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Mine Waste Technology Program

In Situ Source Control Of Acid Generation Using Sulfate-Reducing Bacteria

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Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Risk Management Research Laboratory (NRMRL) is the Agency's center for investigation of technological and management approaches for preventing and reducing risks from pollution that threaten human health and the environment. The focus of the Laboratory's research program is on methods and their cost-effectiveness for prevention and control of pollution to air, land, water, and subsurface resources; protection of water quality in public water systems; remediation of contaminated sites, sediments, and groundwater; prevention and control of indoor air pollution; and restoration of ecosystems. NRMRL collaborates with both public and private sector partners to foster technologies that reduce the cost of compliance and to anticipate emerging problems. NRMRL's research provides solutions to environmental problems by developing and promoting technologies that protect and improve the environment; advancing scientific and engineering information to support regulatory and policy decisions; and providing the technical support and information transfer to ensure implementation of environmental regulations and strategies at the national, state, and community levels.

This project was conducted under the Mine Waste Technology Program. It was funded by the EPA and administered by the U.S. Department of Energy (DOE) in cooperation with various offices and laboratories of the DOE and its contractors. It is made available at www.epa.gov/minewastetechnology by EPA's Office of Research and Development to assist the user community and to link potential users with the researchers.

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Abstract

This report summarizes the results of the Mine Waste Technology Program (MWTP) Activity III, Project 3, *In Situ Source Control of Acid Generation Using Sulfate-Reducing Bacteria*, funded by the U.S. Environmental Protection Agency (EPA) and jointly administered by EPA and the U.S. Department of Energy (DOE). This project addressed EPA's technical issue of Mobile Toxic Constituents – Water through a field demonstration of a water treatment technology based on the use of sulfate-reducing bacteria (SRB) at a remote inactive underground mine.

This project was undertaken to demonstrate the effectiveness of SRB technology to treat metal-laden water flowing through and from an abandoned mine. The Lilly/Orphan Boy Mine, located in the Elliston mining district of Montana near the capital city of Helena, was selected as the site for the field demonstration. The Lilly/Orphan Boy Mine, active in the first part of the 20th century, was a relatively small mine that produced lead ore, which was shipped to a smelter in Helena. After active mining ceased in the 1950s, the mine workings subsequently flooded with groundwater and this eventually resulted in acid rock drainage (ARD) discharging from the mine portal.

Under the MWTP, MSE Technology Applications, Inc. (MSE) demonstrated an innovative, in situ biological technology to treat and control ARD emanating from the Lilly/Orphan Boy Mine. Cables were installed to suspend platforms 30 feet below the static water level in the mineshaft that was open to the surface. Organic matter, primarily cow manure and straw, was placed on the platforms in the shaft, forcing the ARD coming from the mineshaft to pass through the organic matter before exiting the mine through the portal. Dissolved metals were removed from the ARD entering the in situ bioreactor, and the water subsequently flowed out of the mine through the downgradient portal. Because the SRB technology also caused the shaft water pH to rise and the oxidation reduction potential to drop, the amount of acid leaving the mine was substantially decreased. The bioreactor was activated in August 1994, and the water was analyzed for more than a decade (through July 2005). In general, the water has seen a considerable reduction in dissolved metals concentrations, and the discharge pH has been increased from a historic level of near 3 to a more neutral pH close to 6.

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Acronyms and Abbreviations

ARD	acid rock drainage
ATP	adenosine triphosphate
BLAST	Basic Local Alignment Search Tool
BOD	biochemical oxygen demand
COD	chemical oxygen demand
COOH	carboxylic acid group
DMW	drift monitoring well
DNA	deoxyribonucleic acid
DO	dissolved oxygen
DOE	U.S. Department of Energy
EPA	U.S. Environmental Protection Agency
IAG	Interagency Agreement Number
ID	identification
MSE	MSE Technology Applications, Inc.
MWTP	Mine Waste Technology Program
NPV	net present value
NRMRL	National Risk Management Research Laboratory
ORP	oxidation reduction potential
PCR	polymerase chain reaction
pH	negative log of hydrogen ion concentration
PMDTS	passive mine drainage treatment systems
QA	quality assurance
QAPP	quality assurance project plan
SMW	shaft monitoring well
SRB	sulfate-reducing bacteria
VFA	volatile fatty acid

Acknowledgments

This document was prepared by MSE Technology Applications, Inc. (MSE) for the U.S. Environmental Protection Agency's (EPA) Mine Waste Technology Program (MWTP) and the U.S. Department of Energy's (DOE) Environmental Management Consolidated Business Center. For this project, Ms. Diana Bless was EPA's MWTP Program Manager and Mr. Gene Ashby was DOE's Technical Program Officer. Ms. Helen Joyce was MSE's MWTP Program Manager.

The *In Situ Source Control Of Acid Generation Using Sulfate-Reducing Bacteria* project was the result of contributions by over 40 MSE employees. Of these, Ms. Suzzann Nordwick, Ms. Marietta Canty, Mr. Tom McIntyre, and Mr. Creighton Barry made significant contributions.

Special acknowledgment and thanks are extended to the members of the Newman family – owners of the Lilly Lode and Orphan Boy mining claims during this demonstration project.

Executive Summary

The Mine Water Technology Program (MWTP), Activity III, Project 3, *In Situ Source Control Of Acid Generation Using Sulfate-Reducing Bacteria* was funded by the U.S. Environmental Protection Agency (EPA) and jointly administered by EPA and the U.S. Department of Energy (DOE). The project addressed EPA's technical issue of Mobile Toxic Constituents – Water through a field demonstration of an in situ sulfate-reducing bacteria (SRB)-based water treatment technology applicable to acid rock drainage (ARD).

ARD is produced when metal sulfide minerals, particularly iron pyrite, come in contact with oxygen and water. The resulting oxidation of the metal sulfide minerals dramatically increases their solubility in water, causing the formation of an acidic metal-laden stream. Biological sulfate reduction with SRB can be used to treat ARD. The bacteria convert the sulfate dissolved in the ARD to soluble sulfides, which then react with the dissolved metal ions to rapidly precipitate stable metal sulfides.

The main purpose of conducting this field demonstration was to evaluate the use of SRB to mitigate the effects of metal-contaminated ARD in situ. This field demonstration resulted in an effective, relatively long-term test. The performance of the SRB treatment technology was demonstrated through the collection and analysis of samples within the mine tunnel and at the mine portal. Dissolved metals concentrations were the primary parameters monitored. However, periodically collected data also included total metals, alkalinity, temperature, dissolved oxygen, pH, oxidation reduction potential, sulfate, sulfide, biochemical oxygen demand, chemical oxygen demand, and volatile fatty acids. The effects of the treatment were observed in nearly all of the analytical parameters measured.

The Lilly/Orphan Boy Mine, near Helena, Montana was selected as the site for the field demonstration. The flooded subsurface mine workings were turned into an anaerobic biological reactor by suspending an SRB-supporting organic substrate on a platform within the open mineshaft. While SRB technology is commonplace now, its use to treat ARD was a novel concept in the early 1990s. Before the bioreactor was activated in August 1994, concentrations of the major dissolved metals were typical of ARD water. Analytical data taken over the course of the demonstration indicated that dissolved metals concentrations had decreased considerably. In addition, the pH of the discharge was effectively increased from a pre-demonstration value of about 3 to a more neutral value close to 6.

Data evaluation shows that overall metal removal was extremely high for aluminum, cadmium, copper, and zinc, but lower for arsenic and iron. Data also indicates that higher metal removals were obtained within the tunnel than at the portal. The pH of the mine water increased almost immediately after the implementation of the technology. During spring runoffs, the pH was lower in the portal sample, but it stayed near neutral in the tunnel. The spring runoff events influenced the water quality more noticeably at the portal than in the tunnel due to oxygenated surface water runoff penetrating through the ground above the portal and then solubilizing historic metal precipitates. Also, spring water quality was lower at the portal due to a greater amount of ARD infiltration from fractures within the tunnel walls.

This demonstration was one of the pioneering efforts in SRB technology implementation at a mine site. The field work proved the long-term effectiveness of using in situ SRB technology to treat acid rock drainage at remote mine sites. Although new at the time, the technology was shown to significantly improve water quality at an abandoned mine and was also more cost-effective than conventional technologies.

1. Introduction

1.1 Project Overview

This document is the final report for Mine Waste Technology Program (MWTP), Activity III, Project 3, *In Situ Source Control of Acid Generation Using Sulfate-Reducing Bacteria*. The MWTP is a program funded by the U.S. Environmental Protection Agency (EPA) and jointly administered by EPA and the U.S. Department of Energy through an Interagency Agreement (IAG). MSE Technology Applications, Inc. (MSE) is the principal contractor for the MWTP. The intent of this project was to demonstrate the ability of sulfate-reducing bacteria (SRB) technology to treat acid rock drainage (ARD) in situ at the Lilly/Orphan Boy Mine located in the Elliston Mining district near Helena, Montana.

This report presents field results gathered during an 11-year field demonstration from August 1994 to July 2005. During this time, the ability of SRB to treat and control metal-contaminated water was evaluated. SRB technology relies on biologically generated products to treat ARD inside the mine before it discharges naturally from the mine portal and into a surface stream.

The field portion of this project consisted of the installation and operation of in situ bioreactors in the mineshaft and in the portal level mine tunnel. The field application consisted of placing a biological reactor with organic substrate and SRB inside the flooded mineshaft. In general, SRB technology treats ARD by removing dissolved metallic and anionic constituents from the water. The SRB bioreactors biologically reduce sulfate to sulfide and generate alkalinity that precipitates metal sulfides and metal hydroxides, respectively, from the ARD. Both of these reactions increase the pH of the water, which decreases the potential for additional acid.

1.2 Background

Prior to field testing, pilot-scale testing was conducted in the laboratory in eight packed-bed reactors that simulated conditions at the Lilly/Orphan Boy Mine. The results of the column tests are summarized in a previous document (Canty, 1999). The tests were able to demonstrate the effectiveness of SRB technology to treat ARD, by increasing pH and removing most metals. Design parameters were developed from the column tests that were used in the implementation of the field system.

1.3 Project Purpose

Congress charges EPA with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program provides data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The purpose of the in situ source control demonstration was to test and evaluate in situ placement of SRB bioreactors and determine the capabilities of this technique to remove dissolved metals from the ARD emanating from the mine workings. It was proposed that an in situ configuration could allow for sustained SRB growth by maintaining an organic support matrix within the mine workings. Additionally, technical information gained from this project would provide technical and economic information on the capabilities of this innovative application of SRB to treat ARD and improve water quality.

1.4 Scope of the Problem

ARD results when metal sulfide minerals, particularly iron pyrite, come in contact with oxygen and water and the metal sulfide minerals are oxidized and then dissolved into the water. Biological sulfate reduction using SRB can be used to treat ARD. The SRB reduce the sulfate

dissolved in the ARD to form soluble sulfide and the hydrogen sulfide generated by the biological metabolism process react with most dissolved metal ions to rapidly precipitate stable metal sulfides. Besides lowering the concentrations of sulfate and dissolved metals, the SRB process also produces alkalinity in the form of bicarbonate from the oxidation of the organic nutrients. This in turn helps to buffer and decrease the acidity of the ARD.

Acid generation occurs when metal sulfide minerals are oxidized according to the following general overall reaction equation:

$$FeS_2 + 15/4\ O_2 + 7/2\ H_2O <---> Fe(OH)_3 + 2SO_4^{-2} + 4H^+ \quad (1)$$

This reaction is one of many that results in increased metal mobility and increased acidity (lowered pH) of the water.

The oxidation of sulfide minerals is accelerated by bacterial action. *Thiobacillus ferrooxidans* is a naturally occurring bacterium that at pH 3.5 or less can rapidly accelerate the conversion of dissolved Fe^{2+} (ferrous iron) to Fe^{3+} (ferric iron), which can act as an oxidant for the oxidation of FeS_2 (Cohen and Staub, 1992). This bacterial activity may cause up to 80% of the acid production in ARD (Welch, 1980). Ferric ions, as well as other metal ions, and sulfuric acid have a deleterious influence on the biota of streams receiving ARD (Dugan et al., 1968).

1.5 Site Selection

As an initial step in this project, several mine sites were screened and prioritized according to five selection criteria: physical accessibility, legal accessibility, physical shaft characterization, hydrogeologic characterization, and shaft water characterization (Mine Waste Report Activity I Report, 1992). The site selected was the Lilly/Orphan Boy Mine, a relatively small mine with a sulfide-based geology that produced ARD in flooded mine workings with the ARD flowing from the mine portal.

1.5.1 Location

The Lilly/Orphan Boy Mine located in the Elliston Mining District of Powell County, Montana, was selected as the demonstration site. The town of Elliston is located about 20 miles west of Helena and south of the Little Blackfoot River. The mining district includes the town of Elliston but is generally in mountainous, heavily forested terrain. The Lilly/Orphan Boy Mine is situated on patented claims in the Helena National Forest about 11 miles south of Elliston.

1.5.2 Mine Site Geology and History

The bedrock geology of the Elliston Mining District is consistent with the Montana Boulder Batholith, which is composed of intrusive quartz monzonite granitic rocks that intruded into older sedimentary and volcanic rocks such as limestone, shale, quartzite, and andesite.

In the first half of the 20th century, the Elliston District of Powell County, Montana was a small producer of lead-zinc ores with trace values of gold and silver. Impurity metals included iron, arsenic, and antimony. The general geology of the Lilly Lode ore was sulfide mineralization with the major minerals being pyrite, arsenopyrite, galena, sphalerite, tetrahedrite, and chalcopyrite. Production from the district was significant during war years when notable amounts were supplied to neighboring mills. The Grand Republic Mining and Milling Company of Helena first staked the Lilly lode claim in the late 1890s. They sunk a 250-foot shaft. In 1934, the Lindquest family acquired the Lilly Lode, made the Lilly portal, and drove in the Lilly tunnel. The Newman family acquired the claim in 1941. The mine remained active during World War II and even had a special priority road built to transport ore. Records indicated 1,000 tons of ore was shipped with most coming from areas near the shaft.

Chemical properties of untreated Lilly/Orphan Boy Mine water are shown in Table 1-1.

1.5.3 Mine Workings

Prior to the field implementation of the MWTP Demonstration, the Lilly/Orphan Boy Mine

consisted of a 250-foot shaft, four horizontal workings, and some stoping (Figure 3-1). After active operations ceased, the mineshaft naturally flooded with water to the 75-foot level and discharged about 3 gallons per minute (gpm) of pH 3 ARD from the portal or adit associated with the 75-foot level.

1.6 Technology

To treat the ARD discharging from the Lilly/Orphan Boy Mine, an in situ SRB-based system was constructed within the mine workings. The technology consisted of establishing a reactor system to biologically generate sulfide (S^{-2}) and bicarbonate (HCO_3^-) that would react with dissolved metals in the ARD to form metal precipitates and neutralize the water. This system configuration provided good conditions for SRB growth by supplying organic materials and other nutrients. For more detailed information see Technology Description (Section 2).

1.7 Project Objectives

The project objective was to develop technical information on the ability of SRB, as a source control treatment technology, to slow or stop the process of acid generation and, thus, improve water quality at a remote mine waste site. The specific purpose of the field demonstration was to show that SRB technology could treat an in situ acidic aqueous waste by removing toxic dissolved metallic and anionic constituents and neutralizing the pH. The goal of the demonstration was to achieve the effluent parameters summarized in Table 1-2.

The project focus was a technology demonstration, not a remediation project. Since the purpose of the project was purely scientific, the objectives did not attempt to address site remediation considerations. The effluent parameters in Table 1-2 were derived from a State of Montana discharge permit and agreed to by EPA in the project quality assurance project plan (QAPP).

The project work plan specified that appropriate process and environmental information be collected, such as seasonal effects on system operation. The project was not limited to evaluating the effectiveness of SRB technology to control acid generation and treat water, but also focused on the feasibility and appropriateness of using this technology at such a site under specific conditions.

Successful achievement of the project goals was to be quantified by measuring dissolved metals concentrations, which would verify the ability of SRB to treat metal contamination associated with ARD. The drainage emanating from the Lilly/Orphan Boy Mine was initially monitored for reduction of dissolved sulfate, reduction of dissolved heavy metals, and pH. A detailed discussion of the sampling can be found in the project-specific QAPP (MSE, 1994).

Table 1-1. Baseline Lilly/Orphan Boy Portal Water Chemistry

	Fe [milligrams per liter (mg/L)]	Zn (mg/L)	Al (mg/L)	Mn (mg/L)	As (mg/L)	Cd (mg/L)	Cu (mg/L)	SO_4^{-2} (mg/L)	pH
Pre-Treatment Portal Water (average from September 1993 to August 1994)	14.05	19.4	7.36	5.46	0.08	0.24	0.33	213	3.44

Table 1-2. Demonstration Goals

Parameter	Goal
pH	between 6 and 8
Sulfate	reduction of 8%
Dissolved aluminum	< 1.0 mg/L
Dissolved arsenic	< 0.05 mg/L
Dissolved cadmium	< 0.1 mg/L
Dissolved copper	< 0.1 mg/L
Dissolved iron	< 1.0 mg/L
Dissolved zinc	< 4.0 mg/L
Dissolved manganese	< 2.0 mg/L
Biochemical oxygen demand (BOD)	< 4.0 mg/L

2. Technology Description

The following section provides a detailed description of the SRB technology available in the literature that was used as the technical basis for the design of the pilot- and field-scale tests. In addition, a description is presented of the other metal removal mechanisms anticipated to occur within an organic-based system designed to promote SRB activity.

2.1 Metals Removal Mechanisms

Although the purpose of the field testing was to evaluate the use of SRB to mitigate metal-contaminated wastewaters in situ, other metal removal mechanisms are also typically associated with an organic-based system. Wildeman, et al., (1993) list removal processes in the following sequence of decreasing priority: (1) exchange of metals by an organic-rich substrate; (2) biological sulfate reduction with precipitation of metal sulfides; (3) precipitation of metal hydroxides; (4) adsorption of metals by ferric hydroxides; and (5) metal uptake by living plants. The last mechanism (5) can be disregarded for our purposes because plants were not associated with the design. Each of these processes is described below.

2.1.1 Adsorption by Substrate

The binding of metal ions by organic matter can play an important role in removing these ions from solution. Some adsorption most likely occurred at the Lilly/Orphan Boy Mine. Three categories of macromolecular, colloidal, or particulate matter are known to be responsible for metal binding at the solid-solution interface: (1) polymeric organic substances, most of which contain many hydrophilic functional groups that are capable of acting as donor groups for complex formation; (2) colloidal or particulate organic matter; and (3) inorganic solids, especially hydrous oxides (Stumm and Morgan, 1981).

An example of adsorption onto a polymeric organic substance, such as a humic or fulvic acid, can be described by the following reactions. In this example, R represents a complex organic component and M represents a divalent metal.

$$RCOOH \text{ <----------> } RCOO^- + H^+ \quad (2)$$

$$2\ RCOO^- + M^{2+} \text{ <---------> } M(RCOO)_2 \quad (3)$$

Exchange of metals with humic and fulvic acids (RCOOH) in a substrate such as manure or peat is a likely mechanism for temporary retention of metals. Retention in this manner is temporary for two reasons: (1) Equation 2 is pH-dependent, and (2) different metals have diverse affinities for adsorption. The pK_a for acid dissociation of humic materials averages approximately 4.2; therefore, in mine drainage with a pH of 3, the dominant species in solution will be carboxylic acids, which will not complex the metal ion. Therefore, the pH level needs to be at least 4 to allow metal complexes to form to a significant degree (Wildeman et al., 1993).

Even if the pH remains sufficiently high, adsorption is a finite process, dependent on the quantity of organic material present. As the amount of organic acids is depleted, more weakly sorbed metals (such as manganese or zinc) may be released back into solution in exchange for more strongly sorbed metals (such as iron or copper). Consequently, the removal of manganese, zinc, and cadmium by substrate adsorption is difficult (Wildeman et al., 1993).

2.1.2 Biological Sulfate Reduction

Biological sulfate reduction requires SRB, dissolved sulfate as the electron acceptor, and a carbon source as the electron donor. Certain environmental conditions, such as a pH between 5 and 8 and a redox potential (E_H) below -100 millivolts (mV) (Cohen and Staub, 1992) are also helpful for optimal growth. Sulfate reduction generates hydrogen sulfide, which is then available for reaction with metal ions to form metal sulfides. The formation of metal sulfides, most of which are quite insoluble at a low E_H and a neutral pH, is very rapid. Therefore, biological sulfate reduction likely occurred at the Lilly/Orphan Boy Mine. Biological sulfate

reduction is described in more detail in Section 2.2.

2.1.3 Hydroxide Precipitation

Of the metals of interest in the Lilly/Orphan Boy Mine water (zinc, copper, cadmium, aluminum, manganese, iron, and arsenic), metal sulfides are more predominant than hydroxides under the pH (6-8) and E_H (-100 mV) conditions induced on the system by the technology (assuming sufficient hydrogen sulfide produced by the SRB). For example, ferric hydroxide precipitation was viewed as an unlikely occurrence, given the reducing conditions present in the system, which make sulfate reduction and the presence of ferric ion mutually exclusive. Aluminum hydroxide is the only stable hydroxide in this pH and E_H range. Therefore, aluminum removal by hydroxide precipitation most likely occurred at the Lilly/Orphan Boy Mine.

2.2 Biological Sulfate Reduction

Biological sulfate reduction is defined as the chemical reduction of dissolved sulfate by the action of biological processes (Dvorak et al., 1991). When dealing with the treatment of ARD, this process is generally limited to the reduction of dissolved sulfate to hydrogen sulfide and the concomitant oxidation of organic nutrient compounds to bicarbonate within the aqueous solution. Sulfate reduction is accomplished by a group of heterotrophic, anaerobic bacteria known as SRB. To thrive, SRB require reducing conditions. They will not thrive in aerobic conditions for extended periods. Also, as heterotrophic bacteria, SRB need a source of carbon in the form of an organic nutrient.

SRB decompose simple organic compounds using sulfate as the terminal electron acceptor, thus producing hydrogen sulfide. Additionally, other bacteria are capable of reducing less oxidized sulfur compounds (i.e., elemental sulfur and thiosulfate) to produce hydrogen sulfide. Biological sulfate reduction improves the quality of ARD in four ways. First, the hydrogen sulfide that is produced will react with dissolved metals to form insoluble metal sulfides that will precipitate from solution (Equations 4, 5, and 6). Second, the reaction has a neutralizing effect on the pH of the ARD because hydronium ions are consumed by the reduction of sulfate. Third, this reaction produces alkalinity in the form of bicarbonate from the oxidation of the organic nutrients. Finally, sulfate is removed from the aqueous waste stream to produce hydrogen sulfide.

SRB

$$SO_4^{2-} + 2CH_2O \rightarrow H_2S + 2HCO_3^- \quad (4)$$

$$H_2S \rightarrow 2H^+ + S^{2-} \quad (5)$$

$$S^{2-} + M^{+2} \rightarrow MS, \text{ where M = metal} \quad (6)$$

Postgate (1984) reported that lactate, pyruvate, glycerol, ethanol, and the tricarboxylic acids are all converted to acetate and carbon dioxide as major end products by *Desulfovibrio* (a genus of SRB). This process is known to involve the conversion of adenosine triphosphate (ATP) to adenosine monophosphate, the primary way that cells transfer energy (Postgate, 1984).

Several studies have been performed in recent years to research the process by which SRB can remediate metal-contaminated wastewater. These studies range from bench-scale experiments, such as SRB growth in chemostats, to field applications, such as constructed wetlands. The use of wetlands, or passive mine drainage treatment systems (PMDTS), to treat ARD evolved from the observation that the water quality of ARD flowing through natural sphagnum moss bogs improved. The Tennessee Valley Authority has the most experience in constructing wetlands for the treatment of ARD from coal mines, which are typically aerobic systems designed for iron removal (Brodie et al., 1988). Historically, PMDTS were constructed as shallow ponds resembling natural wetlands focusing on plant uptake of metals as an important role in metals removal of these systems (Brodie et al., 1989). However, the most important method of metals removal in PMDTS has become recognized as biological sulfate reduction in the anaerobic

zone of the system. In fact, plant uptake of metals is no longer recognized as a necessary element of a PMDTS. At the Big Five Tunnel PMDTS in Idaho Springs, Colorado, no uptake of metals into the plants could be demonstrated. Consequently, the focus of PMDTS has moved toward metals removal and generation of alkalinity by biological sulfate reduction through optimization of an anaerobic, reducing environment.

2.2.1 Microbial Description

SRB are reported to be present in almost all environments on earth (Young, 1936). For example, bottom muds of seawater were found to contain 100 to 10,000 viable SRB cells per gram (Postgate, 1984). Members of the *Desulfovibrio* genus of bacteria are the principal biological agents that reduce sulfate to sulfide. However, eight genera of SRB are known to exist: *Desulfovibrio*, *Desulfomonas*, *Desulfotomaculum*, *Desulfobacter*, *Desulfobulbus*, *Desulfococcus*, *Desulfosarcina*, and *Desulfonema* (Hunter, 1989). The dominant species of SRB belong to the genera *Desulfotomaculum* and *Desulfovibrio* (Cohen and Staub, 1992).

2.2.2 Growth Parameters

Growth rates of SRB are an important parameter in designing biological reactors, including in situ applications for the treatment of ARD. The required amount of substrate for the reaction can be predicted from experimental growth rates. For example, growth rates can be used to determine the necessary reactor residence time (Lee, 1992 and Middleton and Lawrence, 1977).

Postgate (1984) describes *Desulfovibrio* growth as linear rather than exponential in many media. Middleton and Lawrence (1977) reported that microbial growth of SRB using acetate as the substrate (single substrate model) could most closely be modeled by Monod's Equation for the growth rate of biomass.

Most in situ applications of biological sulfate reduction can be best modeled by a plug-flow model and microbial kinetics. In plug-flow reactors, the fluid retention time in the reactor is an important parameter since it describes the contact time the bacteria will have with the wastewater. A certain portion of the bacteria will be attached to the substrate; however, another portion will be free-floating in the water column. The hydraulic residence time should be at least as long as the doubling time of the organism; such duration ensures the SRB are not "washed out" of the reactor (Lee, 1992). Although in most real systems wash out would not occur because of cell adsorption to surfaces. Residence times required for in situ treatment of ARD have been reported to range from 20-30 hours to 20-30 days (Cohen and Staub, 1992).

An in situ application of biological sulfate reduction would utilize psychrophilic strains of SRB. SRB are comprised of psychrophilic, mesophilic, and thermophilic strains. Mesophilic SRB live in moderate temperatures (30 °C), while thermophilic SRB require higher temperatures (50 °C to 70 °C) for growth. Psychrophilic SRB (live in cool temperatures) have been reported in the literature (Barghoorn and Nichols, 1961), but have been studied to a very limited degree (Postgate, 1984). In addition, growth of mesophilic SRB is considered slow in comparison to typical bacterial growth rates. Postgate (1984) suggested that this slow growth may be the result of H_2S production, which is intrinsically toxic to living systems. However, Postgate (1984) also postulated that H_2S reacting with soluble iron to form insoluble iron sulfide, thus removing iron from availability as a nutrient, may more likely be the cause of slow growth.

2.2.3 Growth Requirements

Temperature, Reduction Potential

SRB are capable of tolerating a wide variety of temperatures, salinities, and pressures and demonstrate considerable adaptability to new conditions of temperature (Postgate, 1984). The major prerequisite for growth is an anaerobic environment with E_H near -100 mV (Postgate, 1984). Mesophilic SRB grow best at temperatures between 30 °C to 42 °C, but tolerate temperature swings between -5 °C to 50 °C (Postgate, 1984).

A temperature of 37 °C was found to be optimum using a bioreactor with wood chips as the organic substrate (Tuttle et al., 1969). On the contrary, low temperatures have been reported to have a considerable suppressing effect on biological sulfate reduction (Davison et al., 1989 and Kuyucak and St-Germain, 1993). At temperatures below 10 °C, SRB performance may be lowered 60 to 80% (Kuyucak et al., 1991). Because an in situ application of biological sulfate reduction would involve a cool environment, SRB effectiveness at a low temperature was of particular relevance during the literature search. Further literature review revealed opposite results on the cold temperature capabilities of SRB. SRB have demonstrated the ability to increase their numbers in cold weather, thus compensating for lower individual activity (Cohen and Staub, 1992). Sulfate reduction was observed in an Antarctic pool (Postgate, 1984). It has been suggested that more SRB in nature function at below 4 °C (psychrophiles) than above 5 °C, largely because of their abundance in ocean sediments (Postgate 1984). Finally, Herlihy and Mills, (1985) reported similar SRB activity rates in both winter and summer. In general, literature information shows that while SRB growth rates may be slower at low temperatures, some growth does occur, and there is ample evidence that a low temperature SRB reactor would function to treat ARD.

pH

SRB tolerate pH values ranging from well below 5 to 9.5 (Postgate, 1984), but the specific bacterial growth rate and the removal rate of metals have been shown to be strongly influenced by pH. A pH of 6 has been reported to be optimum for both SRB growth rate and removal rate of metals (Hunter, 1989). *Desulfovibrio* is reportedly inhibited at pH less than 5 (Postgate, 1984). However, it should be noted that these microorganisms are capable of creating microenvironments conducive to their growth (Hunter, 1989). For example, in a constructed wetland receiving ARD with pH < 3, the pH of the pore water in the substrate ranged from 6 to 7 (Hedin et al., 1989).

Substrate

To determine an appropriate substrate to be used during the pilot-scale testing, a literature review was conducted. Several types of substrates, as well as additives were identified.

SRB require a substrate composed of simple organic compounds (Postgate, 1984). Davison et al. (1989) reported the effects of substrates on SRB activity. The substrates used in these experiments included the following: spent mushroom compost, peat, corn wastes, rice waste, decomposed wood chips, and composted cow manure. Decomposed wood chips and composted cow manure gave the highest activity rates and demonstrated the greatest buffering capacity. The other substrates tested often yielded near zero SRB activity. However, addition of pH raising additives to the poor growth substrates resulted in substantially increased activity. Cohen and Staub (1992) reported that results from the Big Five Tunnel PMDTS indicated that peat was an ineffective substrate even when limestone was added. Additionally, mushroom compost worked well but only in conjunction with very low ARD flow rates. Dvorak et al., (1991) reported that sulfate reduction and metal retention increased in a reactor with the addition of lactate. Postgate (1984) reported that growth of a strain of SRB on an unfamiliar carbon source might require a metabolic adjustment that delays growth.

Another study was performed comparing three cellulosic materials (straw, timothy hay, and alfalfa hay) on their abilities to sustain microbial treatment of ARD (Bechard et al., 1993). Of these three cellulosic materials, alfalfa hay sustained microbial treatment for the longest period of time. However, the study determined that a more readily available carbon source, such as sucrose, was often needed to keep the cellulosic systems operating. Cellulosic materials have been used in conjunction with other substrate materials, such as cow manure, to act as a long-term carbon source, as well as a bulking agent (Cohen and Staub, 1992).

In biochemical reactions under anaerobic conditions, a consortium of "acid forming bacteria" convert complex organic substrates into aliphatic acids. Acetic, propionic, butyric acids, the major aliphatic acids produced, are considered simple organic compounds that can be used by SRB. In other words, substrate effectiveness appears to rely on the presence of other heterotrophic bacteria to convert complex organic compounds into the simple compounds required by SRB. Cohen and Staub (1992) found that a substrate composed of cow manure and decomposed wood chips produced a high SRB activity rate; the data suggested that a consortium of heterotrophic bacteria existed in the substrate that decomposed complex organics into simple ones. In addition, cow manure was found to have the buffering capacity and nutrient composition necessary to enhance SRB activity. Cow manure has been described as the ideal substrate for biological sulfate reduction because of its effectiveness and low cost (Cohen and Staub, 1992).

Other bacteria in anaerobic systems also use simple organic compounds as a food source. For example, "methane-forming" bacteria, or methanogens, are capable of converting the aliphatic acids into methane by cleavage of the carboxylic acid group (COOH) and carbon dioxide reduction (Sundstrom and Klei, 1979). However, in an environment rich in sulfate, SRB effectively compete with methanogenic populations for the available aliphatic acids. While SRB can grow at low concentrations of hydrogen, methanogens are greatly hindered because their hydrogen uptake systems cannot function at low hydrogen concentrations. In addition, SRB have an increased affinity for both acetic acid and hydrogen in comparison to methanogenic populations (Postgate, 1984). Also, a relationship between SRB and methane-producing bacteria has been noted. In natural environments, sediments in which sulfate reduction is actively taking place will often lie above sediments in which methane production is occurring. It has been postulated that SRB may have the capacity to use methane as a substrate (Postgate, 1984).

Although past research has reported the treatment capacity of SRB based on metal loading rates per unit area of substrate (Kleinmann, 1990 and Cohen and Staub, 1992), recent developments have concluded that treatment capacity (flow rate) is more accurately represented on the basis of substrate volume (Euler, 1992).

Sulfate

In biological sulfate reduction, sulfate ions act as an oxidizing agent for the dissimilation of organic matter. SRB assimilate a small amount of reduced sulfide ions, but essentially all sulfide is released into the surrounding fluid. The process is generally less effective at very low concentrations of sulfate (Hedin et al., 1989) and (Kuyucak and St-Germain, 1993). However, as long as sulfate reduction remains the dominant electron acceptor process occurring, methanogenesis would still occur under anaerobic conditions.

Iron

Desulfovibrio shows an exceptionally high requirement for iron. The iron is needed in cell constituents, such as ferredoxin and cytochrome c (Postgate, 1984). The dissolved iron reacts with the H_2S to form iron sulfide, therefore reducing the dissolved iron concentration. Amino acids, which may be a nutritional requirement for SRB growth, are capable of chelating Fe^{2+} and thereby inhibiting iron sulfide precipitation. Consequently, specific amino acids may function to make iron more readily available to SRB (Dvorak et al., 1991).

2.2.4 Growth Inhibition Factors

While the presence of oxygen inhibits SRB activity, SRB can survive long exposure to oxygen and become active again when returned to an anaerobic environment (Postgate, 1984). High metal concentrations, particularly copper, may also inhibit SRB growth. An SRB inhibitory copper concentration of 5-50 mg/L of copper sulfate was reported by Saleh et al. (1964). However, Noboro and Yagisawa (1978) reported rapid bacterial growth at a copper concentration as high as 100 mg/L when a lactate substrate was used.

Lovley and Phillips (1994) reported inhibition of SRB with Fe^{3+} in laboratory experiments. The suggested cause for this inhibition was iron-reducing bacteria, which have an energetic advantage over the sulfate reducers. Few other studies have indicated an inhibitory effect of iron. In fact, several studies have reported significant SRB growth with high concentrations of iron in the wastewater of concern, for example, acid mine water.

Two additional metal ions have been reported to inhibit SRB growth. Postgate (1984) denoted the selenate ion (a competitive antagonist of sulfate reduction) and the molybdate ion (which depletes the organism's ATP pool). However, both ionic species are generally expected to be present in only very small quantities in ARD.

Postgate (1984) reported a cyanide concentration of 1 to 5 mol/mL as being a metabolic inhibitor.

3. Demonstration Description

Demonstration of SRB technology consisted of the following major phases with several sub-phases as described below.

- Phase I – Laboratory testing
- Phase Ia – Bench-scale substrate adsorption study
- Phase II – Field demonstration at a remote mine site
  - Design
  - Implementation
  - Monitoring

3.1 Laboratory Testing

Technical parameters required for the field application were developed from laboratory testing conducted by MSE between January 15 and March 25, 1994 in Butte, Montana. Sediments collected at the Lilly portal were used as the source for the laboratory SRB populations. Water pumped from the Lilly mine shaft was collected in large plastic containers and transported to Butte to be used as the ARD feed for the laboratory test. Following the laboratory testing, the field demonstration commenced in August 1994 and continued through July 2005.

The laboratory tests were designed to support the field demonstration by identifying functional parameters using ambient conditions comparable to the Lilly shaft water. Eight 4-foot vertical Plexiglas organic substrate packed-bed SRB reactors were operated at 8 °C and were fed Lilly mineshaft ARD continuously in an upflow configuration at a reactor hydraulic retention time of 120 hours over 60 days. During this time, numerous physical and chemical parameters were monitored.

The experimental design consisted of a 3x2 full factorial design, allowing for the comparison of two different bacterial preparation methods (no preparation and prepared) and three organic substrate-layering methods (no gravel, mixed, and layered). In the prepared tests, SRB were first grown for two weeks at 20 °C in a sodium sulfate solution.

Laboratory column testing and subsequent laboratory analysis showed that metal removal occurred due to both adsorption and sulfide precipitation, however, the amount of metal removal by either mechanism was not quantified. The total dissolved metal removal efficiencies reached 99% for zinc, 99% for aluminum, 96% for manganese, 98% for cadmium, and 96% for copper. Iron and arsenic removal was not as effective but was slightly more effective in the reactors with prepared bacteria. This was attributed to high levels of iron and arsenic contamination in the organic substrate.

3.2 Substrate Adsorption Studies

Throughout the project, many questions were raised regarding the mechanism for metals removal. Although the majority of metals removal is credited to SRB through sulfide precipitation, some metals removal can be attributed to adsorption by the organic substrate. To help quantify this removal mechanism, exploratory laboratory column testing was conducted with the objective of determining the extent to which metals are removed via adsorption. For this testing, the total metals removal of sterilized organic substrate was compared to that of unsterilized organic substrate. Results indicated that the sterilized substrate was still able to remove aluminum and manganese, so it is likely that these two metals had some removal as a result of initial adsorption onto the substrate.

3.3 Field Design and Construction

The SRB field demonstration was designed to use the flooded subsurface mine workings of the Lilly/Orphan Boy Mine as an "in situ biological reactor" (Figure 3-1). Two platforms were suspended by cables in both sides of the two-compartment shaft 30 feet below the static water level and were secured at the surface. An organic substrate consisting of approximately 70% cow

manure, 20% decomposed wood chips, and 10% alfalfa straw was placed in the shaft and supported by the platforms. The percentages are approximant, as the substrate was prepared by mixing about four parts cow manure, one part wood chips, and one part straw in a concrete mixer while in the field. Some substrate was placed on the platform in the shaft and the remainder was placed in the horizontal adit by drilling holes from the surface and pumping material into the tunnel.

In addition, two injection wells were drilled into the main portal tunnel of the mine (Lilly Tunnel) so that substrate could also be placed into this underground space. Therefore, the ARD flowed upward through the substrate in the shaft (artesian water flow has been observed at the mine) and horizontally through the substrate in the tunnel. The biological reaction took place in the substrate regions, and the treated water subsequently flowed out of the mine through the portal. Because the technology caused the shaft water pH to rise and the E_H to fall, the amount of acid generation within the mine was decreased. Monitoring of the field demonstration began once the substrate was placed in late August 1994 and continued for almost 11 years.

3.3.1 Monitoring Well Installation

Original plans called for sampling water emanating from the mine portal and comparing these samples to historical influent values to determine bioreactor effectiveness. However, as the demonstration progressed, the tunnel was suspected of re-contaminating the water after it passed through the bioreactor. Therefore, monitoring wells were installed to obtain samples from within the mine tunnel to realistically evaluate the bioreactor. One monitoring well was drilled into the tunnel downgradient of the injection well and the injection well was also fitted to be a monitoring well. These monitoring wells allowed for the collection of samples before the water traveled the full length of the tunnel to the portal.

Extensive background testing was performed prior to initiating the field demonstration. This data was used to assess the effectiveness of the treatment technology by comparing effluent parameters to historical influent values. As the demonstration progressed, however, it was decided that this background data was outdated, and a better method would be necessary to monitor influent and effluent at the same time. Therefore, two angled groundwater monitoring wells were installed in September 2003 to monitor the ARD at a point prior to entering the biomass in the shaft. These wells monitor confined and unconfined groundwater in the main shaft and a drift.

Prior to well installation, the mine workings were evaluated based on available maps. However, different subsurface maps placed the adit level at different depths. For example, Aikin (1950) shows a cross-sectional view of the Lilly workings that placed the main underground workings at 102 feet below the collar of the shaft. By contrast, Rankin (1950) showed the same workings 74 feet below the collar.

Elevation data acquired at the site indicated that the elevation difference between the collar of the shaft (6,810.6 feet) and the elevation just above the collapsed adit of the main underground workings (6,746.8) was 63.8 feet. The elevation point above the collapsed adit was at least 7 or 8 feet above the adit. This means that the approximate elevation between the shaft collar and the workings near the adit was 71 feet below the collar of the shaft, similar to Rankin's figure of 74 feet. Therefore, it was determined that the best estimate of vertical drill depth would be based on Rankin's 1950 map of the workings.

Two deviated monitoring wells were installed in September 2003. The shaft monitoring well (SMW) was completed in groundwater associated with the shaft and the drift monitoring well (DMW) was completed in groundwater associated with the main underground drift immediately east of the shaft. The SMW intercepted the shaft at a depth of 123 feet and the DMW intercepts the drift just east of the Lilly shaft 15 feet back.

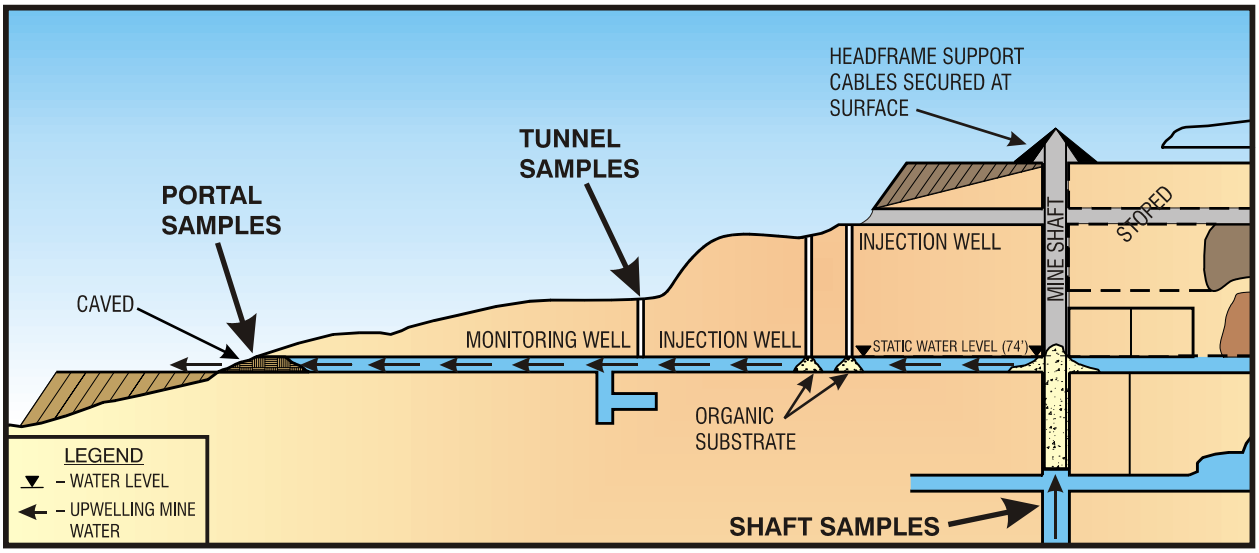

Figure 3-1. Cross-section of underground mine subsurface SRB bioreactor.

4. Field Monitoring Results and Discussion

The first technology evaluation-sampling event was conducted September 6, 1994 after the shaft bioreactor was installed in August 1994. The final sampling was conducted on July 14, 2005. For the first few months of the demonstration, twice-monthly sampling events were conducted. Monthly sampling was then conducted for five and a half years. After the decision was made to extend the project, sampling events were reduced to bimonthly and then to three times per year. Additionally, as the project proceeded, the number of analyses performed on each sample was minimized to reduce project costs. A total of 92 sampling events were conducted. Twice monthly sampling dates are listed in Table 4-1. Monthly sampling events are listed in Table 4-2, and additional sampling events are listed in Table 4-3.

The analytical results of samples taken from within the horizontal mine tunnel and at the portal of the mine are presented within the following sections. Data is presented in graphs and includes dissolved metals, pH, and E_H. A summary of the quality assurance (QA) activities from the project specific QAPP are contained in Appendix A. All data, with the exception of two temperature measurements from 1994, were determined to be usable.

The organic substrate was placed in the mineshaft between August 27 and August 31, 1994. Data prior to this date represents the water chemistry before treatment. The data shows that, for nearly all parameters, tunnel removal efficiencies were better than those for the portal. This is addressed in the discussion of the individual parameter, and has generally been attributed to untreated materials entering the flow between the tunnel sampling point and the adit. The statistical analysis of the metals analysis data is contained in Appendix B along with the QA data summary table.

4.1 Dissolved Metals

As with most acidic mine effluents, the water emanating from the Lilly/Orphan Boy Mine contains significant quantities of metals – both dissolved and contained in particulate matter. The portion of a sample, which passes through a 0.45-micron filter, is considered to be dissolved. Dissolved metals samples were collected from both the tunnel and the portal during the field demonstration and were analyzed for aluminum, arsenic, cadmium, copper, iron, manganese, and zinc. These metals were chosen based on their pre-demonstration concentration in the Lilly/Orphan Boy Mine ARD.

Chemical properties of untreated Lilly/Orphan Boy Mine water are shown in Table 4-4 along with typical post-treatment water chemistries for the tunnel water and mine portal effluent.

4.1.1 Aluminum, Copper, and Cadmium

Concentrations of dissolved aluminum, cadmium, and copper were significantly reduced to below detection limits shortly after the addition of the organic substrate. This would be expected since the solubility of these metals is very dependent on pH. Similar dramatic results were observed in both the tunnel and at the portal for most of the year. However, the portal concentration of all three metals increased for the period of high flow during the spring runoff months of May and June. By contrast, samples from within the mine tunnel showed very little seasonal variation.

As shown in Figure 4-1, the concentration of aluminum in the portal showed regular seasonal variation for the first 6 years of operation. Although data is limited, it appears that the spring runoff values have been going up in recent years. The general trend of the graph is upward, which may indicate that the effectiveness of the SRB reactor is diminishing. After almost 11 years of operation, the nutrient sources are becoming depleted and the ability of SRB to produce sulfide and precipitate metals has decreased. However, since the water was not sampled in winter months for the last years of the demonstration, it is not known if this trend is real. Additional data would be needed to evaluate further. Figures 4-2 and 4-3

for copper and cadmium in the portal show a similar upward trend.

4.1.2 Zinc

Dissolved zinc was not removed as effectively as aluminum, copper, and cadmium. Data shown in Figure 4-4 indicates that zinc is removed to below detection limits in the tunnel, but rebounds prior to the portal. In fact, zinc measured at the portal never reached the target level of 4.0 mg/L. As with other metals, portal zinc concentrations increased considerably during spring runoff. The higher removal of zinc observed in the tunnel of the mine is attributed to the more reduced conditions in the tunnel as compared to the portal.

As with other metals, Figure 4-4 shows an upward trend that may be indicative of the bioreactor's depletion of essential nutrients.

4.1.3 Manganese

Dissolved manganese was not removed as effectively as aluminum, copper, and cadmium. As shown in Figure 4-5, concentrations of manganese in the tunnel were reduced below the target value of 2 mg/L several times, primarily during the first 2 years of operation. Concentrations of manganese at the portal remained at or above pre-demonstration levels showing high correlation to spring runoff times. This same phenomenon was observed during this project's laboratory pilot-scale testing and was attributed to the fact that manganese does not form a solid that can precipitate at the pH and E_H conditions of this system. Effective manganese removal requires oxidizing conditions and a higher pH.

4.1.4 Iron and Arsenic

Dissolved iron data was inconsistent throughout the demonstration and never met the target concentration of 1.0 mg/L (Figure 4-6). During the first few years, dissolved iron was removed more effectively within the tunnel than at the portal. Immediately after the addition of the organic substrate, the dissolved iron concentration actually increased in portal samples. This trend was reversed during the high flow rate that occurred during spring runoff. An increase in dissolved arsenic was observed shortly after the implementation of the technology at the mine as well. Although only a slight increase in arsenic concentration was observed in the tunnel, a large increase was observed at the portal (from near the instrument detection limit of 0.0336 mg/L to a high of 14.7 mg/L). An understanding of iron and arsenic chemistry helps explain these phenomena. Under oxidizing conditions, ferric iron precipitates as $Fe(OH)_3$ (ferrihydrite) and very effectively adsorbs arsenic. However, under reducing conditions, ferrous iron becomes the predominant iron species, which is much more soluble than ferric iron. When the highly soluble ferrous iron was released into solution, arsenic, which had been previously absorbed on to the iron, was released also. This took place during the demonstration when the underground workings of the mine were transformed from an oxidizing environment to a reducing one.

The concentration of arsenic in the mine portal water varied considerably during the demonstration. As shown by Figure 4-7, arsenic in the portal followed the same trend as the iron levels. As with iron concentrations, arsenic concentrations also increased during each spring run off. The system consistently demonstrated that it could recover. Most notable was the 2004 event in which arsenic spiked to 38 parts per million. These increases in iron and arsenic concentrations during spring runoff indicate a reversal of the conditions imposed by the SRB technology and similar to the historical conditions of the mine. As the flow rate increased in the mine system and the retention time decreased, the SRB were likely unable to produce enough soluble sulfide to precipitate the metals, maintain a low reduction potential, and neutralize the water through bicarbonate production. Consequently, the pH fell and the reduction potential increased while the system became more oxygenated. Ferrous iron is oxidized to ferric iron under these conditions, precipitates, and absorbs the arsenic. Conversely, as the system recovered from high spring runoff, the reduction potential appeared to

decrease and the pH and iron concentration appeared to increase.

4.2 Total Metals

In addition to the dissolved metals analyses, samples were collected for total metals analysis on 17 occasions during the first year of the field demonstration. These were obtained from both the tunnel and the portal and analyzed for aluminum, arsenic, cadmium, copper, iron, manganese, and zinc. In comparison with dissolved metals concentrations, total metals results tend to be more variable due to the presence of relatively large particles of organic or other substances containing high concentrations of a particular metal.

Concentrations of total copper and aluminum showed similar trends in that lower, stable values were observed in the tunnel samples compared to higher, more sporadic concentrations in the portal water. Total zinc and cadmium concentrations in the portal samples were higher than those in the tunnel samples. In addition, both total zinc and cadmium concentrations rose in the portal samples during spring runoff, while this trend was not observed within the mine tunnel.

Total manganese concentrations were lower in the tunnel water samples than the portal samples during the first part of the year, then reversed and became higher than the portal samples during the latter part of the year. Similar to the dissolved manganese results, this observation is attributed to the lack of formation of manganese compounds at the pH and E_H of the system.

Total arsenic and iron results were very similar to those for dissolved arsenic and iron. Total arsenic and iron concentrations were both higher in the portal water samples compared to the tunnel water. This phenomenon was previously explained by arsenic and iron chemistry in Section 4.1.

4.3 Alkalinity

Alkalinity typically increases as a result of SRB activity. Therefore, alkalinity, as calcium carbonate ($CaCO_3$), was analyzed in samples taken at the mine portal for the first 3 years of the demonstration. The total alkalinity of the portal water prior to the addition of the organic substrate was less than 10 mg/L as $CaCO_3$. After the addition of the organic substrate, alkalinity concentrations increased, although results were variable. The highest alkalinity concentration (64 mg/L) was measured one month after the implementation of the technology. Overall, effluent alkalinity was not a concern during the demonstration, as other portal water samples did not show alkalinity above the detection limit of 10 mg/L.

4.4 Physical Measurements

Oxidation reduction potential (ORP), pH, dissolved oxygen (DO), flow rate, and temperature were measured on 15 occasions during the first year of the field demonstration.

4.4.1 ORP

ORP is a helpful measurement for assessing SRB growth potential because the organisms require a reducing environment for optimal growth. SRB can also help produce a reducing environment if one does not already exist. ORP is measured in mV, with zero being neither oxidizing nor reducing. Positive values indicate an oxidizing environment while negative numbers specify a reducing environment. Prior to the addition of the organic substrate, the reduction potential in the mine water was about +400 mV. The addition of organic matter caused the E_H to drop sharply in both the tunnel and the portal water samples within the first few weeks to around –50 and +50 mV, respectively. Over the course of the demonstration, it was found that the reduction potential was generally lower in the tunnel than at the mine portal. This has been attributed to the likely possibility that the water eventually exiting the portal becomes contaminated with more oxidized surface water sources. On one occasion the reduction potential within the tunnel rose above that measured at the mine portal. The reduction potential measured within the portal mine water rose sharply during spring runoff due to the addition of fresh oxidized water.

4.4.2 pH

pH is a relatively simple measurement of acidity or alkalinity that can indicate major changes in the condition of the mine water. As stated previously, SRB typically prefer neutral pH, but they can function at lower values and are able to raise the pH of their surroundings by consuming hydronium ions and producing bicarbonate. Within a few weeks of substrate addition to the underground mine workings, the pH increased from about 3 to near neutral within the tunnel and to about 6 at the mine portal. As the demonstration progressed, pH measured at the portal remained neutral for the most part but dropped back to 3 during spring runoff events. Within the tunnel of the mine, however, the pH remained circumneutral at all times, including during spring runoff. The stability of the pH within the tunnel indicates that the technology would be able to provide a stable treatment environment.

4.4.3 Dissolved Oxygen

It was necessary to reduce the level of DO in the mine water since oxygen is detrimental to SRB growth. Heterotrophic bacteria (aerobes and facultative anaerobes) use oxygen as a terminal electron acceptor as they use carbon as an energy source for growth. After available oxygen is consumed, anaerobic organisms, such as SRB can proliferate. Historical DO levels within the portal water were measured at about 6 mg/L prior to the implementation of the technology. Within a few months of substrate addition, DO levels dropped and remained less than 2 mg/L, the ideal condition for SRB growth.

4.4.4 Temperature

Water temperatures recorded in the tunnel and at the portal ranged from 3.5 °C to 12.2 °C and 3.8 °C to 7.5 °C, respectively. Temperatures recorded from tunnel water samples were higher on average than portal samples by 1.3 °C. This difference in temperature may be attributed to the tunnel water mixing with other sources before discharge at the portal. The SRB that thrive in this environment are classified as psychrophilic since they are able to grow at temperatures less than 15 °C. (Morita and Moyer, 2007).

4.4.5 Flow Rate

Due to poor access and the dilapidated condition of the mine portal, the flow rate of ARD was not easily measured. During the first year of the demonstration, field instrumentation was used in an attempt to measure flow. However, this later proved difficult to continue because much of the flow went underground immediately before the portal. During the first year the portal flow rate remained fairly constant at less than 2 gpm. However, spring runoff caused the flow rate to climb to a high of 7.6 gpm during May 1995.

4.5 Other Chemical Measurements

In addition to metals measurements, the following parameters were measured: sulfate, sulfide, BOD, chemical oxygen demand (COD), nitrate, ammonia, and volatile fatty acids (VFA).

4.5.1 Sulfate, Sulfide

As stated previously, SRB reduce sulfate to sulfide during the course of their growth process. These parameters were measured to indirectly verify the existence and proliferation of SRB in the mine water. Sulfate and sulfide samples were measured routinely for the first 6 years of the field demonstration, and then occasionally after that. In general, concentrations of sulfate measured in the tunnel were lower than those at the mine portal. Because organic substrate was located in the shaft and in the tunnel of the mine, these are the regions in which sulfate reduction would occur. Prior to the addition of organic substrate, baseline sulfate concentration in the underground mine workings was analyzed at 274 mg/L. Over the course of the demonstration, sulfate concentrations mostly stayed below this level (Figure 4-8).

Prior to the addition of organic substrate, concentrations of soluble sulfide in the mine water were below the instrument detection limit. After the placement of the cow manure substrate, variable and sporadic concentrations of sulfide were measured in both the tunnel and the portal at the same sampling events in which sulfide was analyzed (Figure 4-9). There was some variability of the concentrations that may have been caused by the highly reactive nature of sulfide and the

analytical difficultly in quantifying this parameter. Sulfide generation is a good indicator of SRB activity because sulfate is not reduced to sulfide simply from a decrease in reduction potential; microbial action is required.

4.5.2 Biochemical Oxygen Demand, Chemical Oxygen Demand

In environmental chemistry, the COD test is commonly used to indirectly measure the amount of organic compounds in water. BOD is a chemical procedure for determining how fast biological organisms use up oxygen in a body of water. It is used in water quality management and assessment, ecology, and environmental science. BOD is not an accurate quantitative test, although it could be considered as an indication of the quality of a water source.

Addition of organic and biological activity can substantially impact oxygen demand. For the first 3 years, BOD and COD samples were collected at the portal to determine the oxygen demand imposed on the mine water and the receiving stream (Telegraph Creek) as a result of the organic substrate. BOD and COD levels rose within a few weeks of substrate addition from background levels of 4 and 83 mg/L reaching highs of 18.5 and 246 mg/L, respectively. Both parameters dropped dramatically within a few months of organic placement to approximately 4 and 40 mg/L, respectively and analysis was discontinued.

BOD concentrations were elevated for six months after technology implementation and then returned to background levels.

4.5.3 Nitrate, Ammonia

Nitrate and ammonia are present in natural organic matter (such as cow manure) and they can be produced as a result of biological activity. Nitrate and ammonia samples were analyzed for the first 3 years to determine discharge levels to Telegraph Creek, which might be subject to state regulatory discharge requirements.

Nitrate levels were measured for the first 4 years of the project. They generally remained low throughout this part of the demonstration. The typical range was 0.05 to 3 mg/L. Ammonia levels rose sharply within the first few weeks after the addition of the organic substrate reaching a high of 11.8 mg/L and gradually declined over the remainder of the 4-year monitoring period, reaching a low of 0.3 mg/L. These results only include the nitrogen component of the nitrate and ammonia for ease of comparison, in keeping with EPA conventions.

Ammonia concentrations were elevated for a longer period, but returned to background concentrations after one year. BOD concentrations dropped more quickly than the ammonia concentrations because the BOD measurement was carbonaceous BOD and not nitrogenous BOD. Oxidation of carbonaceous organics occurs more quickly than nitrogenous organics because of a longer lag phase in the growth of denitrifying bacteria (Barghoorn and Nichols, 1961). After the organic substrate was added to the mine, the BOD was depleted as the abundant heterotrophic microorganisms present in the substrate utilized the carbonaceous organic material. The longer period of time required for the decrease of ammonia was attributed to the limited amount of oxygen available in the system for nitrification of ammonia to nitrite/nitrate. Nitrite/nitrate subsequently underwent anaerobic denitrification.

4.5.4 Volatile Fatty Acids

As discussed previously, VFA are produced by heterotrophic microorganisms by the breakdown of more complex organic substances. According to Kleikemper et al. (2002), SRB are known to use VFAs as a food source, although not all genera are capable of utilizing acetate. It appears that acetate levels rose during the first few months of the demonstration, then enough acetate-utilizing SRB became established, and began to feed on the acetate.

The following VFAs were measured during the first year of the field demonstration: acetate, propionate, iso-butyrate, normal-butyrate, and formate. VFAs were monitored to help determine

which simple organic compounds the SRB were utilizing from the cow manure substrate. Higher concentrations of VFAs were observed in the mine tunnel than at the portal. Acetate concentrations rose sharply in the mine tunnel after the addition of the organic substrate. The concentration of acetate peaked after three months and then began falling. Propionate, iso-butyrate, normal-butyrate, and formate concentrations were variable.

4.6 Molecular Microbiology

At the conclusion of the project, a sample was collected from the shaft bioreactor and examined at Montana State University's Center for Biofilm Engineering using molecular community analytical techniques. Deoxyribonucleic acid (DNA) was extracted from the sample – both from the liquid and from the soil. Method details are given in Appendix C. This included the extraction, purification, and amplification by polymerase chain reaction (PCR) of DNA, assessment of community profiles, and sequencing of amplified DNA to identify species.

Molecular analysis results were significant in that they confirmed the presence of SRBs and indicated a diverse bacterial community. A total of 56 clones had analyzable sequences that were further analyzed using Basic Local Alignment Search Tool (BLAST). Returned sequenced results included two SRB (*Chloroflexi bacterium* and *Flexibacter*) and a sulfur reducing bacteria (*Thermococcales archaeon*). Other identified sequences included three sulfur oxidizers, three compost isolates, four Proteobacteria, and one iron/manganese-reducing anaerobe.

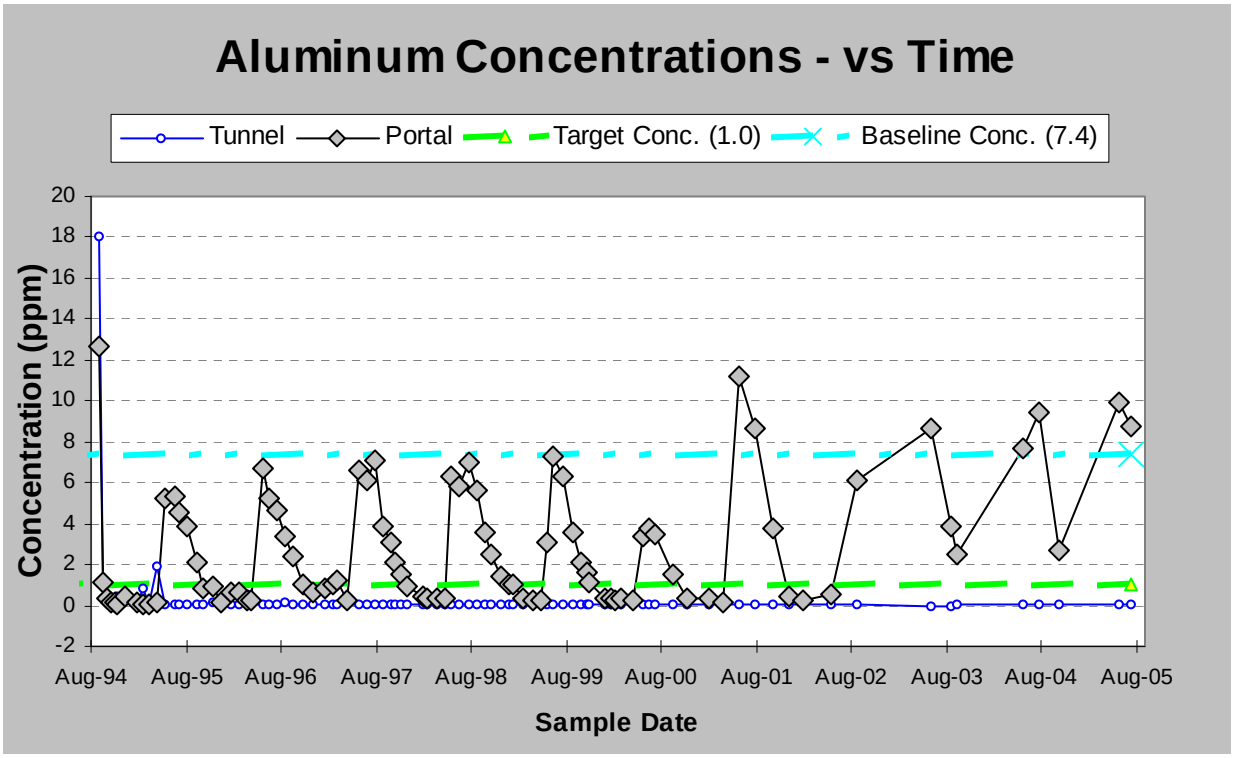

Figure 4-1. Aluminum concentrations.

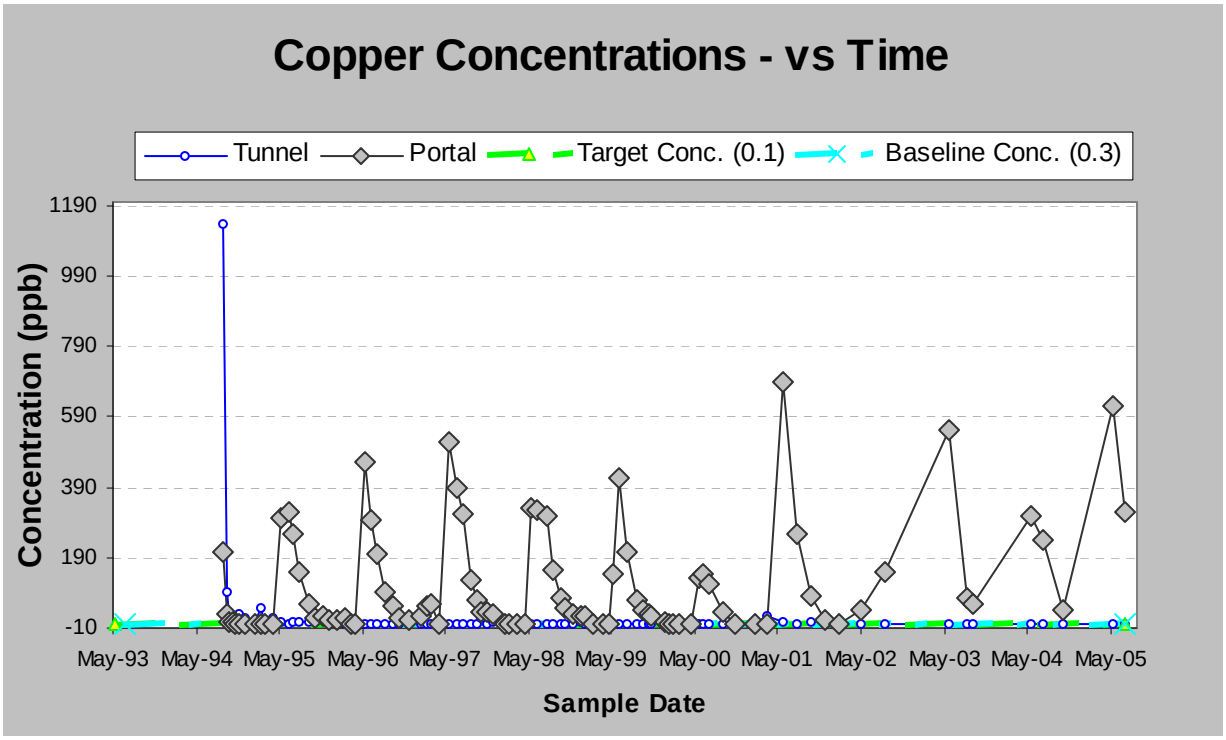

Figure 4-2. Copper concentrations.

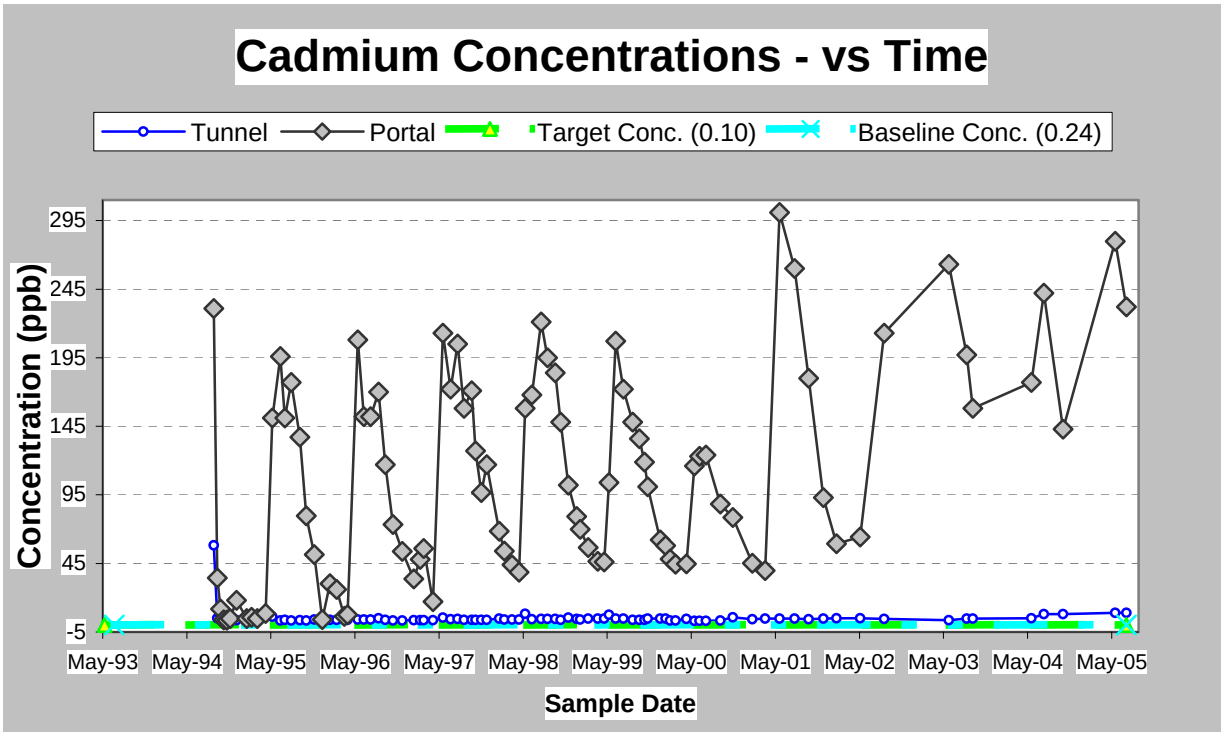

Figure 4-3. Cadmium concentrations.

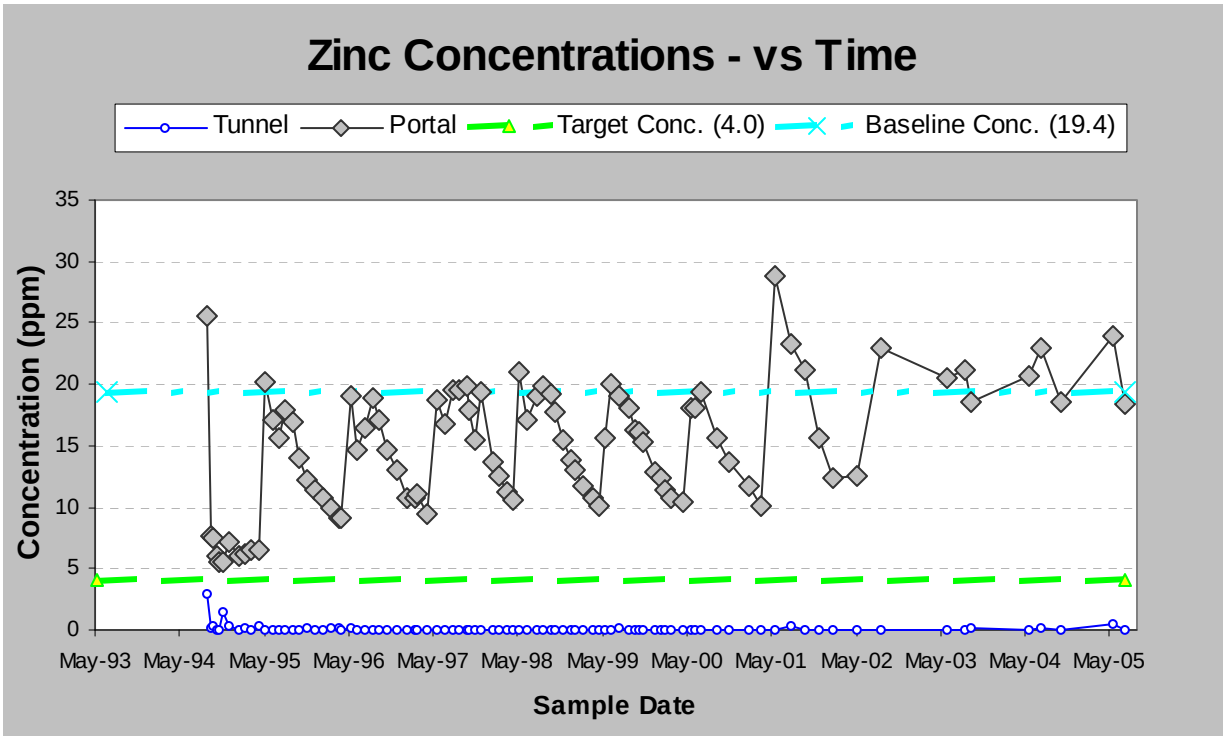

Figure 4-4. Zinc concentrations.

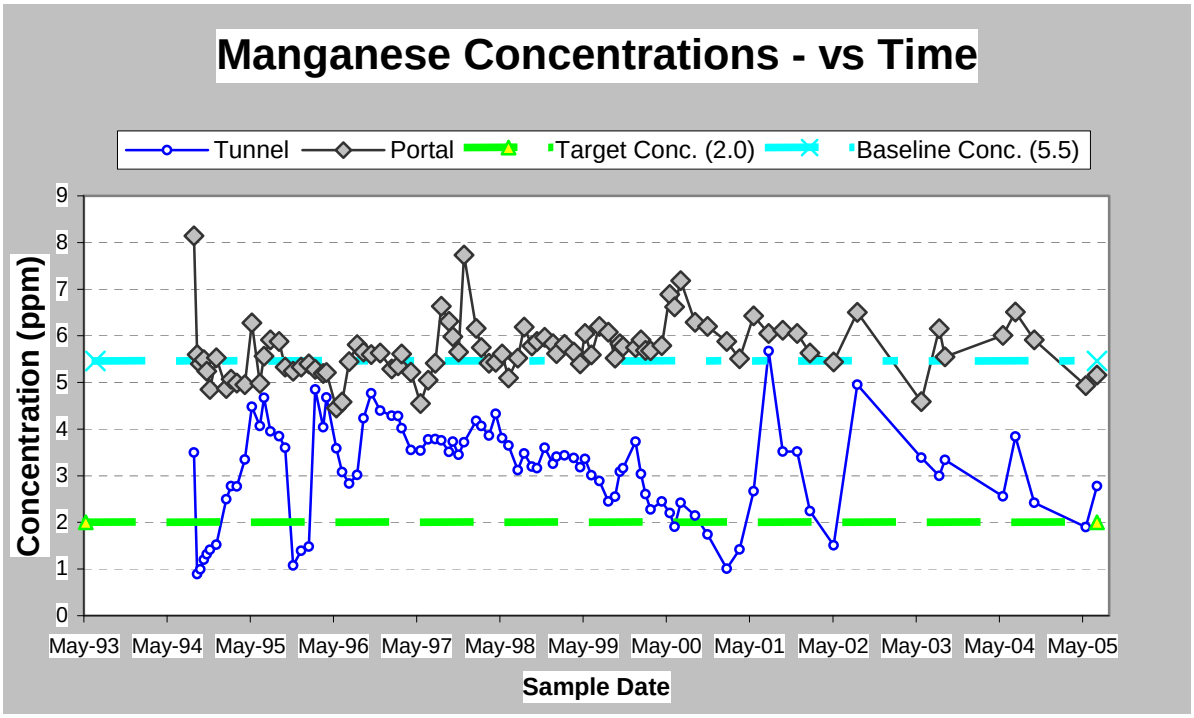

Figure 4-5. Manganese concentrations.

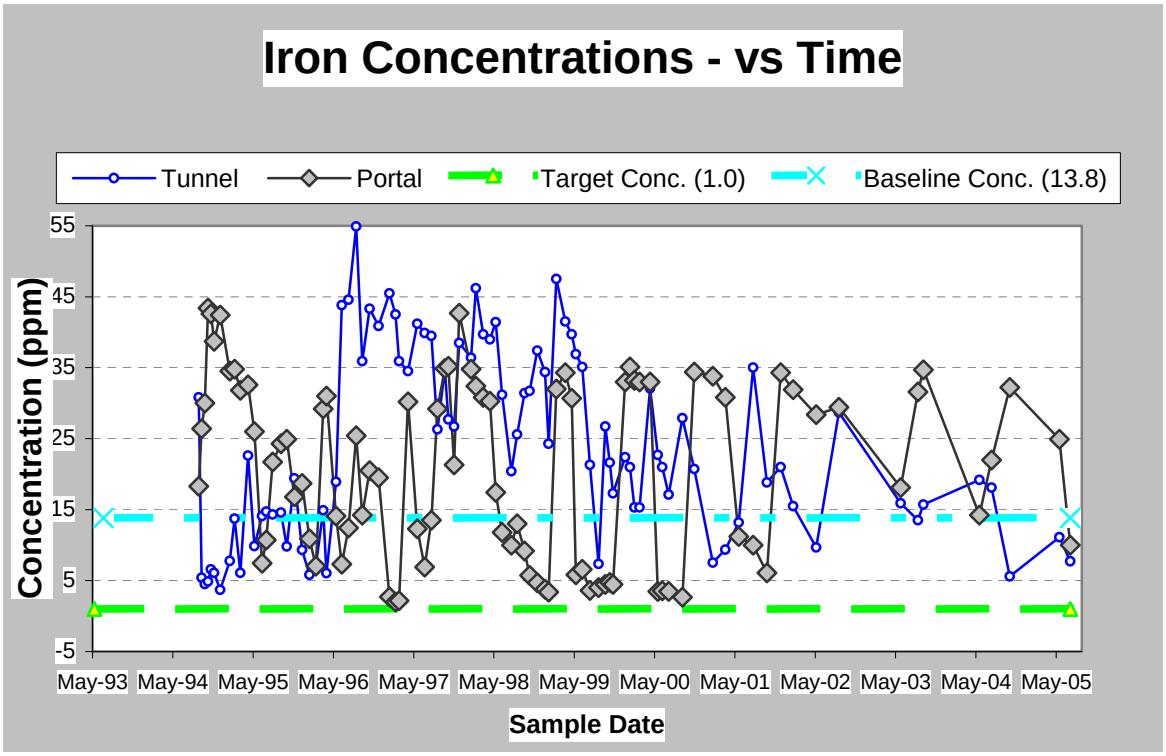

Figure 4-6. Iron concentrations.

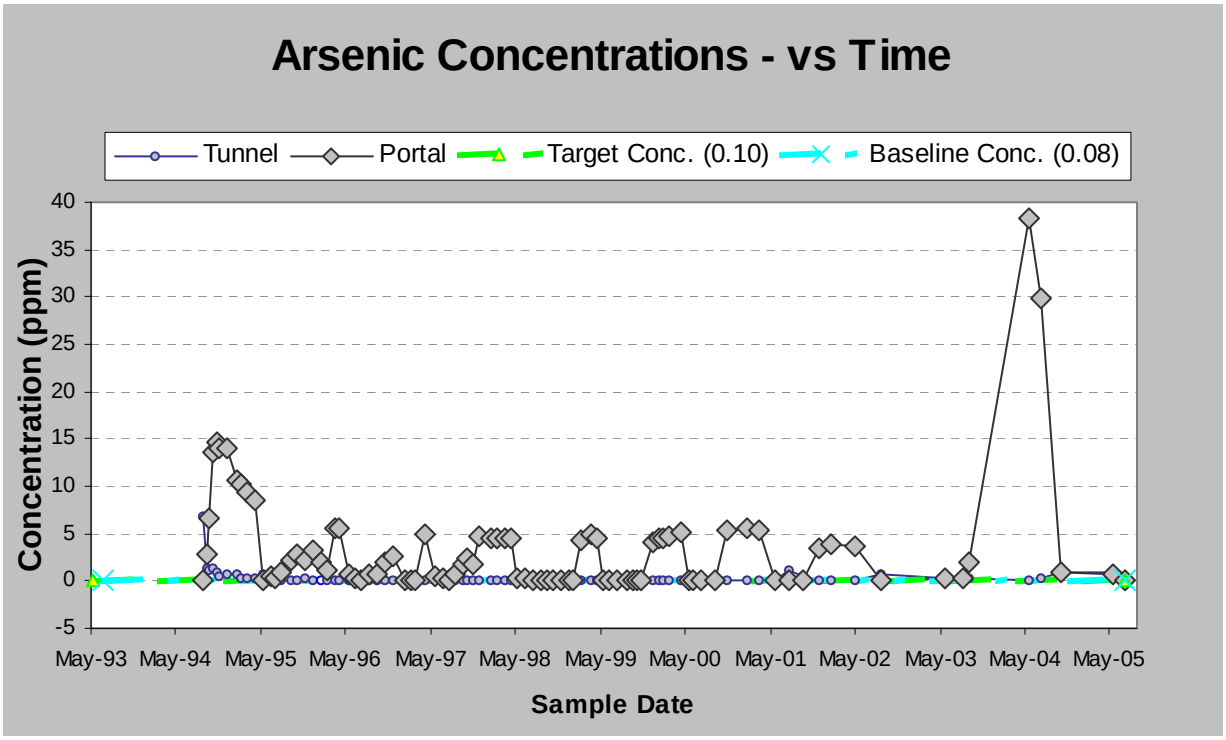

Figure 4-7. Arsenic concentrations.

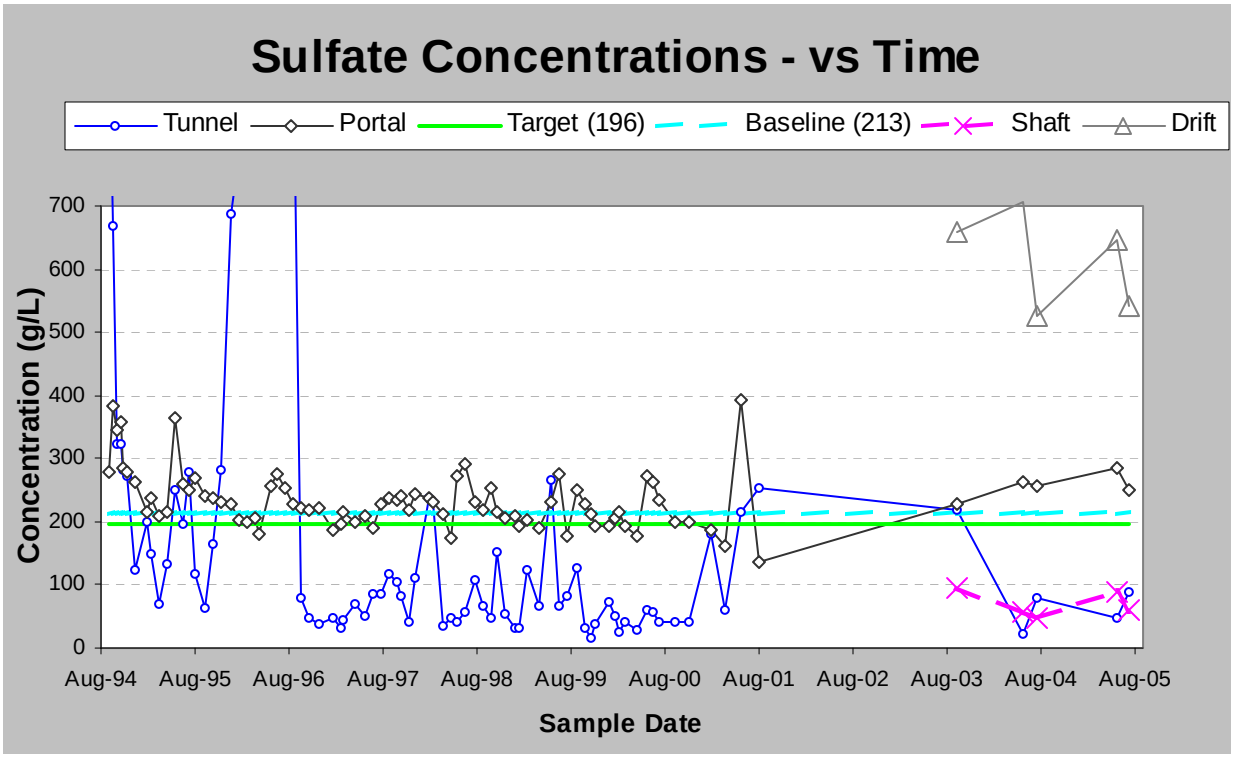

Figure 4-8. Sulfate concentrations.

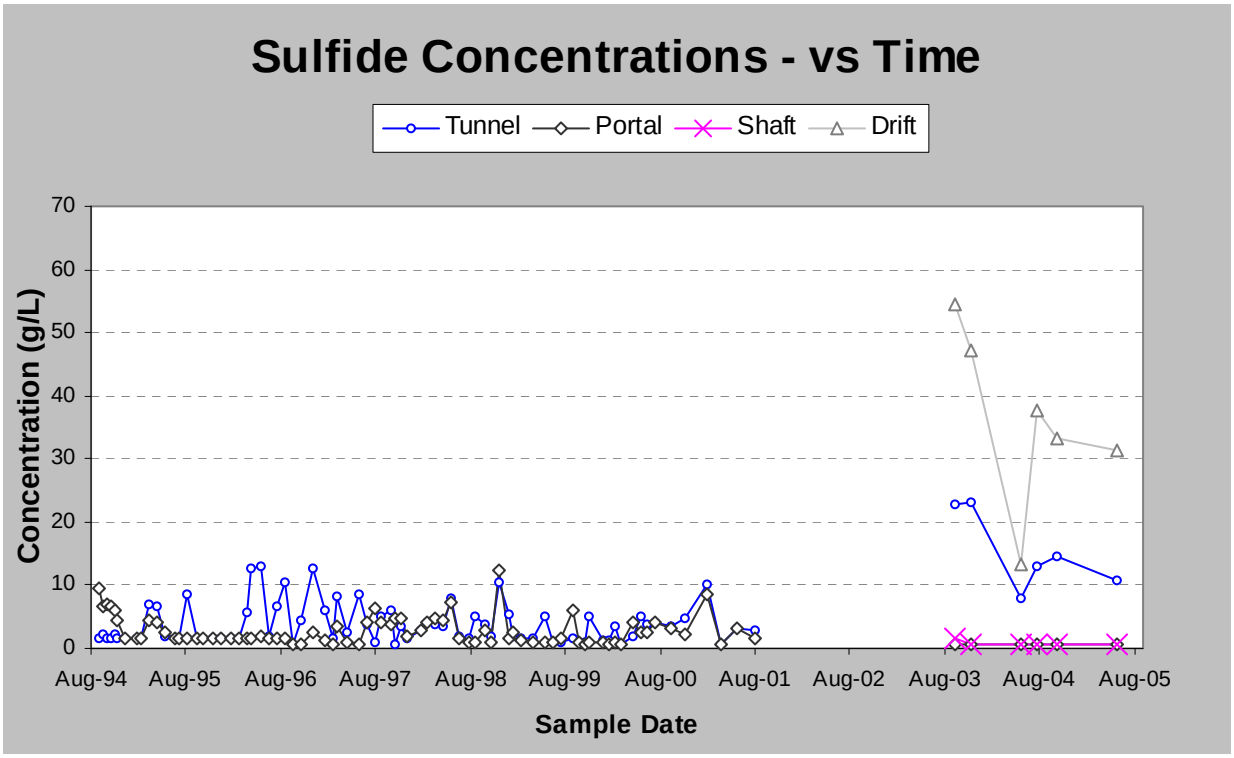

Figure 4-9. Sulfide concentrations.

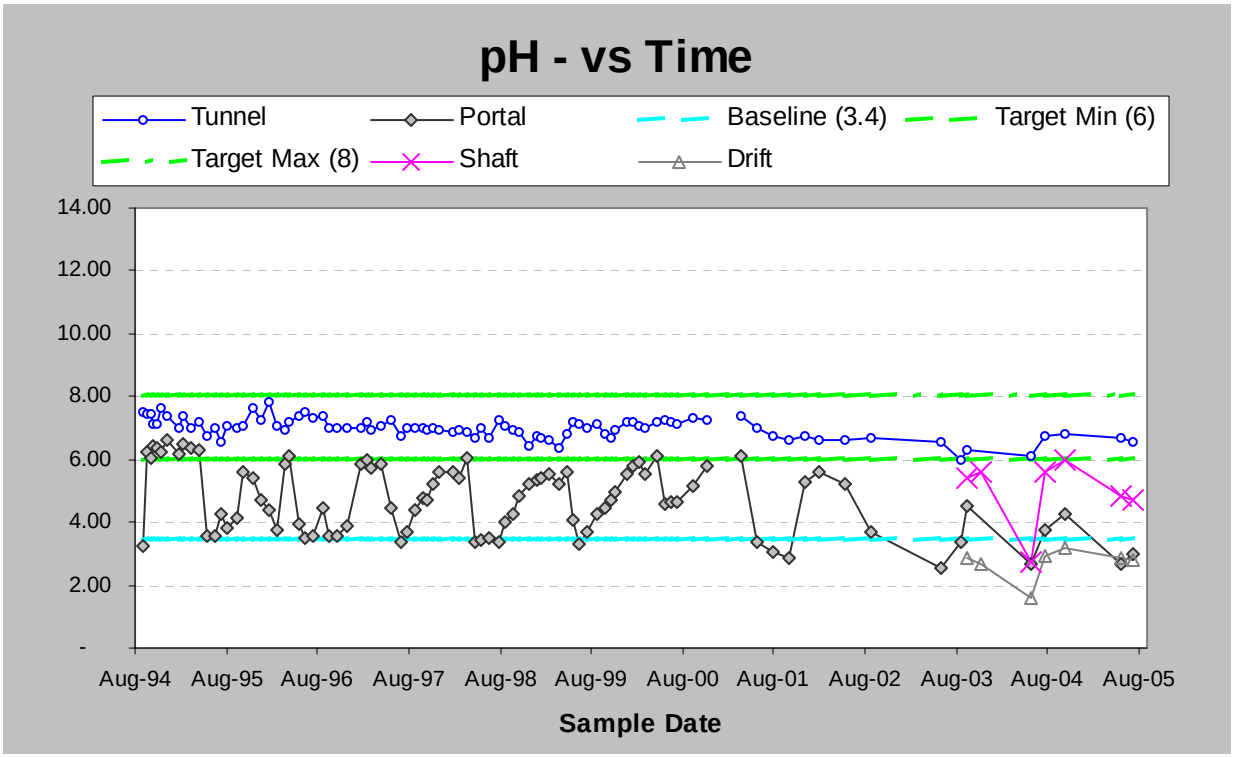

Figure 4-10. pH readings.

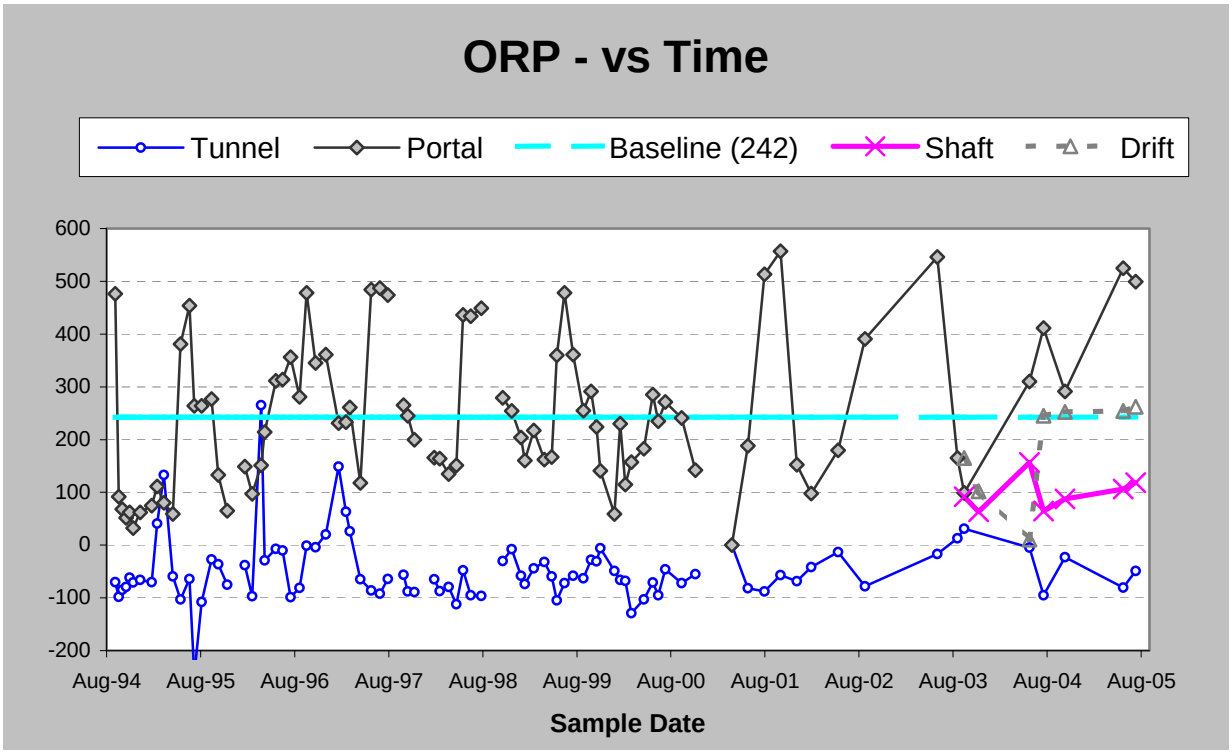

Figure 4-11. ORP readings.

Table 4-1. Initial Sampling Dates

7-Sep-93
20-May93
1-Oct 93
18-May-94
1-Jun-94
14-Jun-94
28-Jun-94
21-Jul-94
8-Aug-94
19-Aug-94
6-Sep-94
20-Sep-94
4-Oct-94
19-Oct-94
1-Nov-94
15-Nov-94

Table 4-2. Monthly Sampling Events

1994	1995	1996	1997	1998	1999	2000
- - - -	26-Jan-95	23-Jan-96	21-Jan-97	27-Jan-98	14-Jan-99	20-Jan-00
- - - -	16-Feb-95	21-Feb-96	18-Feb-97	18-Feb-98	18-Feb-99	9-Feb-00
- - - -	14-Mar-95	26-Mar-96	5-Mar-97	24-Mar-98	31-Mar-99	3-Mar-00
- - - -	18-Apr-95	9-Apr-96	15-Apr-97	23-Apr-98	29-Apr-99	20-Apr-00
- - - -	18-May-95	23-May-96	28-May-97	20-May-98	19-May-99	25-May-00
- - - -	22-Jun-95	18-Jun-96	30-Jun-97	18-Jun-98	17-Jun-99	15-Jun-00
- - - -	11-Jul-95	18-Jul-96	31-Jul-97	29-Jul-98	21-Jul-99	13-Jul-00
- - - -	8-Aug-95	22-Aug-96	27-Aug-97	25-Aug-98	30-Aug-99	- - - -
- - - -	15-Sep-95	19-Sep-96	30-Sep-97	28-Sep-98	29-Sep-99	- - - -
- - - -	12-Oct-95	23-Oct-96	16-Oct-97	21-Oct-98	20-Oct-99	- - - -
- - - -	16-Nov-95	- - - -	11-Nov-97	24-Nov-98	3-Nov-99	- - - -
13-Dec-94	20-Dec-95	2-Dec-96	4-Dec-97	30-Dec-98	28-Dec-99	- - - -

Table 4-3. Additional Sampling Events

2000	2001	2002	2003	2004	2005
14-Sep-00	31-Jan-01	31-Jan-02	4-Jun-03	27-May-04	26-May-05
7-Nov-00	28-Mar-01	15-May-02	21-Aug-03	21-Jul-04	14-Jul-05
	29-May-01	27-Aug-02	16-Sep-03	12-Oct-04	
	2-Aug-01				
	3-Oct-01				
	5-Dec-01				

Table 4-4. Representative Lilly/Orphan Boy Water Chemistry

	Fe (mg/L)	**Zn (mg/L)**	**Al (mg/L)**	**Mn (mg/L)**	**As (mg/L)**	**Cd (mg/L)**	**Cu (mg/L)**	**SO_4^{-2} (mg/L)**	**pH**
Baseline (Average from September 1993 to August 1994)	13.8	19.4	7.36	5.46	0.08	0.24	0.33	213	3.4
Tunnel – May 2002	9.7	<0.01	<0.02	1.51	0.04	<0.005	<0.002	21.0	6.6
Portal – May 2002	28.4	12.5	0.51	5.44	3.66	0.064	0.041	223	5.2

5. Economic Analysis

5.1 Evaluation Summary

As part of the project, an economic analysis was conducted by MSE in 1998. The full report was issued separately as document MWTP-128. The following information has been summarized from that report.

A cost comparison was conducted on the innovative SRB technology installed at the Lilly/Orphan Boy Mine and a baseline lime-addition technology. It was assumed that ARD would be treated for an indefinite amount of time and, therefore, the life-cycle cost analysis for each technology was completed using a 30-year period. SRB technology had higher capital and startup expenses but had lower net present value (NPV) than lime treatment. These up-front costs were diminished over the 30-year period used in calculating the NPV. In comparison, the higher operating expenses of lime addition resulted in a higher overall cost than the passive SRB technology. However, for operating periods of less than 10 years, lime treatment approached the total costs of SRB. Cost analysis was calculated for two flow rates: 3 gpm and 100 gpm. Cost results are presented in Table 5-1 and NPV results are shown in Table 5-2.

Table 5-1. Costs for 3-gpm Systems

Small Scale System: 3 gpm				
Capital/Startup Costs				
Description		**Lime Addition**		**Sulfate Reducing Bacteria**
Materials & Supplies	$	-	$	8,848
Equipment	$	12,623	$	5,805
Installation	$	15,378	$	90,002
Preliminary Laboratory Analysis	$	-	$	28,258
Ponds	$	12,687	$	-
TOTAL	**$**	**40,688**	**$**	**132,913**
Annual Operating & Maintenance Costs				
Description		**Lime Addition**		**Sulfate Reducing Bacteria**
Labor	$	31,200	$	11,760
Laboratory & Field Testing	$	22,500	$	35,275
Materials & Supplies	$	-	$	551
Maintenance/Miscellaneous	$	7,500	$	2,185
Sludge Removal	$	4,500	$	-
Consumables	$	917	$	-
TOTAL	**$**	**66,617**	**$**	**49,771**

Table 5-2. NPV of Costs for Lime Addition and SRB Technologies

NPV of Costs for 30-Year Period		
Technology	**3 gpm**	**100 gpm**
Lime Addition	$1,221,128	$1,826,382
SRB	$1,014,845	$1,527,523

6. Conclusions

The following conclusions were drawn based on the data presented.

- Metals data showed that overall metal removal was higher for aluminum, cadmium, copper, and zinc, than for arsenic, manganese, and iron.
- The data also indicated that higher metal removals were obtained within the tunnel than at the portal. This difference can be explained by either (1) historic precipitates within the tunnel acted as a new source of metals and re-contaminated the water or (2) additional sources of ARD entered the flow as it passed through the tunnel.
- The pH of the mine water increased almost immediately after implementation of the technology, which was attributed to the buffering capacity of the organic substrate.
- During spring runoff periods, pH and water quality were lower in the portal than in the tunnel, where pH remained near neutral. This was likely due to oxygenated surface water runoff penetrating through the ground above the portal, flowing into the tunnel, and then solubilizing historic metal precipitates or becoming re-contaminated as it passed through the tunnel where other ARD infiltration was present.
- The pH of the mine water increased almost immediately after technology implementation. This initial increase in pH was attributed to the buffering capacity of the organic substrate. During the first spring runoff, the pH dropped in samples collected at the mine portal, but the pH remained near neutral in the tunnel. The pH decrease at the portal was attributed to spring runoff influencing the water quality at the portal. Higher spring flow rates allowed larger volumes of water to be re-contaminated by historic precipitates within the tunnel or contaminated from additional fractures within the tunnel. Also, as the portal is closer to the surface, it is possible that more highly oxygenated surface water entered the portal outflow stream and solubilized new metal contaminants as it passed through the ground and tunnel.
- During all spring runoff time frames, metal concentrations generally rose in samples collected at the portal. However, metal concentrations remained steady in samples collected from within the tunnel during the same periods. Again, larger flows of oxygenated surface water percolating into the tunnel and flowing into the portal area likely caused the dissolution of historic precipitates and increased the flow of additional ARD sources.
- At least some of the reduction of sulfate and metals within the underground mine system was caused by the action of SRB. This was evident by measured decreases of sulfate, detection of soluble sulfide within the mine water, and other changes that are typically associated with the action of SRB.
- An increase in iron and arsenic concentrations was observed in the portal discharge water shortly after implementing the technology and regularly throughout the demonstration. This is best explained by the reduction of insoluble ferric iron to the more soluble ferrous form. Historic ferrihydrite within the system was reduced to ferrous iron when the technology induced a reducing environment within the mine water. Arsenic adsorbed to ferrihydrite was also released when the iron was mobilized, increasing its concentration in the effluent.
- BOD concentrations were elevated for six months after technology implementation and then returned to background levels. Ammonia concentrations were elevated for a longer period but returned to background concentrations after one year. This was attributed to the limited amount of oxygen available in the system for nitrification of ammonia to nitrite/nitrate. Nitrite/nitrate

subsequently underwent anaerobic denitrification.

- Although of sufficient capacity to provide significant water treatment much of the year, the system was undersized for high flow run-off conditions and was inconsistent in achieving effluent design goals.
- The upward trend in dissolved metals concentration indicates that the in situ bioreactor is nearing the end of its useful life.

7. Recommendations

The following recommendations were developed after completion of the data analysis from this field demonstration.

- Collect samples from Telegraph Creek upstream and downstream of the portal area and analyze for metals. This data will show the influence of the portal discharge on the receiving stream. In addition, the results will indicate any metal removal occurring in the natural wetlands located just downgradient of the portal. If the wetlands are effectively removing the iron and arsenic remaining in the portal discharge after treatment by the SRB technology, then an oxidizing, polishing step may not be needed.
- Continue to monitor the metals removal efficiency from the mine and within the tunnel every few years to assess the long-term impacts of the organic addition. Sampling should be done during both low and high flow rates every 2 to 3 years. Longevity of SRB-treatment technology is not known because in situ treatments are a relatively recent development. The Lilly/Orphan Boy Mine Demonstration may be the longest running in situ hard rock mine treatment in the nation using SRB technology. Longer-term data would help future projects determine the effective life for substrate and help optimize designs.
- Collect samples from within the organic substrate for molecular microbiological analysis several times in the future. The results could be compared with previous results so that microbial community changes over time could be documented. These changes would help predict microbial behavior in other SRB projects and allow optimization of this technology.
- Future projects that utilize SRB technology should incorporate an oxidation step downstream of the reduction process. This would ensure that metals that are more mobile in the reduced form (i.e., iron and manganese) are oxidized and captured.
- Future projects, that utilize passive treatments in areas that experience large seasonal flow variations, should work to incorporate run-off surge storage capacity, which would control the rate at which these waters enter the system and allow optimal retention times.
- In this study, only the effluent analysis was known. It is recommended that future in situ field technology demonstrations commit the necessary funding to allow for measurement of mine water flows through the treatment system and design, as best as possible, a method to collect feed samples throughout the demonstration. These are needed in order to fully evaluate the treatment efficiency of any system.

The implementation of an SRB technology at the Lilly/Orphan Boy Mine provided an innovative solution.

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Appendix A

Summary of Quality Assurance Activities

Summary of Quality Assurance Activities

Mine Waste Technology Program
Activity III, Project 3, Phase 2
(Field Testing of Sulfate Reducing Bacteria at the Lilly Orphan Boy Mine)

BACKGROUND

On September 6, 1994, sampling officially began for Mine Waste Technology Program (MWTP) Activity III, Project 3, Phase 2 — Field-Testing of Sulfate Reducing Bacteria (SRB) at the Lilly/Orphan Boy Mine. The objective of the project was to investigate the effectiveness of using SRBs to treat the acid mine drainage at a remote mine site and obtain a high quality effluent.

It should be noted that some of the site characterization samples discussed in this report were taken prior to September 6, 1994, and other samples were taken during sampling events that are not outlined in the project specific quality assurance project plan (QAPP); however, all of the field and laboratory data for sampling events from 8/1994 to 9/2003 has been evaluated to determine the usability of the data.

In order to determine the effectiveness of the SRB process being demonstrated, several sampling points were designated, and a variety of analyses were assigned to each point. Just as sampling activities were initiated, however, several of the sampling points were no longer viable due to pipes breaking because of freezing and duplication of results from several different sampling locations. Sampling continued at the three remaining viable points (PT3, PT6, and MW). Several analyses were performed on the collected samples either in the field at the Lilly/Orphan Boy Mine, the MSE Laboratory, HKM Laboratory, or at the State Laboratory, which performed biochemical oxygen demand (BOD) analysis until the MSE Laboratory acquired the capabilities to perform the analysis. In September 2003, two monitoring wells were installed to monitor samples of the water in the shaft and water in the drift. The influent sampling location was destroyed when the technology was deployed.

The analyses to be performed were specified in the QAPP and each analysis was classified as critical or noncritical. A critical analysis is an analysis that must be performed in order to achieve project objectives. A noncritical analysis is an analysis that is performed to provide additional information about the process being tested. Critical analyses for this project were:

- pH;
- temperature;
- flow rate;
- sulfate;
- total suspended solids (TSS);
- nitrate-nitrite as nitrogen;
- total ammonia as nitrogen;
- solid sulfide;
- BOD;
- soluble sulfide; and
- dissolved metals (Al, As, Cd, Cu, Fe, Mn, Zn).

Noncritical analyses for this project were:

- E_H;
- dissolved oxygen (DO);
- alkalinity;
- hydrogenase activity;
- chemical oxygen demand (COD);
- total nitrogen;
- total phosphorous;
- total organic carbon (TOC); and
- total recoverable metals (Al, As, Cd, Cu, Fe, Mn, Zn, Pb and Hg).

The QC objectives for each critical analysis are outlined in the QAPP and were compatible with project objectives and the methods of determination being used. The QC objectives are method detection limits (MDLs), accuracy, precision, and completeness. Control limits for each of these objectives are established for each critical analysis. For noncritical analyses, QC objectives are determined by using standard guidelines that exist, or by applying reasonable control limits in order to determine the usability of the data that was generated in the field or in the laboratory.

VALIDATION PROCEDURES

Data that was generated for all critical and noncritical analyses was validated. The purpose of data validation is to determine the usability of all data that was generated during a project. Data validation consists of two separate evaluations: an analytical evaluation and a program evaluation. An analytical evaluation is performed to determine that:

- all analyses were performed within specified holding times;
- calibration procedures were followed correctly by field and laboratory personnel;
- laboratory analytical blanks contain no significant contamination;
- all necessary independent check standards were prepared and analyzed at the proper frequency and all remained within control limits;
- duplicate sample analysis was performed at the proper frequency and all relative percent differences (RPDs) were within specified control limits;
- matrix spike sample analysis was performed at the proper frequency and all spike recoveries (%R) were within specified control limits; and
- the data in the report submitted by the laboratory to project personnel can be verified from the raw data generated by the laboratory.

Measurements that fall outside of the control limits specified in the QAPP, or for other reasons are judged to be outlier are flagged appropriately to indicate that the data is judged to be estimated or unusable. All QC outliers for the sampling events covered by this report are summarized in Table A-1. In addition to the analytical evaluation, a program evaluation was performed.

Program evaluations include an examination of data generated during the project to determine that:

- all information contained in chain-of-custodies (COCs) is consistent with the sample information in field logs, laboratory raw data, and laboratory reports;

- all samples, including field QC samples were collected, sent to the appropriate laboratory for analysis, and were analyzed and reported by the laboratory for the appropriate analyses;
- all field blanks contain no significant contamination; and
- all field duplicate samples demonstrate precision of field as well as laboratory procedures by remaining within control limits established for RPDs.

Program data that is inconsistent or incomplete and does not meet the QC objectives outlined in the QAPP are viewed as program outliers and are flagged appropriately to indicate the usability of the data. Both the analytical and program evaluations consisted of evaluating the data generated in the field as well as in the laboratory.

ANALYTICAL EVALUATION

Several analytical evaluations of field and laboratory data were performed over the life of this multi-year project.

Field Logbook Evaluation

Field data validation included an examination of the field logbook that was created for this project. The field logbook typically contains all of the information that is available regarding:

- sampling information/conditions; and
- sample treatment/preservation.

Sampling Information/Conditions

Sampling conditions and information such as weather conditions, date of sampling, and time of sampling should be specified in the field logbook for each sampling event. Information should also be provided to specifically identify why a sample could not be collected. An examination of the logbook for this project found that on several occasions information was lacking in some of these areas, particularly with respect to weather information and the time of day the samples were collected. Sampling personnel should also document any additional information about unique conditions that could impact the project data. Information should be complete for each sampling event even if some information must be repeated from previous sampling events. Providing excessive information is better than providing too little information.

Sample Preservation/Treatment

All of the preservatives required for each analysis are clearly listed in the field logbook; therefore, it was assumed that all of the samples were properly treated/preserved prior to delivery to the appropriate laboratory.

Field Data Validation

Field data validation was performed to determine the usability of the data that was generated during field activities. The usability was determined by verifying that correct calibration procedures of field instruments were followed. In addition, the QC parameters of precision and accuracy calculated in the field were compared to those specified in the QAPP. Any data that falls outside of the control limits must be considered outlier and flagged appropriately. The analyses performed in the field were:

- pH (critical);
- temperature (critical);
- flow rate (critical);
- E_H (noncritical); and
- DO (noncritical).

pH

The pH meter was to be calibrated using two known buffer solutions that would bracket the measured pH. To determine the accuracy of the pH meter, a third known buffer in the calibration range was to be measured twice. Accuracy was defined as the absolute difference between the accepted value of the third known buffer solution and the measured value of the third known buffer solution. Precision was defined in the QAPP as the absolute difference between the two measured values of the third known buffer solution. The QC control limits established for pH measurements for both precision and accuracy were 0.1 pH unit.

For each sampling event, calibration of the pH meter was performed correctly. Although sampling personnel either did not calculate the QC control limits or calculated them incorrectly for pH analysis, enough information was available to determine that the pH measurements were within control limits, with one exception. For the first sampling event, no duplicate measurement was taken making it impossible to determine the precision of the measurements; therefore, pH data from this event should be flagged "J" as estimated.

Temperature

The pH meter was also used to determine temperature using the thermistor contained in the pH probe. The thermistor was calibrated against a thermocouple, which was standardized by a National Bureau of Standards (NBS) thermometer. Because of the cost of replacement and the increased likelihood of damage to the NBS thermometer in the field, calibration was performed in MSE's uptown office, prior to departing for the Lilly/Orphan Boy Mine. The thermocouple was standardized against the NBS thermometer at room temperature or roughly 20 °C. The typical temperature measurements in the field varied greatly, but were generally much lower than 20 °C. A suggestion was made to sampling personnel that the thermocouple be standardized against the NBS thermometer at a temperature closer to the temperature that will be measured in the field.

For all sampling events, however, calibration procedures were in compliance with the QAPP, and enough information was provided to determine that all temperature data was within the control limits of 1.0 °C for precision and accuracy as specified in the QAPP, with two exceptions. For the first sampling event (Day 1--09/06/94) no temperature calibration information was provided; therefore, the temperature data generated for the first sampling event should be flagged "R" as unusable. For the second sampling event (Day 8--09/13/94), the precision of the measurements could not be calculated because no duplicate measurement was recorded; therefore, the temperature data generated on 09/13/94 should be flagged "J" as estimated.

Flow Rate

Flow rate was measured by diverting the flow from a weir to a 1-liter or 500-mL graduated cylinder and noting the amount of time it took to reach a certain volume. Flow rates were then calculated by dividing

the volume collected by the amount of time required to reach that volume. The measurement was duplicated to determine the precision of the measurement. The QC control limit established for precision was a RPD between duplicate measurements.

All flow rate data is considered useable with the exception of the first sampling event (Day 1--09/06/94). A duplicate measurement of flow rate was not taken; therefore there is no information about the precision or accuracy of the measurements. Flow rate data from 09/06/94 should be flagged "J" or estimated. Several flow rate measurements throughout the project could not be taken due to the freezing of the water at the mine during the winter months.

E_H

Because E_H was not identified as a critical parameter in the QAPP, there were no specific QC objectives assigned to this analysis; however, the data generated was still examined to determine if the instrument was properly calibrated. The calibration information for E_H was documented in the field logbook for each sampling event, with the exception of the first sampling event (Day 1--09/06/94). Because no calibration information was provided, the E_H data should be flagged "J" or estimated for Day 1.

Dissolved Oxygen

Because DO was not identified as a critical parameter in the QAPP, there were no specific QC objectives assigned to this analysis; however, the DO data that was generated was still examined to determine if the instrument had been properly calibrated. The calibration information was lacking for the majority of the sampling events for DO analysis. Generally, the DO meter is calibrated with sodium sulfite solution, which has a DO of 0%, and then calibrated in air to achieve a DO of 100%; adjustments are then made to account for barometric pressure and salinity. All of this information should have been provided in the logbook. During the Day 52 sampling event, the DO measurement was not performed because of a dead battery; however, the battery probably should have been replaced by this time because on Day 37 and Day 45, sampling personnel noted that the DO meter had a low battery. Because of the lack of calibration information provided, all DO data should be flagged "J" or estimated.

Problems encountered with field measurements were caught early in the project. New data sheets were generated to facilitate collection of all data as well as documentation of calibration. Field data from 1995-2003 had very few problems.

Laboratory Data Validation

Laboratory data validation was performed to determine the usability of the data that was generated at the laboratories analyzing samples for the project. The bulk of the analyses were performed at MSE Laboratory. BOD analysis was performed at the State Laboratory in Helena during the first 8 sampling events until the MSE Laboratory was capable of performing the analysis.

The analyses performed in the laboratory were:

- sulfate (critical);
- TSS (critical);
- nitrate-nitrite as nitrogen (critical);
- total ammonia as nitrogen (critical);

- solid sulfide (critical);
- BOD (critical);
- soluble sulfide (critical);
- alkalinity (noncritical);
- hydrogenase activity (noncritical);
- COD (noncritical);
- total nitrogen (noncritical);
- total phosphorous (noncritical);
- TOC (noncritical); and
- dissolved metals (critical) and total recoverable metals (noncritical).

Laboratory data validation was performed using *Laboratory Data Validation: Functional Guidelines for Evaluating Inorganics Analyses* (USEPA, 1988) as a guide where applicable to each individual analysis. For critical analyses, the QC criteria outlined in the QAPP was also used to identify outlier data and to determine the usability of the data for each analysis. When data validation was initiated, the MSE Laboratory was not sending sufficient information to perform a complete and thorough data validation on the non-metal, wet chemistry analyses. The QA/QC summaries that were submitted with the reports were lacking information about calibration blanks, and the raw data was not submitted, making sample result verification impossible. An informational request was made to the laboratory, and laboratory personnel quickly responded by submitting all of the requested information. Once the information was received, data validation of all wet chemistry analyses was completed. Flagged data is summarized in Table A-1.

Metals Analysis

Dissolved metals analysis was classified as critical in the QAPP, while total metals analysis was classified as noncritical; however, all metals analyses were evaluated using the QC criteria specified in the QAPP for dissolved metals and the *Functional Guidelines for Evaluating Inorganics Analyses* (USEPA, 1988). All metals results are considered usable; however, some samples had to be flagged "J" as estimated for certain analytes. Refer to Table A-1 for a summary of metals data requiring qualification. The amount of qualified data has decreased as the HKM Laboratory became more proficient with the matrix of samples from this project, which was complicated by the addition of organic matter. A sample preparation modification to add hydrogen peroxide to the samples removed the previous interferences caused by the organic matter. For years 1999 through 2003, no metals data, with the exception of a field duplicate in August 2003, required qualification. It should also be noted that, as the project progressed, less samples were collected.

Once the analytical portion of the evaluation was completed, the program evaluation was initiated.

PROGRAM EVALUATION

The program evaluation focused on:

- COC procedures;
- sampling and data completeness;
- field blanks; and
- field duplicates.

Chain-of-Custody Procedures

All information provided in the COC forms for this project was complete and accurate with one notable exception. On the COC from the 09/20/94 sampling event, the field QC sample numbers are transposed. The field blank whose sample ID was 9 was listed with the laboratory ID of W183; however, the sample bottle was labeled W184. As a result, the data for the field blank was reported under the field ID of the field duplicate and vice versa. The samples were checked at the laboratory, and this scenario was verified; therefore, all data was considered usable. A minor finding was that for two sampling events, 10/04/94 and 11/15/94, laboratory personnel signed the COCs as received by the laboratory in the wrong location.

Sampling and Data Completeness

All samples that were scheduled to be collected, were collected when possible. Flow rate measurements could not be taken on several occasions due to pipes freezing at the mine during winter months. A DO measurement was not taken on 10/25/94 because the meter had a dead battery. A solid sulfide sample was not collected during the 11/15/94 sampling event as scheduled due to breaking cables that ran from the surface to the sampling containers immersed in the substrate in the shaft. All samples collected were analyzed and reported for the appropriate parameters in the field or at the laboratory.

Field QC Samples

When data validation was initiated, it became apparent that the number of field QC samples being collected was not sufficient when compared with the field QC sampling scheme outlined in the QAPP. Corrective action was implemented so that additional field QC samples would be taken during the remaining sampling events and the number of field blanks and duplicates would be in compliance with the QAPP. Field blanks and duplicates requiring qualification are summarized in Table A-1 with other flagged data.

Project Reviews

Several project reviews were performed as noted below.

- EPA Field Technical Systems Review—November/December 1994;
- MSE Technical Systems Review at Lilly/Orphan Boy Mine—November 1995;
- MSE Technical Systems Review at Lilly/Orphan Boy Mine—August 2001; and
- MSE Technical Systems Review at Lilly/Orphan Boy Mine—August 2002.

EPA Field Technical Systems Review

From November 29, 1994 to December 1, 1994, EPA conducted a technical systems review (TSR) of field activities for several MWTP projects including Activity III, Project 3. Six concerns listed below were identified during the TSR.

- Field personnel did not calculate QC results;
- The QAPP might need updating;
- There were no BOD QC results;

- The mine drainage was analyzed for lead and mercury, but those parameters were not listed in the QAPP;
- The arsenic objective as stated in the QAPP might not be achievable; and
- Corrective actions needed to be documented.

All of the concerns identified were addressed after the TSR.

MSE Technical Systems Review at Lilly/Orphan Boy Mine – November 1995

An audit of field procedures was performed at the Lilly Orphan Boy Mine in November of 1995. The purpose of the audit was to ensure all corrective actions from the previous year's external audit had been performed and that sampling procedures outlined in the project specific QAPP were being implemented. There were no findings identified during the audit.

MSE Technical Systems Review at Lilly/Orphan Boy Mine – August 2001

An audit of field procedures was performed at the Lilly Orphan Boy Mine on August 2, 2001. The audit focused on procedures outlined in the project-specific QAPP. Six findings were identified:

- The QAPP was deficient because it did not adequately describe sampling locations.
- Sample container was not cleaned in between sample locations. Also, a sample container cap was dropped on the ground and used in this condition rather than replacing or cleaning the cap.
- The meter used to obtain temperature data was not calibrated as outlined in the QAPP.
- Minimum entries required by the MSE SOP for logbooks were not made.
- The flow rate measurement used different equipment than was outlined in the QAPP.
- Groundwater samples were obtained using procedures that may have contaminated the samples (e.g., the retrieving string and bailer were placed on the ground).

MSE Technical Systems Review at Lilly/Orphan Boy Mine – August 2002

An audit of the sampling events at the Lilly Orphan Boy mine was conducted on 8/27/02. The purpose of the audit was to ensure that specified corrective actions resulting from audit findings from a previous audit conducted in August 2001, had been implemented. The audit focus was on the following areas.

- Adequacy of operator training;
- Presence of adequate sample containers;
- Zero head space in sample containers;
- Flow measurement accuracy; and
- Logbook maintenance.

Sampling personnel were John Trudnowski and Travis Hendrickson. Sampling began at 9:30 am. Samples were obtained at three locations: PT6, MW, and PT3. The sampling went smoothly. Decontamination procedures were observed and the sampling personnel appeared to be well trained and experienced. Zero headspace was maintained in the samples. The pH of the samples was taken in order to ensure the adequacy of the preservative. Calibration of field equipment fell within specified limits. Flow measurements at PT3 were not taken due to deterioration of the weir. Sampling was concluded at 1:00 pm.

The only finding was that the logbook and entry data specified in SOP G4 of the QAPP was not followed. Sampling personnel pointed out that to take the master log in the field was not practical, particularly during the winter when snowmobiles must be used get to the site. Therefore, a smaller ring-bound logbook was used. The data was transferred to the master log when sampling personnel returned from the field.

It was recommended that the QAPP be amended by removing SOP G4 and inserting the current logbook practices in Section 4, Site Selection and Sampling Procedures.

Table A-1. Summary Qualified Data for MWTP Activity III, Project 3, Phase 2.

Date[1]	Sample ID or Batch #[2]	Analysis	QC Criteria	Control Limit	Result	Flag[3]	Comment
All events from 8/94 through 12/94	PT3	DO	Calibration	Sodium Sulfite, Air	No calibration documented	J	Flag all DO measurements "J".
08/19/94	PT3	NO_3/NO_2	Accuracy	75-125 %R	54%	UJ	Flag the associated sample "UJ".
08/19/94	C1567	TR Mets-Pb	Accuracy	75-125 %R	132.2	J	Flag all **detectable** samples "J".
08/30/94	PT3	NO_3/NO_2	Accuracy	75-125 %R	48%	UJ	Flag the associated sample "UJ"
09/06/94	PT3	Flow Rate	Precision	≤5% RPD	No duplicate measurement taken	J	Flag the associated sample "J".
09/06/94	PT3,6	E_H	Calibration	Zoebell's solution	No calibration documented	J	Flag the associated sample "J".
09/06/94	Pt1,3& 6	pH	Precision	≤0.1 pH unit	No duplicate measurement taken	J	Flag all associated samples "J".
09/06/94	PT3,6	Temp	Calibration	NBS & Thermocouple	No calibration documented	R	Flag all associated samples "R".
09/06/94	C1585	Diss-As	Accuracy	75-125 %R	72.1	J	Flag all associated samples "J".
09/13/94	PT3,6	Temp	Precision	≤1.0 °C	No duplicate measurement taken	J	Flag all associated samples "J".
09/13/94 09/20/94	C1625 C1625	TR-As Fe Hg	Precision	≤20% RPD	37.6% 25.7% 32.7%	J	Flag all associated samples "J".
09/13/94 09/20/94	C1625 C1625	TR-Al	Accuracy	75-125 %R	39.2	J	Flag all associated samples "J".
09/20/94	C1670	TR Mets (Al, As, Cu, Fe, Pb, Hg)	Field Duplicate	≤20% RPD	Significantly higher (31.3% to 93.3%)	J	Flag the associated samples "J".

Date[1]	Sample ID or Batch #[2]	Analysis	QC Criteria	Control Limit	Result	Flag[3]	Comment
10/04/94 10/11/94 10/18/94 10/25/94	PT3	NO_3/NO_2	Accuracy	75-125 %R	59%	UJ	Flag all associated samples "UJ"
11/01/94 11/08/94	PT3 PT3	NO_3/NO_2	Accuracy	75-125 %R	68%	UJ	Flag the associated samples "UJ".
11/08/94	C1678	TR-Cu	Precision	≤20% RPD	124.9	J	Flag all associated samples "J".
11/22/94	PT3	NO_3/NO_2	Accuracy	75-125 %R	73%	J	Flag the associated sample "J".
12/20/94	PT3	NO_3/NO_2	Accuracy	75-125 %R	66%	UJ	Flag the associated sample "J".
2/7/95	C1740	TR-Fe Mn	Serial Dilution	10% difference	12.6% 11.5%	J	Flag all associated samples "J".
8/8/95	C1886	TR-Cd	Serial Dilution	10% difference	>10% difference	J	Flag all associated samples "J".
12/20/95	C2127	TR-Al	Matrix Spike	75-125% recovery	>10% difference	J	Flag all associated samples "J".
3/26/96	C2245	Diss-CD	Precision	≤CRDL (5ppb)	5.53 ppb	J	Flag all associated samples "J".
3/26/96	C2251	TR-Al As	Field Duplicate	≤35% RPD	41.8% 37.4%	J	Flag all associated samples "J".
4/9/96	C2252	Diss-Cd	Precision	≤CRDL (5ppb)	5.18 ppb	J	Flag all associated samples "J".
5/23/96	C2301	TR-Al As Cu Pb Zn	Field Duplicate	≤35% RPD	71.3% 60.3% 63.4% 81.4% 58.2%	J	Flag all associated samples "J".

Date[1]	Sample ID or Batch #[2]	Analysis	QC Criteria	Control Limit	Result	Flag[3]	Comment
6/18/96	C2358	TR-Zn	Field Blank	≤CRDL (20ppb)	41.8	U	Flag all associated less than 418 ppb "U".
6/18/96	C2358	TR-Al Cu	Field Duplicate	≤35% RPD	92.8% 76.2%	J	Flag all associated samples "J".
6/18/96	C2358	TR-Hg	Field Blank	≤CRDL (0.20 ppb)	0.26	U	Flag all associated less than 2.6 ppb "U".
7/18/96	C2385	Diss-Zn	Field Blank	≤CRDL (20ppb)	21.5 ppb	J	Flag all associated samples "J".
8/22/96	C2465	TR-Al As Cd Cu Pb Zn	Field Duplicate	≤35% RPD	105.4 57.3 41.5 86.2 86.6 70.7	J	Flag all associated samples "J".
9/19/96	PT3 PT6 MW	Sulfide	Matrix Spike	75-125% recovery	130.6	J	Flag all associated samples "J".
9/19/96	PT3 PT6 MW	Diss-Al Pb	Field Duplicate	≤35% RPD	44.3 52.8	J	Flag all associated samples "J".
10/23/96	PT3 PT6 MW	TR-Al Zn	Field Duplicate	≤35% RPD	62.3 35.7	J	Flag all associated samples "J".
10/23/96	PT3 PT6 MW	Sulfate	Field Duplicate	10 ppb absolute difference	13 ppb absolute difference	J	Flag all associated samples "J".

Date[1]	Sample ID or Batch #[2]	Analysis	QC Criteria	Control Limit	Result	Flag[3]	Comment
1/21/97	C2662	TR-Al As Cd Cu Fe Pb Hg Zn	Field Duplicate	≤35% RPD	95.5 71.1 67.4 74.7 29.8 68.0 89.7 64.1	J	Flag all associated samples "J".
2/18/97	C2681	TR-Al As Cu Zn	Field Duplicate	≤35% RPD	57.5 40.3 43.0 28.3	J	Flag all associated samples "J".
2/18/97	PT3	BOD	Test Duration	5 days	6 days	J	Flag all associated samples "J".
3/5/97	PT3 PT6 MW	Sulfide	CCV	90-110% recovery	117.6 113.1 117.9	J	Flag all associated samples "J".
5/29/97	C2787	TR-Al Pb	Field Duplicate	≤35% RPD	41.8 54.4	J	Flag all associated samples "J".
5/29/97	PT3 PT6 MW	Sulfide	Field Duplicate	≤35% RPD	77.7%	J	Flag all associated samples "J".
7/31/97	C2900	TR-Al As Cu Pb Zn	Field Duplicate	≤35% RPD	70.4 38.4 67.1 83.2 51.4	J	Flag all associated samples "J".
8/1/97	PT3	BOD	Blank	Undetectable BOD	Result showed BOD	J	Flag all associated samples "J".

Date[1]	Sample ID or Batch #[2]	Analysis	QC Criteria	Control Limit	Result	Flag[3]	Comment
10/16/97	PT3 PT6 MW	Sulfide	Calibration Verification	90-110% recovery	114.3%	J	Flag all associated samples "J".
10/16/97	PT6	Diss-Zn	Field Blank	≤20 ppb	34.6 ppb	J	Flag all associated samples "J".
12/4/97	PT3 PT6 MW	Diss-Cu Fe Mn	Matrix Spike	75-125% recovery	126.7% 141.1% 139.8%	J	Flag all associated samples "J".
12/4/97	PT3 PT6 MW	Diss-Hg	Field Blank	≤CRDL (0.2 ppb)	0.22 ppb	U	Flag all samples less than 2.2 ppb "U".
5/20/98	PT3 PT6 MW	Sulfide	Duplicate	≤20% RPD	34.5%	J	Flag all associated samples "J".
5/20/98	PT3 MW	Diss-Al Cu Zn	Matrix Spike	75-125% recovery	149.1 129.2 129.5	J	Flag all associated samples "J".
6/19/98	PT6	Diss-Zn	Field Blank	≤20 ppb	36.3 ppb	U	Flag all samples less than 363 ppb "U".
8/26/98	PT3 PT6 MW	Sulfide	Duplicate	≤20% RPD	26.3	J	Flag all associated samples "J".
8/21/03	PT3 MW PT6	Diss As	Field Duplicate	≤35% RPD	56% RPD	J	Flag all samples “J” for arsenic analysis
5/24/04	PT6	Diss/Tot Ca, Mg, and S	Analytical Anomaly	Diss<Tot	Diss>Tot	J	Flag all associated samples "J".

Date[1]	Sample ID or Batch #[2]	Analysis	QC Criteria	Control Limit	Result	Flag[3]	Comment
5/24/04	PT6	Nitrate/ Nitrite	Negative Matrix Interference	No interference	Noticeable interference	J	Flag all associated samples "J".
7/21/04	PT3 MW PT6	Ammonia	Matrix Spike	75-125% recovery	53% recovery	J	Flag all associated samples "J".
10/12/04	Shaft MW	Diss/Tot As and S	Analytical Anomaly	Diss<Tot	Diss>Tot	J	Flag all associated samples "J".

[1] Date that the samples were collected.
[2] MSE Laboratory Batch numbers are listed for the metals analyses early in the project, while Sample IDs are listed for all other analyses.
[3] Data Qualifier Definitions:

U – The material was analyzed for, but was not detected above the level of the associated value (quantitation or detection limit).
J – The sample results are estimated.
R – The sample results are unusable.
UJ – The material was analyzed for, but was not detected, and the associated value is estimated.

SUMMARY

While the majority of the findings of the analytical and program evaluations were minor and were addressed, several lessons can be learned so that mistakes will not be repeated during future projects. The following recommendations are suggested in order to improve future project and MWTP QA/QC.

Laboratory QA/QC:

- QA/QC summaries and raw data were submitted by MSE Laboratory; however, when another laboratory will be performing project analyses, a QA/QC summary and raw data must be requested, particularly if the analysis is considered critical.

Field QA/QC:

- Creating a standardized format for sampling logbooks may help sampling personnel better understand exactly what information they are required to provide to remain in compliance with the QAPP. For example, designating areas for sampling conditions, QC calculations, and calibration information would assist sampling personnel in providing all of the required information. Each logbook should be customized for a particular project by including the appropriate sampling frequency, analyses, and QC requirements outlined in the project specific QAPP.
- Assigning a unique field ID to each sample collected during the project would also facilitate in distinguishing one sample taken at PT3 from a sample taken a week later at PT3 for reporting and validation purposes. Perhaps the date sampled could be included in the field ID, such as, PT3-10/04/94.

Project QA/QC:

- The objective for arsenic in the effluent was stated as <0.03 parts per million (ppm) in the QAPP. The objective cannot be achieved when the instrument detection limit is 0.0336 ppm. Typically, the detection limit should be at least a factor of 5 below the objective to ensure that conclusions made about achieving the objective can be drawn with confidence. Had As been analyzed by Furnace AA (IDL=0.001), this problem could have been avoided.

There was a great volume of data generated for this multi-year project, and while some of the data was considered estimated for various reasons, the fact that the majority of the data was usable, with the exception of one temperature measurement, underlines the fact that quality data has been generated for MWTP projects. Project 3 provided a unique opportunity to apply the MWTP quality system to a long-term project. Because the project evolved over time, the QA requirements also evolved. By the end of the project, several QA/QC challenges were addressed.

Appendix B

Statistical Analysis

STATISTICAL SUPPORT FOR RESEARCH ACTIVITIES

GENERAL INFORMATION

QA ID No.:	**N/A**	Project QA Category:	**N/A**
EPA Technical Lead Person (TLP):		**Diana Bless**	
Title:		**Data Analysis Guidance for MWTP, Activity III, Project 3: In Situ Source Control of Acid Generation Using SRB**	
Support Provided by:		**Neptune & Co.**	
Contract No.	**68-C-03-032**	Date Submitted:	**09/28/04**

REVIEW SUMMARY

Review Distribution Date	01/04/05	Endorsement Status	**N/A**
NRMRL-STD QA Manager	Lauren Drees	No. of Findings	**N/A**
Telephone No.	569-7087	No. of Observations	**N/A**

The data provided to EPA were analyzed by a statistician with respect to the project objectives. Since the associated QAPP was not specific regarding data analysis procedures, one recommended approach is provided in the attachment.

If you have any questions or need additional information, please contact the STD QA Manager.

cc: Helen Joyce
Suzzann Nordwick
Michelle Lee

Data Handling

All data were used as reported regardless of the qualifier. Each metal had a total of 89 observations from both locations (Point 3 and 6) with the exception of As which had only 88 observations from Point 6. All data were used in the analyses even though the data from the last two sampling events for As at Point 3 and the first sampling event for Al at Point 6 appear to be outlying values. Since these three concentrations fall outside the main body of the data (see Figures B-1 and B-3), it is recommended they be checked for assignable causes.

Data Analysis

Table B-1 presents descriptive statistics for the data provided. Figures B-1 through B-7 display concentrations for each of the seven metals over time.

The results for Al, Cd, Cu, and Zn followed similar patterns. At Point 3, the pattern was cyclical with the highest concentrations occurring in the summer months and the lowest in the winter months (see Figures B-1, B-2, B-5 and B-7). At Point 6, the concentrations were independent of the change in season and remained relatively constant throughout the duration of the investigation. The percentage of the 89 observations that were below the target effluent levels varied for the four metals. At Point 3, 0% of Zn, 44% of Al, 51% of Cd, and 68% of Cu observations were below their target effluent levels. At Point 6, 98% of Al, 99% of Cu, and 100% of both Cd and Zn observations were below their target effluent levels.

With the exception of the last two sampling events in 2004, As displayed a cyclical pattern at Point 3 that was opposite from the pattern displayed by Al, Cd, Cu and Zn. The highest concentrations of As occurred in the winter months and the lowest in the summer months (see Figure B-3). At Point 6, the concentrations were independent of the change in season and remained relatively constant throughout the investigation. At Point 3, 27% of the observations were below the target effluent level; this increased to 43% at Point 6.

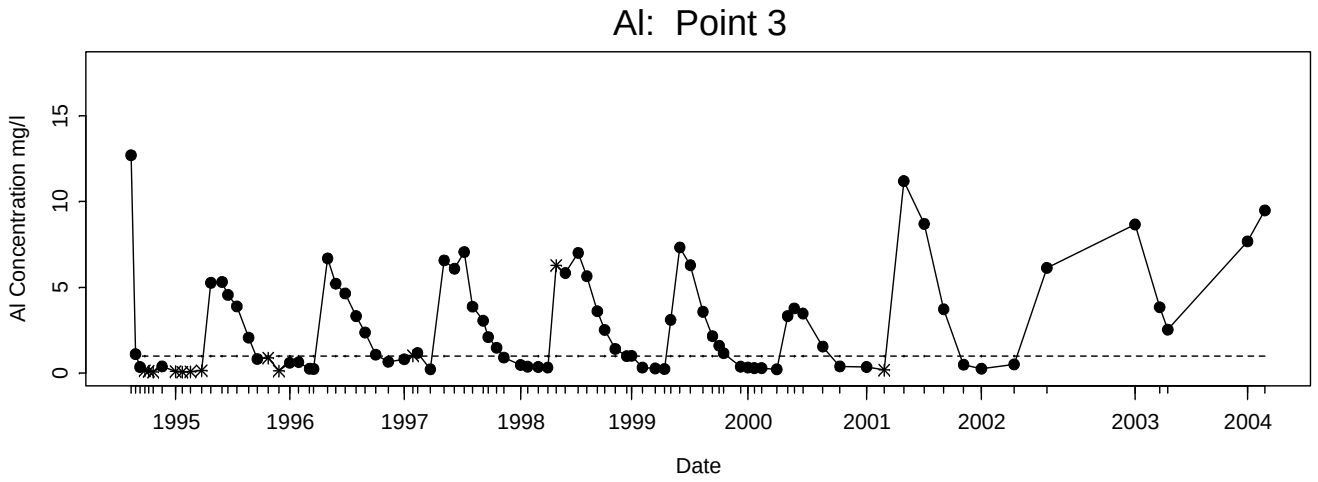
Fe and Mn (see Figures B-4 and B-6) are similar in that their concentrations at Point 6 were not constant like the other five metals but fluctuated throughout the investigation. There is no apparent seasonal trend at either Point 3 or 6. None of the Fe concentrations at either location were below the target effluent level. None of the Mn concentrations were below the target effluent level at Point 3, whereas16% were below the target level at Point 6.

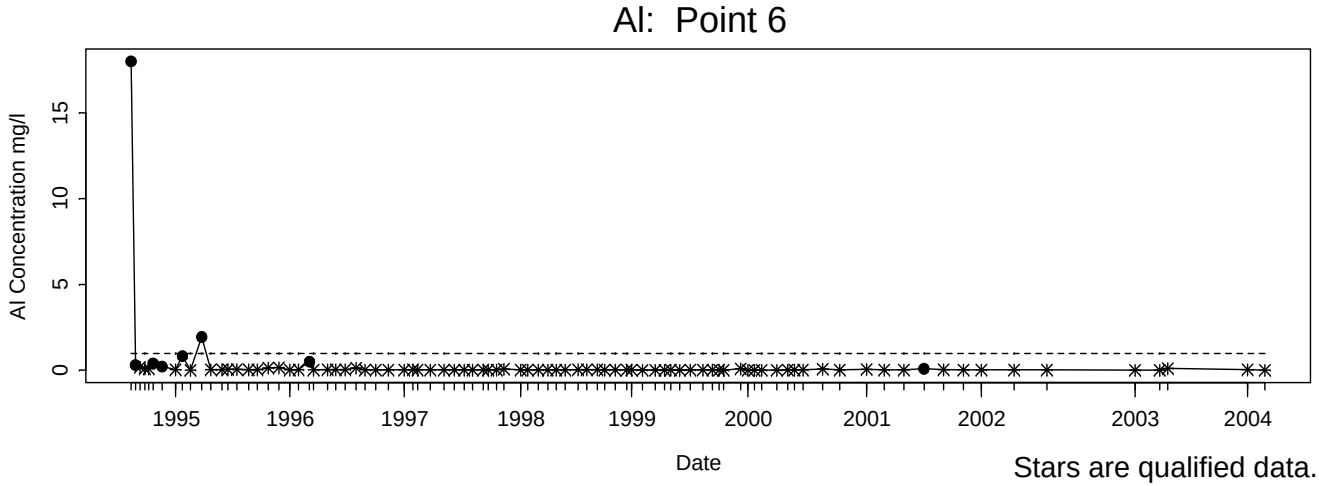
Table B-1. Descriptive Statistics (ppm) by Location

Metal	Point	Target	Q*	UQ	Minimum	Median	Mean	Maximum	Std. Dev.
Al	3	1.0	12	77	0.07	1.18	2.68	12.70	2.90
	6	1.0	81	8	0.00	0.02	0.28	18.00	1.91
Cd	3	0.1	6	83	3.7	96.7	106	301	74.7
	6	0.1	77	12	3.2	4.3	5.1	58.3	5.8
As	3	0.05	24	65	0.02	1.69	3.48	38.4	5.90
	6	0.05	41	47	0.01	0.06	0.26	6.76	0.76
Fe	3	1.0	0	89	2.0	21.3	20.7	43.4	12.6
	6	1.0	0	89	3.7	21.6	24.2	54.9	13.0
Cu	3	0.1	38	51	1.5	33.3	105.7	696.0	147.4
	6	0.1	81	8	1.1	2.6	18.9	1140.0	120.8
Mn	3	2.0	0	89	4.45	5.63	5.71	8.14	0.61
	6	2.0	0	89	0.89	3.36	3.15	5.67	1.03
Zn	3	4.0	0	89	5.57	15.40	14.86	28.80	5.01
	6	4.0	0	89	0.00	0.02	0.09	2.98	0.35

*Q = Number of qualified observations; UQ = the number of unqualified observations.

Figure B-1. Al Concentrations Over Time for Points 3 and 6 (target maximum dissolved effluent 1.0 ppm).





Stars are qualified data.

Figure B-2. Cd Concentrations Over Time for Points 3 and 6 (target maximum dissolved effluent 0.1 ppm).

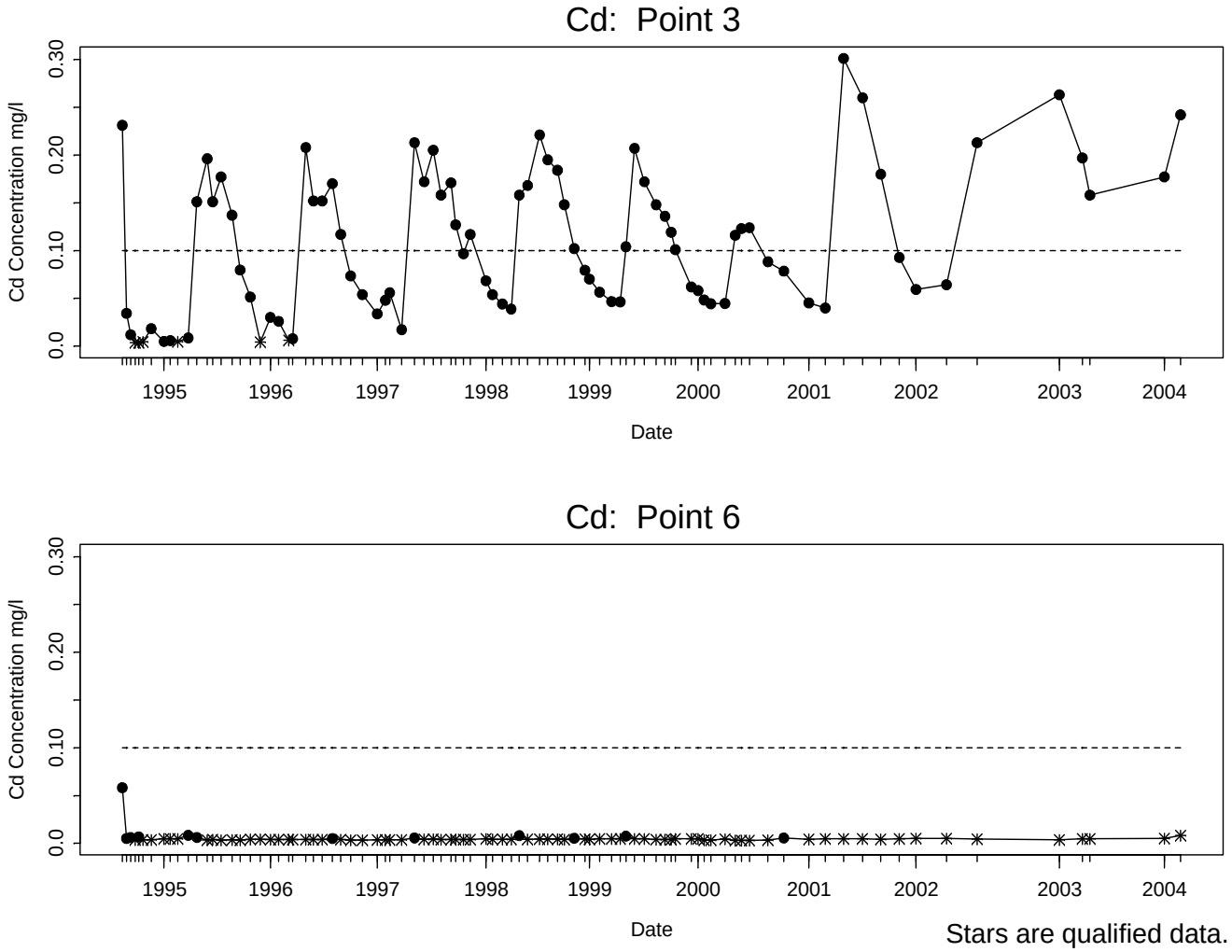


Figure B-3. As Concentrations Over Time for Points 3 and 6 (target maximum dissolved effluent < 0.05 ppm).

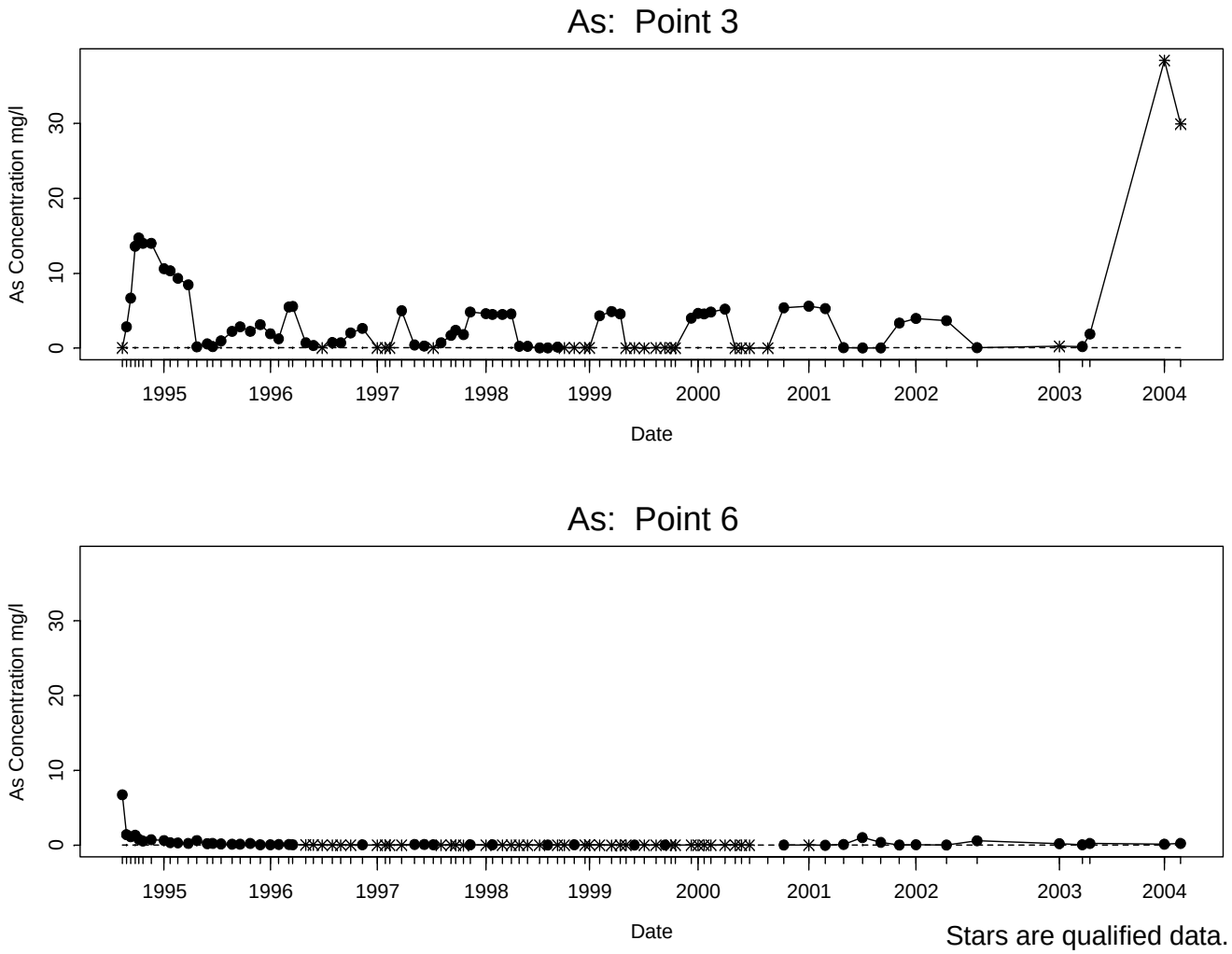


Figure B-4. Fe Concentrations Over Time for Points 3 and 6 (target maximum dissolved effluent 1.0 ppm).

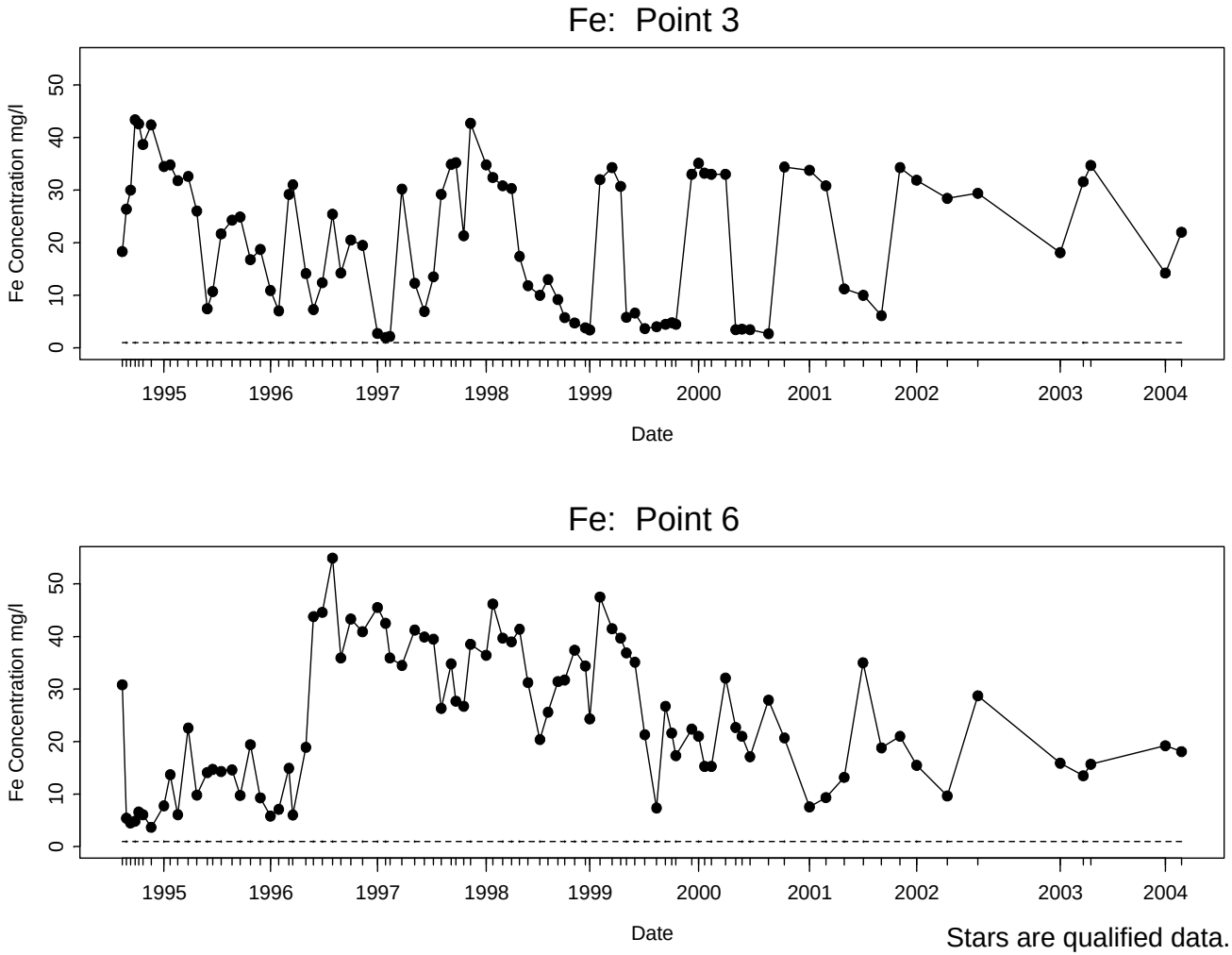


Figure B-5. Cu Concentrations Over Time for Points 3 and 6 (target maximum dissolved effluent 0.1 ppm).

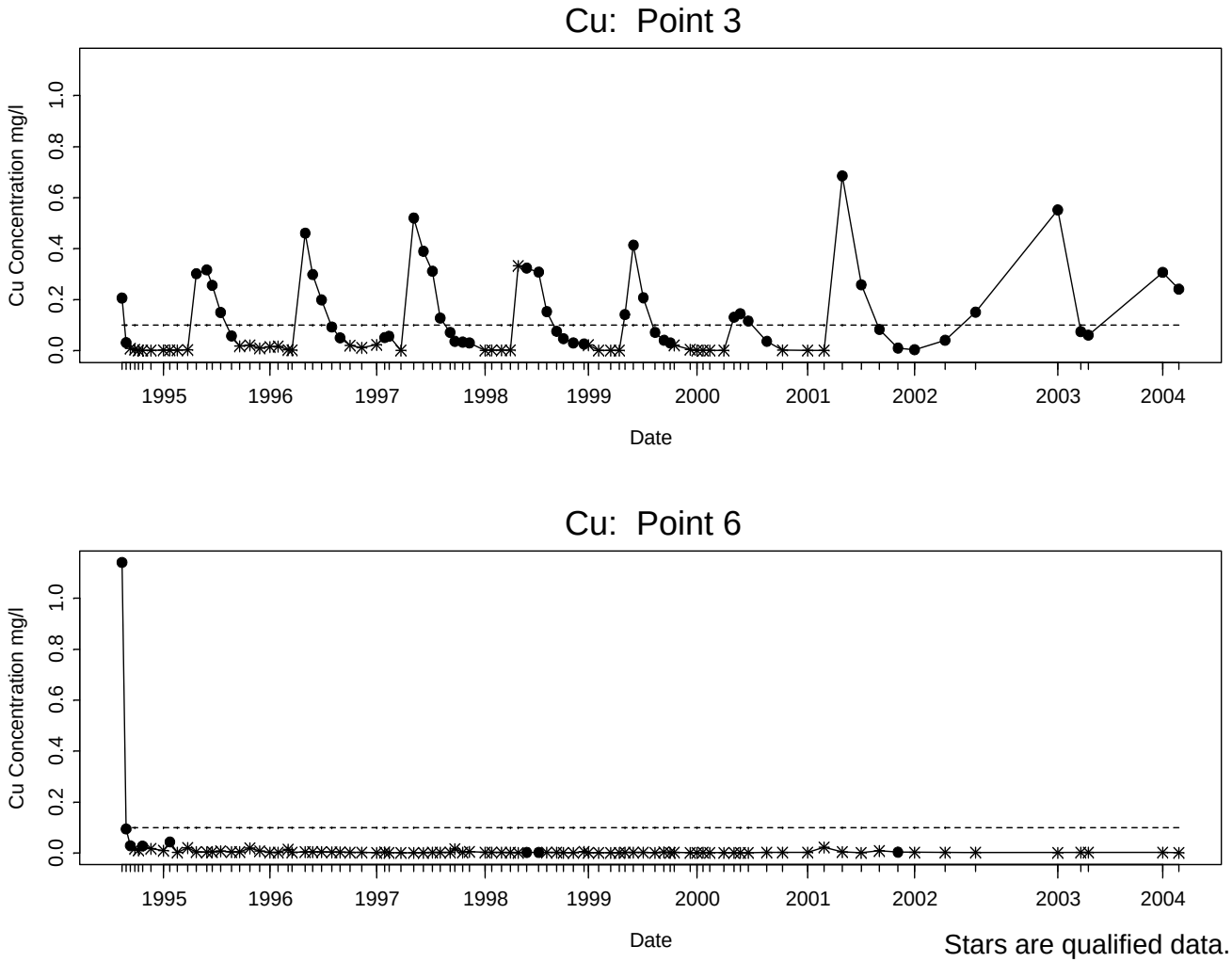
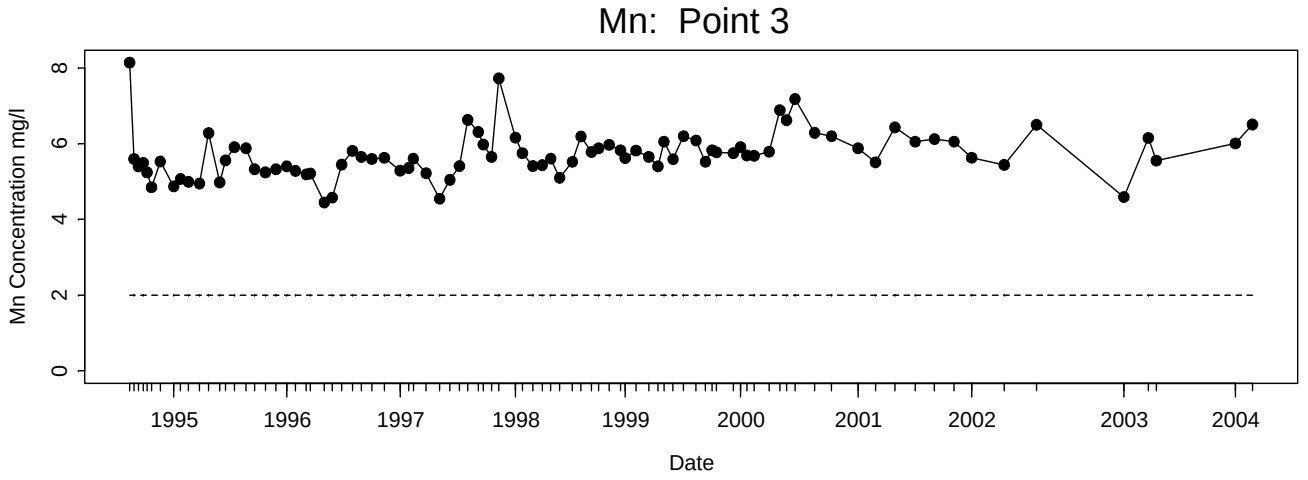
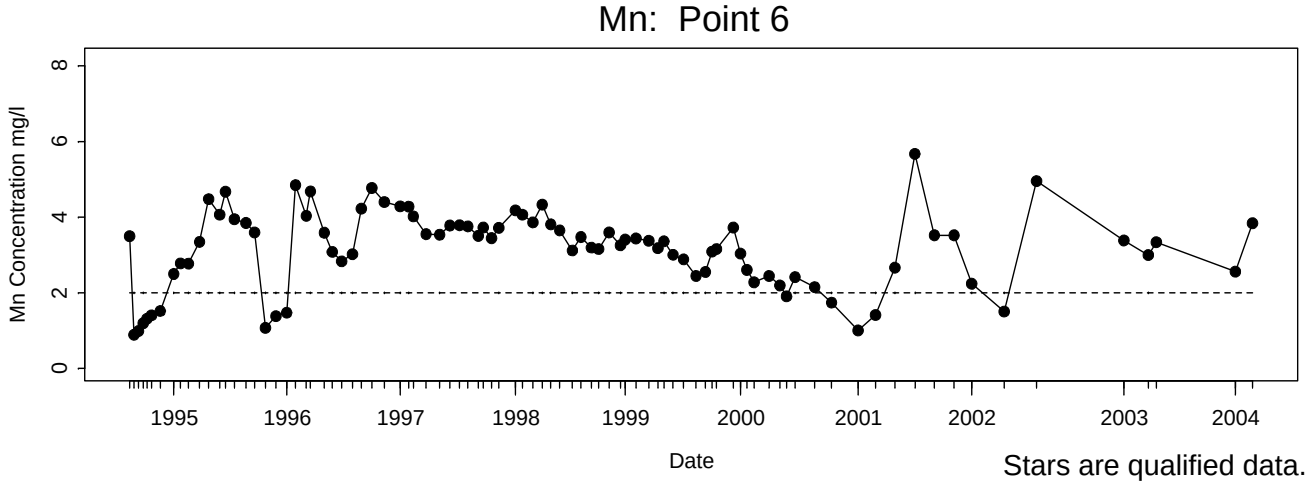


Figure B-6. Mn Concentrations Over Time for Points 3 and 6 (target maximum dissolved effluent 2.0 ppm).





Stars are qualified data.

Figure B-7. Zn Concentrations Over Time for Points 3 and 6 (target maximum dissolved effluent 4.0 ppm).

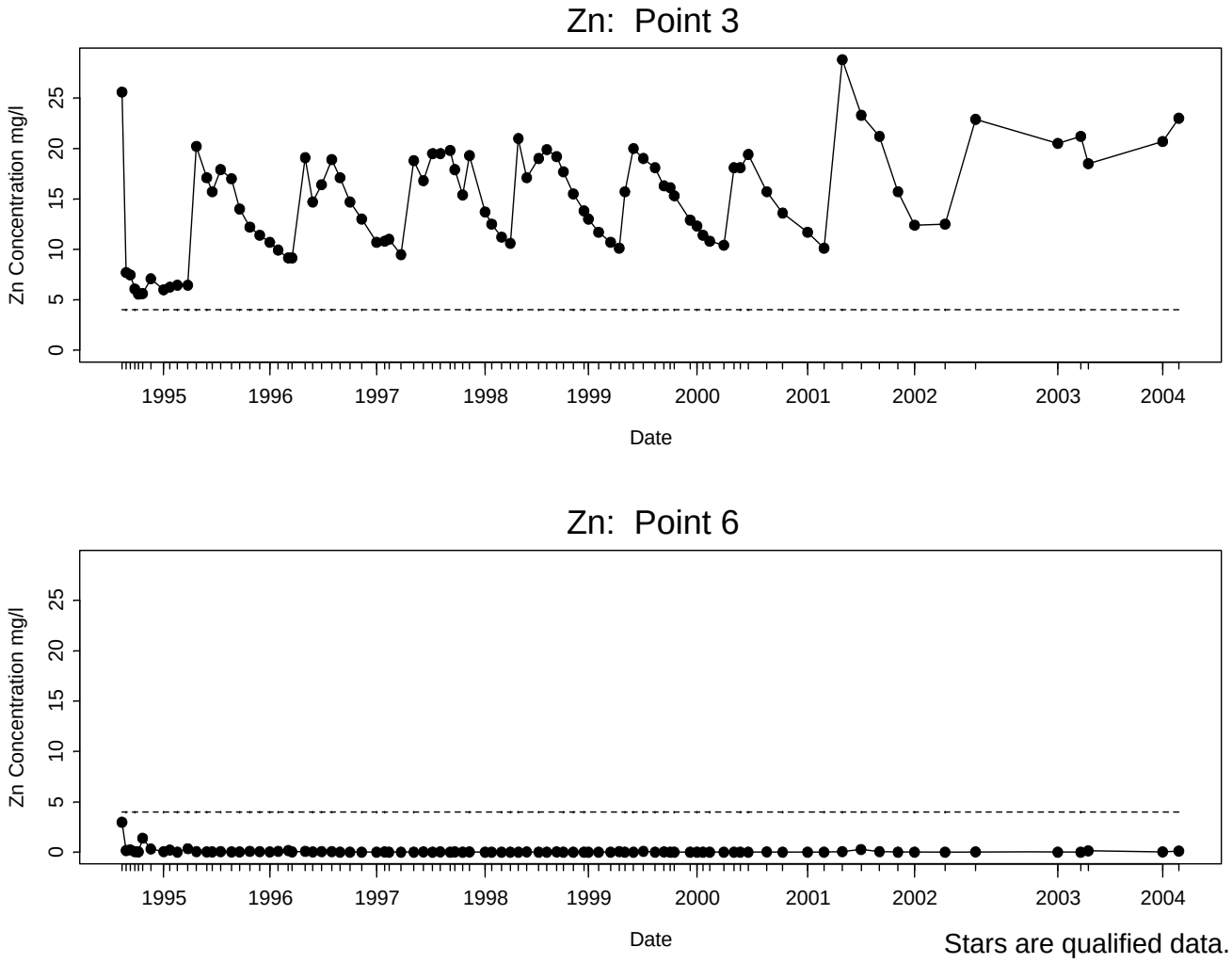


Table B-2. QA Data Summary of Dissolved Metals Analysis

Metal	Point	Target	Q*	UQ	Minimum	Median	Mean	Maximum	Std. Dev.	% Under Target
Al	Portal	1.00	12	80	0.07	1.46	2.82	12.70	3.02	42%
	Tunnel	1.00	84	8	0.00	0.02	0.27	18.00	1.88	98%
Cd	Portal	0.10	12	86	3.70	101.50	109.69	301.00	76.85	0%
	Tunnel	0.10	84	12	3.20	4.40	5.21	58.30	5.74	0%
As	Portal	0.05	25	67	0.02	1.10	3.38	38.40	5.83	27%
	Tunnel	0.05	41	51	0.00	0.06	0.27	6.76	0.75	42%
Fe	Portal	1.00	0	92	1.97	21.50	20.73	43.40	12.53	0%
	Tunnel	1.00	0	92	3.71	21.15	23.72	54.90	13.16	0%
Cu	Portal	0.10	38	54	1.50	36.20	112.93	686.00	156.37	0%
	Tunnel	0.10	84	8	1.10	2.45	18.36	1140.00	118.80	0%
Mn	Portal	2.00	0	92	4.45	5.63	5.70	8.14	0.61	0%
	Tunnel	2.00	0	92	0.89	3.35	3.13	5.67	1.02	16%
Zn	Portal	4.00	0	92	5.57	15.60	15.01	28.80	5.04	0%
	Tunnel	4.00	3	89	0.00	0.02	0.10	2.98	0.34	100%

*Q = Number of qualified observations; UQ = number of unqualified observations.

Appendix C

Microbial Analysis Report

Methods

DNA was extracted from the sample (both from the liquid and from the soil), from a positive control (*P. aeruginosa*) and a negative control (sterile water) using the Bio-101 DNA Fast Prep Kit (QBioGene) using a Savant 101 bead beater (Fast Prep).

Since different primers may preferentially prime different species, two Eubacterial primers were used: 1070F (5' ATG GCT GTC GTC AGC T 3') and 1392R (5' ACG GGC GGR GRG TAC 3') and using 518R (5'GTA TTA CCG CGG CTG CTG G 3') and 357F (5" CTA CGG GAG GCA GCA G 3') (Integrated DNA Technologies). Primer reactions and DNA amplification will be performed using a PTC-100 Programmable Thermal Controller (MJ Research) using the following parameters: 94 ºC for 2 minutes, 15 cycles of 94 ºC for 45 seconds, 55 ºC for 45 seconds, 72 ºC for 45 seconds with a final extension step of 72 ºC for 7 minutes. Verification of the presence of DNA was assessed in 1.5% agarose gels stained with ethidium bromide.

PCR products were cloned using TOPO TA Cloning Kit (Invitrogen). Manufacturer's protocols were followed using 4 ul of PCR product in the initial reaction. 40ul of the transformation mix was plated on Luria Broth agar plates supplemented with kanamycin (LB+kan) and IPTG (isopropyl-beta-D-thiogalactopyranoside). IPTG induces activity of beta-galactosidase, an enzyme that promotes lactose utilization, by binding and inhibiting the *lac* repressor and is used to induce *lacZ* gene expression in cloning experiments which is seen as blue versus white colonies on agar plates. Since it would be unlikely that a contaminant would have both antibiotic resistance genes, white colonies were transferred from the LB+kan plates to Luria Broth supplemented with ampicillin (LB+amp). Those tubes that became turbid were used for the plasmid prep using the Wizard Plus SV Miniprep DNA Purification System (Promega) following manufacturer protocols.

Template DNA was prepared for sequencing using the QIAprep Spin Kit (Qiagen) following manufacturer's protocols. Samples were labeled, frozen and shipped overnight to Retrogen Inc, (http://www.retrogen.com/) for sequencing using the M13 primer (5-CAC GAC GTT GTA AAA CGA C-3'). This allows for better amplification of the PCR product for sequencing since it primes approximately 30 to 40 bases inside the template DNA, thereby removing poorly amplified DNA at the beginning of the sequence. Sequencing data were received from Retrogen Inc. via email and were analyzed with BLAST sequence searches utilizing (www.ncbi.nlm.nih.gov) to identify bacterial species.

Results

A total of 64 clones were sent to Retrogen Inc.for sequencing. Sixty of the 64 clones returned analyzable sequences; four of the sequence reactions gave multiple sequences and were not analyzed using BLAST. Possible reasons given by Retrogen Inc. for multiple sequences are heterogeneous DNA templates, multiple priming sites for the primer, GC compression and repeated sequences in the DNA template.

Species were determined based on the following parameters:

- The sequences had to contain the TOPO vectors (the DNA sequences adjacent to the PCR product insert).
- The deposited sequences had to have a minimum 90% match to the DNA sequences that were analyzed through BLAST.
- Sequences that matched with less than 300 base pairs were eliminated.

- Sequences that matched greater than 400 base pairs were eliminated.

All sequences analyzed with BLAST returned as soil microorganisms, there were no clinical isolates.

Sequence results:

Sulfate-reducing bacteria

Uncultured Chloroflexi bacterium isolate WB-7 16S ribosomal RNA
295/328 (89%) (Note: This one does not fit the exclusion criteria)

Uncultured Flexibacter sp. partial 16S rRNA gene, clone 150
348/351 (99%)

Sulfur reducer

Thermococcales archaeon T30a-17 partial 16S rRNA gene, clone T30a-17
310/310 (100%)

Thiosulfate oxidation

Uncultured Bacteroidetes bacterium partial 16S rRNA gene, clone
299/311 (96%)

Uncultured Bacteroidetes bacterium clone BPC3_E09 16S ribosomal RNA gene, partial sequence
340/341 (99%)

Uncultured Bacteroidetes bacterium partial 16S rRNA gene, clone JG35+U2A-AG10
295/307 (96%)

Compost Isolates

Anoxybacillus toebii NS1-1 16S ribosomal RNA gene, partial sequence
327/343 (95%)

Planifilum fulgidum gene for 16S rRNA, partial sequence, strain:C0170
348/352 (98%)

Uncultured bacterium pPD12 16S ribosomal RNA gene, partial sequence
321/339 (94%)

Proteobacteria

Uncultured beta proteobacterium clone DS087 16S ribosomal RNA gene, partial sequence
340/340 (100%)

Uncultured gamma proteobacterium clone C-CS3 16S ribosomal RNA
336/343 (97%)

Uncultured gamma proteobacterium clone C-CS3 16S ribosomal RNA
350/354 (98%)

Uncultured beta proteobacterium partial 16S rRNA gene, clone NE62
348/351 (99%)

Other

Thermoactinomyces sp. LA5 16S ribosomal RNA gene, partial sequence
337/341 (98%)

Uncultured Acidobacteria bacterium clone BSR3LG05 16S ribosomal RNA gene
331/334 (99%)

Uncultured bacterium NoosaAW69 16S ribosomal RNA gene, partial sequence
317/330 (96%)

Uncultured bacterium clone S-Jos_62 16S ribosomal RNA gene, partial sequence
345/352 (98%)

Methylotenera mobila strain JLW8 16S ribosomal RNA gene, partial sequence
350/351 (99%)

Uncultured soil bacterium clone PAH-Bio-17 16S ribosomal RNA gene, partial sequence
302/303 (99%)

Uncultured bacterium clone CD207F01 16S ribosomal RNA gene, partial sequence
306/340 (90%)

Uncultured bacterium clone DUNssu095 (+1B) (OTU#107) 16S ribosomal RNA gene, partial sequence
350/351 (99%)

Uncultured bacterium NoosaAW69 16S ribosomal RNA gene, partial sequence
324/337 (96%)

Legionella donaldsonii gene for ribosomal RNA, small subunit
331/342 (96%)

Uncultured bacterium clone KD4-59 16S ribosomal RNA gene, partial sequence
341/343 (99%)

Uncultured bacterium clone DUNssu095 (+1B) (OTU#107) 16S ribosomal RNA gene, partial sequence
349/351 (99%)

Pseudomonas spp from soil/root environments
Pseudomonas sp. NRS243 partial 16S rRNA gene, isolate NRS243
314/341 (92%)

Microbiology of a wetland ecosystem constructed to remediate mine drainage from a heavy metal mine
Frateuria sp. WJ64 16S ribosomal RNA gene, partial sequence
342/342 (100%)

Fe(III) and Mn(IV)-reducing anaerobe

Bacillus infernus TH-22 16S small subunit rRNA gene, partial sequence
325/339 (95%)