siphon hoses.

A comparable t-test could not be carried out for desorption because the one sample of dead insects held for 24 hours in fresh water showed too much tissue degradation. The $\bar{X}$ weight of the dead insects after 24 hours exposure in the fresh water tank was 53% of the $\bar{X}$ weight of the live insects under the same circumstances. As bacterial decay began to break down the fleshy tissue in the dead insects, the concentration of Cd in the dead subjects increased. The body tissue actually sloughed away, leaving the hollow exoskeleton holding the Cd. The anomalous increase in Cd concentration in the dead subjects was accompanied by a decrease in total Cd burden. The concentration increased 1.6 times, whereas, the actual total amount of Cd dropped 3.2 ug.

Toxic Effects

After the insects were exposed to Cd 72 hours ($\bar{X}$ water level of Cd = 20.21 ppb) they had difficulty moulting.

Similar observations were made by Clubb et al. (1975). They described insects in this predicament as "...ecologically dead, since the ability to molt or emerge was inhibited". However, after Cd-exposed insects were placed in the fresh water tank for 48 hours, they were able to effect a moult again. Clubb et al. (1975) reported a similar recovery after placing Cd-exposed insects into fresh water. During the period of time when the insects could not moult, they crawled around the tanks with their exoskeletons attached along the abdomen or legs. Many times, just the head emerged through the exoskeleton. All of these individuals eventually died. A condition similar to this occurred in the Slate River (Summer 1980) where Cinygmula sp. were unable to moult or emerge. The heads of Cinygmula spp. were exposed, projecting dorsally through the prothoracic exoskeleton and flexed backward. There was no separation through the head and abdomen from which to emerge. The level of Cd in the Slate River at that time was 9.5 ppb.

It should be noted that after placing my arms in the Cd exposure tank for two days they became very irritated. A distinct line of demarcation where the water level ended was visible. The skin looked and felt like it was "chemically burned," probably from the Cd binding with the organic sulfur compounds ordinarily found in epidermal tissue. To prevent further skin damage, shoulder length obstetrical gloves were used when siphons had to be shifted in the Cd tank.

VI PART III: FIELD APPLICATION OF THE FCF METHOD

The original plan for this part of the research included a broad geographical sampling of rivers and streams in Colorado for Cd and Mo to prove the practicality of the FCF Method. Later, for several reasons, the sampling was restricted to the East River - Upper Gunnison River drainage (Fig. 18). The gasoline shortage, increased cost of travel, and the extra time required for long distance travel were considered. However, the primary reason for confining the geographical study to Gunnison County was a human health concern. Levels of Cd in lower Coal Creek and the Slate River exceeded safe levels for human consumption (10 ppb). If the levels of Cd in upper Coal Creek and Slate River (Table 5) are plotted against hardness on the graphs (Fig. 19, 20), the concentrations fall far beyond the limits for safe concentrations of Cd for fresh water aquatic life to either chronic or acute exposure. Consequently, efforts focused on the East River - Upper Gunnison River drainage. A practical application of the FCF Method could be tested in Gunnison County and perhaps the conclusions reached would assist local planners in water resource management decisions.

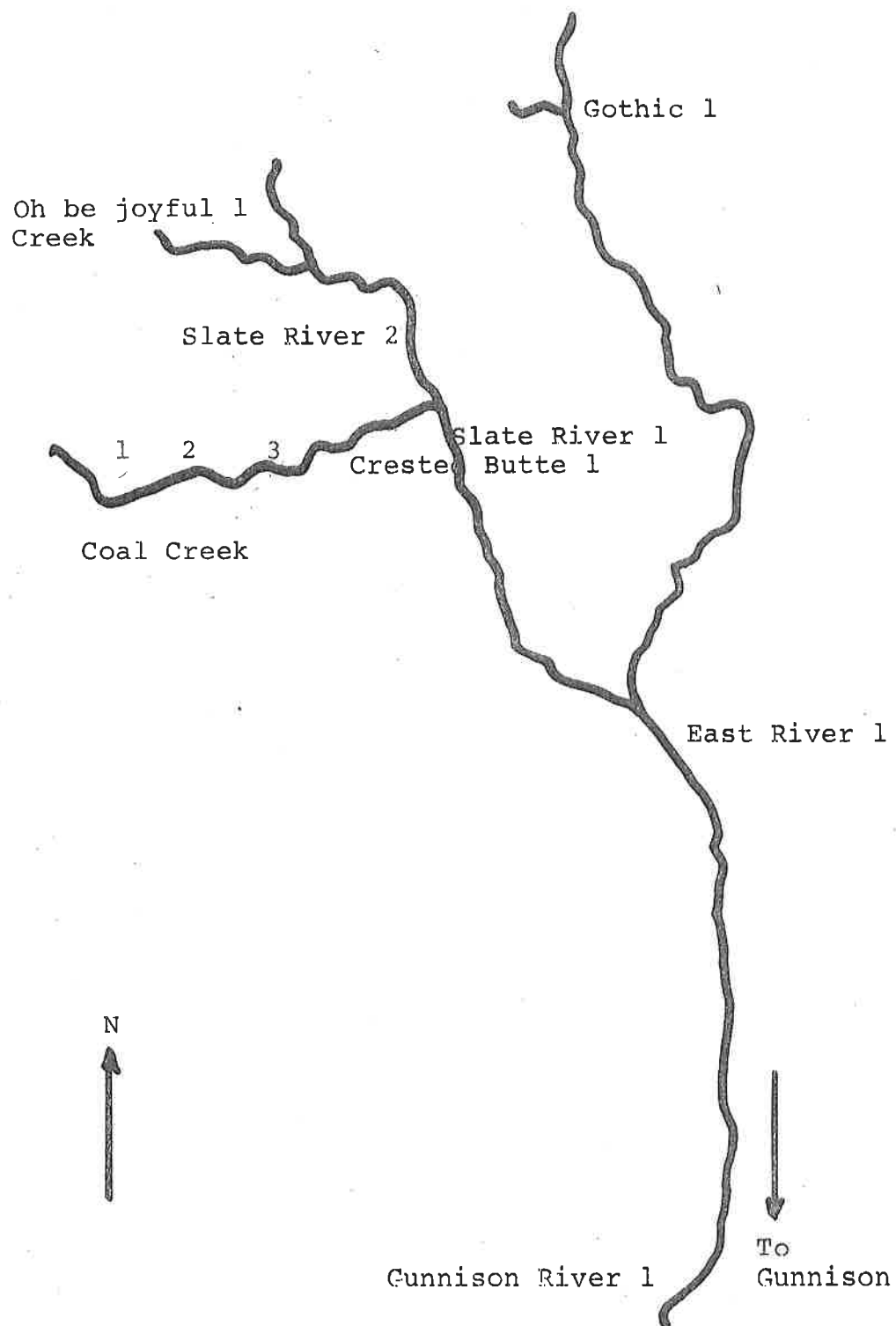


Fig. 18. Nine sampling stations on the East River - Upper Gunnison River drainage.

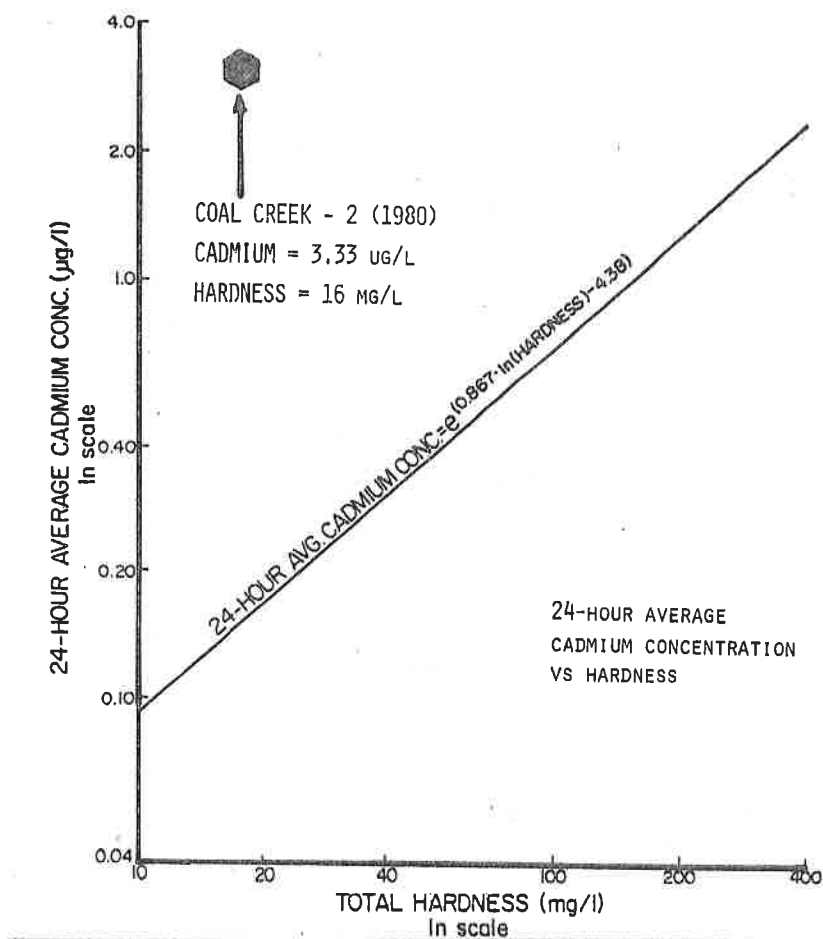


Fig. 19. EPA graph (modified) showing relationship of Cd at CC-2, 1980, to criterion "to protect fresh water aquatic life ... as a 24-hour average".

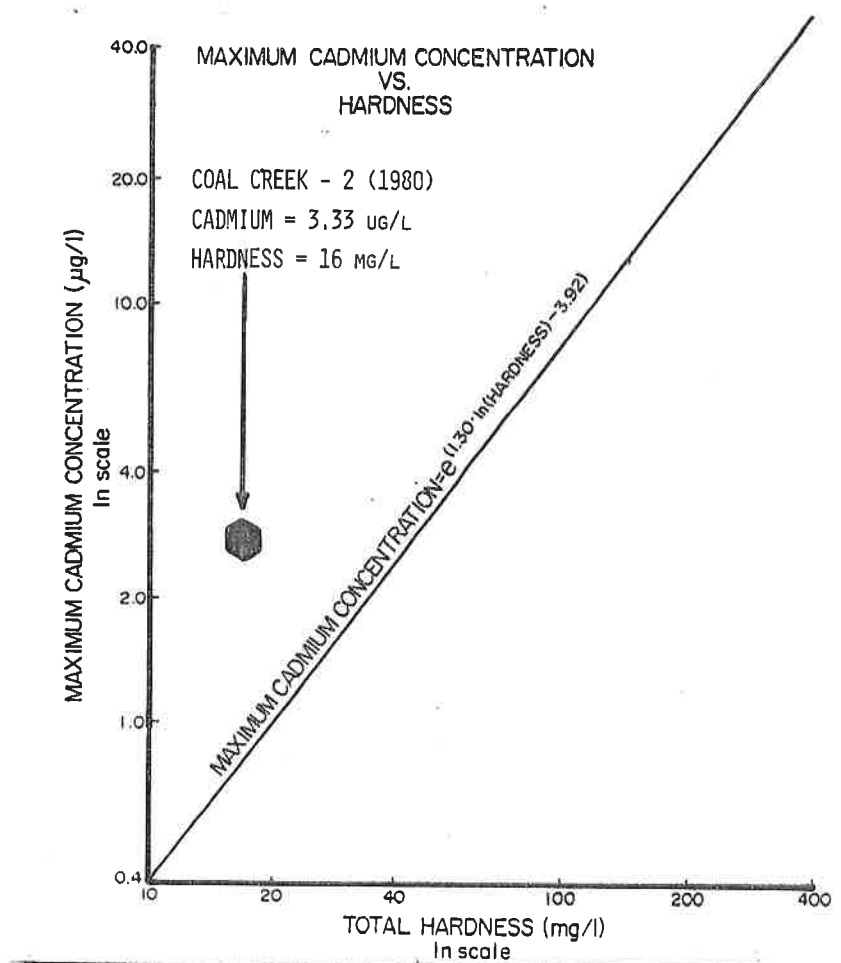


Fig. 20. EPA graph showing relationship of Cd at CC-2, 1980, to criterion "to protect fresh water aquatic life ... at maximum Cd concentration".

METHODS

Nine stations were selected in the East River - Upper Gunnison River drainage (Fig. 18; Table 14). Five water samples were collected at each station (Appendix A). At the same time, aquatic insects were collected, air-dried, stored in plastic bags and kept in a freezer until analyzed. Enough insects to provide a dried weight of 100 mg per sample were collected. In every instance, the more common larger larvae were used. Water samples and insects were sent to Colorado Analytical Laboratory, Brighton, Colorado for atomic absorption spectrophotometric analysis.

Station Selection

Gothic-1 (G-1) (Fig. 21)

In an attempt to find the actual background levels of Cd and Mo in the waters of Gunnison County, water at altitudes high enough to be above human activity were tested. The summer community at Rocky Mountain Biological Laboratory (RMBL), Gothic, Colorado gets its water from the base of Gothic Mountain. The water flows from a 3848 m granite outcropping. The slope is very steep, heavily grown over with willow (Salix spp.), scattered with talus rock screes, and the scene of many avalanches every winter. There has been no mining or timbering near the source of the water. Perhaps an occasional stray cow or wild animal might create a disturbance. Because of the steep slope, it has no human disturbance other than hikers seeking a

TABLE 14

Station	Description	Insect Samples *	Sampling Date	pH
Gothic-1	T 12 S; R 86 W; on a stream	10 M. <u>signata</u>	7 18 80	7.75
G-1	west to east directly be- tween NE 1/4 and SE 1/4 of NE 1/4 of Sect. 4. Elevation: 3505m	" " " " "		
Oh be joyful-1	T 13 S; R 87 W; NE 1/4 of	30 E. <u>doddsi</u>	8 12 80	-
OBJ-1	SE 1/4 of Sect. 24. Unsurveyed.	27 " 33 "		
	Elevation: 2926m	23 " 23 "		
		36 <u>Hydropsyche</u>		
Slate River-2	T 13 S; R 86 W; NE 1/4 of SW 1/4 of Sect. 34. Elevation: 2682m	40 <u>Cinygmula</u> sp. " " "	8 29 80	7.0
		8 E. <u>doddsi</u>		

* Number of insects per sample

TABLE 14-Continued

Station	Description	Insect Samples	Sampling Date	pH
Slate River-2 cont.		9 E. <u>doddsi</u>		
Coal Creek-1	T 14 S; R 87 W; SW 1/4 of NE 1/4 of Sect. 7.	10 E. <u>doddsi</u>	7 21 80	6.62
CC-1	Unsurveyed.	" "		
	Elevation: 2956m	" "		
		" "		
Coal Creek-2	T 14 S; R 87 W; between the NE 1/4 and SE 1/4 of NE 1/4 of Sect. 7.	13 E. <u>grandis</u>		
CC-2	Unsurveyed.	12 E. <u>doddsi</u>	7 26 80	7.0
	Elevation: 2915m	18 "		
		12 "		
		17 "		
		15 "		
Coal Creek-3	T 14 S; R 86 W; SE 1/4 of NE 1/4 of Sect. 5.	19 E. <u>grandis</u>		
	Elevation: 2820m	10 E. <u>grandis</u>	8 2 80	7.3
		" "		
		" "		

TABLE 14-Continued

Station	Description	Insect Samples	Sampling Date	pH
Coal Creek-3 cont.		10 <u>E. grandis</u>		
		" "		
		9 <u>Hydropsyche</u>		
		25 <u>E. grandis</u>		
East River-1	T 14 S; R 85 W; center	35 <u>Hydropsyche</u>	7 28 80	7.3
	point of SW 1/4 of Sect.	47 "		
	27.	52 "		
	Elevation: 2602m	46 "		
		38 "		
		7 <u>P. badia</u>		
		7 "		
		5 "		
		8 "		
		10 "		
		21 <u>E. grandis</u>		
		12 "		

TABLE 14-Continued

Station	Description	Insect Samples	Sampling Date	pH
Gunnison River-1	T 50 N; R 1 W; center	11 <u>P. badia</u>	7 24 80	7.68
GR-1	point of Sect. 24. Elevation: 2365m	8 "		
		8 "		
		9 "		
		8 "		
		8 "		
		16 <u>Hydropsyche</u>		
		16 "		
		27 "		

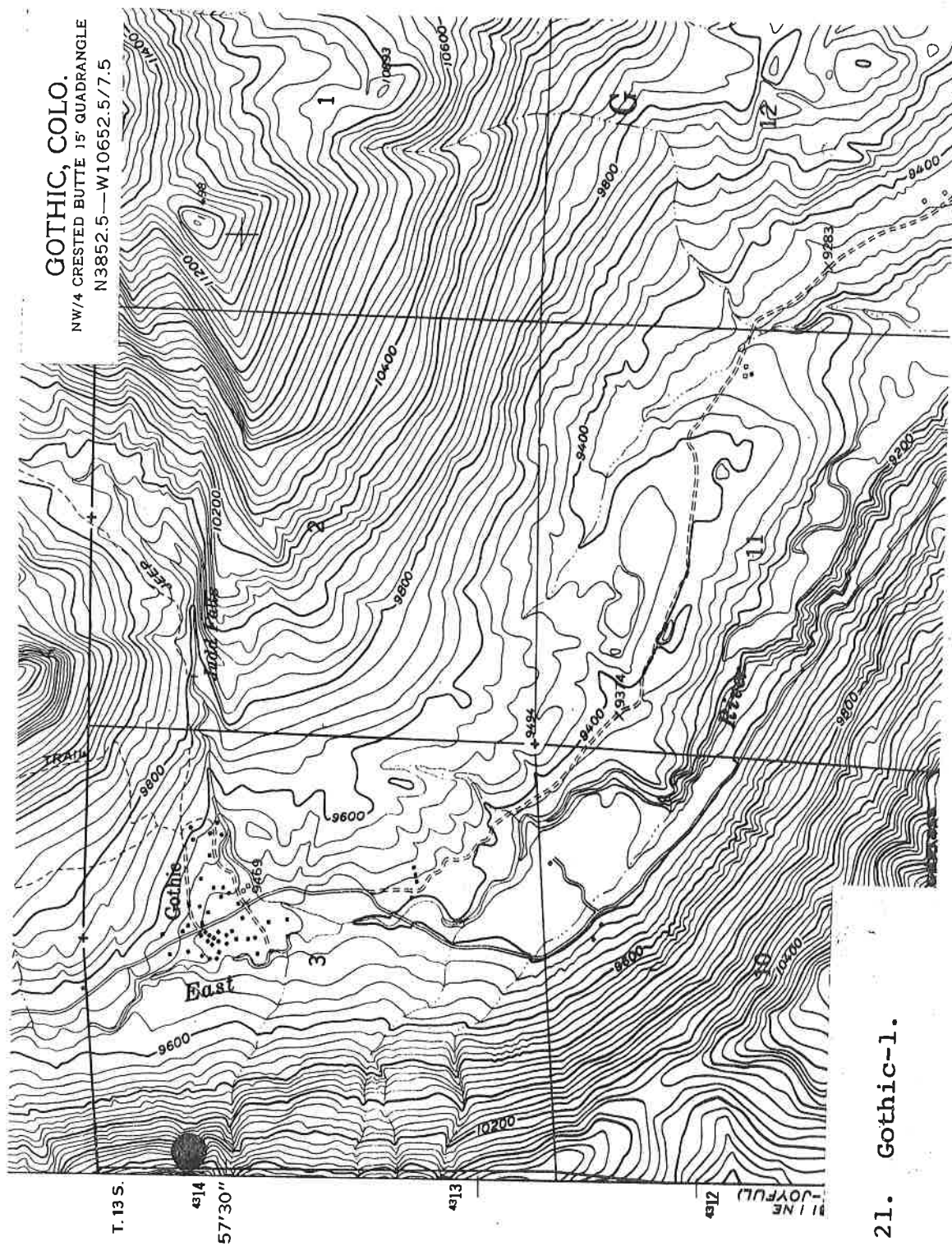


Fig. 21. Gothic-1.

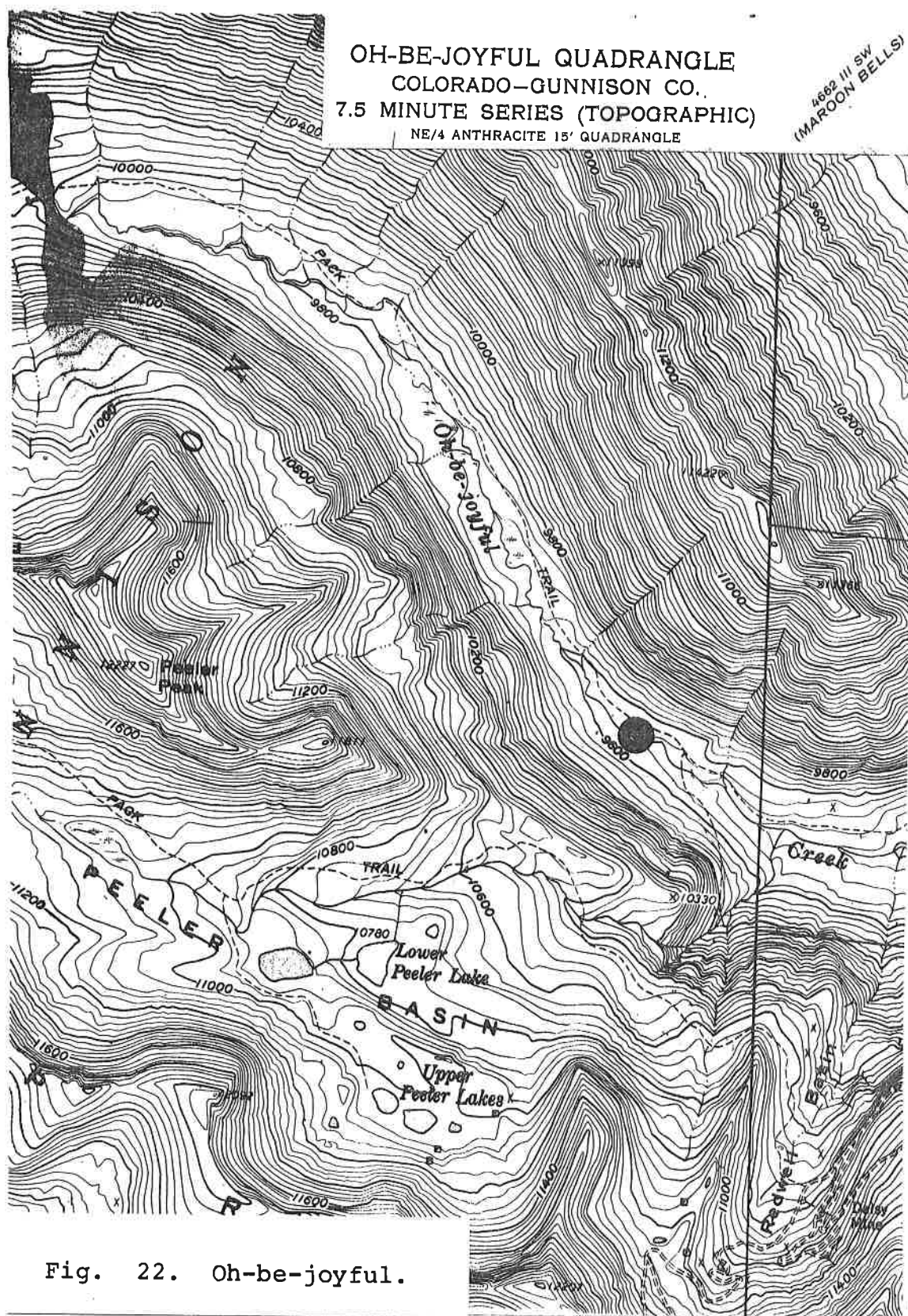
challenge. RMBL personnel divert a small volume of water from one of the larger low order streams flowing out of Gothic Mountain. A small impoundment backs up the water before it spills into a pipe carrying the water by gravity to the town of Gothic. It was here, at 3505 m, G-1 water samples were taken. Other than ambient air effects, such as dryfall or acid-rain, little or no anthropogenic influence could be assigned to this station.

Oh-be-joyful-1 (OBJ-1) (Fig. 22)

Another kind of pristine area was selected to compare with G-1. This station was at 2896 m on the Oh-be-joyful Creek. Since it has been suggested that Crested Butte consider getting its domestic water from this drainage rather than Coal Creek, data from this location could also serve a double purpose. Although this station was very inaccessible, there was an active drill rig functioning around the clock approximately 0.5 km downstream. Unlike G-1, however, a very poor road followed the reaches of the creek, the meadows and surrounding steep mountainsides were being used for grazing, and there was evidence of old mining activity. Also, the beauty of the area, i.e.; waterfalls, a picturesque stream, and access to high mountain lakes, and excellent fishing attracts backpackers and sportsmen.

Slate River-2 (SR-2) (Fig. 23)

In order to conclude whether all the Cd in the Slate River was coming from Coal Creek, a sampling upstream was



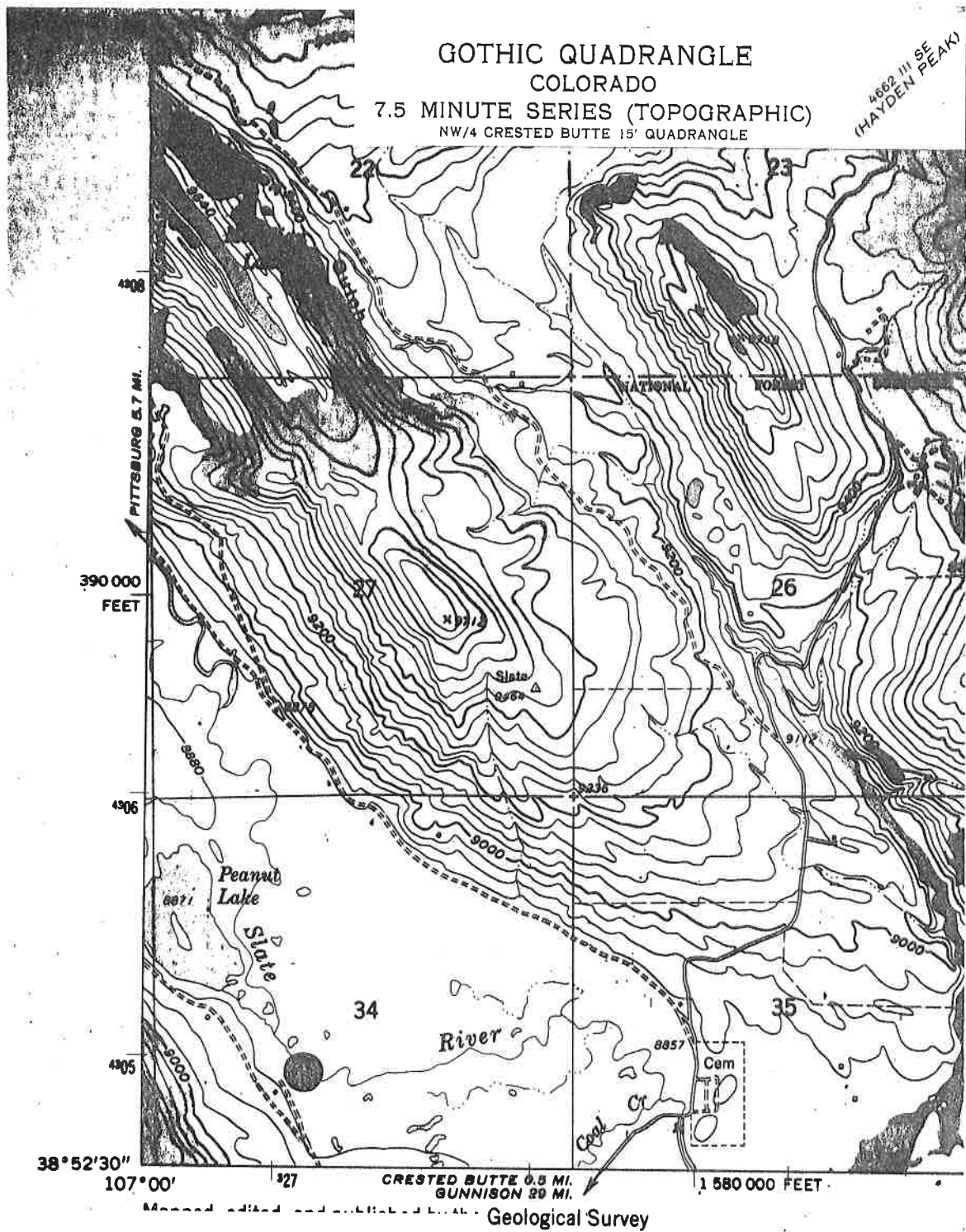


Fig. 23. Slate River-2.

from aerial
 1961



needed. Furthermore, it has also been suggested that Crested Butte consider pumping groundwater from this area for domestic purposes. Therefore, a sampling from the Slate River was taken upstream from SR-1 just below the Peanut Lake influence on the river. Evidence of past mining activity could be seen to the west and north from this station where abandoned gob piles, buildings, and equipment stretch along the base of Mt. Emmons. Access was gained to this station from the west side of the river.

Coal Creek-1, 2, 3 (CC-1, CC-2, CC-3) (Fig. 24)

Since CC-1 and CC-2 are a source of domestic water for the town of Crested Butte, it was most important to test them again in 1980. Reference insects from 1979 at CC-2 showed an increase in Cd over CC-1. In order to see if the Cd concentration in Coal Creek continued to increase downstream, a third Coal Creek Station was selected farther downstream at the mouth of Crested Butte's diversion from Coal Creek just above the Keystone Mine discharge into the creek. From here the water travels through a culvert to the town reservoir. Like Gothic, the water reaches the town entirely by gravity flow. It is this stretch that could be drastically affected by the mining activities at Mt. Emmons and the reason why it has been suggested that Crested Butte look at other sources of water.

Crested Butte-1 (CB-1) (Fig. 2)

Work in 1979 showed levels of Cd increasing in Coal Creek as the water flowed downstream toward Crested Butte.

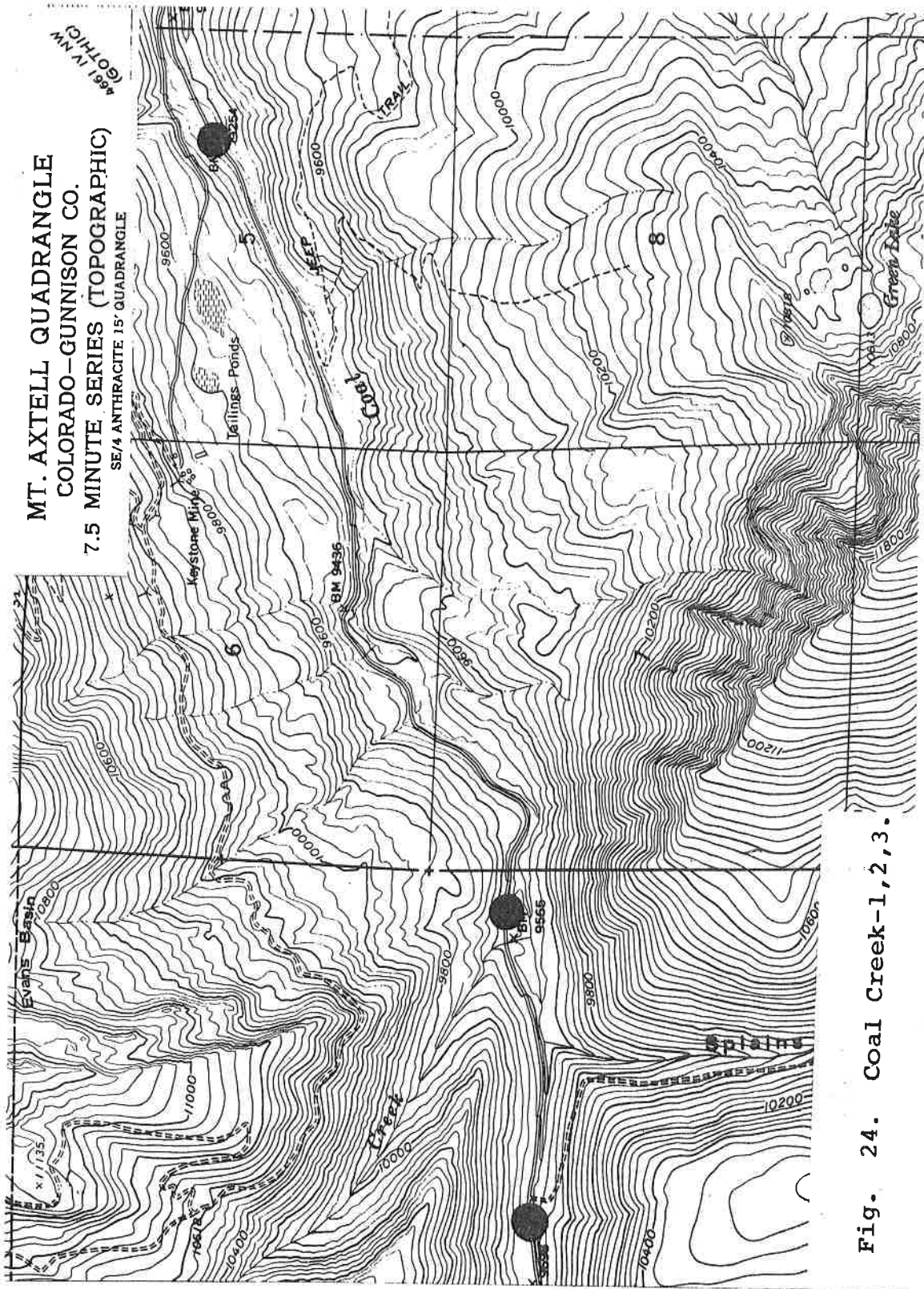
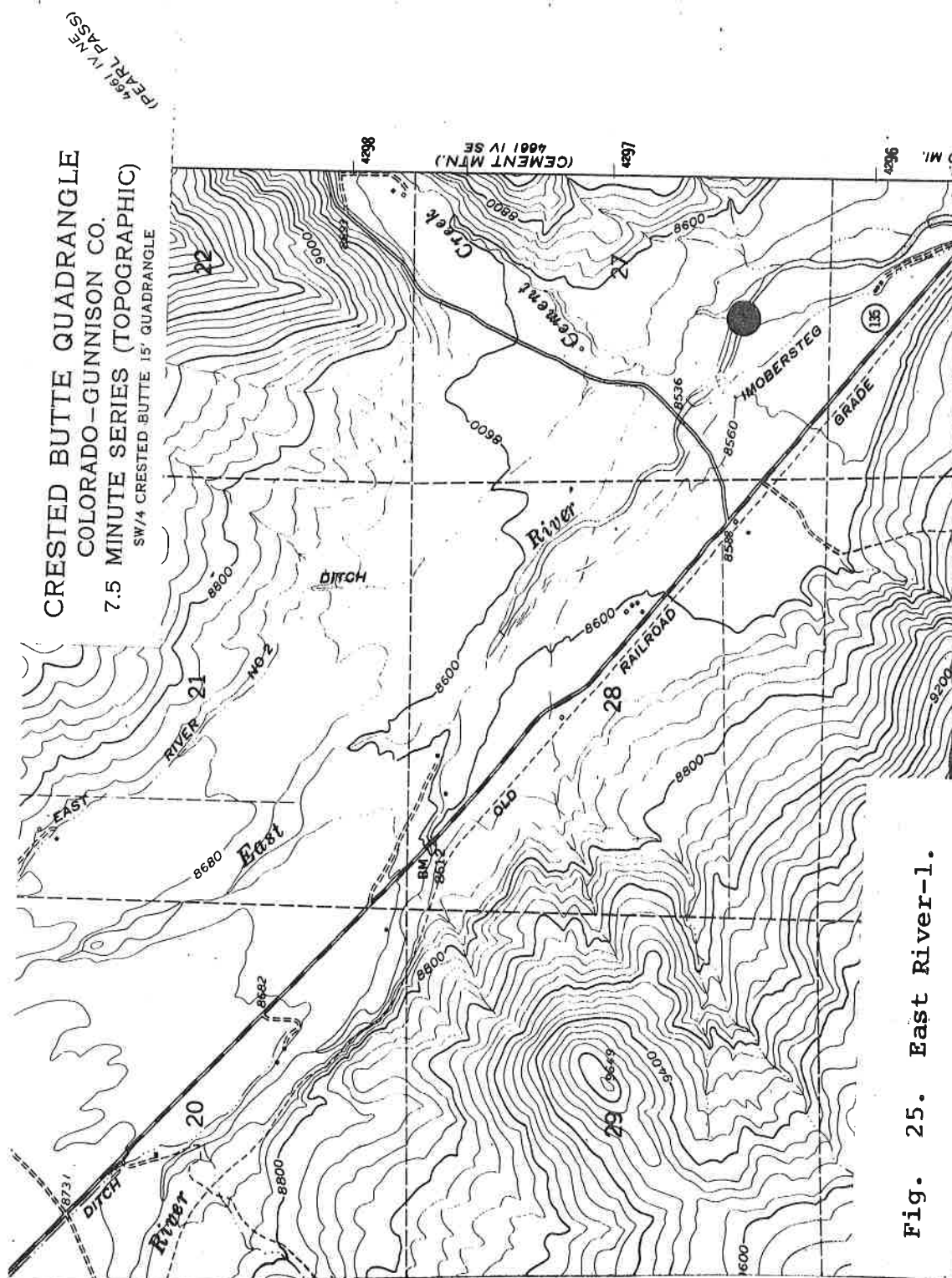


Fig. 24. Coal Creek-1, 2, 3.

It was important to see if the Cd reached the taps in town, or was being detained between the creek and town. The water travels through a culvert approximately 2.4 km to the town reservoir where it is impounded. The bottom of the reservoir is rich in sediment containing large quantities of organic allochthonous material. In the presence of the humic material in the lake, the Cd could be delayed and not enter the flow to town. Consequently, a water sample was taken from a tap in Crested Butte. For obvious reasons, no insects accompanied this sampling.

East River-1 (ER-1) (Fig. 25)

This station was chosen for many reasons. In 1979, high levels of Cd were found upstream in the Slate River. Recently, subdivision growth has placed large numbers of people along this stretch of river. It was important to know if the Slate River levels of Cd extend this far downstream. According to media coverage (Gunnison Country Times 7/11/80, 7/18/80; The Crested Butte Chronicle 7/18/80), raw sewage, sewage sludge, and sewage waste disposal have been dumped upstream. La Plante (1980) has been doing monthly bacteriological and chemical water sampling at this station. The additional analysis done by La Plante may provide more background and insight into the evaluation of the results of this study. Access was gained to the East River from the end of lower Allen Road.



Gunnison River-1 (GR-1) (Fig. 26)

GR-1 lies downstream from all the other stations and above the most densely populated area of Gunnison County. If there is any influence from the river on groundwater and wells in the environment below the river, this would be the proper place to sample. GR-1 was the source of P. badia for the pilot study, March 1980; providing a comparison of Cd levels in the insect for seasonal effects, if any. Samples were taken approximately 20 m upstream from Highway 135 bridge on the north side of the river. The altitude is 2365 m.

RESULTS AND DISCUSSIONCadmiumStations

G-1 Of all the stations under study, G-1 exhibited the lowest level of Cd in both water and insects (Table 15; Fig. 27). The insects had to have hatched at, or very near, the collection site. The insect Cd level, therefore, was under no other influence than the Cd in the immediate ecosystem. The water Cd level represented a very low order stream. The FCF of 9500 may be the equilibrated result for Cd and M. signata.

OBJ-1 The mean level of Cd in the water at this station was 4.77 times higher than the mean level of Cd at G-1. E. doddsi showed a FCF of 13000. Hydropsyche exhibited a FCF of 4300. This station provided the densest naiad

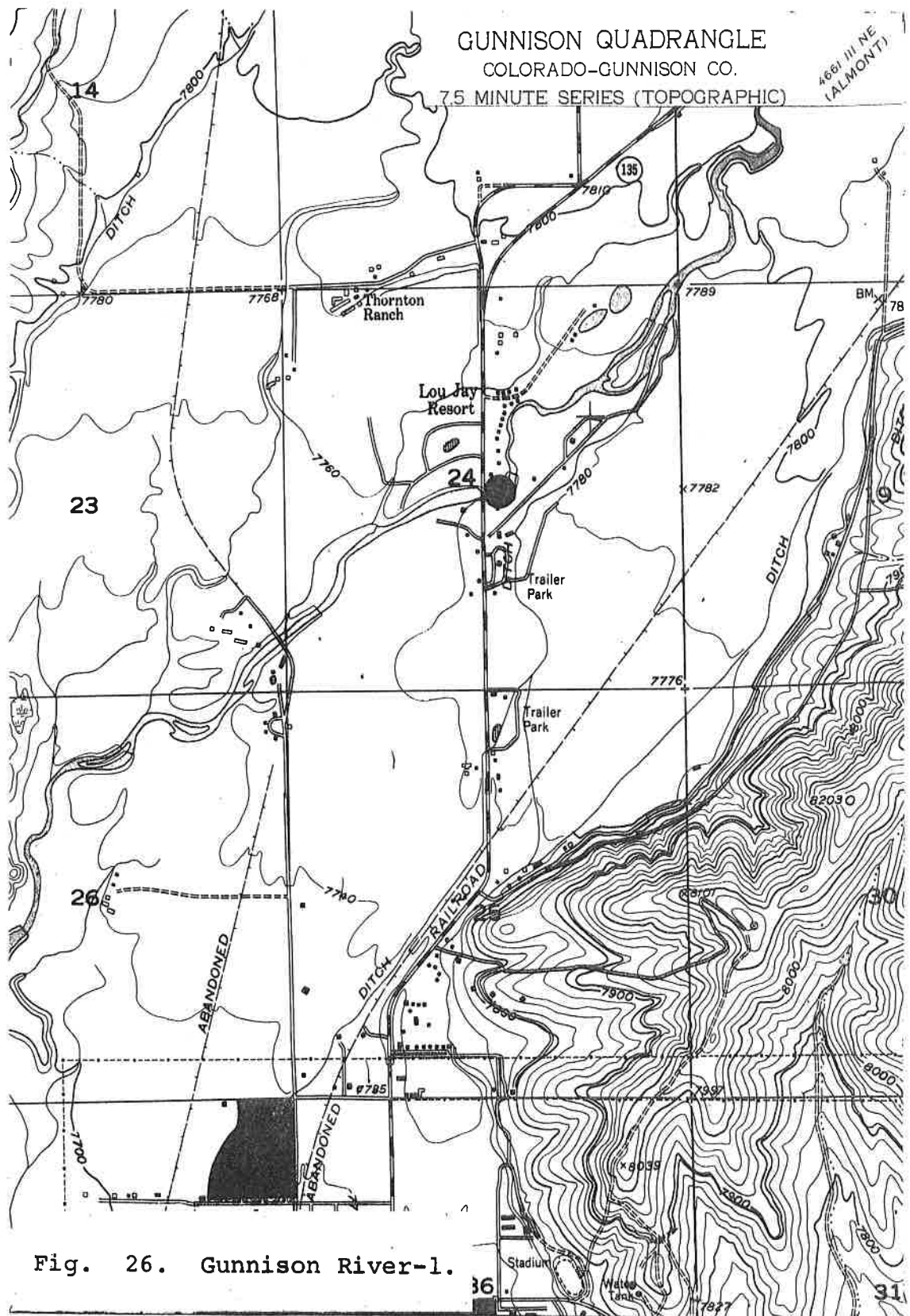


TABLE 15

INSECT AND WATER SAMPLE LEVELS OF CADMIUM AND MOLYBDENUM

Station	Insect	Insect Cd ppm	Insect Cd $\bar{X}$	Water Cd ppb	Water Cd $\bar{X}$	FCF	Insect Mo ppm	Insect Mo $\bar{X}$
G-1	<u>M. signata</u>	3.2		0.21			0.26	
		1.7		0.22			0.11	
		2.1		0.22			0.17	
		2.2		0.23			0.33	
		1.2	2.1	0.22	0.22	9500	0.12	0.20
OBJ-1	<u>E. doddsi</u>	10.3		1.31			0.56	
		16.1		0.84			0.85	
		10.8		1.09			0.83	
		16.8		0.83			0.64	
		16.2	14.0	1.18	1.05	13000	0.62	0.70
SR-2	<u>Hydropsyche</u>	4.5	4.5			4300	0.35	0.35
	<u>Cinygmula</u>	75.5		7.34			0.95	
		118.1		11.22			1.60	

TABLE 15-Continued

Station	Insect	Insect Cd ppm	Insect Cd $\bar{X}$	Water Cd ppb	Water Cd $\bar{X}$	FCF	Insect Mo ppm	Insect Mo $\bar{X}$
CC-1	<u>E. doddsi</u>	97.7	97.1	8.61		10000	1.65	1.40
		49.4		9.06			0.94	
		32.0	40.7	11.26	9.50	4300	1.41	1.18
	<u>E. doddsi</u>	7.8		1.07			0.57	
		5.3		1.24			0.27	
		4.2		1.26			0.21	
CC-2	<u>E. grandis</u>	4.1		1.06			0.27	
		6.9	5.7	1.09	1.14	5000	0.32	0.33
		7.5	7.5			6600	0.29	0.29
	<u>E. doddsi</u>	31.9		3.41			1.41	
		29.2		2.46			0.82	
		31.1		4.27			1.08	
		26.2		3.58			0.59	
		22.3	28.1	2.95	3.33	8500	0.69	0.92

TABLE 15-Continued

Station	Insect	Insect Cd ppm	Insect Cd $\bar{X}$	Water Cd ppb	Water Cd $\bar{X}$	FCF	Insect Mo ppm	Insect Mo $\bar{X}$
CC-3	<u>E. grandis</u>	27.8	27.8			8300	0.97	0.97
	<u>E. doddsi</u>	13.5		1.52			0.64	
		13.3		1.31			0.35	
		11.5		1.51			0.40	
		14.1		1.57			0.89	
ER-1	<u>Hydropsyche</u>	18.6	14.2	1.51	1.48	9600	0.74	0.60
		12.2	12.2			8200	0.68	0.68
	<u>E. grandis</u>	23.1	23.1			16000	0.82	0.82
	<u>Hydropsyche</u>	3.6		0.50			0.39	
		3.4		0.74			0.33	
P. badia		3.5		0.47			0.38	
		4.7		0.46			0.35	
		6.2	4.3	0.67	0.56	7600	0.66	0.42
		8.0					0.44	
		3.5					0.49	

TABLE 15-Continued

Station	Insect	Insect Cd ppm	Insect Cd $\bar{X}$	Water Cd ppb	Water Cd $\bar{X}$	FCF	Insect Mo ppm	Insect Mo $\bar{X}$
		7.2					0.32	
		6.5					0.25	
		3.4	5.7			10000	0.25	0.35
	<u>E. grandis</u>	23.5	23.5			42000	0.27	0.27
	<u>E. doddsi</u>	12.5	12.5			22000	0.38	0.38
GR-1	<u>P. badia</u>	2.3		0.23			0.17	
		3.0		0.23			0.13	
		2.5		0.24			0.09	
		2.5		0.24			0.18	
		2.3	2.5	0.24	0.23	11000	0.11	0.14
	<u>Hydropsyche</u>	3.0					0.30	
		2.0					0.29	
		2.9	2.6			11000	0.18	0.26

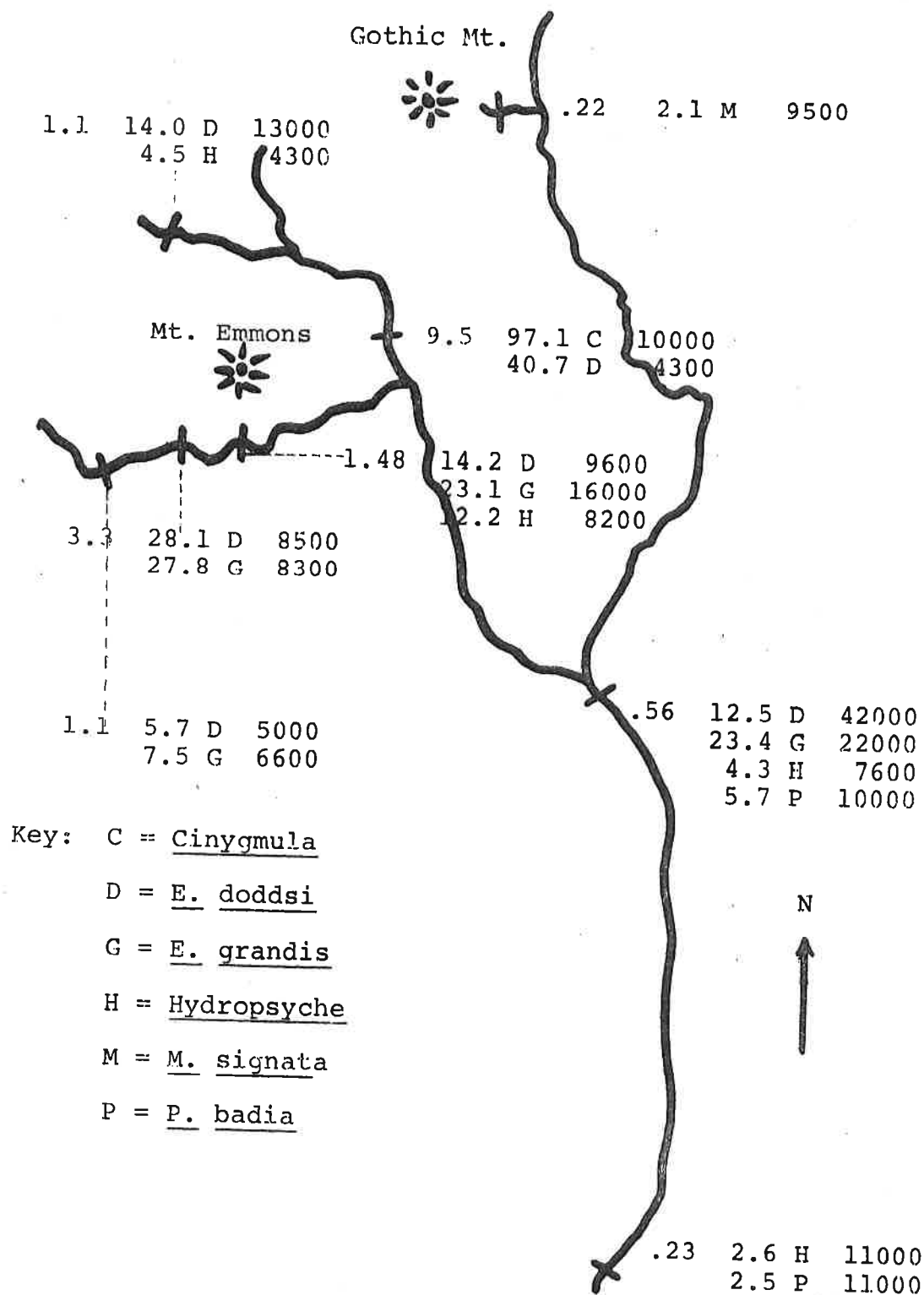


Fig. 27. Stream profile of water (ppb) and insect (ppm) Cd concentrations and FCF of the East River - Upper Gunnison River drainage (1980).

population of all the sites. It took less than an hour to harvest the insect samples.

SR-2 The highest level of Cd in all the water samples was found here (Table 15; Fig 27). The substrate was very poor. Flat sheets of splintered slate less than 8 cm in diameter made up the majority of the river bottom. There were very few cobbles or rocks. The water had a milky cast. Collecting a sufficient number of insects was almost impossible. A long stretch of river was examined for insects before enough were collected. The paucity of insects, even 1.0 km upstream, suggests that conditions were intolerable to living systems. Other than several large leeches (Hirudinae), and the insect samples taken, no other living organisms were found. All 17 E. doddsi were collected upstream as far as 1.0 km above the water sampling site. These had to be insects that drifted from upstream for several reasons: (1) they were always alone, never in the usual clusters (2) the low Cd FCF of 4300, (3) and the abiotic condition of the river precluded their hatching and maturing here.

The Cinygmula sp. were collected from just below the water sampling site and as far upstream as the E. doddsi. All of the Cinygmula sp. collected were in their last instar, had dark wingpads, and had started to emerge. Many showed an abnormal dorsal extrusion of the head through the first thoracic segment with the head flexed posteriorly. The frontal and coronal sutures of the head were not split.

The Cinygmula sp. were often found in groups and showed a Cd FCF of 10000 which would indicate they had spent more time in this part of the river than the E. doddsi.

CC-1 E. doddsi showed a 5 times increase in Cd content at this station over the reference samples collected in 1979. The FCF for E. doddsi was 600 in 1979 and 5000 in 1980. Water level of Cd was 1 ppb lower in 1980 than 1979, which could be sampling error and is insignificant. The higher FCF, however, suggested that more Cd is accumulating in the system.

CC-2 The insects (Ephemerella) at this station exhibited 7.9 times more Cd over the year before. The FCF for E. doddsi increased from 1200 in 1979 to 8400 in 1980. Again, there was little change in the water Cd level. Water level was 2.9 ppb in 1979 and 3.33 in 1980. Several hypotheses could explain the increase in Cd in the insects here: (1) Elk Creek may be imposing a Cd influence on Coal Creek, (2) a wier box was installed approximately 100 m above this station on Elk Creek within the last year, disturbing the substrate during installation, (3) natural weathering phenomena could be exhibiting an effect, (4) or acid precipitation (snow, rainfall, and dryfall) is releasing more and more Cd into the system. The latter could be contributing a large part to the increase, or the entire loading, since a similar increase was seen at CC-1.

CC-3 Blackfly (Simuliidae), midge (Chironomidae) and caddis fly (Hydropsyche) larvae were the predominant genera

at this station. The increased presence of these species signifies a less pristine condition than at OBJ-1, G-1, CC-1, and CC-2. As the creek flowed downstream from CC-1, the FCF increased at each station, although water levels of Cd remained almost constant. Again, E. doddsi with an FCF of 9600 showed a lower concentration factor than E. grandis with 16000, and the Hydropsyche had the lowest FCF which was 8200.

CB-1 The tapwater in the town of Crested Butte assayed for 0.44 ppb Cd. It appears as though the diversion pipe from the creek to the reservoir and the reservoir itself are serving as excellent filters for the Cd before it reaches town. The pH of the reservoir ranged between 5.43 to 5.75 as determined by Hall and Colborn, 7/3/80. Despite the acid conditions in the reservoir it was able to support some small-mouth bass. This reservoir should be monitored closely as acid precipitation affects the dynamics of the metals within the aquatic system.

ER-1 Despite the high levels of Cd in the Slate River upstream, the water level of Cd was 0.564 ppb at this station. However, the FCF increases significantly at this point. E. grandis exhibited a FCF of 43000, E. doddsi 22000, Hydropsyche 7600 and P. badia 11000. The Hydropsyche were most often attached to the large cobbles and rocks at this station, and were the dominant genus. Again, it took a long time to collect enough larvae other than Hydropsyche at this station.

GR-1 A water level of 0.23 ppb Cd at this station was similar to G-1. Unfortunately, there were no insects of the same genus collected at both G-1 and SR-1. Transplants would be the most effective way to overcome the species voids and establish a true stream profile of metals.

P. badia and Hydropsyche both had an FCF of 11000. This suggests that this stretch of river is as highly loaded with Cd as ER-1 since the P. badia showed the same FCF and the Hydropsyche level was even higher.

The mean level of 2.54 ppm Cd in the P. badia was almost identical with the level found in the single insect P. badia sample in March 1980, which was 2.46 ppm. The triple insect sample of the pilot run assayed 1.83 ppm Cd. These results suggest a steady state of Cd availability downstream with no seasonal variation. The possible acid-rain effect seen upstream on Coal Creek could not be expected at this reach of the river because of the high buffering capacity present.

Practical Application of Cd Results

For many years the USGS and EPA have been trying to establish a generalized model of the metal distribution within aquatic systems affected by mine discharge. Because "The composition of mine drainage is complex and may be expected to vary for each mine or mining area" (USEPA 1978) this goal has not been achieved. By using the concentration of metals in insects combined with concentration of metals in water, one can obtain a much better feel for metals that

are present in an aquatic system than by measuring the levels of metal in the water alone. The continuum map (Fig. 28) is just the beginning of the type of mapping that can be done.

Vannote et al. (1980) describe the River Continuum Concept as a method to predict and understand the natural and unnatural dynamics of lotic water. They describe the possibility of using a biological analogy comparable to the river's kinetic energy. By applying Cd-FCF data to the continuum profile, a new method exists to analyze Cd stress on aquatic ecosystems. Through the charting of the level of Cd sorbed by insects vs water level of Cd and FCF, the spatial distribution of Cd from the headwaters to the lowest reaches of a stream becomes clear (Fig. 28). At the ends of the valley, there appears to be a balance between insect and water Cd level in relation to the FCF (Fig. 28). However, the stretches of drainage 22.8 km above GR-1 show Cd stress. The collection of many more Cd-insect-water data sets between G-1 and GR-1 will give a better understanding of the dynamics of Cd in the river system.

After mapping the results of this research, it is reasonable to conclude that the town of Crested Butte should consider protecting its Coal Creek watershed. The results at SR-2 preclude using the Slate River as a source for its water. The Oh-be-joyful assayed higher for both Cd and Mo than CC-1. Also, the FCF for Cd at OBJ-1

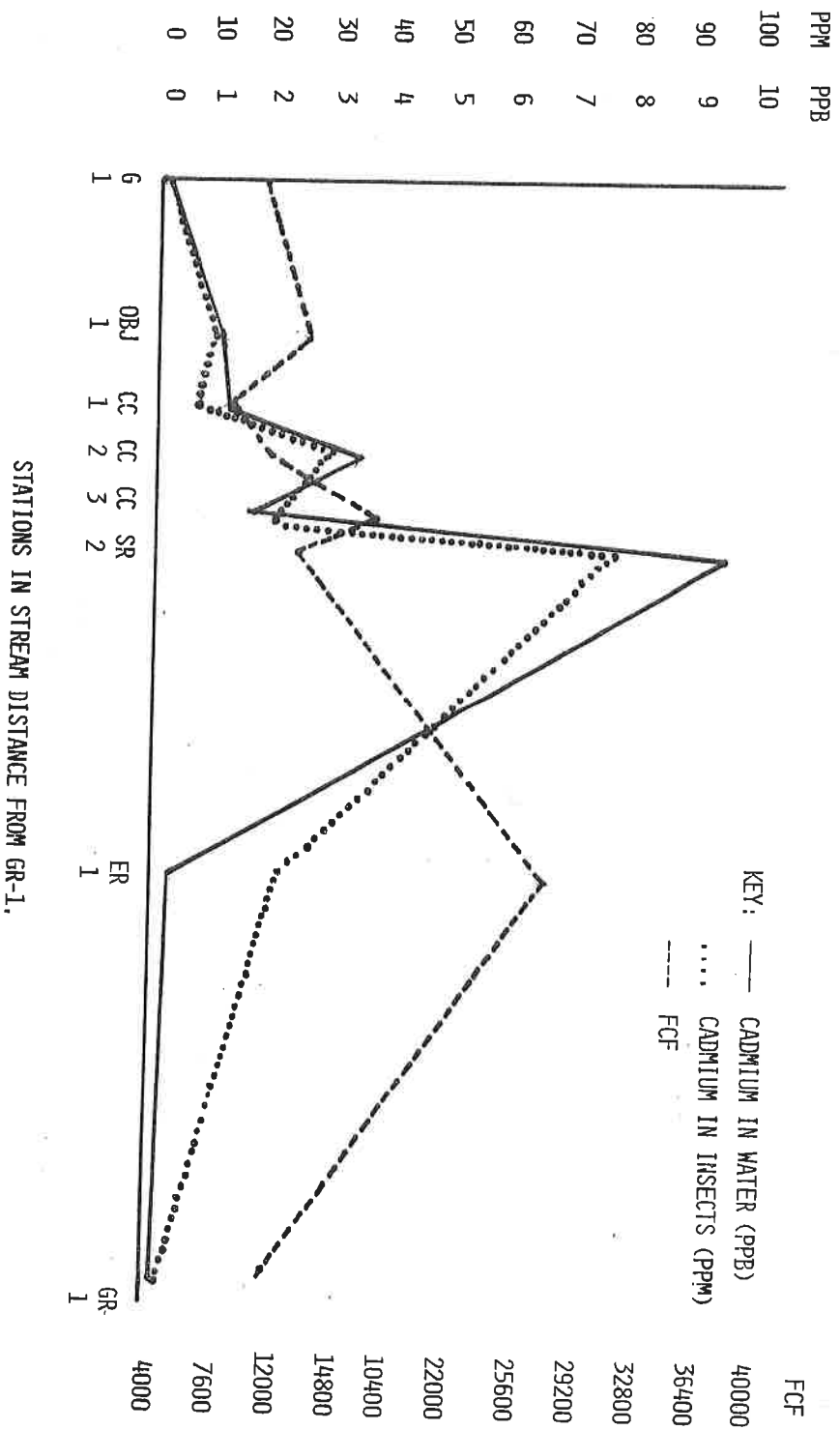


Fig. 28. Distribution of Cd through the river continuum from headwaters at G-1 and OBJ-1 to GR-1. Graph shows the mean Cd in insects and water and mean FCF values for each station.

suggests there is some Cd loading of the aquatic system. At this time, it would seem prudent for the town of Crested Butte to commence exploring the possibility of diverting its water farther up Coal Creek to avoid the Cd and Mo loading as exhibited by the results of CC-2 and CC-3. By diverting the water farther upstream in an enclosed pipe, mobilization of metals will be decreased as acid-rain persists.

Using the FCF Method, it appears that the lower East River - Upper Gunnison River drainage is becoming a sink for the Cd spill at the Keystone Mine and any additional Cd being introduced upstream. It should be noted that the general increase in use of fertilizers by ranchers and the USFS could be contributing part of the Cd found in this stretch of river. In all probability, the dumping of sewage material into the river provides the organic material needed to bind the Cd and remove it from solution; and in some instances, the sewage material itself may bear heavy loads of Cd. Although raw sewage in a river is not desirable, it may have one unusual benefit if, in fact, it binds Cd and removes it from the water.

Continued downstream migration of Cd must be monitored closely as mining, industrialization, recreation, and the resultant population impacts increase Cd loading of the riverine system. Consideration must be given to doing a hazard evaluation of the presence of Cd in this environment. The effect of water being diverted for irrigation from the

lower Slate River on Cd bioaccumulation in vegetation and ultimately on the livestock and wildlife grazing in the areas must be monitored. The high concentrations of Cd building up in the high order streams and impoundments may remain locked in the substrates. On the other hand, the anthropogenic effect of acid-rain may release the element to enter the domestic water delivery and irrigation systems. The effects of acid-rain may be seen in sporadic releases of Cd from this stretch of river or perhaps only upstream where there is less buffering capacity. Direct users of water from Coal Creek, Oh-be-joyful, Slate River, and lower East River should be monitored for human health effects related to high levels of Cd in the environment. Comparative epidemiological studies of other mineralized high altitude valleys with population centers should be concurrent while monitoring for mobilization effects of metals by acid-rain.

It is clear that future work must be done with transplants in order to remove species variability and voids. In many instances, stretches of rivers that need to come under observation have no viable insect communities. At the same time, the entire spectrum of trace elements needs to be studied. Continuation of all the streams feeding the Gunnison River should be plotted using live transplants of one species (P. californica) remaining at stations for five days. The stations should be no more

than 5 km apart, extending as far as and including the Blue Mesa Reservoir.

Molybdenum

As with all previous experimentation, no Mo was found in any of the water samples, even after concentrating the ions in water 40X. The levels of Mo in the different insect species showed far less spread than Cd levels between the same species. There is a very narrow range of $\bar{X}$ Mo levels at each station (Fig. 29).

The results of the transplants in the field work of 1979 (Table 10) showed Mo moving in and out of the insects. In order to decide if insect Mo accumulation is metabolic or exoskeletal, flow-through studies using controlled measurable levels of Mo are needed. By controlling ambient temperatures during the studies, moulting could be affected and the exuviae and moultees analyzed. The results of this analysis should reveal how Mo is sorbed and as with Cd, if the process is primarily exoskeletal.

P. badia at G-1 exhibited 0.198 ppm Mo. Similarly, this was one of the lowest insect values for Mo just as P. badia held the lowest level of Cd for all stations.

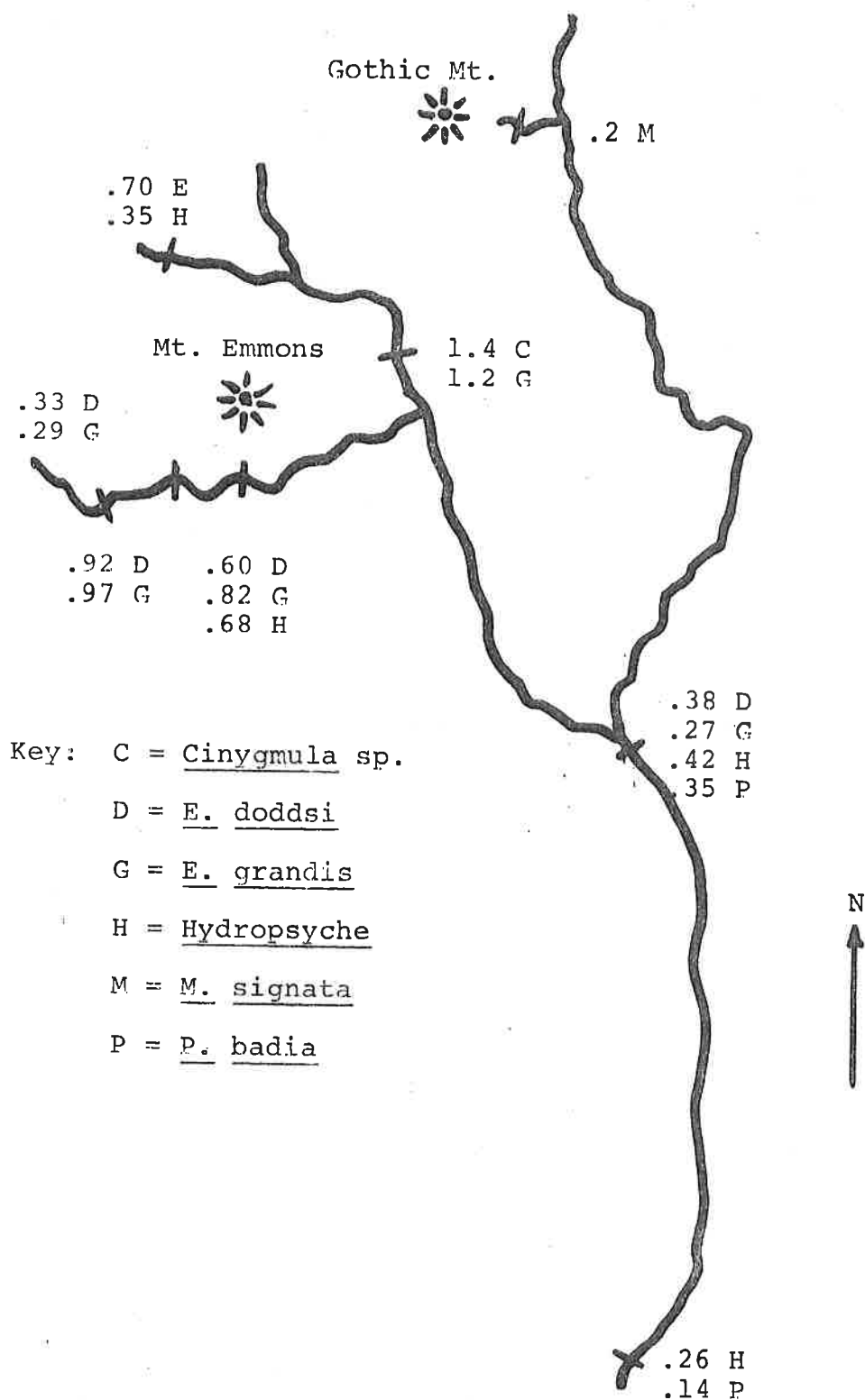


Fig. 29. Stream profile of insect Mo concentration (ppm) of the East River - Upper Gunnison River drainage (1980).

Perhaps, this level of Mo represents the essential amount of Mo required to support life.

The E. doddsi at OBJ-1 held twice as much Mo as the Hydropsyche; 0.7 to 0.35 ppm. The Slate River E. doddsi assayed for 1.18 ppm Mo and the Cinygmula sp., 1.4 ppm. Here, as with the Cd, the insects accumulated more Mo than at any other station. This might reflect the impact of the old abandoned Peanut Mine which tunnels under Mt. Emmons.

On the south side of Mt. Emmons, CC-1 held the least amount of Mo of all the stations on Coal Creek (0.33 ppm) (Fig. 29). CC-2 concentrated three times more than CC-1, whereas, CC-3 concentrated only twice as much as CC-1. Compared with 1979, CC-1 and CC-2 showed less Mo content in 1980. This drop in Mo accompanied by the increase in Cd at CC-1 and CC-2 could be reflecting the effect of acid-rain. The acid-rain would mobilize the Cd and immobilize the Mo giving the resultant change. This trend should be monitored again in 1981 and subsequent years. This may be another example of the "see-saw" effect between Cd and Mo found in Part I (1979).

Farther downstream, away from the ore body, ER-1 shows approximately the same Mo content as CC-1. GR-1 drops as low as 0.26 and 0.135 ppm, much like G-1 (Fig. 29).

Practical Application of Mo Results

The geographical profile of insect-Mo in the East River - Upper Gunnison River drainage starts very low at

Gothic, increases at all stations around Mt. Emmons, peaks at SR-2, and then phases out again as the riverine system flows farther away from the main ore body (Fig. 30). The mapping of insect Mo content raises the possibility of using aquatic insects to explore for Mo. It would be interesting to collect insects above, near, and below other suspected Mo lodes.

As the mining of the Mt. Emmons lode commences and large amounts of mineralized earth are to be disturbed, every option must be employed to protect Gunnison County from the release of Mo as well as other toxic trace metals. The latest state of the art should include totally confined systems including the mining, hauling, treatment, and disposal of the 360 million tons of waste material. As this technology is incorporated, the insects can serve as monitors for the release of Mo by airborne fine particulate matter, and water contamination into the ecosystems of Gunnison County.

Simultaneously, background or baseline monitoring of Mo should be started now in those areas of known uranium lodes. The alkaline flotation systems used to process the uranium ore increase the possibility of Mo entering the aquatic ecosystems in those areas.

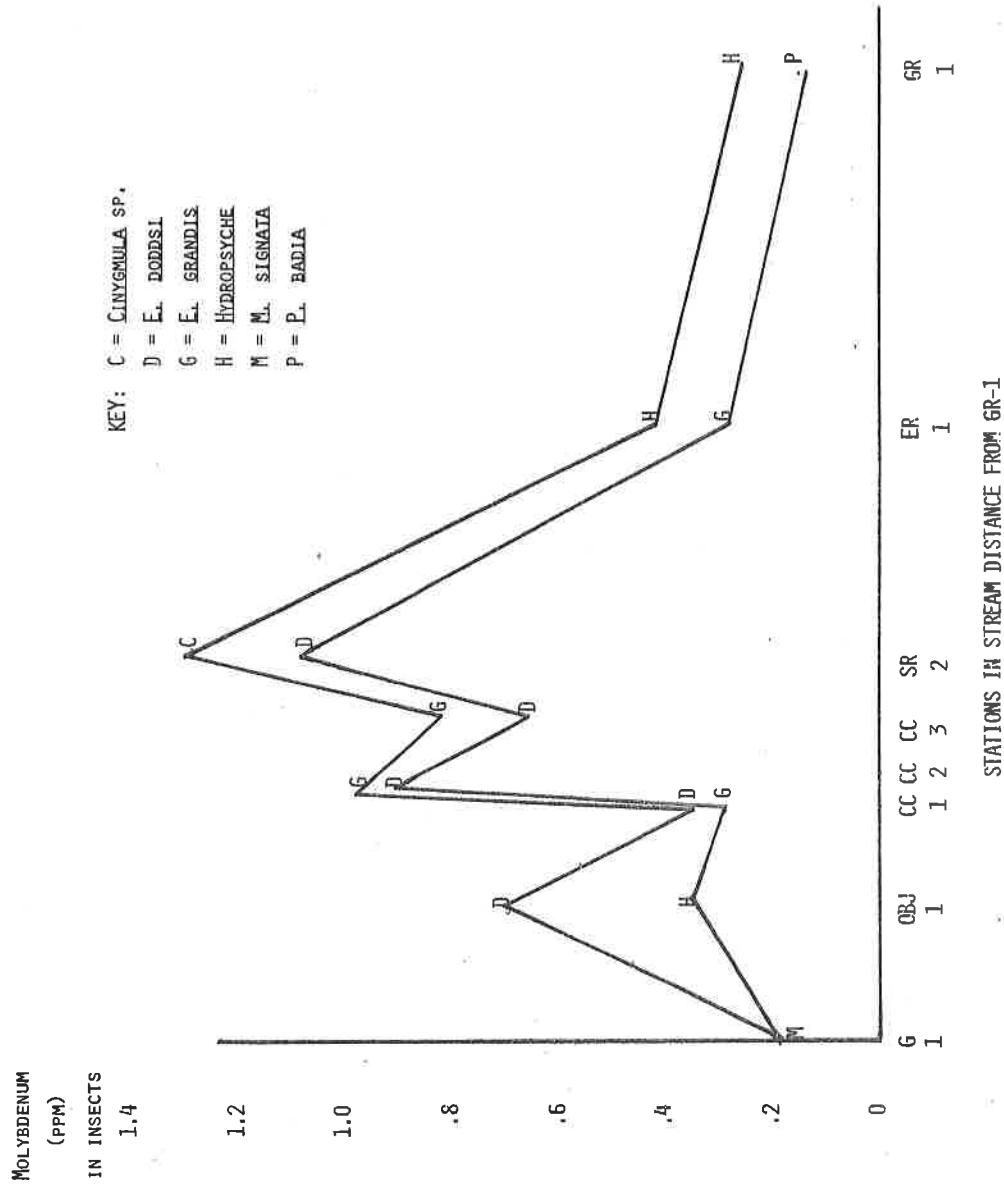


Fig. 30. Distribution of Mo through the river continuum from headwaters at G-1 and OBJ-1 to GR-1. Graph shows lowest and highest mean Mo insect values (per species) for each station.

VII SUMMARY

This research demonstrated that aquatic insects concentrate Cd at 2 to 4 orders of magnitude. Mayflies concentrate Cd more than stoneflies, and caddis flies take up the least. Of the mayflies, E. grandis accumulates more than E. doddsi. Cinygmula sp. binds more Cd than any other species observed in the study. The exoskeleton sorbs the largest part of the Cd, and in the process of moulting, the insect cleanses itself of Cd. Live and dead insects sorb Cd at the same rate, which indicates the process is not physiological. Cd can shift in an aquatic system and at the same time, not exhibit high levels in the water. Because of its labile nature, Cd will be accumulated by aquatic insects if it is present in an aquatic ecosystem and not go undetected. The practical application of this research has been demonstrated through the continuum mapping of Cd-FCF information. Despite low levels of Cd in the water itself, the shadow effect of the FCF allows one to discern where substrate Cd loading occurs.

When live insects shed their outer integument, they provide riverine systems a continuous source for purifying and detoxifying the water. At each shedding, two new exoskeleton surface areas are added to the system: (1) the

inside surface of the exuviae, and (2) the virgin, outside soft surface of the integument on the moulted. The Cd-laden chitinous material eventually settles to the bottom of the stream and gets buried. In this anoxic substrate, the Cd binds with S and becomes fixed (Gardiner 1974b).

In the case of Mo, nothing has been clarified concerning where and how it binds in the insect. Nor has it been established that the amount of Mo assayed in the insect is related to the level of Mo in the water. Like Cd, the Mo in the insect may reflect more than water content. Nevertheless, the mapping of insect Mo concentrations correlated with a known lode of Mo, and provided baseline data preceding commencement of mining and milling of an ore body.

The complexity and interdependence of an organism's role in a community and total ecosystem has again been demonstrated. We now find aquatic insects serving not only as shredders, collectors, grazers, detritivores, and predators assisting in the partial breakdown of organic material by preparing it for bacterial degradation (Stanford and Ward 1979), and as energy for organisms higher in the food chain, but as efficient, detoxifying filtering systems capable of removing Cd from water. Through this process, the insects provide the FCF Method for detecting Cd in an aquatic ecosystem.

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APPENDIX A

Procedure for Concentrating Heavy-metal Ions on Site by Ion Exchange

Gregory Lockett

- I. Laboratory or Car Step for Collecting Water Sample
 - A. Using a 400 ml beaker* which has been rinsed three times with the water to be sampled, fill a one liter volumetric flask to the one liter mark.
 - B. Rinse a 25 ml graduated cylinder twice with a small portion of ammonium acetate buffer (pH 5.2). Add 20 ml ammonium acetate buffer to water sample in one liter volumetric flask.
 - C. Rinse a one liter Nalgene plastic separatory funnel three times with deionized distilled water. Leave valve open so that the lower stem, adapted with a rubber connector, and Pasteur Pipette are also cleaned.

*All glassware should be pretreated with a 1N HCL + 1N HNO₃ solution. Volumetric flask, separatory funnel, and collection bottles should be stored full of the above acid mix. All equipment should be wrapped in Saran paper to reduce exposure to contamination.

- D. Rinse vent (screw top with plastic tubing inserted) on separatory funnel. Let deionized distilled water run through the tube, then thoroughly wash outside.
- E. Release any rinse water which has accumulated at the base of the reservoir and CLOSE VALVE. Transfer buffered water sample quantitatively to reservoir. Rinse volumetric flask twice with 15 ml portions of distilled water and add to separatory funnel.
- F. Make sure that valve on the bottom of the 1 x 10 cm resin column packed with Cheelex 100 (50-100 mesh) in sodium form is closed. Seat head firmly into column and then raise column so that the Pasteur pipette on the separatory funnel fits firmly in the head.
- G. Add water sample from reservoir to resin column. Try to open valves simultaneously. It is important that water does not drop directly into the resin during the exchange step. The direct action of water on resin creates air bubbles which then migrate deeper into the resin. This effectively decreases the amount of resin to which the buffered water sample is exposed.
- H. Adjust flow to a rate of 11 ml per minute.
- I. Allow column to run dry.

- J. Separate resin column from separatory funnel. Place small yellow cover firmly over head and place column in an upright position until it can be eluted. Resin column can be stored indefinitely at this stage without deterioration of sample.

II. Laboratory Elution Step

- A. Flush column with 20 ml ammonium acetate buffer.
- B. Pass 25 ml 4N nitric acid through column at a rate of 1.5 ml per minute and collect in a 30 ml Sybron Nalgene Polyethylene #2002 bottle. In this step, resin will shrink to approximately 50% of its volume.
- C. Pass 25 ml 4N ammonium hydroxide through column at 1.5 ml per minute and collect in another 30 ml Nalgene bottle. In this step, the resin will re-expand to former volume.
- D. Store 30 ml sample bottles in freezer for transport to analysis site for atomic adsorption spectrophotometric analysis.

III. Laboratory Regeneration Step

- A. Pass 20 ml 1N hydrochloric acid through column.
- B. Rinse resin column with 50 ml deionized distilled water.

- C. Pass 20 ml 1N sodium hydroxide through column.
(Volume changes associated with elution will occur here.)
- D. Rinse resin with 50 ml deionized distilled water. Column ready for reuse.

APPENDIX B

Exoskeleton Dwgt 7.8 mg

Ash (Cd^{++}) 0.15 in 5 ml

Moultee Dwgt 82.1 mg

Ash (Cd^{++}) 0.05 in 5 ml

$$\frac{(0.15 + 0.05) \times 5}{0.0078 + 0.0821} = \frac{11.12 \text{ ppm in total}}{\text{insect at time of moult}}$$

Exposure $120/48 \times (\text{Cd}^{++}) = 14.5 \pm 6.02$

Exoskeleton Dwgt 10.4 mg

Ash (Cd^{++}) 0.28 in 5 ml

Moultee Dwgt 130.6 mg

Ash (Cd^{++}) 0.06 in 5 ml

$$\frac{(0.28 + 0.06) \times 5}{0.0104 + 0.1306} = \frac{12.05 \text{ ppm in total}}{\text{insect at time of moult}}$$

Exposure $96/72 \times (\text{Cd}^{++}) = 13.83 \pm 5.77$

APPENDIX C

RAW DATA FROM LABORATORY ANALYSES

Colorado Analytical Laboratory Results on Run #1, 1979,
April 17, 1980.

Insect Sample ID				Dwgt	Cd Sol	Cd	Mo Sol	Mo
				Grams	Ppm	Net	Ppm	Net
1	Control	CCl ₁	8 2 79	0.0757	0.017	2.25	0.078	2.06
2	"	CCl ₂	"	0.1612	0.043	2.67	0.063	0.78
3	"	CCl ₂	"	0.1888	0.065	3.44	0.122	1.30
4	"	CClA*	"	0.1303	0.041	3.15	0.057	0.87
5	24 hrs	CClL	8 3 79	0.0314	0.026	8.28	0.061	3.90
6	"	CClD	"	0.0576	0.030	5.21	0.011	0.38
7	48 hrs	CClD	8 4 79	0.0804	0.051	6.34	0.012	0.30
8	"	CClL	"	0.1628	0.263	16.20	0.022	0.27
9	"	CClAL	"	0.0867	0.058	6.69	0.038	0.88
10	24 hrs	CClAD	8 3 79	0.1603	0.078	4.87	0.120	1.50
11	72 hrs	CClAD	8 5 79	0.0364	0.030	8.24	0.071	3.90
12	24 hrs	CClD	8 3 79	0.0601	0.055	9.15	0.260	8.60
13	72 hrs	CClD ²	8 5 79	0.0573	0.045	7.85	0.063	2.20
14	48 hrs	CClAD	8 4 79	0.2365	0.157	6.64	0.100	0.85
15	72 hrs	CClL	8 5 79	0.0568	0.070	12.32	0.067	2.40
16	"	CClAL	"	0.1504	0.092	6.12	0.052	0.69
17	24 hrs	CClAD	8 3 79	0.1532	0.110	7.18	0.068	0.89
18	72 hrs	CClL	8 5 79	0.1201	0.217	18.10	0.044	0.73
19	24 "	CClD	8 3 79	0.1533	0.121	7.89	0.053	0.69
20	"	CClL	"	0.1528	0.161	10.50	0.013	0.17
21	"	CClAL	"	0.1557	0.098	6.29	0.015	0.19

1 = E. longisimus

2 = Cinygmula sp.

* = Brachycentrus

In the original report of April 17, 1980, the Cd figures were reported erroneously. On February 19, 1981, John Appel and Dean Lansing, chemists at the above laboratory, confirmed that the results for the net Cd in the insects had been reported 2X higher than they should have been. Figures in Chapter IV reflect the corrected Cd levels in the insects.

Gregory Lockett, University of California, Berkeley Results
of Water Analysis on Run #1. March, 1980. Cadmium.

Water Sample ID	Cd Sol Ppm	Sample Size Ml
22 CC-1 8 2 79	0.065	50
24 SR-1 8 2 79	0.211	"
26 " 8 3 79	0.267	"
28 " 8 4 79	0.269	"
30 " 8 5 79	0.291	"
Blank 8 5 79	0.012	"

Samples were not analyzed for Mo.

Colorado Analytical Laboratory Results on Run #2, 1979.
February 28, 1980.

Insect Sample ID						Dwgt Grams	Cd Ppm	Mo Ppm
1	Control	CC2	8	9	79	0.112	3.55	1.3
2	"	CC2A*	"	"	"	0.123	2.80	2.0
3	24 hrs	CC2L**	8	10	79	0.096	4.80	2.6
4	"	CC2D***	"	"	"	0.169	5.05	0.9
5	"	CC2AL	"	"	"	0.054	2.80	1.8
6	"	CC2AD	"	"	"	0.071	4.25	0.7
7	48 hrs	CC2AD	8	11	79	0.095	3.70	1.6
8	"	CC2D	"	"	"	0.125	7.20	1.2
9	"	CC2L	8	12	79	0.133	9.40	0.4
10	"	CC2L	"	"	"	0.053	13.20	<0.1
11	"	CC2AL	"	"	"	-	-	-
12	"	CC2AD	"	"	"	0.074	3.40	0.7
13	"	CC2D	"	"	"	0.059	11.85	1.7
14	"	CC2D	"	"	"	0.092	5.45	1.6
15	72 hrs	CC2D	"	"	"	0.100	8.50	2.5
16	"	CC2L	"	"	"	0.056	12.50	4.5
17	"	CC2L	"	"	"	0.084	10.10	1.8
18	"	CC2AL	"	"	"	0.096	3.65	1.6
19	"	CC2AD	"	"	"	0.066	9.10	1.5

* = Brachycentrus

** = Live insects

*** = Dead insects

Water Sample ID						Cd Sol Ppm	Sample Size Ml
2	SR-1	8	9	79		0.41	25
4	CC-2	8	10	79		0.14	"
6	SR-1	"	"	"		0.45	"
8	SR-1	8	11	79		0.57	"
10	SR-1	8	12	79		0.49	"
Blank	8	5	79*			0.012	50

* = Analysis done by Lockett, Berkeley

Colorado Analytical Laboratory Results for Pilot Run
March, 1980. Cadmium.

Insect Sample ID						Dwgt Gms	Cd Sol Ppm	Insect Cd ppm
7	Holding	0 hr	1 3 14	80		0.0631	0.031	2.46
8	"	" "	3	"		0.1448	0.053	1.83
9	Cadmium	72 hr	1 3 17	80		0.0774	0.154	9.94
10	"	" "	3	"		0.0688	0.258	18.78
11	Fresh	6 hr	1	"		0.0435	0.062	7.13
12	"	" "	3	"		0.1537	0.437	14.22
13	"	48 hr	1 3 19	80		0.0822	0.077	4.68
14	"	" "	3	"		0.1230	0.175	7.11

Water Sample ID					Cd Sol Ppm	Sample Size Ml
1	Holding	0 hr	3 14	80	0.028	25.0
2	Cadmium	" "	"		3.780	26.1
3	Fresh	72 hr	3 17	80	0.052	25.9
4	Cadmium	" "	"		0.040	25.0
5	Fresh	6 hr	"		0.042	25.0
6	"	48 hr	3 19	80	0.084	25.5
15	Blank		"		0.033	29.9

Colorado Analytical Laboratory Results on Flow Through
Experimentation. Date: July 10, 1980. Cadmium.

Insect Samples ID					Dwgt Grams	Soln Ppm	(x5/wt) Net Ppm
1	Control	6	2	80	0.0701	0.02	1.4
2	"	"	"	"	0.0882	0.03	1.7
3	"	"	"	"	0.0930	0.02	1.1
4	"	"	"	"	0.1070	0.04	1.9
5	"	"	"	"	0.1451	0.05	1.7
6	"	"	"	"	0.0603	0.03	2.5
7	"	"	"	"	0.0648	0.02	1.5
8	"	"	"	"	0.0772	0.04	2.6
9	"	"	"	"	0.0862	0.04	2.3
10	"	"	"	"	0.0875	0.04	2.3
11	Cd Live	6	5	80	0.0852	0.34	20.0
12	"	"	"	"	0.1221	0.62	25.4
13	"	"	"	"	0.1220	0.47	19.3
14	"	"	"	"	0.1411	0.03	1.1
15	"	"	"	"	0.0837	0.38	22.7
16	"	"	"	"	0.1346	0.73	27.1
17	"	"	"	"	0.0709	0.03	2.1
18	"	"	"	"	0.1104	0.46	20.8
19	"	"	"	"	0.0856	0.65	38.0
20	"	"	"	"	0.0765	0.05	3.3
21	Cd Dead	6	5	80	0.0871	0.49	28.1
22	"	"	"	"	0.0763	0.49	32.1
23	"	"	"	"	0.1016	0.67	33.0
24	"	"	"	"	0.1044	0.40	19.2
25	"	"	"	"	0.0783	0.44	28.1
26	"	"	"	"	0.0580	0.33	28.4
27	"	"	"	"	0.0455	0.32	35.2
28	"	"	"	"	0.0827	0.26	15.7
29	"	"	"	"	0.0566	0.39	34.5
30	"	"	"	"	0.0769	0.44	28.6
31	Live I	6	6	80	0.1015	0.06	3.0
32	"	"	"	"	0.1141	0.19	8.3
33	"	"	"	"	0.1188	0.02	0.8
34	"	"	"	"	0.1138	0.26	11.4
35	"	"	"	"	0.1005	0.24	11.9
36	"	"	"	"	0.1237	0.28	11.3
37	"	"	"	"	0.0782	0.28	17.9
38	"	"	"	"	0.1081	0.13	6.0
39	"	"	"	"	0.1191	0.44	18.5
40	"	"	"	"	0.0947	0.16	8.4

41	Dead I	6	6	80	0.0118	0.48	203.4
42	"	"	"	"	0.0556	0.39	35.1
43	"	"	"	"	0.0566	0.42	37.1
44	"	"	"	"	0.0798	0.43	26.9
45	"	"	"	"	0.0867	0.35	20.2
46	"	"	"	"	0.0688	0.37	26.9
47	"	"	"	"	0.0455	0.25	27.5
48	"	"	"	"	0.0435	0.36	41.4
49	"	"	"	"	0.0839	0.29	17.3
50	"	"	"	"	0.0363	0.19	26.2
51	Cd Live	6	6	80	0.0970	1.07	55.2
52	"	"	"	"	0.0806	1.65	102.4
53	"	"	"	"	0.1288	0.63	24.5
54	"	"	"	"	0.1326	0.85	32.1
55	"	"	"	"	0.0991	1.26	63.6
56	"	"	"	"	0.0802	0.80	49.9
57	"	"	"	"	0.0756	0.88	58.2
58	"	"	"	"	0.1059	0.58	27.4
59	"	"	"	"	0.0751	0.81	53.9
60	"	"	"	"	0.0765	0.11	7.2
61	Live I	6	7	80	0.0834	0.03	1.8
62	"	"	"	"	0.0761	0.12	7.9
63	"	"	"	"	0.0876	0.15	8.6
64	"	"	"	"	0.0925	0.19	10.3
65	"	"	"	"	0.1038	0.38	18.3
66	"	"	"	"	0.0850	0.19	11.2
67	"	"	"	"	0.1182	1.06	44.8
68	"	"	"	"	0.0788	0.20	12.7
69	"	"	"	"	0.1495	0.26	8.7
70	"	"	"	"	0.1168	0.21	9.0
71	Live II	6	7	80	0.1190	0.78	32.8
72	"	"	"	"	0.1236	0.16	6.5
73	"	"	"	"	0.1049	0.81	38.6
74	"	"	"	"	0.1075	0.53	24.7
75	"	"	"	"	0.1138	0.41	18.0
76	"	"	"	"	0.1229	0.57	23.2
77	"	"	"	"	0.1014	0.07	3.5
78	"	"	"	"	0.0643	0.51	39.7
79	"	"	"	"	0.1274	0.45	17.7
80	"	"	"	"	0.0757	0.36	23.8
81	Cd Live	6	7	80	0.0785	0.47	29.9
82	"	"	"	"	0.0740	3.50	236.5
83	"	"	"	"	0.0643	2.40	186.6
84	"	"	"	"	0.0882	3.30	187.1
85	"	"	"	"	0.0740	0.96	64.9
86	"	"	"	"	0.1073	2.20	102.5
87	"	"	"	"	0.1239	6.00	242.1
88	"	"	"	"	0.0684	0.53	38.7
89	"	"	"	"	0.0918	0.26	14.2
90	"	"	"	"	0.1001	0.15	7.5

91	Live I	6	8	80	0.0696	0.47	33.8
92	"		"		0.0989	0.72	36.4
93	"		"		0.0785	0.34	21.7
94	"		"		0.0765	1.11	72.5
95	"		"		0.1461	1.36	46.5
96	"		"		0.1241	0.07	2.8
97	"		"		0.0881	0.32	18.2
98	"		"		0.0788	0.13	8.2
99	"		"		0.1227	0.27	11.0
100	"		"		0.0899	0.21	11.7
101	Live II	6	8	80	0.1087	0.23	10.6
102	"		"		0.1093	0.15	6.9
103	"		"		0.1184	0.32	13.5
104	"		"		0.0807	0.44	27.3
105	"		"		0.1150	0.33	14.3
106	"		"		0.0915	0.16	8.7
107	"		"		0.0652	0.16	12.3
108	"		"		0.0977	0.21	10.7
109	"		"		0.1034	0.37	17.9
110	"		"		0.1117	0.36	16.1
111	Live III	6	8	80	0.0831	0.51	30.7
112	"		"		0.1145	1.26	55.0
113	"		"		0.1214	0.26	10.7
114	"		"		0.1116	0.02	0.9
115	"		"		0.1237	1.30	52.5
116	"		"		0.1113	0.24	10.8
117	"		"		0.0883	0.42	23.8
118	"		"		0.0868	0.78	44.9
119	"		"		0.0892	1.98	111.0
120	"		"		0.1096	0.08	3.6
121	Live II	6	9	80	0.1262	1.40	55.5
122	"		"		0.1476	0.38	12.9
123	"		"		0.1472	0.21	7.1
124	"		"		0.0603	0.21	17.4
125	"		"		0.0839	0.24	14.3
126	"		"		0.0873	0.60	34.4
127	"		"		0.0945	0.33	17.5
128	"		"		0.1066	2.16	101.3
129	"		"		0.1449	1.24	42.8
130	"		"		0.1139	0.79	34.7
131	Live III	6	9	80	0.0920	0.47	25.5
132	"		"		0.0928	0.16	8.6
133	"		"		0.0696	0.11	7.9
134	"		"		0.1288	0.63	24.5
135	"		"		0.1259	0.29	11.5
136	"		"		0.1373	0.38	13.8
137	"		"		0.1094	0.02	0.9
138	"		"		0.1155	0.24	10.4
139	"		"		0.1121	0.33	14.7
140	"		"		0.1068	0.29	13.6

141	Live	III	6	10	80	0.1051	0.15	7.1
142	"	"	"	"	"	0.0827	0.38	23.0
143	"	"	"	"	"	0.1235	0.27	10.9
144	"	"	"	"	"	0.0819	0.06	3.7
145	"	"	"	"	"	0.0682	0.11	8.1
146	"	"	"	"	"	0.0941	0.13	6.9
147	"	"	"	"	"	0.0711	0.07	4.9
148	"	"	"	"	"	0.0637	0.10	7.8
149	"	"	"	"	"	0.1170	0.13	5.6
150	"	"	"	"	"	0.0987	0.25	12.7
167	Live	IIM	6	8	80 *	0.1306	0.06	2.3
168	Live	IIX	"	"	*	0.0104	0.28	134.6
170	Live	IIIM	"	"	**	0.0821	0.05	3.0
171	Live	IIIX	"	"	**	0.0078	0.15	96.1

* M = Moultee

* X = Exoskeleton from same individual

** From same individual

Water Sample ID					Cd ppm	Sample size ml
Holding	6	2	80		0.00	25.0
"	"	"	"		0.00	19.2
"	"	"	"		0.00	25.0
"	"	"	"		0.00	25.0
"	"	"	"		0.00	27.8
Cd	"	"	"		0.78	25.9
"	"	"	"		0.84	25.0
"	"	"	"		0.84	25.0
"	"	"	"		0.83	25.0
"	"	"	"		0.83	25.0
Cd	6	3	80		0.64	25.0
"	"	"	"		0.64	25.0
"	"	"	"		0.64	25.0
"	"	"	"		0.63	25.0
"	"	"	"		0.63	25.8
Cd	6	4	80		0.94	25.5
"	"	"	"		0.95	25.0
"	"	"	"		0.95	25.0
"	"	"	"		0.94	25.0
"	"	"	"		0.94	25.9
Freshwater	6	5	80		0.00	25.8
"	"	"	"		0.00	26.4
"	"	"	"		0.00	25.6
"	"	"	"		0.00	25.5
"	"	"	"		0.00	25.8
Cd	"	"	"		1.13	25.9
"	"	"	"		1.15	25.6
"	"	"	"		1.14	25.5
"	"	"	"		1.14	25.1
"	"	"	"		1.15	25.2

Freshwater	6	6	80	0.02	25.3
"	"	"	"	0.00	25.0
"	"	"	"	0.00	25.4
"	"	"	"	0.01	25.3
"	"	"	"	0.00	25.0
Cd	"	"	"	1.23	25.0
"	"	"	"	1.15	25.0
"	"	"	"	1.26	25.0
"	"	"	"	1.25	25.0
"	"	"	"	1.28	24.7
Freshwater	6	7	80	0.02	25.0
"	"	"	"	0.00	25.0
"	"	"	"	0.00	25.3
"	"	"	"	0.01	25.4
"	"	"	"	0.00	25.8
Cd	"	"	"	1.15	25.1
"	"	"	"	1.23	25.0
"	"	"	"	1.20	25.0
"	"	"	"	1.19	25.1
"	"	"	"	1.21	25.0
Freshwater	6	8	80	0.00	25.0
"	"	"	"	0.00	25.7
"	"	"	"	0.00	25.8
"	"	"	"	0.00	25.4
"	"	"	"	0.00	25.3
"	6	9	80	0.00	25.0
"	"	"	"	0.00	25.8
"	"	"	"	0.00	25.5
"	"	"	"	0.00	25.4
"	"	"	"	0.00	25.0
"	6	10	80	0.00	25.1
"	"	"	"	0.00	25.3
"	"	"	"	0.00	25.4
"	"	"	"	0.00	25.8
"	"	"	"	0.00	25.4
Blank	6	8	80	0.00	30.0
"	"	"	"	0.00	30.0
"	"	"	"	0.00	30.0
"	"	"	"	0.00	30.6
"	"	"	"	0.00	29.4

Agricultural Consultants, Inc. Results for Field Work 1980.

October 22, 1980. Cadmium.

Sample ID						Grams Weight	Soln Ppm	(x5/wt) Net Ppm
1	G-1	7	18	80	<u>M. signata</u>	0.0775	0.05	3.2
2	"	"	"	"	"	0.0881	0.03	1.7
3	"	"	"	"	"	0.1181	0.05	2.1
4	"	"	"	"	"	0.0902	0.04	2.2
5	"	"	"	"	"	0.0856	0.02	1.2
6	SR-2	8	29	80	<u>Cinygmula sp.</u>	0.0629	0.95	75.5
7	"	"	"	"	"	0.0500	1.18	118.1
8	"	"	"	"	"	0.0486	0.95	97.7
9	"	"	"	"	<u>E. doddsi</u>	0.0748	0.74	49.4
10	"	"	"	"	"	0.0640	0.41	32.0
11	GR-1	7	24	80	<u>P. badia</u>	0.3023	0.14	2.3
12	"	"	"	"	"	0.3151	0.19	3.0
13	"	"	"	"	"	0.3347	0.17	2.5
14	"	"	"	"	"	0.3350	0.17	2.5
15	"	"	"	"	"	0.3746	0.17	2.3
16	"	"	"	"	<u>Hydropsyche</u>	0.1654	0.10	3.0
17	"	"	"	"	"	0.1740	0.07	2.0
18	"	"	"	"	"	0.3316	0.19	2.9
19	CC-1	7	21	80	<u>E. doddsi</u>	0.0705	0.11	7.8
20	"	"	"	"	"	0.1132	0.12	5.3
21	"	"	"	"	"	0.1441	0.12	4.2
22	"	"	"	"	"	0.0736	0.06	4.1
23	"	"	"	"	"	0.0937	0.13	6.9
24	"	"	"	"	<u>E. grandis</u>	0.1401	0.21	7.5
25	CC-2	7	26	80	<u>E. doddsi</u>	0.1208	0.77	31.9
26	"	"	"	"	"	0.1095	0.64	29.2
27	"	"	"	"	"	0.0837	0.52	31.1
28	"	"	"	"	"	0.1353	0.71	26.2
29	"	"	"	"	"	0.1010	0.45	22.3
30	"	"	"	"	<u>E. grandis</u>	0.0828	0.46	27.8
31	CC-3	8	2	80	<u>E. doddsi</u>	0.0779	0.21	13.5
32	"	"	"	"	"	0.1128	0.30	13.3
33	"	"	"	"	"	0.1264	0.29	11.5
34	"	"	"	"	"	0.0673	0.19	14.1
35	"	"	"	"	"	0.0672	0.25	18.6
36	"	"	"	"	<u>Hydropsyche</u>	0.0738	0.18	12.2
37	"	"	"	"	<u>E. grandis</u>	0.0736	0.34	23.1
38	ER-1	7	28	80	<u>Hydropsyche</u>	0.4307	0.31	3.6
39	"	"	"	"	"	0.4886	0.33	3.4
40	"	"	"	"	"	0.4953	0.35	3.5
41	"	"	"	"	"	0.4038	0.38	4.7
42	"	"	"	"	"	0.2426	0.30	6.2
43	"	"	"	"	<u>P. badia</u>	0.1370	0.22	8.0
44	"	"	"	"	"	0.1011	0.07	3.5
45	"	"	"	"	"	0.1587	0.23	7.2
46	"	"	"	"	"	0.2373	0.31	6.5
47	"	"	"	"	"	0.1625	0.11	3.4

48	ER-1	7	28	80	E. grandis	0.1876	0.88	23.5
49	"	"	"	"	E. doddsi	0.1043	0.26	12.5
50	OBJ-1	8	12	80	E. doddsi	0.1595	0.33	10.3
51	"	"	"	"	"	0.1054	0.34	16.1
52	"	"	"	"	"	0.1939	0.42	10.8
53	"	"	"	"	"	0.1250	0.42	16.8
54	"	"	"	"	"	0.1454	0.47	16.2
55	"	"	"	"	<u>Hydropsyche</u>	0.3678	0.33	4.5

Water Samples

Sample ID	Cd, Ppm	Sample Size Ml
1 G-1 7 18 80	0.01	29.2
2 " " "	0.01	28.2
3 " " "	0.01	28.9
4 " " "	0.01	27.5
5 " " "	0.01	28.2
6 SR-2 8 29 80	0.33	28.1
7 " " "	0.51	28.4
8 " " "	0.38	27.6
9 " " "	0.40	27.6
10 " " "	0.51	28.3
11 GR-1 7 24 80	0.01	26.8
12 " " "	0.01	27.0
13 " " "	0.01	25.6
14 " " "	0.01	26.6
15 " " "	0.01	26.3
16 CC-1 7 21 80	0.05	29.3
17 " " "	0.06	30.3
18 " " "	0.06	29.7
19 " " "	0.05	29.5
20 " " "	0.05	28.7
21 CC-2 7 26 80	0.15	27.5
22 " " "	0.11	28.0
23 " " "	0.19	27.8
24 " " "	0.16	27.9
25 " " "	0.13	27.5
26 CC-3 8 2 80	0.07	28.8
27 " " "	0.06	28.6
28 " " "	0.07	28.9
29 " " "	0.07	27.9
30 " " "	0.07	28.9
31 ER-1 7 28 80	0.02	25.6
32 " " "	0.03	25.5
33 " " "	0.02	26.6
34 " " "	0.02	27.0
35 " " "	0.03	28.2
36 CB-1 8 10 80	0.02	28.5
37 " " "	0.02	28.2
38 " " "	0.02	28.9
39 " " "	0.02	27.5
40 " " "	0.02	30.1

41	OBJ-1	8	12	80	0.06	28.6
42	"		"		0.04	29.9
43	"		"		0.05	28.6
44	"		"		0.04	30.0
45	"		"		0.04	21.1
46	Blank	8	19	80	0.00	24.7
47	"		"		0.00	25.4
48	"		"		0.00	25.0
49	"		"		0.00	24.9
50	"		"		0.00	25.2

Agricultural Consultants, Inc. Results for Field Work 1980.

October 22, 1980. Molybdenum.

Sample ID						Grams Weight	Soln Ppm	(x5/5/wt) Net Ppm
1	G-1	7	18	80	<u>M. signata</u>	0.0775	0.02	0.26
2	"	"	"	"	"	0.0881	0.01	0.11
3	"	"	"	"	"	0.1181	0.02	0.17
4	"	"	"	"	"	0.0902	0.03	0.33
5	"	"	"	"	"	0.0856	0.01	0.12
6	SR-2	8	29	80	<u>Cinygmula sp.</u>	0.0629	0.06	0.95
7	"	"	"	"	"	0.0500	0.08	1.60
8	"	"	"	"	"	0.0486	0.08	1.65
9	"	"	"	"	<u>E. doddsi</u>	0.0748	0.07	0.94
10	"	"	"	"	"	0.0640	0.09	1.41
11	GR-1	7	24	80	<u>P. badia</u>	0.3023	0.05	0.17
12	"	"	"	"	"	0.3151	0.04	0.13
13	"	"	"	"	"	0.3347	0.03	0.09
14	"	"	"	"	"	0.3350	0.06	0.18
15	"	"	"	"	"	0.3746	0.04	0.11
16	"	"	"	"	<u>Hydropsyche</u>	0.1654	0.05	0.30
17	"	"	"	"	"	0.1740	0.05	0.29
18	"	"	"	"	"	0.3316	0.06	0.18
19	CC-1	7	21	80	<u>E. doddsi</u>	0.0705	0.04	0.57
20	"	"	"	"	"	0.1132	0.03	0.27
21	"	"	"	"	"	0.1441	0.03	0.21
22	"	"	"	"	"	0.0736	0.02	0.27
23	"	"	"	"	"	0.0937	0.03	0.32
24	"	"	"	"	<u>E. grandis</u>	0.1401	0.04	0.29
25	CC-2	7	26	80	<u>E. doddsi</u>	0.1208	0.17	1.41
26	"	"	"	"	"	0.1095	0.09	0.82
27	"	"	"	"	"	0.0837	0.09	1.08
28	"	"	"	"	"	0.1353	0.08	0.59
29	"	"	"	"	"	0.1010	0.07	0.69
30	"	"	"	"	<u>E. grandis</u>	0.0828	0.08	0.97
31	CC-3	8	2	80	<u>E. doddsi</u>	0.0779	0.05	0.64
32	"	"	"	"	"	0.1128	0.04	0.35
33	"	"	"	"	"	0.1264	0.05	0.40
34	"	"	"	"	"	0.0673	0.06	0.89
35	"	"	"	"	"	0.0672	0.05	0.74
36	"	"	"	"	<u>Hydropsyche</u>	0.0738	0.05	0.68
37	"	"	"	"	<u>E. grandis</u>	0.0736	0.06	0.82
38	ER-1	7	28	80	<u>Hydropsyche</u>	0.4307	0.17	0.39
39	"	"	"	"	"	0.4886	0.16	0.33
40	"	"	"	"	"	0.4953	0.19	0.38
41	"	"	"	"	"	0.4038	0.14	0.35
42	"	"	"	"	"	0.2426	0.16	0.66
43	"	"	"	"	<u>P. badia</u>	0.1370	0.06	0.44
44	"	"	"	"	"	0.1011	0.05	0.49
45	"	"	"	"	"	0.1587	0.05	0.32
46	"	"	"	"	"	0.2373	0.06	0.25
47	"	"	"	"	"	0.1625	0.04	0.25

48	ER-1	7	28	80	<u>E. grandis</u>	0.1876	0.05	0.27
49	"	"	"	"	<u>E. doddsi</u>	0.1043	0.04	0.38
50	OBJ-1	8	12	80	<u>E. doddsi</u>	0.1595	0.09	0.56
51	"	"	"	"	"	0.1054	0.09	0.85
52	"	"	"	"	"	0.1939	0.16	0.83
53	"	"	"	"	"	0.1250	0.08	0.64
54	"	"	"	"	"	0.1454	0.09	0.62
55	"	"	"	"	<u>Hydropsyche</u>	0.3678	0.13	0.35

Water Samples

No measurable Mo in any water sample.

APPENDIX D

STATISTICAL ANALYSES

Complete computer printouts of the statistics for the flow-through study and the field work of 1980 are on file in the main office of Hurst Hall, Division of Natural Sciences and Mathematics, Western State College of Colorado, Gunnison, Colorado, 81230.

ANALYSIS OF VARIANCE

72/0-24-48-72

Source	Sum of squares	D.F.	Mean Square	F	Sig
Between groups	1680.278	3	560.093	3.247	0.0357
Within groups	5001.984	29	172.482		

96/0-24-48-72

Between groups	6024.062	3	2008.021	4.790	0.0068
Within groups	14673.455	35	419.242		

120/0-24-48-72

Between groups	63160.197	3	21053.399	7.783	0.0005
Within groups	86562.218	32	2705.069		

24/72-96-120

Between groups	3797.682	2	1898.841	4.909	0.0171
Within groups	8510.384	22	386.836		

ANALYSIS OF VARIANCE

48/72-96-120

Source	Sum of Squares	D.F.	Mean Square	F	Sig.
Between Groups	3.440	2	1.720	0.025	0.9635
Within Groups	1733.370	25	69.335		

72/72-96-120

Between groups	3016.609	2	1508.304	3.452	0.0465
Within groups	10923.898	25	436.956		

Adsorption/Desorption

Between groups	12268.223	2	6134.111	3.637	0.0291
Within groups	177102.194	105	1686.688		

Desorption/Adsorption

Between groups	40735.350	3	13578.450	9.501	0.0000
Within groups	148635.067	104	1429.183		

SCHEFFE'S TEST

$$\frac{(\bar{X}_1 - \bar{X}_2)^2}{MS_{wg} \times (N_1 + N_2) / (N_1 \times N_2)} = \text{Scheffe's F ratio}$$

$$F^1 = F(\text{table value}) \times (K-1)$$

72/24	$\frac{(24.8 - 11.7)^2}{172.48((7 + 8)/(7 \times 8))}$	= 3.71	$F^1 = 8.79$
72/48	$\frac{(24.8 - 14.6)^2}{172.48((7 + 9)/(7 \times 9))}$	= 2.38	
72/72	$\frac{(24.8 - 28.9)^2}{172.48((7 + 9)/(7 \times 9))}$	= 0.38	

Fail to reject H_0 for 72/24, 72/48 and 72/72.

120/24	$\frac{(111 - 42.4)^2}{2705((8 + 10)/(8 \times 10))}$	= 7.73	$F^1 = 7.73$
120/48	$\frac{(111 - 14.5)^2}{2705((9 + 10)/(9 \times 10))}$	= 16.3	
120/72	$\frac{(111 - 9.7)^2}{2705((9 + 10)/(9 \times 10))}$	= 17.97	

Fail to reject H_0 for 120/24.

ANOVA
CD IN INSECT BY ADSORPTION AND DESORPTION

Source of variation	Sum of squares	DF	Mean Square	F	Sig.
Main effects	50665.629	5	10133.126	9.157	0.000
AHR	9930.283	2	4965.142	4.487	0.014
RHR	38397.406	3	12799.136	11.566	0.000
2-way interactions	32467.129	6	5411.188	4.890	0.000
AHR RHR	32467.129	6	5411.188	4.890	0.000
Explained	83132.758	11	7557.523	6.829	0.000
Residual	106237.664	96	1106.642		
Total	189370.422	107	1769.817		

ANALYSIS OF VARIANCE

CD IN INSECT BY STATION AND SPECIES

Source	Sum of squares	DF	Mean Square	F	Sig.
Between groups	21500.286	7	3071.469	22.505	0.0000
Within groups	5049.844	37	136.482		

CD IN INSECT BY SPECIES AND STATION

Between groups	22841.238	4	5710.310	61.585	0.0000
Within groups	3708.892	40	92.722		

FCF BY STATION AND SPECIES

Between groups	205869984.840	7	*****	4.147	0.0019
Within groups	262416699.479	37	*****		

FCF BY SPECIES AND STATION

Between groups	41180639.440	4	*****	0.964	0.4389
Within groups	427106044.541	40	*****		

REGRESSION ANALYSIS

 CD IN INSECT WITH CD IN WATER (VARIED SPECIES)
 FROM G-1 TO GR-1

Correlation (r)	0.89502
r squared	0.80106
Significance	0.00001
Std err of est	11.08294
Intercept (A)	1.18878
Slope (B)	7774.26659
Plotted values	45

 CD IN E. DODDSI WITH CD IN WATER (FROM OBJ-1
 TO CC-3)

Correlation (r)	0.83426
r squared	0.69599
Significance	0.00001
Std err of est	6.46398
Intercept (A)	8.24917
Slope (B)	3891.08034
Plotted values	22