THE EFFECTS OF SURFACE DISTURBANCES
ON THE LEACHING OF HEAVY METALS

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ABSTRACT

The harmful effects of heavy metal contamination of surface waters impacted by gold mining activity are well documented. An examination was conducted on the effects of surface disturbances in Wade Creek on the concentrations of heavy metals in solution, and whether Thiobacillus ferrooxidans, a bacteria found in heavy metal contaminated drainages from placer mines, is found in the drainage.

Thiobacillus ferrooxidans was not detected in this particular setting. The effects of mining activity and re-landscaping of stockpiled tailings showed in a short distance, a net increase of dissolved arsenic, copper, zinc, and iron. However, the long distance impact of dissolved metals was minimal. Generally, it seems that the dampening of the total suspended solids had a direct effect on the removal of metals dissolved in solution.

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PREFACE

"They're cowboys who would mine their grandmother's mouth if there was some gold in it."

B. Cohen, 1986

ACKNOWLEDGMENTS

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LITERATURE REVIEW

CHAPTER I

Introduction

Deleterious effects of mine drainage on water quality
have been known for years. As early as 1868, a report to
the River Pollution Commission in Britain described the
injury to livestock and killing of herbage caused by mine
drainage of toxic metals from lead, zinc, and arsenic
mines (Forstner and Wittman, 1981). Recently, significant
deleterious impacts caused by mine drainage from coal
fields in the eastern United States have become public,
and the difficulties faced with trying to rehabilitate
these impacted areas have become known.

Unfortunately, mine drainage does not only come from
the mine but also from the mine's refuse (tailings).
Tailings often have high concentrations of metal sulfides
with the most commonly occurring iron sulfides being in
the form of pyrites, pyrrohotites , and marcasites
(Forstner and Wittman, 1981). Metals and reduced sulfur in
tailings can oxidize causing dissolution of the metals at
such a slow rate that tailings are able to leach metals
for decades.

This primary mine effluent is commonly known as acid

mine drainage. This acidic effluent is indicated by a very rapid pH decrease which can enhance the leaching of metals into the water system. The pH decrease is attributed to the slow oxidation of sulfides and/or sulfo-salts which are associated with ore and coal deposits. The oxidation process can be aided by the acidophilic bacterium Thiobacillus ferrooxidans, which assists in the oxidation of exposed pyrites to produce ferrous iron, sulfate, hydrogen ions, and an indirect leaching of metals that accumulate in the iron sulfides.

Since the presence of T. ferrooxidans and the leaching of heavy metals by acidic water from coal mines has been described by numerous scientists,it is possible that the same environmental conditions that had arisen in coal mines could arise in gold mines that had sulfide formations associated with them (Brown et al., 1982). In fact recent studies in Alaska and Canada have identified acidic subarctic streams and the presence of T. ferrooxidans in areas affected by gold mine drainage. Hence, areas of Alaska may provide unique opportunities to study problems of acid mine drainage from gold mines because of the long history and wide dispersal of gold mining in Alaska.

The objective of this project was to determine if the presence of T. ferrooxidans in the waters affected by gold mining in Alaska can be used as a biological indicator for

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heavy metal contamination.

The objective was approached by first reviewing the literature that describes the history, geography, and geology of a historic mining area in Alaska. I chose the Wade Creek area of the Fortymile District. Next, I reviewed the nature of metal pollution in aquatic environments in general, and the environmental affects of metals on the growth of T. ferrooxidans. Finally, I undertook a field study to determine the presence of T. ferrooxidans and heavy metals at several sites along Wade Creek throughout the summer of 1986.

CHAPTER II

History

The Fortymile district is the oldest mining district
in the interior of Alaska. Gold was first discovered in
1886 on the bars of the Fortymile river by Howard Franklin
and shortly thereafter on numerous stream tributariesto
the Fortymile river (Mertie,1930). In 1899, the placers of
Wade Creek were first discovered by Jack Wade and within a
short period bench and creek claims were actively being
worked. Work continued through the early 1900's mostly
between 4 and 9 miles above the mouth, with production of
16,231 ounces of gold between 1904-07 (Prindle, 1908).
Because of favorable working conditions, the small mining
parties turned Wade Creek into one of the major gold
producing creeks in the district. The Fortymile district
produced 2 percent of the total placer gold of Alaska as
of 1973 (Cobb, 1973).

Historically, a hydraulic plant was installed on the
upper end of Wade Creek to begin drift mining operations,
and to expand the winter mining activity in 1922. However,
by 1924, lack of water slowed the operation and the miners
went back to scrappers (Brooks and Capps, 1924). In 1934,
a extensive drilling program began to test prospective
dredging grounds and by 1936 preparations for the transfer

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of a dredge from Franklin Creek to Wade Creek started. By the end of 1936, the dredge was installed near the mouth and operated and worked the ground until 1940 where it stopped operating one half mile below Ophelia Creek and still remains today.

During the 1950's mining on Wade Creek was hampered by the war effort and most of the miners found employment in road construction.

More recently, smaller placer mining operations are still the common method of mining on Wade Creek (e.g. 1-2 people working a claim). Yet, the miners have gone beyond the pick and shovel method and employ bulldozers and gas powered generators and pumps. The mining techniques are still basically the same however; remove the over burden; sluice the placer; recover the gold; and stock pile the tailings.

In summary, the majority of the mining on Wade Creek has been large scale open cuts in the upper valley, while downstream, most of the impact that is seen was caused by shallow drift mining, open cuts by dozers and dredging. In fact, the mining activity has been so extensive that today the original stream bed that Jack Wade prospected cannot be identified.

CHAPTER III

Physical Setting

Location

The Wade Creek drainage system is located at a longitude between 64 degrees 5 minutes and 64 degrees 10 minutes north, at a latitude between 141 degrees 25 minutes and 141 degrees 37 minutes west in Section 26, Township 28 North, Range 20 East from the Copper River Meridian, in the Yukon basin, near the townsites of Chicken and Boundary, Alaska.

The drainage system encompasses an area of 23.1 square miles. The headwaters are formed at the confluence of Grace Creek and Warner Creek. With the headwaters at an elevation of 3,300 feet, Wade Creek flows until its confluence with the Walker Fork at an elevation of 2,400 feet. The total length of the creek is 10 miles, with an average slope of less than two percent. The valley is entrenched about 1,500 feet from the plateau, and flows in a nearly straight southwestern direction. Three primary stream tributaries flow into Wade Creek: those of Ophelia Creek, Robinson Creek, and Gilliland Creek.

The vegetation types surrounding the undisturbed areas of Wade Creek are typical of boreal taiga forests, typical in interior Alaska and the Yukon basin. Where

surface disturbances by mining activity have occurred, natural revegetation of the tailings seems to be occurring with the growth of alder and grasses.

Permafrost is frequent and close to the surface of north facing slopes and undisturbed valley bottoms. The gravel and tailings adjacent to Wade Creek are usually permafrost free.

The region experiences a subpolar climate with severe subarctic winters and seasonal summers. Periods of -50° C. temperatures in the winter are common with summer temperatures reaching as high as 32° C.. Freeze up usually occurs in October and by early May Wade Creek may be ice-free.

Developments in the Wade Creek basin include the old townsite of Jack Wade, an airstrip that was used to supply goods to the townsite, the Taylor highway which runs parallel to Wade Creek, three active gold mining operations, and a Bureau of Land Management campground at its mouth. One of the mining operations is located at milepost 89.9 of the Taylor highway and operates at milepost 87.8. Another of the operations is located at and operates at the confluence of Gilliland Creek and Wade Creek. The third operation is located at the airstrip and operates on Grace Creek.

During the study period, employees of the Alaska Department of Transportation were engaged in a re-

landscaping project of some of the tailings areas on the lower four miles of Wade Creek.

Geology

The Fortymile area is underlain primarily by metamorphic rocks which have been intruded by a wide variety of igneous rocks (Foster and Clark, 1970). The metamorphic rocks consist of a wide variety of metasedimentary and metaigneous rocks which have a range from lower greenschist to upper amphibolite facies (Foster, Weber, et. al., 1973). All of the rocks are highly deformed and faulted. Some greenschist facies are known to be of Paleozoic age (Wasserburg, et. al., 1963). The igneous rock occurs in lava flows and in large and small intrusive bodies, such as batholiths, stocks, dikes, and sills.

It is likely that the anomalies in some areas are due to sulfide minerals in greenschist and greenstone, and are not indications of mineral deposits. Some other metamorphic rocks such as quartzite have locally abundant speaks of sulfide minerals. (Foster and Clark 1970).

The bedrock in which Wade Creek is incised includes several varieties of schist, and some ferroigneous rock, and thin bedded limestone, which are apparently interbedded with the schist (garnetiferous). The gravels

vary from one to three feet in thickness and are composed of rocks that are found outcropping the valley (Prindle, 1905).

The Taylor highway, which runs parallel to Wade Creek, cuts through numerous metamorphic rock types. On the east side of the highway towards the mouth of Wade Creek, white quartzite and limy quartzite show complex folding characteristic of medium- high- grade metamorphic. At about milepost 84.4, gray banded garnetiferous quartz-biotite gneiss is found on the west side which is characteristic metamorphic rock of the area. Appromately 2.6 miles further along the highway a low cliff on the west side has crinkled quartz-biotite gneiss exposed, which was isotopically aged and shown to be 177-182 million years old (Wasserburg, et. al., 1963). Indicative of the complex structure of all metamorphic rock in the area is a small plunging anticline in quartzite in the streambed at milepost 89.0. The lower 2/3 of the valley shows a variety of quartz-biotite gneiss, and the upper 1/3 is of marble (green, pink, and white) and quartzite (Foster and Keith, 1969).

The fact that no foreign wash material has been observed means that the minerals are derived from the drainage itself. Many quartz seams and stringers occur in the schist, some of considerable thickness, and seem more abundant towards the head of the creek. Yet most

importantly, both bedrock and quartz veins in the Wade
Creek drainage contain, in places, considerable amounts
of pyrite (Prindle, 1905).

Mineralogy

Presently, gold is the only metallic mineral of
economic significance being mined in the Fortymile region.
Nearly all of this gold has been derived from placer
deposits. No lode gold has been located in sufficient
quantities to mine (Foster and Clark, 1970).

Within Wade Creek, the placer pay streak extends
intermittently from Gilliland Creek to Walker Fork, and
the placer is deposited on and in the bedrock crevices;
some in the lowest 1-1 1/2 feet of gravel. The pay streak
seems to follow the valley, sometimes on one side of the
creek and sometimes on the other (U.S. GeologicSurvey,
1977).

Stream sediments and rock samples have been evaluated
for other metallic minerals, and (Table 1) lists
the concentrations on a dry weight basis of samples
collected from the stream sediments. High concentrations
of lead and barium may be attributed to localized
anamolous deposits in the surrounding geology.
Concentrates containing barite, garnet, cinnabar,
cassiterite, sheelite, and iron-oxide minerals have been

Table 1. Concentrations of metals collected from Wade
         Creek sediments.*

METAL (ppm)

STREAM	Ba	Be	Bi	Co	Cr	Cu	La	Mo	Nb
Gilliland	1000	1.0	**N	N	N	5.0	N	N	N
Gilliland	1000	1.0	N	5.0	5.0	7.0	N	N	N
Confluence	1000	1.0	300	N	10	5.0	N	70	N

	Ni	Pd	Sb	Sc	Sn	Sr	V	W	Y
Gilliland	N	10.0	N	N	N	.5	10	N	N
Gilliland	N	15.0	N	N	N	.5	10	N	N
Confluence	N	1500	N	N	N	150	10	N	N

	Zn	Zr
Gilliland	N	N
Gilliland	N	20
Confluence	N	N

* From U.S. Geologic Survey Open-file Report 77-845.
** N = not detectable.
*** Based on a dry weight basis.

identified within Wade Creek (Martin, 1919; Mertie, 1931; Joesting, 1942). Fluorite was also found in a prospect pit in the fourth tributary above the mouth (Nelson et al., 1954). Yet, as of today, none of these minerals have been mined (U.S. Geologic Survey, 1977; Foster and Clark, 1970).

Geochemistry

The distribution of the elements throughout the Fortymile River area seem to be very consistent with a few anomalous areas having been located (Smith, 1968). The geochemistry studies have shown the presence of copper and zinc at levels that may indicate weak anomalies (Saunders, 1968). Yet, weak anomalies may be due to scattered sulfide minerals in low grade metamorphic rocks, and not indicate extensive mineralization (Foster ans Clark,1970). Therefore, the metallic values foundmay be coming from the scattered specks of sulfide minerals in greenschist and greenstone, or from the mineralization of the quartz veins cutting the metamorphic rock that have locally abundant sulfide minerals (Foster ans Clark, 1970).

Generally, minerals that have been identified in placer concentrations include cassiterite (tin oxide) and scheelite (calcium tungstate; Saunders, 1968). Weak anomalous molybdenum areas have been located in small

granitic intrusions into amphibolites and marbles. Also, areas with anomalous amounts of lead, copper, zinc, and nickel have been found. However, the nickel may be from ultrabasic bodies, or they may be derived from the greenstones (Saunders, 1968).

The sulfide minerals have been found to be abundant locally in the diorite and adjacent metamorphic rocks (Foster and Clark, 1970). Also, narrow veinlets of cuprite have been found in the region (Saunders, 1968).

Within Wade Creek, flourite, casserite, scheelite, barite, and cinnabar have all been identified. Geochemical sediment studies have found concentrations of copper greater than 30 ppm, lead near 10 ppm, zinc as high as 130 ppm, molybdenum from 2 - 3 ppm, manganese as high as 2,000 ppm, mercury as high as 1500 ppm, and barium as high as 1000 ppm. (Foster and Clark, 1966; Saunders, 1968).

Although three studies on the geochemistry have been completed in the area, none identified any anomalous mineral areas. Several conditions are thought to suppress the values of the stream sediment concentrations. One of the most important is the presence of large amounts of limestone and marble in the area. Thus, the metals tend not to migrate as far as they might in noncarbonaceous areas (Smith, 1942).

Summary

In summary, the stream channel of Wade Creek has been
extensively mined for almost an entire century. As a
consequence, the whole length of the creek has been mined
at some point leaving the tailings as a present reminder
of past activities.

Some revegetation of these tailings seems to be
occuring with the growth of alder and grasses. Yet, the
landscape bordering the mined areas is an ever present
reminder of how it might still be if gold had never been
discovered in Wade Creek. However, because of the geologic
formations, gold was deposited, attracted mining, and the
mining activity left the area so disturbed that the
original channel can not be identified. Because of this
extensive working of the creek for its placer, greater
amounts of possible sulfide minerals may have been exposed
to the physical, chemical, and biological weathering
processes.

CHAPTER IV

Heavy metals

Introduction

The term metals is a broad one, covering elements of atomic weight greater than calcium and having specific gravities in excess of 5.0 (Coleman and Piotrowski, 1980). The definition can therefore apply to over 70 metallic elements of which only a few have been recognized as having environmental concerns; a few of those are listed in Table 2. Of the heavy metals listed, 8 are essential in human diets. All 12 elements occur in the noncontaminated environment. Toxic effects to living organisms may occur when any metal exceeds a certain critical level in the environment (Coleman and Piotrowski, 1980).

To cause acute toxic effects doses of relatively high amounts are necessary for most toxic metals. The chronic toxicity is the long term, low concentration exposure of toxic metals, and is dependent upon the progressive accumulation of the toxic metals in an organism. In assessing the potential impact of levels of metals, the terms critical concentrations, threshold concentrations, and no detectable effect level are used. The first two refer to the lowest acute level of exposure

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Table 2. Heavy metals of environmental concern.*

Metals	Essential in Mammals
Copper	Yes
Cadmium	No
Mercury	No
Tin	Yes
Lead	No
Antinomy	No
Vanadium	Yes
Chromium	Yes
Molybdenum	Yes
Manganese	Yes
Cobalt	Yes
Nickel	Yes

* From Piotrowski and Coleman, 1980.

which produce detectable adverse effects. The effects may appear at first at the molecular level causing biochemical damage. The latter refers to the highest acute level of exposure at which no adverse effects can be detected. Therefore, it is assumed that when the critical or threshold level is exceeded, both the frequency and intensity of adverse effects will be likely to increase, and with certain metals these effects may be carcinogenic or mutagenic (Coleman and Piotrowski, 1980).

Bioassay testing is used to determine critical levels of toxicity. Usually by exposing a test organism to various concentrations for a 24-96 hour period, and determining the concentration at which 50 percent of the test organisms perish, the critical or threshold level is derived. The level is expressed as the LC_{50} concentration.

Environmental Impact

The ecological impacts of metals are determined by the amounts released into the environment, persistence and fate of the metals, the toxicity of the metals to organisms, and the extent to which the metals can be bioaccumulated or degraded in the ecosystem (McIntyre and Mills, 1974). The impact may be reflected in the population dynamics, and the ability of the species to survive as a population. The resistance of a species to a particular metal may result from natural selection for

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resistant strains within the population that have developed because of mutations (Forstner and Wittman, 1981).

Release into the Environment

Through infiltration of waters through the ground surface, receiving waters serve as an important route for heavy metals to the environment. Once there, the fate and distribution varies according to pH, hardness, salinity, organic matter, and nutrient content of the receiving waters. Physical changes of heavy metals are possible and residence times will vary according to the physical, chemical, and biological parameters. Yet ultimately, a finite receiving capacity for each metal is reached in the water (de Groot et al., 1973).

Persistence

Primarily, the persistence of a metal in the environment is dependent upon the attractive forces between the metal ions, and organics and inorganics present in the aquatic system. The distribution and fate is highly dependent on the grain size composition of sediments to which a metal can adsorb.Apart from grain size, the characteristics of the suspended matter is of next most importance since the bulk of the heavy metals in

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the streams are being transported by the particulates in the turbid material (R & M Consultants, 1982). Hence, it has been suggested that heavy metals associate with the particulates by means of adsorptive bonding, coprecipitation by hydrous iron and manganese oxide, complexation with organic molecules, and being incorporated in crystalline minerals (Gibb, 1977). Thus, the movement of metals must then be divided into transport by adherence to a suspended material or in a dissolved form.

Adsorption and Complexation

The mechanisms which results in cation exchange of metals are based on the sorptive properties of negatively charged anionic sites in clay minerals. The balance is a selective process which accounts for preferential adsorption of specific cations. In the case of the organic constituents, humic acids exert a particularly high exchange capacity for metal cations when they are present in the the water (Forstner and Wittman, 1981). A sequence for complex stability between metals and humic complexes has been established and is similar to the one established by Irving and Williams (1948) for soil-metal complexation with:

Pb > Cu > Ni > Co > Zn > Cd > Fe > Mn > Mg

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In the adsorptive process of heavy metals and clay, pH may dominate the process. In some streams, hydrogen ions compete with heavy metals for exchange sites on the clay's lattice structure, thereby partially releasing the heavy metals. The metals are completely released from the particulates under extremely acidic conditions (Forstner and Wittman, 1981). Metals which are adsorbed or included in structural units of inorganic sediments can be transformed from these inactive sites through the process of reduction and oxidation. The resulting redox reactions can lead to metals being kept in the colloidal state or in solution by complex formation with organic compounds. Under normal conditions metals would be found in trace amounts, but because of the extended period the metals are held in solution the metals become more readily available to the aquatic and biotic environments in greater concentrations.

Sorption can also occur through the metal's strong affinity for hydroxyl groups of iron and manganese hydroxides. In turn, the metals are incorporated in the hydroxide structure and hydrogen ions are exchanged. This type of sorption is generally not important in water with a the pH range of 5 to 6 (Forstner and Wittman, 1981).

Biomagnification

Once metals reach the environment they may be bioaccumulated so that the concentrations within tissues of an organism are greater than the surrounding environment. The primary consumers and subsequent secondary consumers may further concentrate metals with resulting concentration increases at successive trophic levels. Within the food web, most of the fluctuations in the concentrations of metals depend, to a great extent, upon microbial activities in the water and sediment. In some cases, microorganisms are involved in the transfer, while at other times they convert metals of less availability or toxicity to those of more availability or toxicity.

Depending upon the uptake rate of metals by root systems, plants often translocate metals to areas where the metals will usually be incorporated into connective tissues and proteins (Epstein and Chaney, 1981).

The accumulation of metals in individuals and transfer through the food web are the most important processes to evaluate in order to predict the effects metals have in a given natural environment. The uptake and excretion of many metals are controlled by physiological processes. For example, when a metal is introduced into an empty stomach of a average human, the pH of the stomach

has a range from 1 to 3. This low pH partially solublizes the metal. However, metal absorption is negligible in the stomach (Luckey and Vungepal, 1977). The majority of absorption occurs in the small intestine. The percent absorbed for each metal and the absorption mechanics may change the values according to the ion's characteristics, pH level, time, microbial metabolism in the intestines, amount and type of food eaten, chelating compounds present, physiological condition of the human, and the biochemical mechanisms involved in the absorption of each metal (Luckey and Vungepal, 1977). These physiological processes can result in homestatic control which involves absorption, transportation, and distribution of each metal to various tissues. Because of the requirement by some tissues for certain metals, prime targets for toxic actions and/or metal excretion are somewhat selective (Luckey and Vungel, 1977), and are also dependent on the individual differences in retention and elimination rates, possibly even in the capacity to detoxify the toxic metal (McIntyre and Mills, 1974).

Various vitamins and coenzymes frequently contain metal ions in active sites. Some examples include hemoglobin, ferredoxins, and cytochromes. Essential metal ions can sometimes be displaced from active sites by other metal ions leading to toxic affects. In addition to active sites of vitamins and coenzymes, other electron donor

groups in macromolecules will bind metal ions. For example, disulfide peptide groups of proteins, and phosphate groups on nucleic acids will bind various metals. Therefore, the same metal ion, by binding with different sites at various concentrations, can have beneficial and adverse effects in the same organism (McIntyre and Mills, 1974).

A typical mechanism of absorption for most metals entering into living cells is through chelation. By a toxic metal chelating to a ligand molecule for example, two or more electron donating atoms bind to the metal to form a stable covalently bonded ring. This chelation increases the lipid solubility in some cases and allows the metal to be carried into the cell (otherwise the metal would be left on the outside cell surface; Luckey and Vungepal, 1977). Once inside the cell, the chelated metal will hydrolyze and then combine with the subcellular components and exert its toxic effect.

Summary

In general the physical and chemical characteristics of soil will influence the distribution and fate of heavy metals in the system, and control the transportation of these elements through leaching, plant uptake, and

erosion. As a consequence, the extent to which compounds persist and stabilize to particulates or organisms or are metabolized by organisms will account for the accumulation in the food web.

CHAPTER V

Thiobacillus ferrooxidans

Introduction

For many years the only microorganism thought to be important in the leaching of metals from ores was a rod shaped bacterium known as Thiobacillus ferrooxidans. The microorganism was first discovered in acidic waters draining from coal mines in the eastern United States. It was not until 1947 that a correlation was recognized between the presence of T. ferrooxidans and the dissolution of metals in copper leaching operations (Bryner et al., 1954). Since then a great deal of information has been collected on the role of the bacterium in leaching metals, and recently it has been identified to occur in subarctic streams affected by gold mining activities (Brown et al., 1982)

Ninety percent of the streams affected by gold mining that Brown et al. (1983) sampled had T. ferrooxidans. Some of those streams also had moderate to high concentrations of dissolved arsenic. This suggests that T. ferrooxidans may be directly involved in the leaching of heavy metals from sulfide deposits disturbed through placer gold mining

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in Alaska. Sulfides and heavy metals are associated with placer lodes (Wilson and Hawkins, 1978).

Physiology and Ecology

Thiobacillus ferrooxidans is acidophilic and will thrive in sulfide ore deposits that have high concentrations of self-generated sulfuric acid. The majority of investigations of T. ferrooxidans have been under pH conditions ranging from 1 - 5. Optimum pH values for oxidation of iron have been reported to lie between 2.3 - 2.5 for chalcopyrite, zinc sulfide, chalcocite, covellite, and ferrous iron (Torma, 1976).

However, T. ferrooxidans is capable of colonizing and producing acid in near neutral pH environments (Kleinmann and Crerar, 1979). Thiobacillus ferrooxidans is consistently able to establish itself at pH values greater than 4.0 and can tolerate pH levels up to 6.98 (Ehrlich, 1962).

Purified T. ferrooxidans cell enzymes have optimum pH values ranging from 5.0 to 9.0 (Apel and Dugan, 1978), which is significantly higher than the pH of the organisms environment. Hence, the acidophilic thiobacilli possess cell membranes which are impermeable to high concentrations of protons. Therefore, a natural proton gradient is developed. Through chemiosmotic coupling,

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cells pump protons through membrane channels resulting in both a proton and electropotential gradient across the membrane. The cell then utilizes the energy associated with the gradient to form ATP (Apel and Dugan, 1978). The ATP energy is utilized by the cell for transport work, mechanical work, and biosynthetic work. In this process the ATP is hydrolyzed to ADP and inorganic phosphate (Sakaguchi et al., 1976).

The temperature range for optimum productivity of T. ferrooxidans is limited. Two competing process can occur as the temperature changes: the rise in temperature increases the reaction rate, but at the same time, an increase of cell lysis caused by the denaturing of the proteins occurs. An increase in temperature from 25^0 to 35^0 C results in an increase of more than double the oxidation rate of substrate (Sakaguchi et al., 1976). Most strains are mesophilic with the optimum temperature range varying from 25^0 - 45^0 C depending on the strain of T. ferrooxidans. Both ferrous iron oxidation and sulfide leaching are optimum at a temperature of 35^0 C (Torma, 1976). The biological oxidation ceases at 55^0 C , and at 0^0 C (Torma, 1976). No minimum temperature for T. ferrooxidans has been determined but generally activity should stop at the freezing point of water. In addition, the activity of acidophilic iron-oxidizing bacteria decreases extremely at a water temperature of 10^0 C (Noike

et al., 1980). Consequently, distinct seasonal variations in the number of iron-oxidizing bacteria have been observed in river water with seasonal changes in water temperature (Wakao et al., 1978). Thus, water temperature is an environmental factor influencing iron oxidation due to bacterial growth.

The nutrient requirements for T. ferrooxidans are those which are normal for any chemosynthetic autotroph. It synthesizes its cell material from inorganic sources which are: carbon dioxide (carbon source for the cell growth), ammonium sulfate and dipotassium hydrogen phosphate (as nitrogen and phosphate sources), and potassium chloride, magnesium sulfate, and calcium nitrate (as growth factors) (Torma, 1976). The minor nutrients required are usually in sufficient amounts for the bacterium's growth simply by their occurrence as impurities in the salts and sulfides, and their concentrations have no detectable effect on the rate of substrate oxidation (Torma, 1976).

Growth depends on the availability of carbon dioxide as its carbon source. Carbon dioxide is transferred from the air into the water as a function of its partial pressure and concentration in the water. The bacterium then fixes the carbon dioxide using energy provided by the oxidation of the inorganic substrate. Maciag and Lundgren

(1964) were the first to suggest that the Calvin cycle, as the possible pathway for carbon dioxide fixation (Torma and Habashi, 1972), and ATP provides the bacterium with the energy for carbon dioxide assimilation (Trudinger and Swaine, 1979). Torma et al. (1972) identified that additional oxidation can occur when carbon dioxide enriched air is supplied until other factors becomes limiting. Thiobacillus ferrooxidans is a member of the Thiobacillus group of sulfur bacteria, and can be classified as a chemolithotroph since the bacterium generate its ATP from electrons released during the oxidation of iron in the ferrous form, or oxidizing sulfur in the form of soluble and insoluble sulfides (Brierly, 1982), and obtains its carbon from atmospheric carbon dioxide. It is also a strict aerobe because of the high demand for oxygen for oxidation.

Thiobacillus ferrooxidans is responsible for leaching of heavy metals from sulfide deposits by two means; one being direct leaching and the other being indirect leaching (Brierly, 1982). Under normal air-aeration conditions, the leaching rate is limited by the solid surface substrate per volume, with the best oxidation occurring on particles in the size range of 42 to 220 micrometers in diameter (Torma, 1976; Torma et al., 1972). Direct microbiological leaching of metal sulfides can be defined as a biochemical oxidation process

catalyzed by living organisms. This process can be
simplified to the following equation:

$$MS + 2O_2 \longrightarrow MSO_4 \tag{1}$$

where the M is a divalent metal and the action is mediated
by T. ferrooxidans where the sulfide is oxidized to
sulfate by sulfide oxidase and the energy produced being
available from the removal of eight electrons from the
sulfide. However, the leaching process is a complex
interaction of microorganisms, substrates, and nutrient
concentrations, which are not fully understood (Torma,
1976). The rate of oxidation varies from strain to strain
and is not just an adaptation to the sulfide minerals
(Groudeva et al., 1983).

The sulfide oxidation process is only valid for
synthetic metal sulfides, since the crystal lattice of
most natural metal sulfides contain some iron.
Consequently, some iron is oxidized and dissolved during
metal leaching; thus producing ferric iron which attacks
the minute sulfur substrates as illustrated:

$$MS + 2Fe^{+3} \longrightarrow M^{+2} + 2Fe^{+2} + S \tag{2}$$

or

$$MS + 2Fe(SO_4)_3 \longrightarrow MSO_4 + 2FeSO_4 + S^0 \tag{3}$$
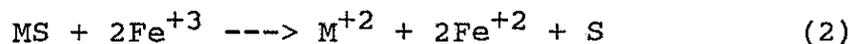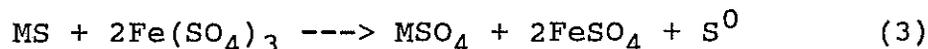
Then the elemental sulfur which is set free is oxidized

by T. ferrooxidans,

$$S^0 + 1.5\ O_2 + H_2O \longrightarrow H_2SO_4 \qquad\qquad (4)$$
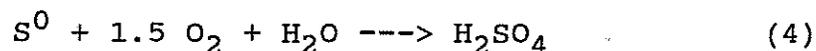
and ferrous iron is also then reoxidized by the bacterium (Torma, 1976).

The bacteria play an indirect role in this reaction as they regulate the ferric leaching agent by oxidizing the ferrous iron to a trivalent state (Groudev, 1980). Ferric iron being in a powerful oxidizing agent reacts with other metals, increasing their solubility, and oxidative state. All of the indirect oxidation occurs in a sulfuric acid solution, and usually with T. ferrooxidans present. The reaction is greatly accelerated by the bacterium oxidizing sulfide, thiosulfate, or tetrathionate in an acid environment. Luong et al. (1984) showed that the iron complex produced by the bacterial oxidation is more stable than the abiotic ferric complex because of an exchange barrier created by the sulfate ions making the oxidized iron less reactive chemically. Nevertheless, the leaching of metal sulfides is accelerated in the presence of ferric iron which is an oxidizing agent. The metal is then freed to the environment, with bacterial activity contributing to trace metal and sulfate leaching in areas that are conducive to the process (Olson et al., 1981).

In instances of high concentrations of ferrous iron

and a pH level in the range of 2.0 - 3.5 (Touvinen and Kelly, 1974) , the optimum growth conditions for T. ferrooxidans are generated. Thus Torma's (1976) equation describes the biological oxidation of ferrous iron:

$$4FeSO_4 + 2H_2SO_4 + O_2 ---> 2Fe_2(SO_4)_3 + 2H_2O \qquad (5)$$

$$2Fe_2(SO_4)_3 + 12 H_2O ---> 4Fe(OH)_3 + 6H_2SO_4. \qquad (6)$$
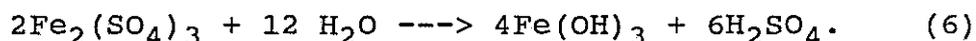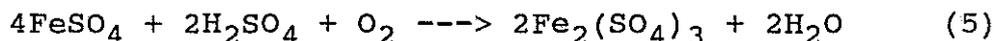
The organism consumes oxygen in a ratio of one mole oxygen per four mole of ferrous iron and oxidized product ferric iron. The energy (ATP) is derived when electrons liberated from the iron are coupled to cytochrome c.

$$Fe^{+2} ---> Fe^{+3} + e^- \qquad (7)$$
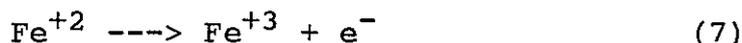
The electron(s) is then transported from cytochrome c to cytochrome a via cytochrome oxidase and is subsequently accepted by oxygen, and eventually forms water (Lundgren and Dean, 1979)

$$1.5 \ O_2 + 2 \ e^- + 2 \ H^+ ---> H_2O \qquad (8)$$
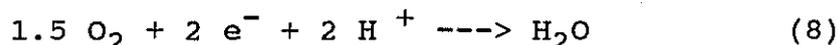
The ferric sulfate, the product of the oxidation, reacts with water to form ferric hydroxide and sulfuric acid (equation 6). This is spontaneous and leads to an increase in acid formation in the environment.

Various ferric hydroxide complexes can result as the reaction occurs. These ferric hydroxyl- sulfate complexes have a buffering capacity which can control the pH of the

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environment. Thus, the complexes compete with the reaction and decrease the sulfuric acid production (Torma, 1976).

Though direct oxidation of pyrite by T. ferrooxidans has been demonstrated, indirect oxidation is 7 times faster than direct oxidation (Singer and Stum, 1970). Therefore, direct mechanisms have not been considered as significant of aspect to the acid mine drainage problem as indirect mechanisms (Kleinmann and Crerar, 1979).

Common types of reduced iron which serve as inorganic substrates are pyrite (FeS_2), chalcopyrite ($CuFeS_2$), and arsenopyrite (AsFeS; Torma, 1976). However Olson et al.(1981) suggests that some forms of pyrite may not be susceptible to microbiological attack even under favorable environmental conditions. Some other sulfide metals that the bacterium metabolizes include nickel sulfide (NiS), zinc sulfide (ZnS), cobalt sulfide (CoS), covellite (CuS), galena (Cu_2S), and lead sulfide (PbS; Silver and Torma, 1974; Sakaguchi et al., 1976).

While the organism is intolerant to low concentrations of silver, molybdenum, and uranium (Roy and Mishra, 1981), the growth of populations may develop metal tolerances in the molar concentration of iron, zinc, cobalt, and nickel (Roy and Mishra, 1981), and selection for metal tolerant strains may occur (Torma 1976; Braddock, 1984).

Though T. ferrooxidans is normally found in acidic

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waters draining from mines. They have also been found in
near neutral and alkaline waters draining from mines in
Montana (Olson et al., 1981). The presence of T.
ferrooxidans in neutral pH mining streams reveals the
probability that heavy metals leaching from mineral
sources is enhanced biologically (Brown et al., 1983).
Yet, the most common areas for T. ferrooxidans to occur
are areas where pyrite is exposed. Which suggests, that T.
ferrooxidans is not growing in the stream draining the
mines but that they are being washed down with
particulates which contain heavy metals (Brown et al.,
1982). Therefore, if the particulates contain heavy metals
associated with sulfides, the bacterium may contribute to
the release of heavy metals from the materials.

However, these heavy metals should eventually be
immobilized by precipitation or by complexing with ferric
hydroxides, and this is dependent on turbidity and time
(Hounslow, 1980; Brown et al., 1982). Fortunately, in
Alaska, wastes from most placer gold mines are only
sometimes high in sulfide minerals, and the affected
streams and groundwaters are rarely acidic (Luong et al.,
1984).

Though there is a great deal of variation in the
physiological and biochemical behavior of strains of T.
ferrooxidans, these organisms are tolerant of many

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metallic ions (Roy and Mishra, 1981). Yet silver shows the highest toxicity, inhibiting activity at 0.1 parts / 10^6, molybdenum at 5 parts / 10^6, and uranium at 250 parts / 10^6 (Roy and Mishra, 1981), and mercury: of which no concentration has been determined (Ingledew, 1982).

At sites where rainfall is limited and soils freeze during the winter, T. ferrooxidans growth may become stressed because of the water potential. The activity of the bacterium is controlled by the water availability which is determined by osmotic and absorptive properties of the substrate. The water potential can be reduced by the water absorbing to a surface and forming a matrix, or it can be reduced by the presence of solutes in the water. In natural soil systems, the potential is negative since it measures the suction that must be applied for the bacterium it draw water from the matrix. Brock (1975) found T. ferrooxidans to be completely inhibited at -23 bars. This describes the maximum amount of pressure the bacterium can exert to draw water from the matrix . Water potential in natural systems are always less than that of pure water and are expressed as negative bar (a suction). Olson et al. (1981) suggest that some environments suitable for the growth of the organism may not attain moisture levels high enough for their growth. This may indicate that water stress could limit T. ferrooxidans populations. Yet, Kleinmann and Cater (1979) found that T.

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ferrooxidans could survive extended periods between rainfall, and its activity increased sharply following water infiltration. Even so, Olson et al. (1981) found that in wet spring months spoils contained relatively low acidophilic iron and sulfur-oxidizing bacteria, and that large population increases were not detected.

There is inhibition by a variety of low molecular weight organic compounds (humic acids), and their effect are relative to their electronegativity. The compounds are most inhibitory in their protonated state (Tuttle and Dugan, 1976). Data from Tuttle and Dugan (1976) suggests the organics effect the enzyme systems, react abiotically with ferrous iron outside the cell, interfere with the role of phosphate and sulfate in iron oxidation, and disrupt the cell membrane. Some organics seem to only exhibit their inhibition when they approach or exceed the ferrous iron concentration (Tuttle and Dugan, 1976).

Also, particulate matter of fluorapatite in concentrations of 0.1 - 4.0 percent have inhibitory effects on the cells when trying to oxidize iron. However, concentrations above these levels have little effect for some unknown reason (Dispirito et al., 1981).

Summary

From the ecological point of view, the presence of T.

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ferrooxidans in the tailings material would indicate the possibility of acid and heavy metal drainage. Since placer, suction, and lode gold mining have increased in Alaska recently, many new areas have the possibility of contaminating the environment for years to come. Contaminated stream waters, stream sediments, and groundwaters are all sites which could be impacted, and in turn, affecting the basic environment from which the food chain begins.

Since the location, distribution, and diversity of the organisms growing in various natural environments are continuously changing, steps to apply information from in vitro experimentation need to be taken if we are to make progress in remedying the problems associated with ecotoxicity caused by biologically enhanced heavy metals leaching and acid drainage from mining sites.

Summary and Objectives

The literature review was completed in order to gather information on the growth of T. ferrooxidans, the physical setting of Wade Creek, and the means by which heavy metals can move through the environment.

Knowing that T. ferrooxidans could be a potential biological indicator of acid mine drainage, and heavy metal leaching from sufide deposits associated with gold mining operations, the objective is to determine if a correlation exists between the presence of T. ferrooxidans and select heavy metals which deposit with sulfide and/or absorb to settable solids that could be leached into solution. The correlation will be based upon whether T. ferrooxidans is present in Wade Creek and the analysis of dissolved heavy metals concentrations from water samples collected from Wade Creek. Inevitably, if T. ferrooxidans is present in this particular setting, it may play an important role in the formation of acidic waters and leach heavy metals into the Wade Creek drainage.

MATERIALS AND METHODS

Materials

Growth medium: The growth medium used for the most
probable number (MPN) test for T. ferrooxidans was the
same as that used by Braddock et al.(1984) and contained:
$MgSO_4 - 7H_2O$ (0.4 g/l), $(NH_4)_2SO_4$ (0.4 g/l), KH_2PO_4 (0.1
g/l), and 10N H_2SO_4 to bring the medium to a pH between
1.8 - 2.0. Reduced iron as the sole energy source was
supplied as $FeSO_4 - 7H_2O$ at a concentration of 15.0 g/l.

To eliminate iron precipitation, the components of
the medium were autoclaved separately in two different
solutions and then were mixed after the solutions had
cooled to room temperature. Solution 1 contained the
$FeSO_4$. Solution 2 contained the remaining salts in de-
ionized water and 1-2 ml. of 10N H_2SO_4.

After mixing the two solutions, the final solution
was placed aseptically into test tubes which were then
sealed to reduce the chance of the medium becoming
contaminated.

pH: The pH of streams was measured using a Hach
visual color comparison with color discs field testing
kit, and VWR digital mini pH meter model 49 with a 0.1 pH
resolution and + or - 0.05 pH reproducibility.

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Temperature: A gas-filled mercury glass thermometer
incremented to 0.1^0 C. was used to measure temperature of
streams.

Total Suspended Solids: Total suspended solids were
measured using a 500 ml. Erlenmeyer flask, filter cup,
membrane filter paper (0.45 um), and an oral vacuum.

Water Samples: 200 ml. Nalgene plastic bottles
containing 1.0 - 3.0 ml. of concentrated nitric or
hydrochloric acid were used to store water samples for
later analysis of heavy metals.

Methods

Field

At each sampling site, the temperature, pH, total
suspended solids, and water samples were taken. The direct
count MPN test was taken sporadically for each trip into
the field and number of samples taken for T. ferrooxidans
varied from two on May 31, 1986 to four on July 3-4, 1986.

Five field trips were made throughout the summer: May
31, June 21, July 3-4, August 2, and September 2, 1986.

The temperature was taken by placing the thermometer
directly in the creek for a minimum of 10 minutes before
recording. The pH was measured from grab samples from Wade

Creek and was determined by color comparison using the Hach test kit. On one occasion, the VWR pH probe was used to determine the pH of the water samples by placing it directly into the stream. Water samples for total suspended solids (TSS) and heavy metals were taken.

For TSS and metal analysis, water samples were collected by pouring the water through a filter cup and allowing it to drain through a 0.45 um membrane filter into a 500 ml. flask. Half of the water sample was then fixed with nitric acid and the other half was fixed with hydrochloric acid for later laboratory analysis.

The TSS was determined by differences in pre and post weighings of the filter paper per volume of water passed through the filter.

Microbial Biomass: Thiobacillus ferrooxidans numbers were measured by aseptically removing 1 ml. aliquots directly from Wade Creek with a 1 ml. pipette.The sample was then diluted to 10 ml. with sterile medium in a test tube. Serial dilutions of .1, .01, .001 ml. were completed. Finally, each dilution was placed in five growth tubes. The growth tubes contained, 10 ml. of T. ferrooxidans medium. The growth tubes were incubated for four weeks at room temperature before recording the observations.

Laboratory

Microbial Biomass: Medium tests were completed prior
to and following the field season using lab isolates of T.
ferrooxidans.

Total Suspended Solids: Filter papers were dried and
preweighed for the field season. Following the field
season, samples were dried and weighed again, and the
difference between the two weighings minus the weight of
the filter paper determined the TSS.

Heavy Metals:

Total Dissolved Arsenic (ppb): Arsenic was analyzed
by graphite furnace atomic absorption using an
Electrodeless Discharge Lamp (EDL) at 193.7 nm., with a
sensitivity of 0.01 ppb, as described for the Perkin and
Elmer Model 4000 atomic absorption spectrophotometer
(Perkin-Elmer, 1982).

Total Dissolved Copper (ppm): Copper was analyzed by
flame atomic absorption method using an Hollow Cathode
Lamp (CRT) at 324.8 nm., with a sensitivity of 0.001 ppm,
as described for the Perkin and Elmer Model 4000 atomic
absorption spectrophotometer (Perkin-Elmer, 1982).

Total Dissolved Iron (ppm): Iron was analyzed by
flame atomic absorption method using an CRT at 248.3 nm.,

with a sensitivity of 0.001 ppm, as described for the Perkin and Elmer Model 4000 atomic absorption spectrophotometer (Perkin-Elmer, 1982).

Total Dissolved Zinc (ppm): Zinc was analyzed by flame atomic absorption method using an CRT at 213.9 nm., with a sensitivity of 0.001 ppm, as described for the Perkin and Elmer Model 4000 atomic absorption spectrophotometer (Perkin-Elmer, 1982).

Total Dissolved Cadmium (ppm): Cadmium was analyzed by flame atomic absorption method using an CRT at 228.8 nm., with a sensitivity of 0.001 ppm, as described for the Perkin and Elmer Model 4000 atomic absorption spectrophotometer (Perkin-Elmer, 1982).

Figure 1 shows the locations of the studies sites along Wade Creek and the mileposts along the river corridor.

Table 3 shows results of field samplings for T. ferrooxidans, and two physical descriptors; temperature, and pH. During the second field sampling (6/21/86), a pH probe was used to determine the pH level. The probe was discarded because of instrumentation problems with the probe, and the complications explain the reason for the low pH levels recorded in Table 3 on the date of 6/21/86. Throughout the summer, no T. ferrooxidans were identified, pH was near neutral, the temperature average was 13.0° C (range from 4.5° to 23.0° C), at all of the sites water samples were taken.

Table 4 is a compilation of all the metal concentrations determined from the water samples taken throughout the summer along with the total suspended solids. The metals measured include zinc, cadmium, copper, arsenic, and iron. The range of concentrations for these metals was very dispersed: arsenic had a range from 0.55 ppb (ug/L) to 633.0 ppb; copper had a range from 4 ppb (ug/L) to 58 ppb; iron had a range from 52 ppb to 14,800 ppb; zinc had a range from 0 ppb to 1,258 ppb, and cadmium was below detection limits for the flame techique of

atomic absorption. Total suspended solids ranged from 1.0 to 88.0 mg/L. Table 4 shows the results of sampling on May 31 of 1986 before any mining or re-landscaping activity had begun in the Wade Creek drainage. The metal concentrations at all of the sites are similar. There are no wide variations in the metal concentrations except in the lower section of Wade Creek where a dissolved iron concentration of 1687.0 ppb was collected. However, dissolved zinc varied only 45.0 ppb, arsenic only 0.69 ppb, and copper only 26.0 ppb over the 10 mile length of the creek.

In June the Alaska Department of Transportation (D.O.T.) began a re-landscaping project and Figures 2 and 3 illustrate a change in iron and arsenic concentrations in samples collected at sites directly before the activity (site 9), directly after (site 10), and 0.5 miles farther down the creek (site 11). The plots neglect to show zinc and copper concentrations but they both decreased below the activity; copper by 11.0 ppb, and zinc by 75.0 ppb.

July saw the first noticeable signs of gold mining activity. Mining at the third mine located 4.5 miles down Wade Creek began by setting up operation, moving material, and sluicing some pay dirt to find the value of the gravel. At this time, water samples showed increases in dissolved copper, iron, and arsenic (Figure 4 and Figure 5).

WADE CREEK

Site # 2
m.p. 0.0

Mine #2
Site

Site # 3
Mine #1

M.P. 2.0
Site # 4

Site # 5

M.P. 4.0

Site # 6

Mine #3

Site # 7
M.P. 6.0

Site # 8

Site # 9

Site # 10
Site # 11

Site # 12
M.P. 8.0

Site # 13

M.P. 10.0

WALKER FORK
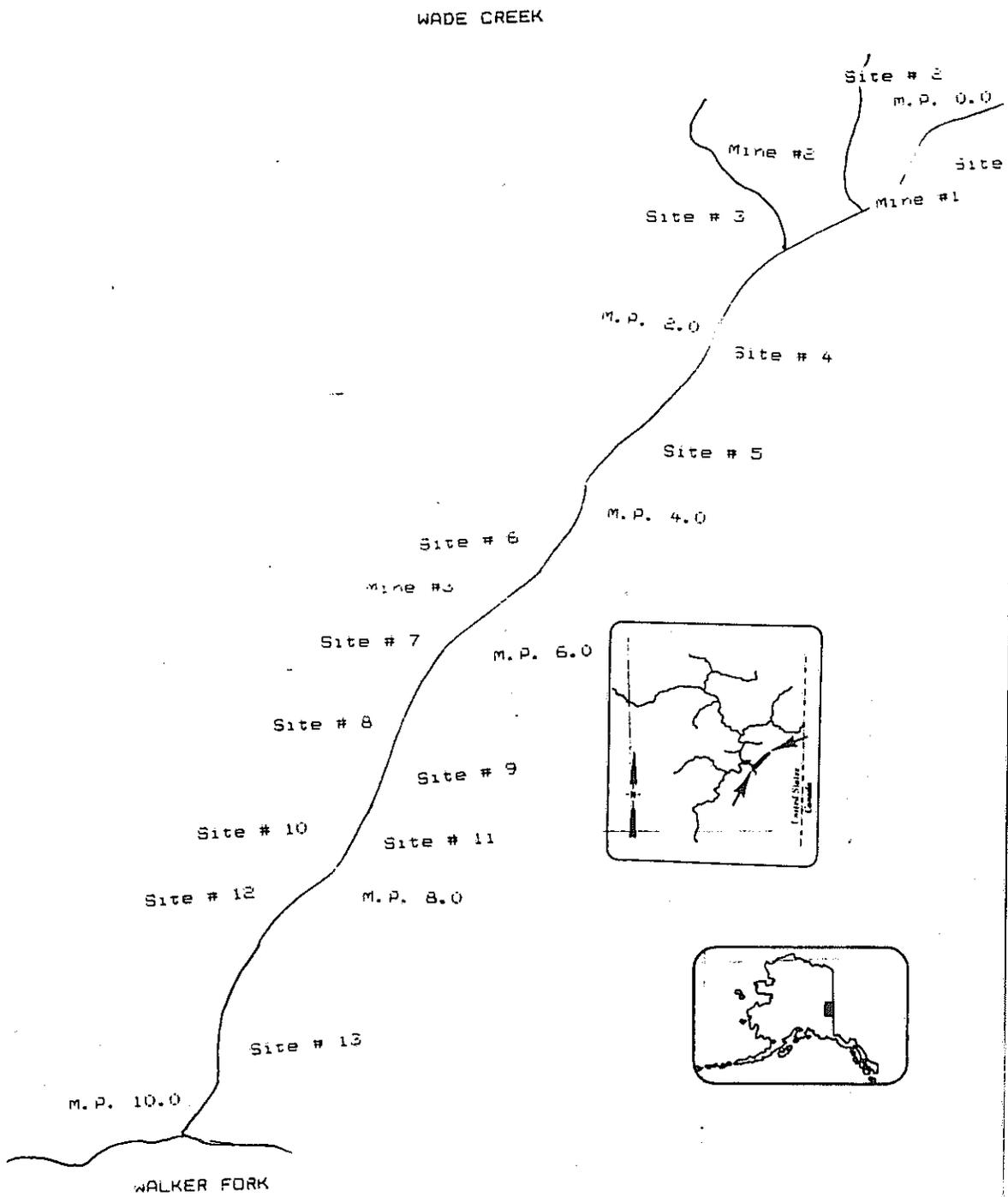
Figure 1 Map of Wade Creek.

Table 3. Temperature, pH, and MPN of Thiobacillus
ferrooxidans in Wade Creek throughout the summer of 1986.

	DESCRIPTION		
	Temperature (^{O}C.)	pH	No. Bacteria*
Date	Site#			
5/31/86				
	1	5.5	7.5	NS
	6	7.0	7.0	NS
	8	8.0	7.6	ND
	12	10.0	7.0	ND
6/21/86				
	9	10.0	5.4	NS
	10	12.5	6.1	ND
	10	13.0	6.3	ND
	11	14.5	6.4	ND
7/3-4/86				
	1	12.6	7.5	NS
	2	20.0	8.0	NS
	4	15.0	7.5	ND
	5	15.5	7.8	NS
	6	16.0	7.5	ND
	7	16.5	7.0	ND
	10	18.0	7.5	ND
	11	16.0	6.5	ND
	13	23.0	8.0	ND

	Temperature (^{O}C.)	pH	No. Bacteria*
8/2/86			
1	7.0	7.0	NS
2	9.5	7.5	NS
3	11.0	7.5	ND
6	11.0	7.5	NS
7	14.0	7.5	ND
8	16.0	7.5	ND
9	13.5	8.0	ND
12	16.5	7.5	NS
9/1/86			
1	10.0	7.5	NS
2	6.0	7.5	ND
3	6.0	7.5	NS
6	8.0	7.5	ND
7	4.5	7.5	NS
9	8.0	7.5	NS
12	8.0	7.5	ND

* NS = No Sample Taken   ND = Not Detectable

Table 4. Total suspended solids,arsenic, copper, iron,

and zinc in Wade Creek throughout the summer of 1986.*

		TSS [mg/l]	As (ppb)	Cu (ppb)	Fe (ppb)	Zn (ppb)
			DESCRIPTION			
Date	Site#					
5/31/86						
	1	4.0	1.80	34.0	322.	71.0
	6	8.0	1.07	29.0	604.	96.0
	8	4.6	1.68	55.0	441.	64.0
	12	30.0	1.73	52.0	1687.	116.
6/21/86						
	9	-1.9	2.28	41.0	725.	133.
	10	16.0	7.62	38.0	793.	58.0
	10	9.0	5.53	41.0	594.	54.0
	11	26.0	1.70	34.0	738.	54.0
7/3-4/86						
	1	1.3	0.79	34.0	404.	1298.
	2	3.3	1.64	36.0	292.	298.
	4	50.0	0.55	32.0	297.	94.0
	5	1.3	0.92	46.0	52.0	65.0
	6	3.0	0.90	38.0	336.	89.0
	7	36.0	1.57	58.0	730.	27.0
	10	13.0	2.06	43.0	636.	26.0
	11	66.0	1.23	47.0	879.	56.0
	13	13.0	0.65	57.0	451.	54.0
8/2/86						
	1	3.0	1.57	39.0	728.	23.0

		TSS[mg/l]	As (ppb)	Cu (ppm)	Fe (ppm)	Zn (ppm)
	2	8.8	2.52	49.0	1322.	79.0
	3	7.3	26.37	49.0	1690.	61.0
	6	6.0	1.42	52.0	385.	24.0
	7	55.0	633.00	63.0	14800.	74.0
	8	2.6	2.32	65.0	992.	27.0
	9	2.6	1.95	65.0	992.	74.0
	12	2.6	2.12	60.0	431.	14.0
9/1/86	1	32.0	1.86	63.0	546.	000.
	2	1.0	2.18	58.0	854.	002.
	3	36.0	1.51	55.0	502.	49.0
	6	5.0	1.23	51.0	485.	29.0
	7	18.0	2.12	56.0	528.	21.0
	9	8.0	5.93	52.0	1308.	26.0
	12	6.0	2.51	57.0	2252.	18.0

* Cd [mg/l]: all samples below detection limits for the
flame method of atomic absorption.
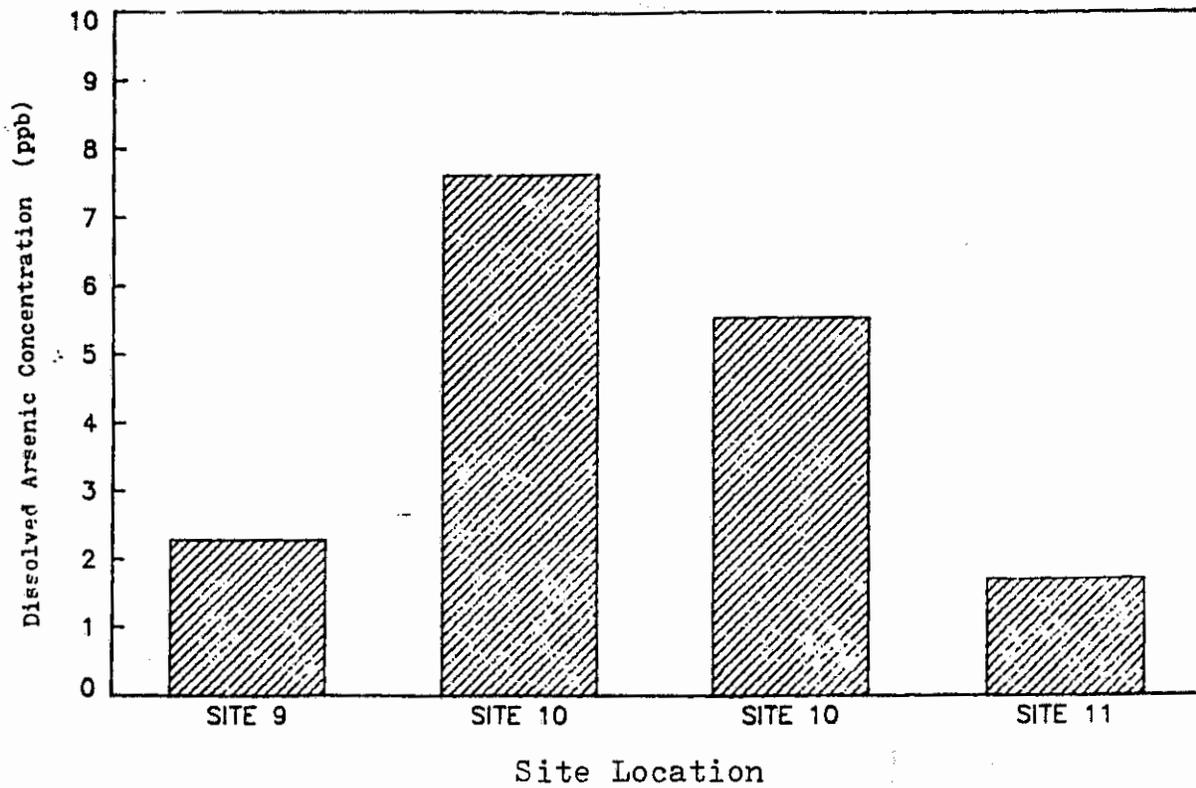
Figure 2. Arsenic concentrations after re-landscaping of
used tailings in Wade Creek. Site 9 was located
above the area of activity, Site 10 was within
the area, and Site 11 was approximately 0.5
miles below the area.

Figure 3. Iron concentrations after re-landscaping
activity of used tailings in Wade Creek. Site 9
was located above the area of activity, Site 10
within the area, and Site 11 was approximately
0.5 miles below the area.
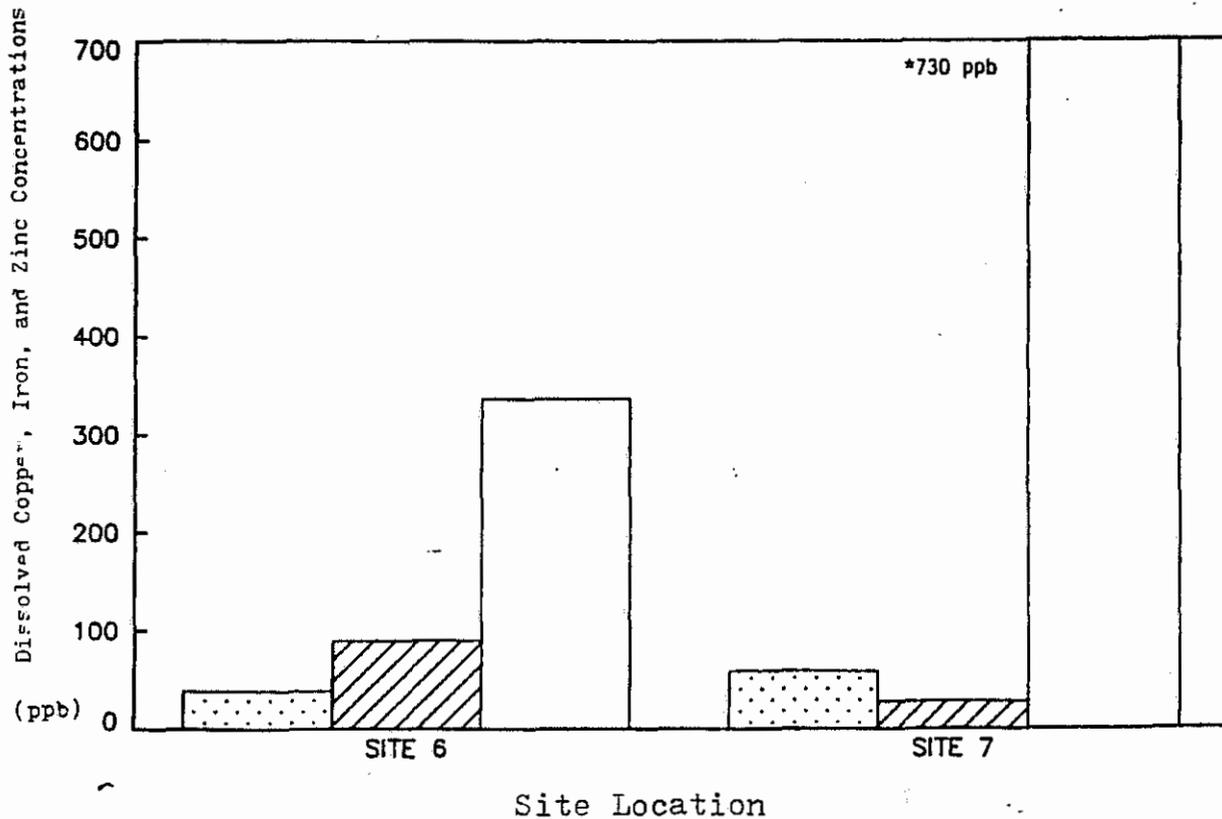
Figure 4. Copper (⊙), iron (⊘), and zinc (◯)
concentrations after a mining operation had
been moving material. Site 6 was located
directly above the operation and Site 7 was
directly below the operation.
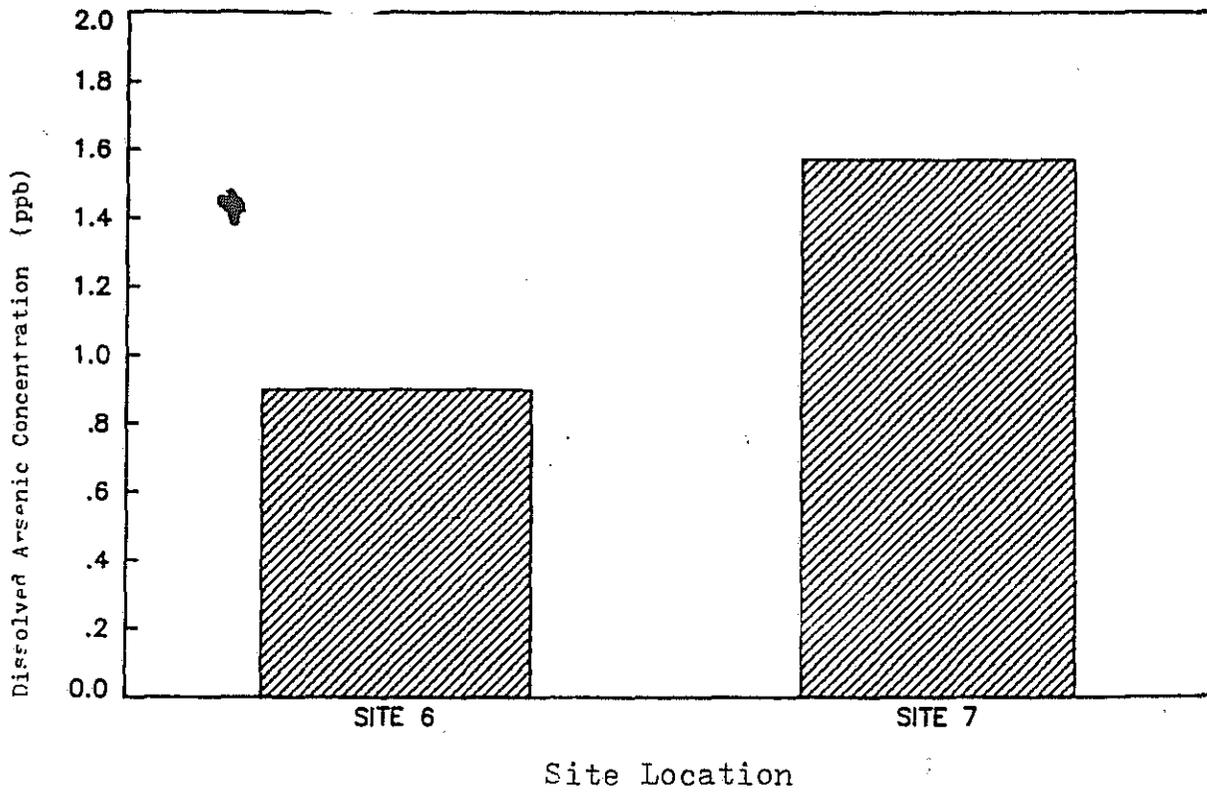
Figure 5. Arsenic concentrations after a mining operation
had been moving material. Site 6 was located
directly above the operation and Site 7 was
directly below the operation.

Again the samples were collected directly before the mine (site 6), and directly below the last settling pond (site 7). This mine was operating a partial recycling system for water. There was a net decrease in total dissolved zinc as shown in Figure 12, site 6-7 (1).

In August, the first two mines were operating in tandem on separate tributaries which both fed into Wade Creek at miles 0.5 and 0.75. They both were moving material and sluicing but not at a significant volume. On the particular day of sampling, the first mine had only been moving material, while the second mine had been sluicing, and Figure 6, Figure 7, and Figure 8 show the effects on total dissolved iron, arsenic, copper, and zinc from the tandem disturbances. Generally, the arsenic increased by approximately 26.0 ppb, iron 1000.0 ppb, copper by 10.0 ppb, and zinc by 60.0 ppb. The zinc then decreased while all the others remained constant or increased.

On this same date in August, samples were collected both before and after the third mining operation at a time when actual sluicing was occurring. There was a 632.0 ppb increase in dissolved arsenic (Figure 10), a 14500.0 ppb increase in dissolved iron (Figure 11), and copper and zinc did not have as significant of increase (Figure 9); copper increased by 16.0 ppb, and zinc by 50.0 ppb. An
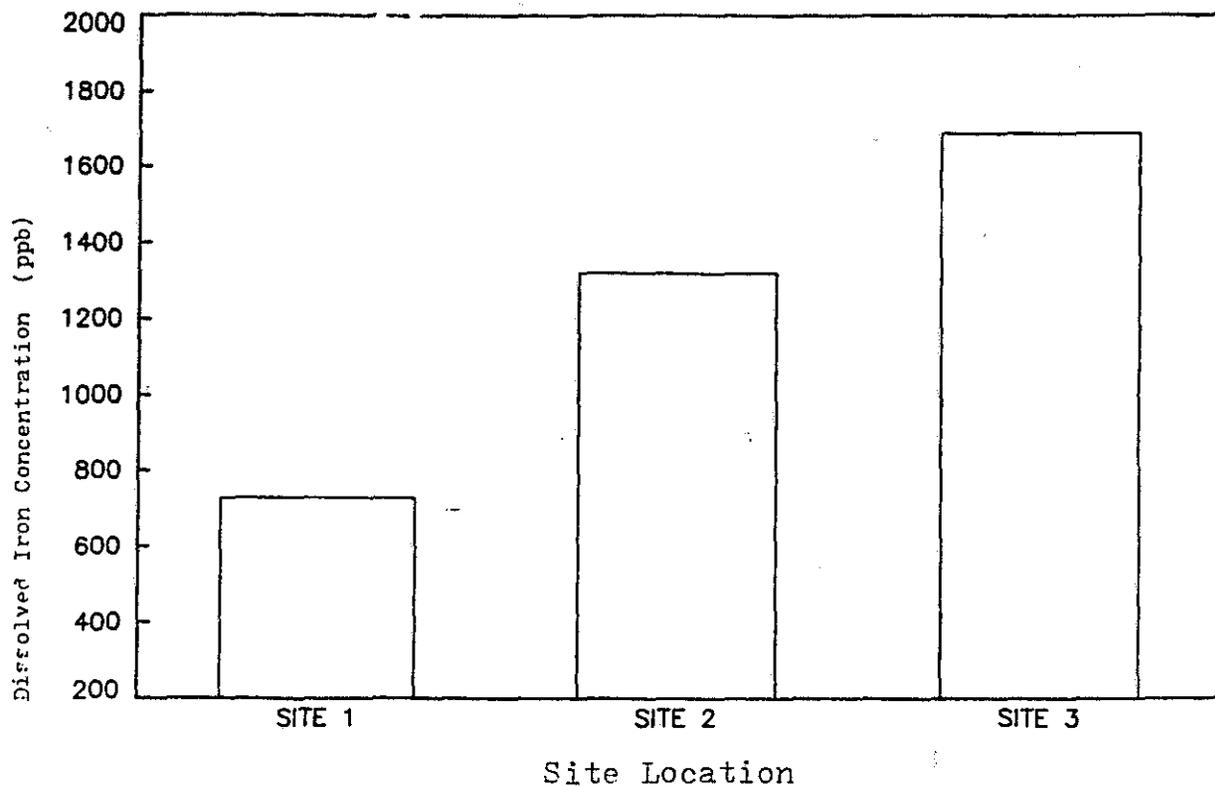
Figure 6. Iron concentrations after two mines operating
in tandem. Site1 was before any kind of
disturbance, Site 2 was below the first mine
located on a tributary to Wade Creek, Site 3
was below the second mine located on the second
tributary to Wade Creek.
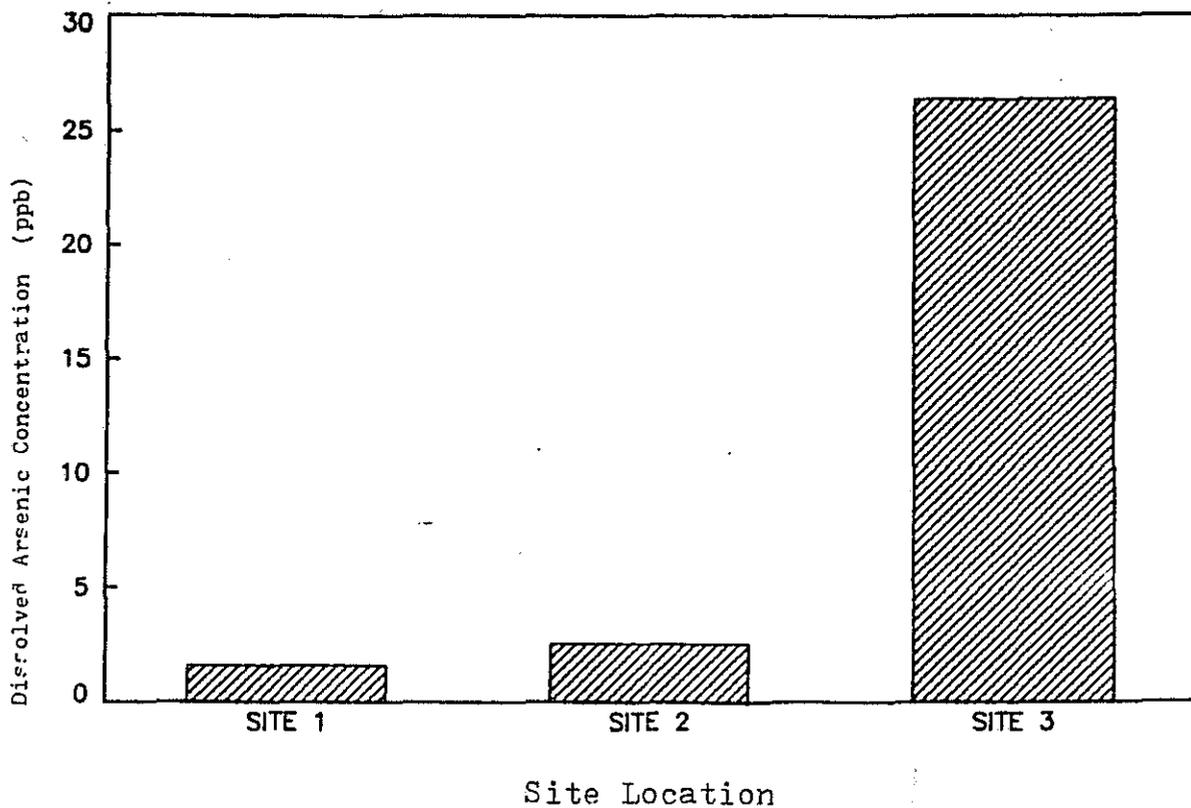
Figure 7. Arsenic concentrations after two mines operating
    in tandem. Site 1 was before any kind of
    disturbance, Site 2 was below the first mine
    operating on the first tributary to Wade Creek,
    Site 3 was below the second mine located on the
    second tributary to Wade Creek.
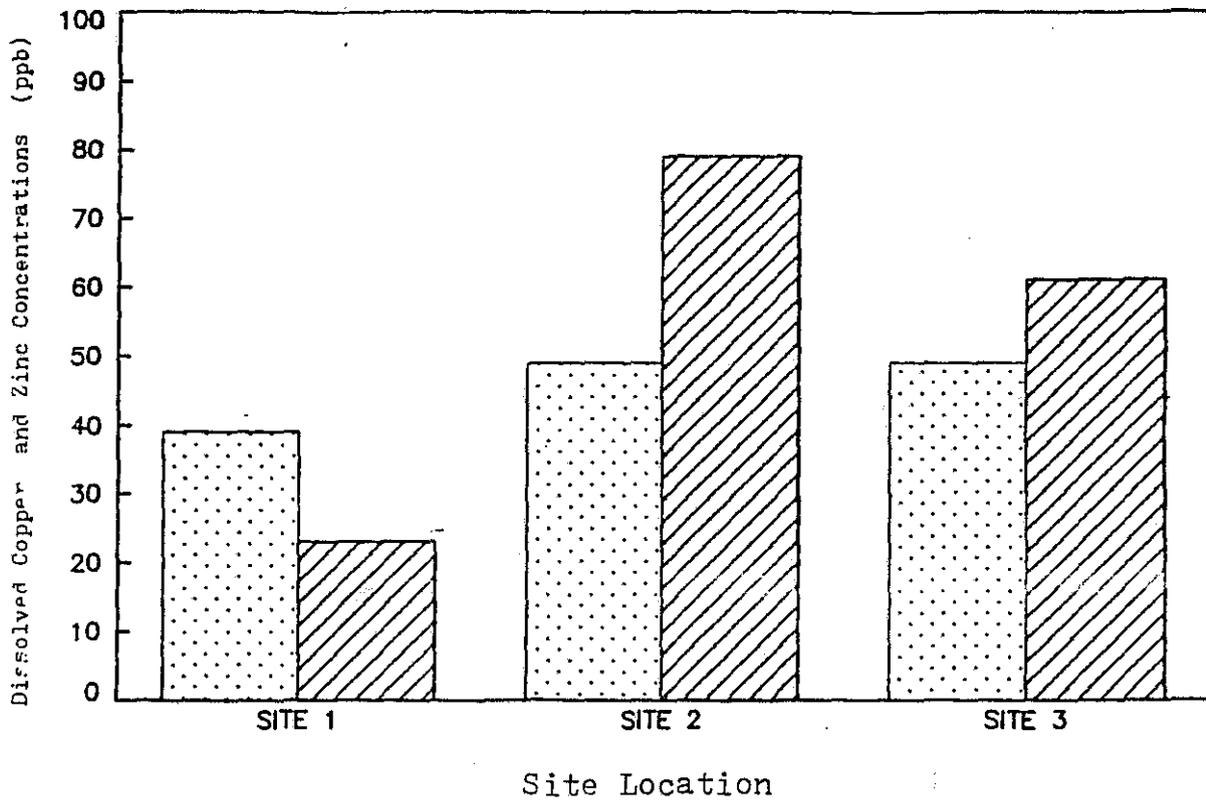
Figure 8. Copper (⊙) and zinc (⊘) concentrations
after two mines operating in tandem. Site 1
was before any kind of disturbance, Site 2 was
below the first mine operating on the first
tributary to Wade Creek, Site 3 was below
the second mine located on the second tributary
to Wade Creek.
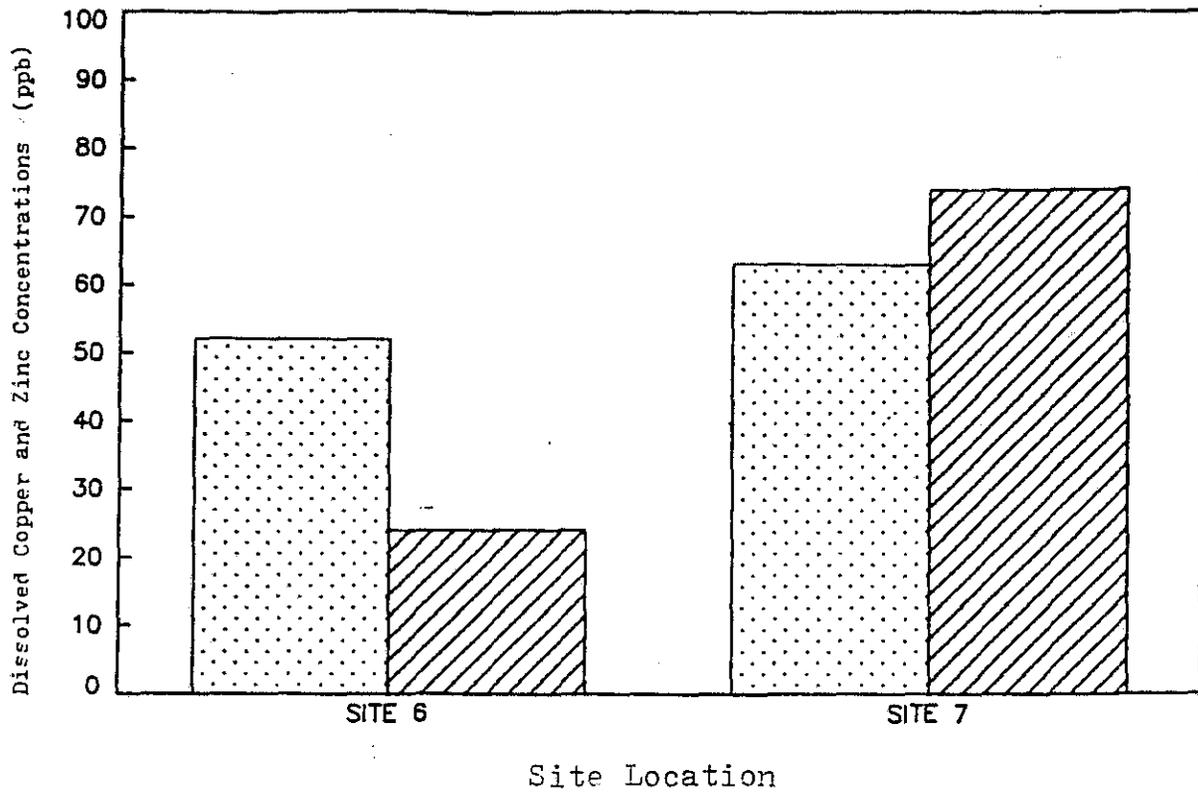
Figure 9. Copper (⊙) and zinc (⊘) concentrations
after sluicing at the third mine. Site 6 is
before the mine. Site 7 is after the mine
while the operator had been sluicing material.
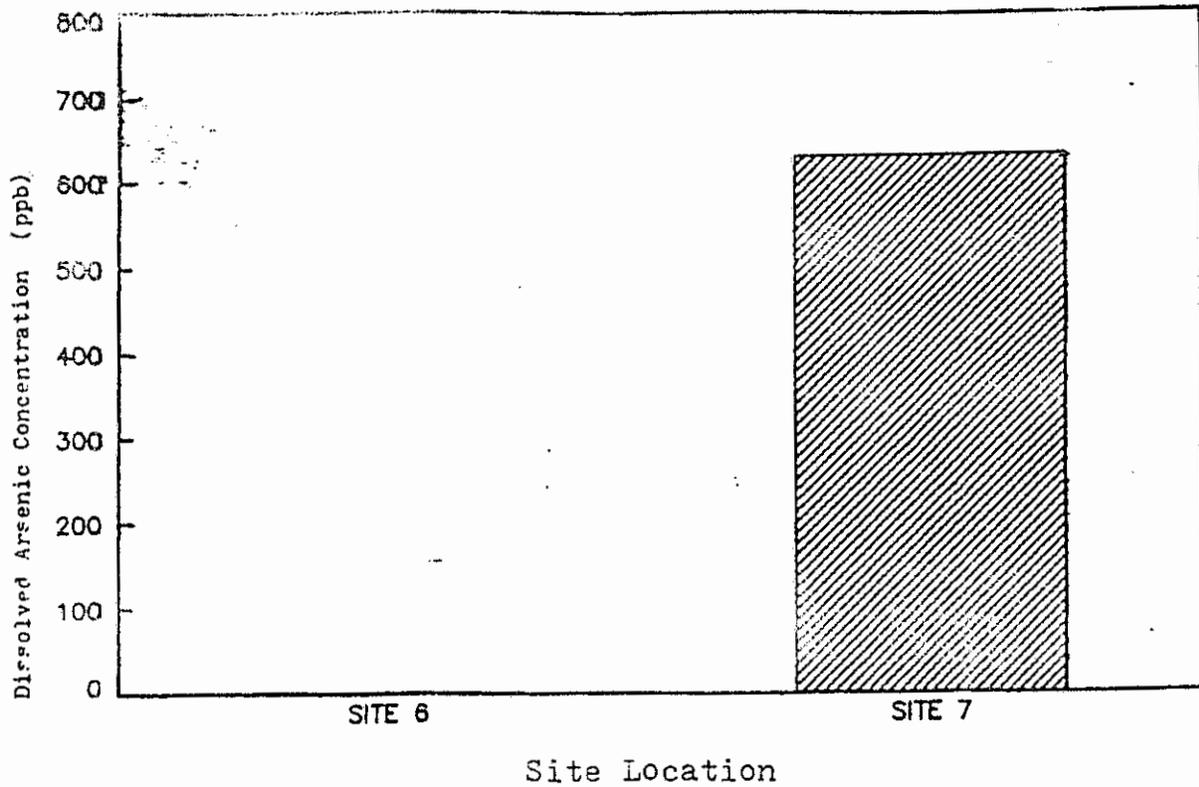
Figure 10. Arsenic concentrations after sluicing at the
third mining operation. Site 6 is before the
third mine. Site 7 is after the third mine
while the operator had been sluicing material.
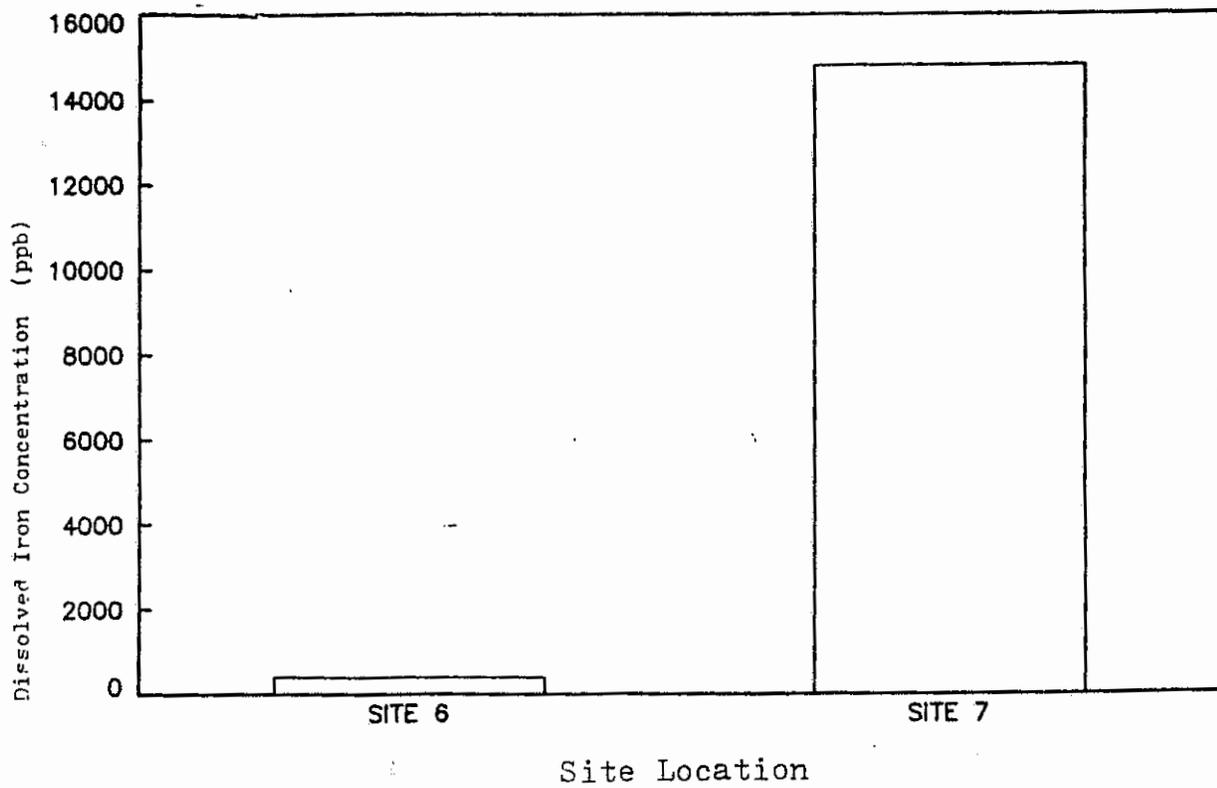
Figure 11. Iron concentrations after sluicing at the
third mine. Site 6 is before the mine. Site 7
is after the mine while the operator was
sluicing material.

interesting point here is that this was one of the few times zinc increased below an area of disturbance as Figure 12, site 6-7 (2) shows.

Figure 12 illustrates the changes in zinc concentrations. Generally a decrease in total dissolved zinc below these areas of surface disturbance was observed.

The total dissolved arsenic and iron concentrations are illustrated in Figure 13 and Figure 14. These figures show the increase in total dissolved arsenic beyond the third mine. The third mine was the most active operation over the season. These figures also show that when there was no activity in May dissolved iron and arsenic concentrations were low.

Finally, Figures 15 - 18 show the changes in concentrations of dissolved metals from the headwaters (site 1) down to the mouth of Wade Creek. Overall, the trend seems to be little net change. The iron tended to pick up through the lower section of Wade Creek as it traveled through the large tailings area. However, even iron was fairly constant until that point. The metals, nevertheless, seem to be constant with the deviation being minimal: arsenic averaging 1.0 - 2.0 ppb. copper 40.0 - 60.0 ppb, zinc 10.0 - 20.0 ppb at the confluence of Wade Creek and Walker Fork where all activity ceases.
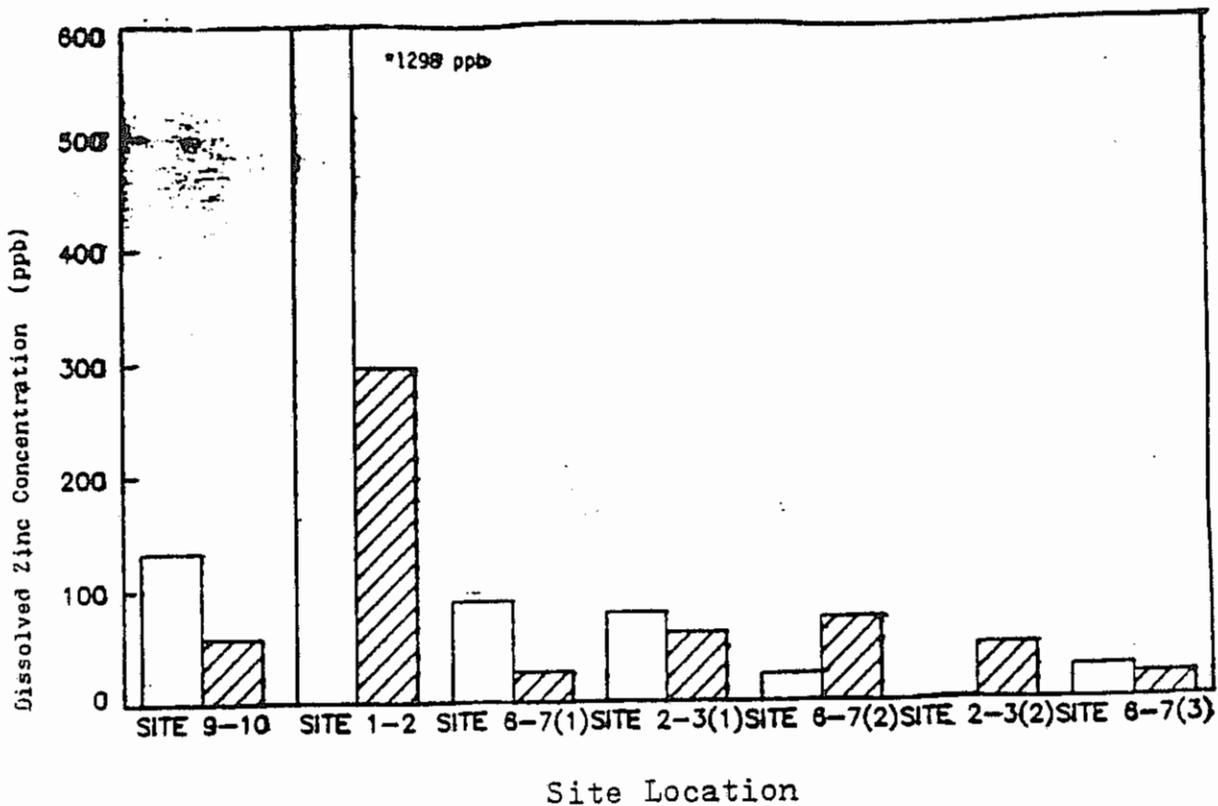
Figure 12. Zinc concentrations after areas of surface
disturbance. Zinc concentrations before ($\bigcirc$)
areas of activity are Sites 1, 2, 6, and 9. Zinc
concentrations after ($\oslash$) areas of activity are
Sites 2, 3, 7, and 10. Site 9-10 was an area
of re-landscaping. Site 2-3 was an area of a
mining operation on the second tributary. Site
1-2 was an area of a mining operation on the
first tributary to Wade Creek. Site 6-7 was
at an area of a mining operation working
directly on Wade Creek (the third mine).

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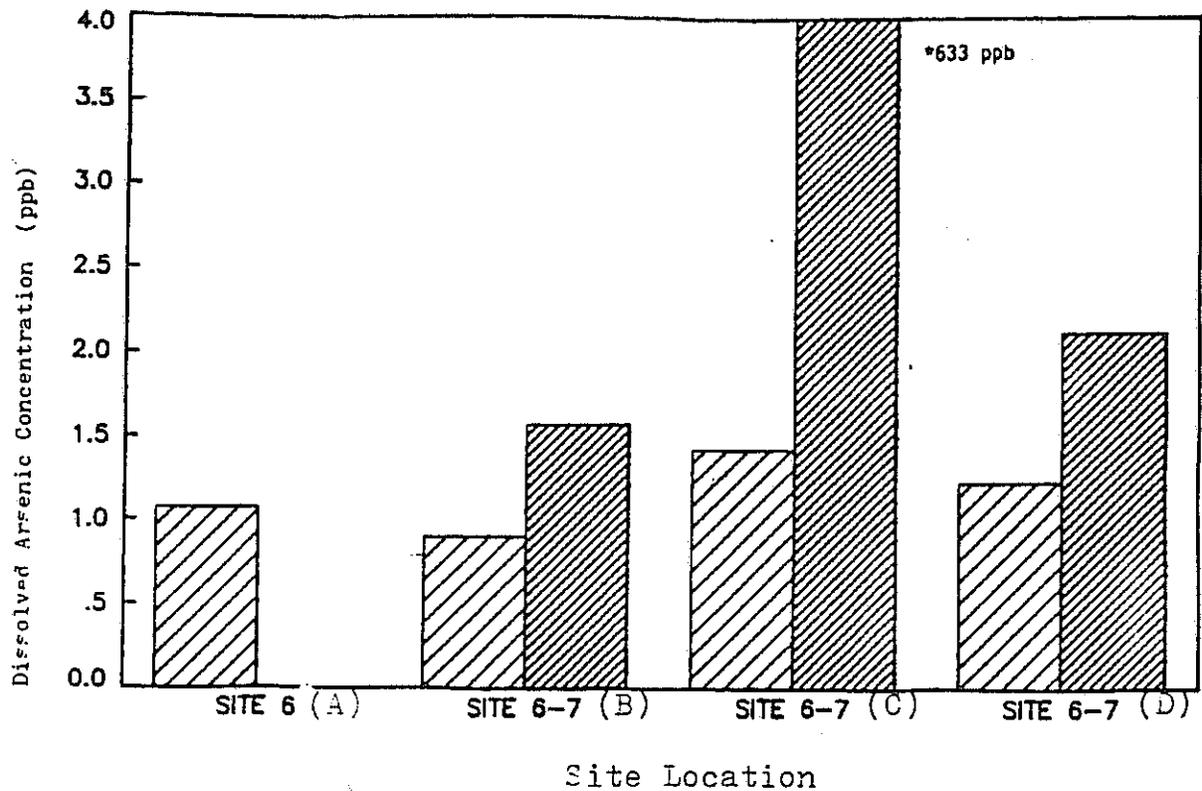
Figure 13. Arsenic concentrations after a mining area
over the summer. Site 6 was located above the
third mining operation. Site 7 was located
below the third mining operation. Samples
were collected on 5/31/86 (A), 7/3-4/86 (B),
8/2/86 (C), and 9/1/86 (D).
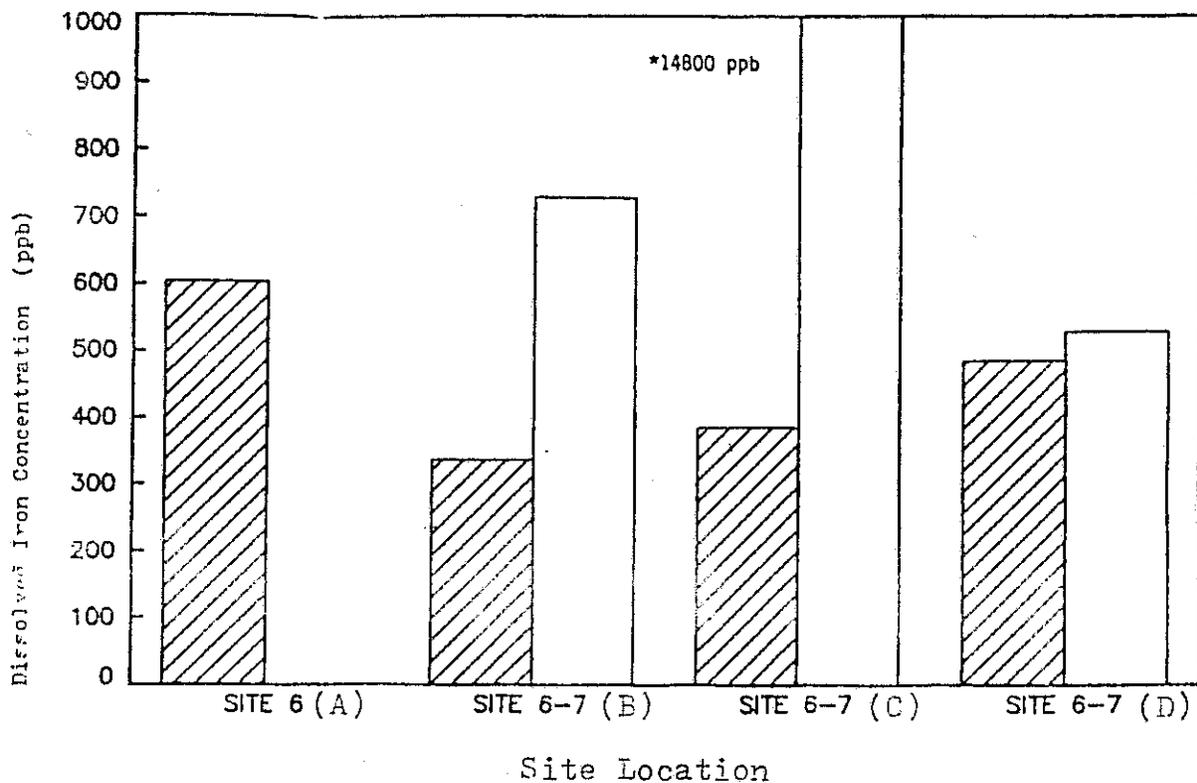
Figure 14. Iron concentrations after a mining area over the summer. Samples were collect on 5/31/86 (A), 7/3-4/86 (B), 8/2/86 (C), and 9/1/86 (D) at the third mining operation on Wade Creek.
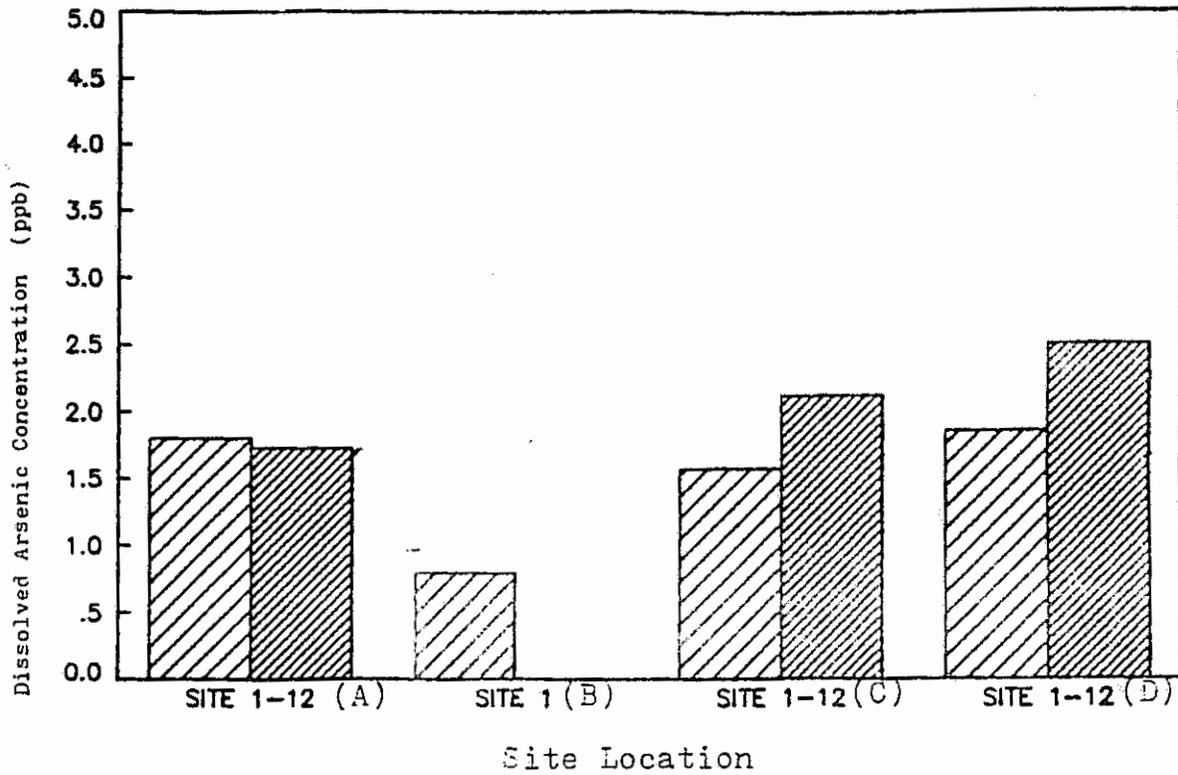
Figure 15. Arsenic concentrations at the end of Wade
          Creek over the summer. Site 1 (⊘) was
          located at the headwaters of Wade Creek while
          Site 12 (⊘) was located at the mouth of
          Wade Creek. Samples were collected on 5/31/86
          (A), 7/3-4/86 (B), 8/2/86 (C), and 9/1/86
          (D).
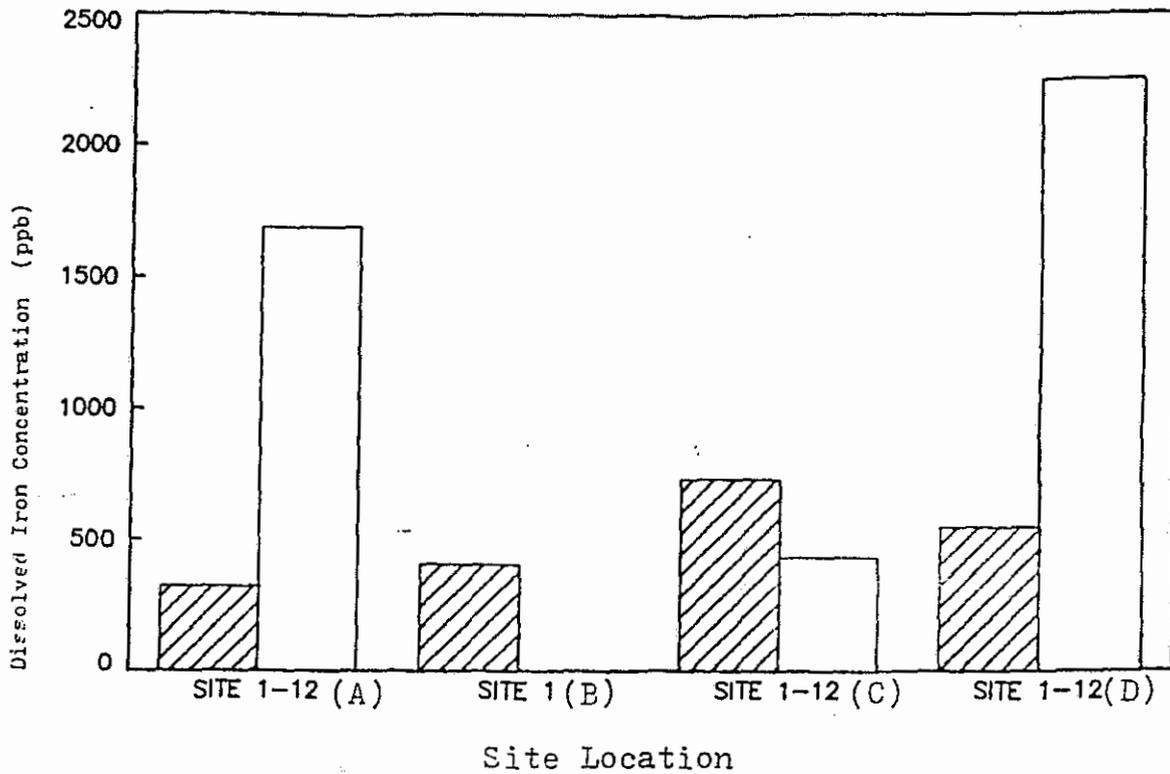
Figure 16. Iron concentrations at the end of Wade
Creek over the summer. Site 1 (⊘) was
located at the headwaters of Wade Creek
while Site 12 (◯) was located at the
mouth of Wade Creek. Samples were collected
on 5/31/86 (A), 7/3-4/86 (B), 8/2/86 (C),
and 9/1/86 (D).

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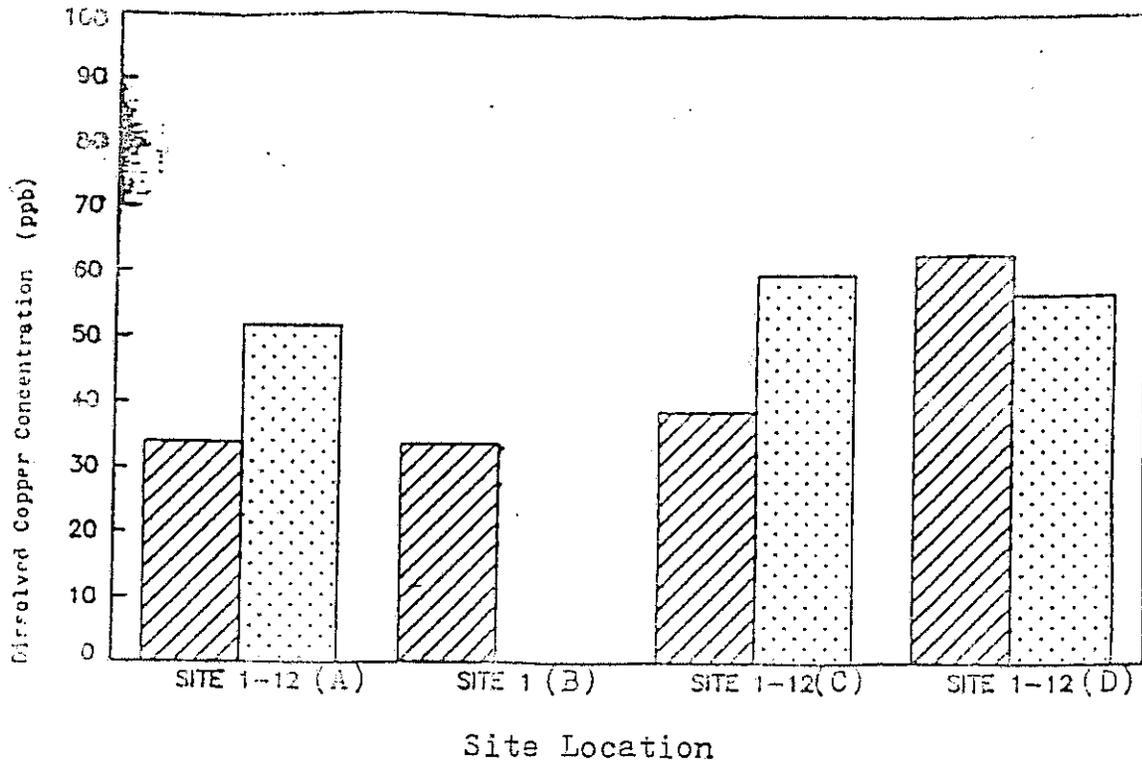
Figure 17. Copper concentrations at the end of Wade Creek over the summer. Site 1 (⊘) was located at the headwaters of Wade Creek while Site 12 (⊙) was located at the mouth of Wade Creek. Samples were collected on 5/31/86 (A), 7/3-4/86 (B), 8/2/86 (C), and 9/1/86 (D).
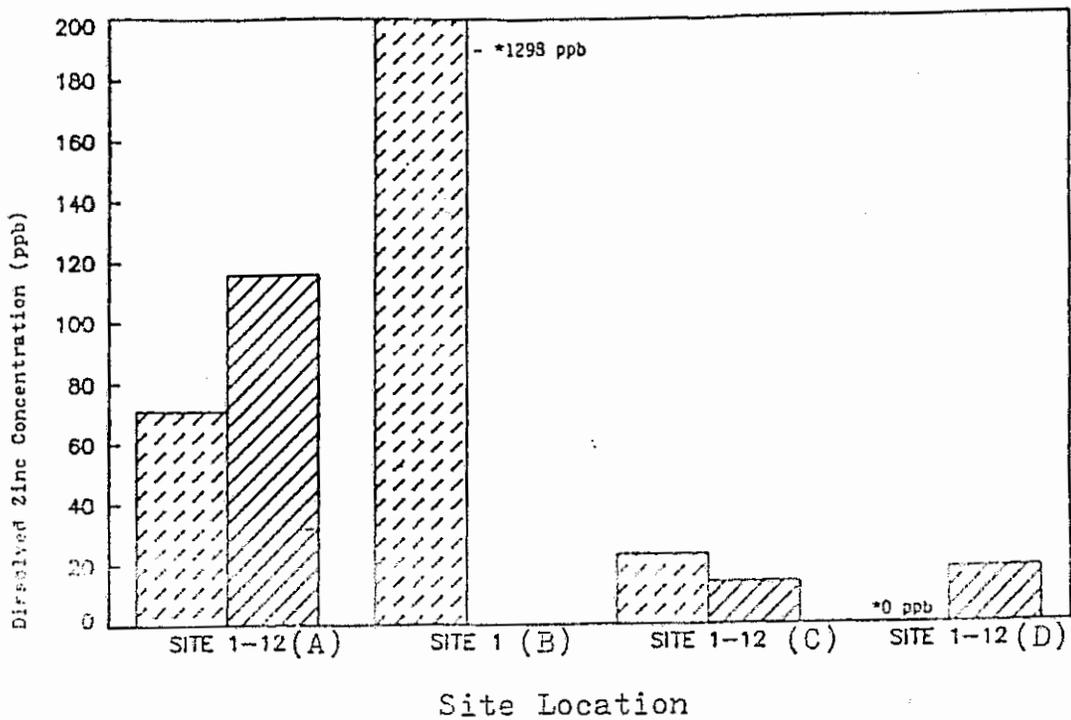
Figure 18. Zinc concentrations at the end of Wade
Creek over the summer. Site 1 (⊘) was
located at the headwaters of Wade Creek
while Site 12 (⊘) was located at the
mouth of Wade Creek. Samples were
collected on 5/31/86 (A), 7/3-4/86 (B),
8/2/86 (C), and 9/1/86 (D).

DISCUSSION

Thiobacillus ferrooxidans

No T. ferrooxidans was isolated from the Wade Creek drainage over the course of the summer. This suggests that T. ferrooxidans did not exist in this drainage, that it was below detection limits to establish its presence using the MPN testing procedure, or that my testing procedure was inadequate. The lack of, or suppressed number of T. ferrooxidans may be because of a lack of concentrated sulfide-mineral deposits in the drainage, or inhibition of its growth due to high levels of organic acids released during disturbance of the soil organic layer, and a general lack of environmental conditions conducive to its growth (e. g. sub-optimal temperatures and low moisture levels).

In discussing possible areas where problems may have arisen in testing for T. ferrooxidans, the possibility of the medium having spoiled, or not being selective for strains of T. ferrooxidans is probably invalid since both pre- and post-field tests were conducted using the same batch of sample medium inoculated with laboratory strains of T. ferrooxidans. Growth did occur in both instances. The possibility that T. ferrooxidans was out competed for nutrients in this test medium is probably invalid because,

except for occassional growth of a filamentous organism nothing grew in this MPN medium inoculated with water samples.

While deleterious effects to streams (associated with the leaching of metals and enhanced acidic conditions) are known to occur when T. ferrooxidans is present, there is no indication that the traditional biogeochemical scenerio described in the literature review is occurringin this drainage. Therefore, it can be concluded that no appreciable amounts of dissolved metals occur because of microbial leaching by T. ferrooxidans, and that increases in total dissolved metals can be attributed to surface disruption of minerals by the mining activity on Wade Creek.

Dissolved vs. Total Metal Concentrations

The fact that the water samples collected for metal analysis were filtered and then fixed in an acid is important since there is generally a concentration factor of 0.01 - 0.001 of dissolved metals in comparison to the amount of metals sorbed on to particulates (R & M Consultants, 1982). This also means that the total dissolved metal concentrations could be higher than the concentrations of metal sorbed to suspended particulates in areas where there is little disturbance, or areas where

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the disturbance area's effluent has been dampened sufficiently to remove most of the suspended metals (R & M Consultants, 1982).

Metals

Iron, arsenic, zinc, copper, and cadmium were five metals selected as indicators for metal contamination since each, except cadmium, are deposited as a sulfide mineral. Cadmium was selected because it is often a metal that can bioaccumulate. Metals which deposit as sulfide minerals were selected primarily because of their ability to be oxidized by physical, chemical, and biological means, and because anomalous sulfide deposits like sphalerite (zinc sulfide), iron pyrite, arsenopyrite, and chalcopyrite are known to occur in areas of gold placer or lode deposits, and/or in the Wade Creek drainage.

Arsenic: In all cases of surface disturbances, whether it was relandscaping, moving material, or actual sluicing, increase in total dissolved arsenic occured. The arsenic concentration increase was greatest where it seemed that activity was occurring at that time, and that sluicing activity resulted in the release of more arsenic into solution than other types of land disturbances.Any activity which allows a greater surface area of arsenic

containing minerals to be exposed to oxidative processes, could lead to arsenic solubilization. Active sluicing also increases water flow into settling ponds which shortens detention time, increases the surface overflow rate, and thus could increase the levels of arsenic in the final discharge.

Once arsenic is in solution it is thought to oxidize to arsenate. Consequently, dissolved arsenic is anionic and will not readily sorb on to anionic clay particulates or humic acids. Therefore, arsenic is carried in solution until the arsenate forms a complex or precipates (e.g. $Fe(AsO_4)$) and settles out. This is reflected by the fact that where any surface disturbing activity was occurring, both TSS and total dissolved arsenic increased. This indicates that the arsenic is not settling out, and that arsenic may not be sorbing onto the TSS. Another factor is that other metals may have a stronger affinity to form complexes with anionic particulates and acids than arsenic. In that case, arsenic may simply be out competed for binding sites, and not be able to sorb to sites. Consequently, arsenic remains in solution.

Zinc - Copper: Dissolved zinc, which exists as divalent cation in solution, would tend to absorb on to negatively charged clay mineral colloidal matter. This probably explains why, in most cases, the total dissolved

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zinc concentrations decreased below areas where disturbances had occurred, and the TSS was not extremely high. Since colloidal matter is brought into solution below these areas of disturbance, this allows for sorption of zinc (a cation) to the clays and humic acids (anions), and as the particules settle out the zinc is carried out with it. In cases where total dissolved zinc concentrations increased, this may occur simply because of the detention time in the ponds was not long enough for the zinc to sorb to the colloidal particulates, or a greater volume of zinc is brought into solution because of the volume of material being worked. This is reflected by increases in dissolved zinc concentrations where TSS concentrations are unusually high. Therefore, it would indicate that there has not been enough time for settling and sorption to occur.

Dissolved copper, also a divalent cation in solution, should tend to sorb on to the anionic colloidal matter and humic acids, as well. It too reflects the fact that any type of disturbance increases the concentration of dissolved copper. In cases where copper has increased, other divalent cationic metals have a higher affinity and are more competitive for the available sorbing sites than the dissolved copper. The copper is then held in solution rather than sorbing and settling out. Another factor which

can not be over looked is that the disturbance may occur in an area where there are higher than normal copper deposits. In general, where there was surface disturbance dissolved copper concentrations tended to increase. However, the only time copper decreased after a disturbance was when dissolved copper was in substantially greater concentrations than dissolved zinc. This would indicate that there may be competition for absorption sites between copper and zinc.

Iron: Dissolved iron concentrations measured were either ferric or ferrous iron. Since the pH level of the water was always near-neutral, ferric iron should not exit in solution since it is nearly insoluble at neutral pH. Therefore, the iron concentration is an indication of dissolved ferrous iron for the most part because any ferric iron would normally form complexes and quickly settle out.

The increase in iron below areas of activity occurs as greater amounts of insoluble iron as pyrite are exposed to chemical oxidation because of the disturbance, and become soluble. Once in solution, the largest fraction of soluble iron is lost within minutes, and a large fraction of trace element constituents associated with colloidal matter; with copper and lead concentrations decreasing rapidly as colloidal ferric iron precipitates (Babb et

al., 1985). The iron and arsenic generally mirror each other in increases and decreases which indicate their source may be from arsenopyrite. By disturbing mineralized areas more arsenopyrite would be exposed to chemical and physical processes.

Once the majority of ferric iron has settled out, the remainder is in the ferrous state. The settling of ferrous iron is dependent upon anion adsorption sites to which it can absorb. Through surface disturbances, organic acids and particulates, sulfide, inorganic particles, and calcium carbonate are brought into solution to which ferrous iron can bind. As was shown (Figure 11), in the areas with large disturbances total dissolved iron increased below the sites especially when a mine was sluicing material. The release may occur because of a short detention time in settling ponds, the sluiced material itself is rich in iron, or a combination of both. Because of the naturally high iron levels in the soil and rocks, another phenomena arises as illustrated by the results from the lower section of Wade Creek. Because of the long history of mining activity in the lower section of Wade Creek, there is a large volume of stockpiled tailings. Dissolved iron concentrations increased significantly as water flows through these areas indicating the effects of natural weathering of the

stockpiled tailings. Thus stockpiled tailings may present a long term problem of metal leaching if iron and sulfide minerals are present.

Activity effects

The overall environmental impacts due to heavy metal leaching from surface disturbances in the Wade Creek appear short term since the dissolved metal concentrations showed no major increase either from consecutive sampling sites or from the most downstream sampling site of a given day when compared to the concentration of the dissolved metal at the headwaters for that day.

The only time there was any major net increase at the most downstream sampling site compared to the most upstrean sampling site was when the Alaska Department of Transportation (D.O.T.) was re-landscaping the lower section of Wade Creek in June. Even then, TSS was the only parameter which had any appreciable increase. In July, failure of a D.O.T. constructed settling pond during heavy rains could have been responsible for causing an increase of 53.0 mg/L TSS between sites 10 and 11. As a further consequence to increases in TSS, all the metals that had settled out in the pond were brought back into solution which may explain why copper, iron, and zinc all increased below site 10. The reason arsenic did not increase may be because much of the arsenic in this pond was still in

solution. Consequentally, with increased flow, the arsenic that is in solution would be diluted (Table 4, 7/3-4/86, Site # 10 - 11).

The effects of a tandem of mining operations seem to be as one would expect with greater concentrations occurring as the operation's effluent mixes with the creek, and as more mines operated and discharge into the creek, the dissolved metal concentrations increased. It also seems as one would expect that the more significant the activity; whether it is the volume of material being moved or the type of activity occurring, will directly effect the amount of metals to be brought into solution.

It appears that the dissolved metals do not remain in solution for an extended distance but the metals may reach short term concentrations that exceed environmentally sound discharge levels for iron, arsenic, copper, zinc, and TSS when the individual sites are moving material or sluicing. These high concentrations of dissolved metals could create a significant site specific problem during periods of reclamation.

Over the length of Wade Creek, the net change in dissolved metal concentrations were not appreciable since variation in concentations for most of the metals from the headwaters of Wade Creek compared to the mouth of Wade Creek were minimal. Therefore, it could be assumed that

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the variations were generally insignificant, and that the
long range impact of dissolved metals is minimal from site
specific surface disturbances that occurred on Wade Creek.

SUMMARY AND CONCLUSIONS

The history of gold mining in Alaska is long and rich
with adventure. Yet, in the last few years, the means of
regulating gold mining by government agencies has been
based upon the effectiveness of the mines to meet total
settleable solids discharge limits which has dampened a
lot of the mining activity. Presently, the miners feel
that the discharge limit for turbidity is often impossible
to meet, and the regulating agencies feel that it is often
impossible to enforce. Therefore, it seems probable that
in the future if new economically reasonable techniques
are not developed, the governing agencies may change their
means of regulating and enforcement, and one possibility
may be discharge limitations for certain dissolved or
suspended metals and require the posting of a bond.

Here an examination describing the discharge of
dissolved zinc, iron, copper, arsenic, and total suspended
solids caused by gold mining, relandscaping of gold mine
tailings, and natural weathering processes in Wade Creek
has been conducted. It was determined that T. ferrooxidans
did not occur in high concentrations in this mining area
which allows for the conclusion that the variations in
dissolved metals can not be attributed to the presence of
large numbers of this bacterium but can be attributed to
the site disturbances and natural weathering processes.

The importance of this study illustrates the fact that in the Wade Creek drainage, discharge of dissolved metals associated with mining or surface disturbance is a short term and a site specific problem, and the long range impact of the dissolved metals caused by these activities will be negligible. Futhermore, since a consequence of mining is the stockpiled tailings and the settling ponds filled with settleable solids which have heavy metals sorb on to them, these two types of mining refuse may lead to long term impacts on the environment over time if the heavy metals are able to leach from the tailings or the settled solids. Additional research may be needed to determine what are the long term effects on the environment from these two forms of mining refuse.

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APPENDIX A

ACTIVITY OCCURRING ON WADE CREEK

May 31,1986:

   -No activity was occurring along Wade Creek.

June 21, 1986:

   -There was activity in the lower section of Wade Creek.
   The State Department of Transportation was starting
   their re-landscaping project of an abandoned mine
   (Site # 7).

July 3-4, 1986:

   -The state had continued its re-landscaping project.
   The third mine had been active (the mine was operating
   with a recirculation system).

August 2, 1986:

   -Activity at the second mining operation they had set
   up their ponds for recirculation and were working.
   Third mine was sluicing material at site # 7. The
   state had not been working on the lower area.

September 1, 1986:

   -There was activity at third mine, they had actively
   been sluicing materials.

APPENDIX B

SITE DESCRIPTION

Site #1, (M.P. 0.0): All samples were taken at the headwaters of Wade Creek.

Site #2, (M.P. 0.5): All samples were taken above the mining operation operating on a tributary to Wade Creek.

Site#3, (M.P. 0.75): All samples were taken below the mining operation operating on the second tributary to Wade Creek.

Site #4, (M.P. 2.4): One sample was taken in a drainage area of an old settling pond surrounded by tailings.

Site #5, (M.P. 3.0): Site below tailings that were worked 10-20 years ago. Site had good vegetation on tailings.

Site 6, (M.P. 4.5): All samples were taken from area above a mining operation, located within some tailings.

Site # 7, (M.P. 5.3): All samples were taken below mining operation, below last settling pond.

Site # 8, (M.P. 6.0): The two samples were taken below an

old dredge located below Ophelia Creek.

Site # 9, (M.P. 7.5): All samples were taken above an abandoned mining operation that State Department of Transportationwas re-landscaping during the summer. The mine had been operating up until last summer. This was the area of their final cut from the summer of 1985.

Site # 10, (M.P. 8.2): All samples were taken approximately 25 meters below the area that was sluiced in the summer of 1985. The site was approximately 25 meters below the sluice box.

Site # 11, (M.P. 8.8): The two samples were taken below one of the settling ponds from the 1985 operation. This was the area that the state concentrated most of their re-landscaping project in for the summer.

Site # 12, (M.P. 9.0): These samples were taken below the area that was mined. The site were among tailings and were impacted by the state's activities was well.

Site # 13, (M.P. 9.8): Sample was taken below all of the activity occurring on Wade Creek. The samples were taken from among some of the old tailings.

APPENDIX C

THE WEATHER CONDITIONS ON THE SAMPLING DATES

The sampling dates for each site are listed:

Sites 1, 6, 8, and 12 : May 31, 1986, the weather had been dry but had rained the day before.

Sites 9, 10, 10, and 11 : June 21, 1986, the weather was clear on the day of the sampling but had been raining the three previous days in the afternoon.

Sites 1, 2, 4, 5, 6, 7, 10, 11, and 13 : July 3-4, 1986, the weather was clear for the previous week and was raining at the time the samples were taken.

Sites 1, 2, 3, 6, 7, 8, 9 and 12 : August 2, 1986, the weather had had occasional rains for the previous week.

Sites 1, 2, 3, 6, 7, 9, and 12: September 1, 1986, the weather was clear and had been clear for the previous two days.