# *IN SITU* MICROBIAL REDUCTION OF SELENATE IN BACKFILLED PHOSPHATE MINE WASTE, S.E. IDAHO

by

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of

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in

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This dissertation has been read by each member of the dissertation committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistency, and is ready for submission to The Graduate School.

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# DEDICATION

To those who make things work….and those who make it worth working.

Most especially, to

My beloved husband, Allan and our daughters, Meghan and Molly Kirk

With gratitude for all of your patient support,
without which this would not have been possible.

"Whatever you do, or dream you can, begin it.
Boldness has genius and power and magic in it."
*J.W. von Goethe*

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# ABSTRACT

The reduction of selenium (Se) by microbes is controlled by oxygen ($O_2$)-availability within mixed deposits of shale, chert, and mudstone mined from the Phosphoria Formation in S.E. Idaho. Waste rock and groundwater from backfilled mine pits, which have been studied using geochemical, microbial cultivation, and molecular methods, host native populations of selenate-($SeO_4^{2-}$) and selenite-($SeO_3^{2-}$) reducing bacteria that are highly similar to the genera *Dechloromonas*, *Stenotrophomonas*, *Anaeromyxobacter*, and *Ralstonia*. These bacteria rapidly reduced more than 95% of soluble $SeO_4^{2-}$ concentrations. Reduction occurred within a consortium of slow-growing, cold-tolerant, hydrocarbon-degrading, and nitrate-($NO_3^-$), iron-($Fe^{3+}$), and manganese-($Mn^{4+}$) reducing bacteria, including the genera *Polaromonas* and *Rhodoferax*, which appeared to use the naturally-occurring hydrocarbon present in the rock. Most-probable number estimates of $SeO_4^{2-}$-reducers were highest in saturated sediments and in unsaturated shale, and were very low in unsaturated chert and mudstone. Selenium reduction was studied in microaerophilic, saturated native chert, shale, and mixed run-of-mine sediments inoculated with live groundwater cultures, with sampling and analysis of total Se, Fe, Mn; Se speciation; $NO_3^-$ and sulfate ($SO_4^{2-}$); dissolved organic carbon and total nitrogen(N); and mineralogy. Following an $O_2$- and N-dependent lag, $SeO_4^{2-}$ was reduced within 100 hours under saturated, suboxic conditions at rates that varied depending on lithotype and temperature. The microbial community shifted during reduction as well, from phylotypes associated with the Fe-reducing *Rhodoferax* and HC-degrading *Sphingomonas* and $SeO_4^{2-}$-reducing *Dechloromonas* genera to include members of the $SeO_3^{2-}$-reducing genus *Ralstonia*. A unique biogeochemical Se reduction pathway was suggested in chert experiments, where Se reduction proceeded more rapidly and produced $SeO_3^{2-}$ and elemental Se products, relative to the shale, wherein reduction was slower and produced more reduced selenide minerals. Results of these experiments offer insight into the results of *in situ* monitoring in backfill at multiple locations in S.E. Idaho, and potentially explain differences in Se solubility at these locations. Strategic management of rock and water in constructed mine wastefacilities to limit $O_2$ recharge can thus promote $SeO_4^{2-}$ reduction by communities of indigenous organisms using available carbon and other electron donors. This offers a sustainable, design-based approach to natural attenuation of Se in mined rock.

# CHAPTER ONE

## INTRODUCTION

Selenium (Se) release associated with weathering of phosphate mine waste is recognized as a risk for human health and the environment in the S.E. Idaho Phosphate Resource Area (Figure 1). Bioaccumulation of Se released by mined phosphate waste rock has resulted in toxicosis in horses and sheep grazed on affected vegetation, and increased concentrations of Se water have been measured at some locations [3, 4]. Awareness of this risk has prompted significant efforts on the part of phosphate producers, state and federal agencies, and other investigators to describe mechanisms of Se release and attenuation associated with mined phosphate wastes. Various research initiatives have addressed questions of health and environmental risk [5], mineralogy [6-10], Se speciation [11-13], and microbial communities in Se-affected sediments [14] of the S.E. Idaho Phosphate Resource Area. Results of these studies have offered insight into the biogeochemical processes that influence Se release from surficial deposits of phosphate waste rock. This study describes the Se biogeochemistry of mined phosphate overburden under subsurface conditions and evaluates factors influencing the extent of native microbial reduction of selenate ($SeO_4^{2-}$) as a potential method of operational source control in backfilled mine waste.

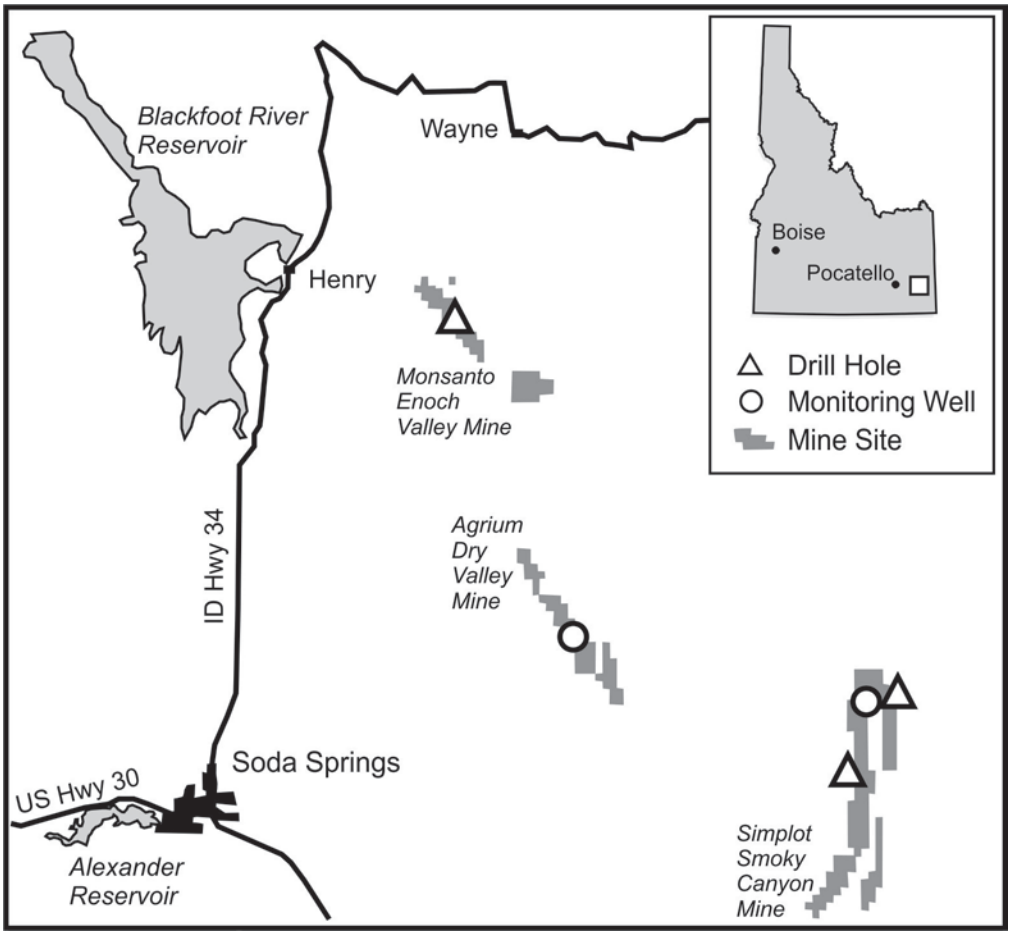



Figure 1. Location of S.E. Idaho Phosphate Resource Area, showing Enoch Valley, Dry Valley and Smoky Canyon mines with studied drillholes and monitoring wells.

## Project History and Location

Results of *in situ* monitoring and laboratory testing show that Se hydrogeochemistry in the S.E. Idaho Phosphate Resource Area varies, depending on waste rock mineralogy and composition, placement and location of waste rock, site hydrology and geochemical weathering processes [2, 15, 16]. The central finding that prompted this research was that concentrations of Se in groundwater within backfilled mine waste at Agrium Nu-West Industries, Inc.'s (Agrium) Dry Valley Mine are

relatively low, in contrast to values measured in surface seeps from external mine waste rock dumps [17], near surface lysimeters [2], and shallow monitoring wells [2], as well as backfill monitored at other mine sites [15].

At the Dry Valley Mine (Figure 1), low Se concentrations were measured in a monitoring well that was placed in randomly-distributed mine backfill [2]. The mine waste backfill deposit at this location had been reclaimed and covered with a vegetated cover, but had been intermittatly saturation with nitrate ($NO_3^-$)- and $SeO_4^{2-}$-bearing water that was pumped out of an active mine pit over a period of a few years. Nitrate and $SeO_4^{2-}$ concentrations monitored in the well increased initially, but dropped quickly following each application of pit water (Figure 2). Following the discharge of water onto the backfill, the groundwater returned to its original elevation, leaving rock above the water table in an unsaturated state. In spite of the lack of saturated conditions in the upper backfill, groundwater Se concentrations in monitoring well GW7D have remained at or below the Idaho groundwater standard of 50 µg/L. (http://adm.idaho.gov/adminrules/rules/idapa58/0102.pdf).

Data from Dry Valley (Figure 2) show low concentrations of $SeO_4^{2-}$, $NO_3^-$, and total dissolved iron (Fe) at consistent pH, with elevated concentrations of $SO_4^{2-}$ and total dissolved manganese (Mn). Low concentrations of dissolved oxygen ($O_2$) were measured at this location (see Chapter 3). In contrast, groundwater samples collected from a monitoring well (GW11), completed in comparable mixed (run-of-mine) backfilled waste rock at J.R. Simplot Company's (J.R. Simplot) Smoky Canyon Mine, showed higher concentrations of $SeO_4^{2-}$, with measurable dissolved $O_2$, under variably saturated

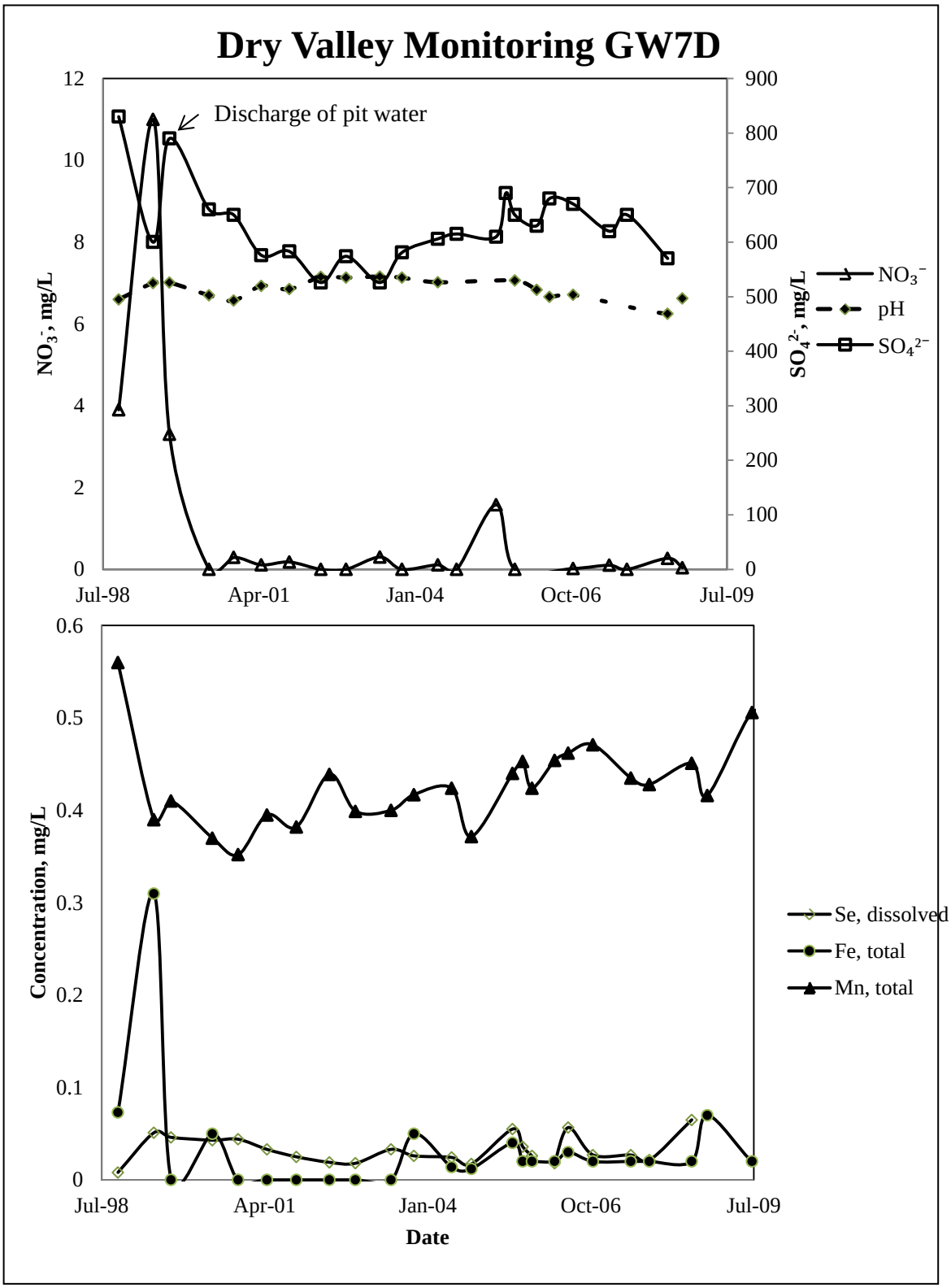



Figure 2. Monitored chemistry in B panel backfill at Dry Valley, groundwater well GW7D [18].

conditions.

Efforts to explain the greater $SeO_4^{2-}$ release from backfill monitored at Smoky Canyon, relative to that observed at Dry Valley, based solely on abiotic mechanisms were unsuccessful and led to the hypothesis that microbial reduction of $SeO_4^{2-}$ to more reduced, less soluble $SeO_3^{2-}$, $Se^0$, or $Se^{2-}$ minerals by indigenous organisms, using native carbon (C), may play an important role in controlling $SeO_4^{2-}$ mobility in backfilled phosphate mine waste deposits. Improved understanding of how extensive and/or consistent this process might be within the S.E. Idaho Phosphate Resource Area, and whether microbial reduction of $SeO_4^{2-}$ within backfills can be promoted to reduce impact on downgradient water resources, will benefit operational Se management strategies. Development of operational source control strategies that make effective use of microbial ecology to stabilize waste *in situ* by controlling the flux of water and $O_2$ to promote the formation of suboxic zones, through placement of key lithotypes to control texture and geochemistry, thereby influencing the potential for stabilization of solutes, has significant implications for sustainable mine waste management across a variety of mineral commodity sectors, well beyond Se control or phosphate production.

## Research Goals

The research presented in this dissertation seeks to address the following questions:

- Which, and how many, $SeO_4^{2-}$-reducing microbes are present in phosphate mine waste? In groundwater from backfilled mine waste?
- Which lithologies support native communities of $SeO_4^{2-}$-reducing organisms?

- What moisture, oxygen, and temperature conditions support $SeO_4^{2-}$ reduction by native organisms using naturally available C?
- How do moisture, oxygen, and temperature conditions affect the microbial community diversity and capacity for $SeO_4^{2-}$ reduction?
- How fast does $SeO_4^{2-}$ reduction proceed under saturated anaerobic conditions in mine waste? What variables control the rate of Se reduction *in situ*?
- What concentration and species of Se, C, N, S, Fe, Mn, as well as microbial community changes, are observed during $SeO_4^{2-}$ reduction?
- What are the end products of $SeO_4^{2-}$ reduction?
- Can indigenous microbes, using native C, reliably support operational source control of Se in mine waste?

## Scope of Investigation

This document begins with a review of relevant literature (Chapter 2), in the context of a conceptual model for addressing the questions listed above. This is followed by a description in Chapter 3 of the sites and historical data available describing the S.E. Idaho Phosphate Resource Area. The sampling and analysis of groundwater and waste rock from sonic drill holes and monitoring wells is also reviewed in Chapter 3, supported by *in situ* measurement of $O_2$, carbon dioxide, moisture content, temperature, and geochemistry [15]. These samples were collected from three mine sites: Agrium's Dry Valley Mine, J.R. Simplot's Smoky Canyon Mine, and Monsanto Company's (Monsanto) Enoch Valley Mine (Figure 1), and were studied to:

1. Identify and enumerate $SeO_4^{2-}$-reducing microbes, using (a) cultivation-dependent methods and (b) molecular methods of identification using the (c) most probable number method (Chapter 4)

2. Evaluate environmental factors, including lithology, $O_2$, temperature, and moisture content, that influence the chemistry, extent and rate of microbial $SeO_4^{2-}$ reduction (Chapter 5).

3. Review environmental conditions needed for conceptual design of operational mine facilities to promote $SeO_4^{2-}$ reduction (Chapter 6) and identify questions to be addressed in future work.

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# CHAPTER TWO

## CONCEPTUAL MODEL OF PHOSPHATE BACKFILL BIOGEOCHEMISTRY

Release of selenium (Se) associated with phosphate mining in the S.E. Idaho Phosphate Resource Area has important environmental and economic consequences. Selenium has a low crustal abundance of 0.05 mg/kg and is measured in low (1-5 µg/L) concentrations in natural water except in association with Se-rich soils or rock [1]. It is readily bio-accumulated from water and sediment via synthesis of organic-Se compounds, including selenocysteine, the 21$^{st}$ amino acid [2, 3]. Selenium is required in amino acids and proteins used in mammals for intracellular signaling, redox homeostasis, and thyroid metabolism [4], as well as production of antioxidant enzymes [5]. Selenium is also potentially toxic, at levels of exposure that vary between receptor organisms [6, 7] and are strongly affected by Se speciation [8, 9]. Selenate ($SeO_4^{2-}$) is less toxic than the more reduced biselenite ($HSeO_3^-$) and selenite ($SeO_3^{2-}$) forms, and many organisms methylate Se to further reduce its toxicity. Selenium is therefore known as an "essential toxin," due to the small difference between necessary and toxic concentrations in mammals, and diseases related to both Se-deficiency and acute or chronic Se-exposure are known [1]. One disease related to Se-deficiency is Keshan disease, a lethal form of cardiomyopathy named for the province in China where soils depleted in Se led to thousands of deaths until the need for supplementation was recognized [10]. Conversely, exposure to elevated concentrations of Se from industrial activities or leaching of naturally elevated Se from soils is known to produce a range of toxicosis symptoms

including gastrointestinal disorders, loss of hair and nails, fatigue, irritability and neurological damage [1]. The ecotoxicology of Se varies significantly between organisms, in part due to differences in detoxification mechanisms [9].

Reduction of soluble and toxic $SeO_4^{2-}$ to $SeO_3^{2-}$/$HSeO_3^-$ or insoluble elemental selenium ($Se^0$) and selenide ($Se^{2-}$) compounds significantly reduces Se mobility and bioavailability. While this reduction does occur abiotically, it is slow, especially in the conversion of $SeO_4^{2-}$ to $SeO_3^{2-}$. This reduction is thus most readily accomplished by a variety of heterotrophic organisms that couple the reduction of Se with the oxidation of a broad spectrum of carbon (C) sources. Much attention has been focused on the study of microbial reduction of Se in promotion of bioremediation strategies for impacted water and sediment, with considerable focus on agriculture-affected settings like the Kesterson Reservoir, California [11]. These processes have also been incorporated into a variety of passive and active water treatment systems that rely on significant C and nutrient amendment [12]. Volatilization of $Se^{2-}$ and phytoremediation have also received considerable attention, and some believe that these methods offer superior remediation capacity as gaseous Se mixes into the atmosphere and does not have potential for reoxidation in sedimentary or aqueous environments [11, 13]. The potential influence of volatilization on mass transfer and sequestration within subsurface phosphate overburden backfill deposits is likely to be relatively low, however. Use of microbial Se reduction in subsurface biobarriers designed for groundwater remediation has also been suggested in recent investigations [14, 15]. The focus of the present research was an evaluation of options for design of backfilled facilities that promote solid phase biomineralization, but

concurrent production of organic and volatile forms of Se in relation to biomineralization processes have also been considered.

## Se Biogeochemistry at the Facility Scale

Integration of the hydrogeochemical and biological processes that together influence the mobility of Se within weathering mine waste requires a conceptual understanding of their influence on Se speciation at both the field ("mine facility") and the micro ("pore") scales, as shown in Figures 3 and 4.

Figure 3 illustrates a phosphate mine pit where mineable phosphorite deposits are exposed in the upper and lower portions of the Meade Peak Member of the Phosphoria Formation. The mine pit is partially backfilled with mixed ("run-of-mine") waste rock (Figure 3) and extends below the groundwater table at this location. An arrow is drawn through the mixed backfill to illustrate infiltration of precipitation through the unsaturated rock, with flow towards saturated rock below the groundwater table (Figure 3). Changes in oxygen ($O_2$) concentration and moisture content are anticipated along this flow path, under the influence of changing lithology, particle size, compaction, and $O_2$ demand within the facility, resulting in transition from oxidizing and atmospheric to reduced, subsurface conditions. A constructed lift (bench) of mined waste rock is shown in Figure 3 as a conceptual reactive barrier designed to promote the reduction of the most oxidized form Se(VI) which occurs as $SeO_4^{2-}$ , to less soluble Se(IV), Se(0) and or Se(II) forms. The facility scale conceptual model thus considers hydrologic, geologic and

geochemical conditions relevant to subsurface Se reduction within a backfilled phosphate mine pit.

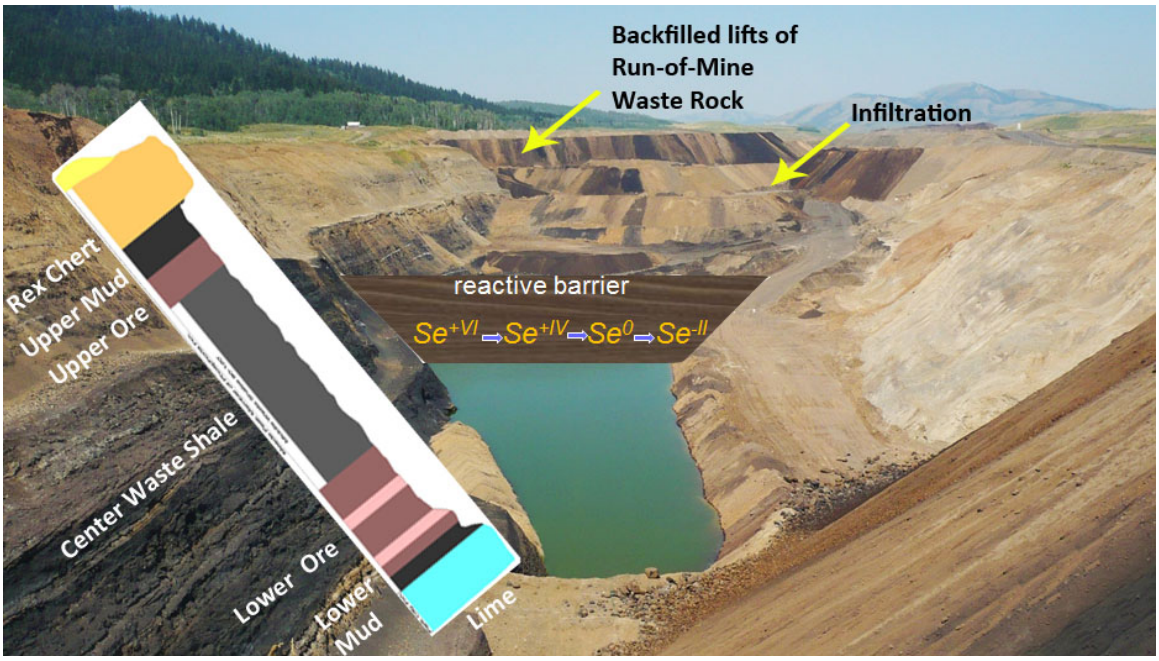



Figure 3. Facility scale conceptual model showing a mined section of the Phosphoria Formation in the S.E. Idaho Phosphate Resource Area in a partially backfilled panel. Geologic section indicates upper and lower phosphorite ore zones with chert, mud, and shale waste rock lithotypes. Lifts of mixed run-of-mine backfill are contrasted with a conceptual biobarrier placed to promote Se reduction within the groundwater flowpath in the middle of the mined panel. Sorted, end-dumped waste rock in foreground.

Phosphate is mined from the Meade Peak Member of the Permian Phosphoria Formation in the S.E. Idaho Phosphate Resource Area, within a section of reduced, fine-grained and organic-rich clastic shale and carbonate sediments [16]. This stratigraphy is illustrated in the column included in Figure 3. These sediments were deposited at the margin of a biologically productive, isolated marine basin, where $O_2$-depleted, denitrifying conditions allowed preservation of the organic C and phosphorous deposits [17, 18]. Elevated concentrations of biogenic copper (Cu), zinc (Zn), Se, cadmium (Cd),

and molybdenum (Mo), relative to mean crustal abundance, occur in the shale and mudstone of the Meade Peak member [19] and are mobilized to varying degrees when mined rock weathers under oxidizing surface conditions [20].

The Meade Peak shale is comprised of quartz; clay (muscovite, illite); feldspar (albite/orthoclase/buddingtonite), and sulfides (pyrite and sphalerite, containing as much as 5% total sulfide) [21]. Trace element, carbonate, and organic C content of the shale varies depending upon weathering history, with loss of carbonate and organic C, and changes in trace element ratios associated with near-surface alteration by meteoric water over geologic time [17]. Selenium content of the shale ranges from 1 to 1040 mg/kg and averages 65 mg/kg; it occurs as $Se^0$ or substituted for S in the sulfide host-rock minerals pyrite ($FeS_2$), vaesite ($NiS_2$), sphalerite (ZnS), and sulvanite ($Cu_3VS_4$) [22]. The mineral dzarkenite, $FeSe_2$, has also been identified as a Se-bearing mineral in the S.E. Idaho Phosphate Resource Area mine waste [23]. Selenium has also been shown to occur as organo-Se compounds [22, 23] and as sorbed $SeO_3^{2-}$ complexes on mineral surfaces [23] in weathered portions of the geologic section [24]. Iron oxides occur locally in more oxidized and altered sediments, but no green rust has been reported [17, 22].

Phosphatic sediments are exposed within the S.E. Idaho Phosphate Resource Area along major north-northwest trending fold structures within the Meade Peak overthrust regional structure [16, 17, 25-27]. Waste rock is mined principally from the overlying Rex chert member, the Meade Peak shale member known locally as the “center waste shale” between the upper and lower phosphorite zones, and mudstone mined from the upper and lower contacts of the Meade Peak with the overlying Rex chert (Figure 3).

Locally, the underlying limestone/dolomites of the Wells and Park Formations are also mined (not shown). Mined chert overburden is randomly placed as backfill into mined-out pits with shale and lesser amounts of mudstone. Mixed overburden deposits of black shale, brown mud, and tan chert are stacked in backfill lift deposits at the back of the panel, immediately to the left of the infiltration arrow in Figure 3. The mixed "run-of-mine" composition is approximately 35% chert, 55% shale, and 10% mudstone, which varies locally based on deposit geometry and mining practices. Together, the phosphate overburden lithologies create an alkaline geochemical setting that hosts Ca-$HCO_3$-$SO_4$ type groundwater with elevated concentrations of nitrate ($NO_3^-$) and variable amounts of dissolved organic carbon (see Chapter 3). Leaching rates determined in field and laboratory studies of these mixed waste rock deposits indicate values consistent with the oxidation of $Se^0$ reported elsewhere, with initially high concentrations that decline to low, steady state levels [28].

Rock is generally placed randomly into backfilled mine pits, without selective handling, compacting, or control of influent water until closure. In some locations, backfill is built in lifts (e.g., benches from the bottom up) and is compacted by haul traffic; in other places, revegetated moisture store-and-release covers are placed as dumps are constructed. Alternatively, waste rock material can be end-dumped along steep embankments over vertical distances of more than 100 feet, where it falls in loose blankets of rock with pronounced sorting as a function of down slope distance [29]. The coarsest rock accumulates at the dump toe, creating zones with greater capacity for air flow. Precipitation infiltrating through the waste rock also transports $O_2$ into the waste

rock, promoting oxidation of minerals that host Se as a trace element; heat rising within the interior of the dump pulls $O_2$ into the dump through the coarser toe deposits. Under these conditions, reduced forms of Se are oxidized to the mobile form, $SeO_4^{2-}$, which persists in alkaline groundwater.

Mined rock typically has a low moisture content of 2 to 4% (weight) water, so that unsaturated conditions within waste backfills are expected to dominate when waste rock is first deposited. Local zones of preferential flow with higher moisture contents are common within fine-grained and compacted material in mine waste, however, and overall moisture content is expected to increase (over tens to hundreds of years, depending upon water management strategies, climate conditions and sediment storage capacity) until unsaturated flow begins. Some backfill deposits in the S.E. Idaho Phosphate Resource Area are located within panels that extend below the local groundwater table, while others confine water within perched aquifers in fine-grained sediments above the regional groundwater table. Air temperature ranges from seasonal highs of 30°C to below freezing, with subsurface temperatures ranging from 8 to 12°C at depths of up to 300 feet. [29].

It is plausible, based on observed conditions within existing backfills which support $SeO_4^{2-}$ reduction, that conditions equally supportive of *in situ* Se stabilization could be intentionally developed on an operational basis within constructed reactive biobarriers. These passive reactive barriers would rely on materials and organisms already present within the backfilled mine waste, with a goal of reducing soluble (and toxic) $SeO_4^{2-}$ to the more strongly sorbed $SeO_3^{2-}$/$HSeO_3^-$ forms (at neutral pH) or

insoluble $Se^0$ and $Se^{2-}$ minerals. The potential for bioremediation of organic and metal compounds is well established [30], particularly for Se, and a variety of approaches have been taken to create suitable conditions to promote biological immobilization of contaminants within reactive barriers [31, 32]. Passive reactive barriers have been used to remediate groundwater in a variety of settings, including groundwater with excess $NO_3^-$ [33], $SeO_4^{2-}$ [34], and $SeO_3^{2-}$ [15]. To determine the residence time required for contaminant reduction, and the mass of C required, it is necessary to understand the biogeochemical processes that operate within the barrier. This study examines Se transformation within subsurface overburden deposits in locations like the Dry Valley, Smoky Canyon, and Enoch Valley mines, where reduction is observed, as analogs for possible constructed biobarriers.

## Se Biogeochemistry at the Micro-Scale

A conceptual model for Se release and attenuation at the pore scale, where the microbial and abiotic geochemical processes that control mobility and bioavailability occur at the mineral surface, is shown in Figure 4. Se speciation within the pore space is directly influenced by pH, temperature, presence of $O_2$ and carbon dioxide ($CO_2$) gas, and biological activity within groundwater and biofilms developed on mineral surfaces. These micro-scale factors are controlled by macro-scale processes that must be described at the facility level. Based on the conditions described within the facility scale conceptual model, pH is expected to remain circumneutral, with temperature ranging between 10 and 25°C. Abiotic factors and biogenic processes likely to influence Se environmental geochemistry under these conditions are discussed below in the context of a conceptual

microscale model.

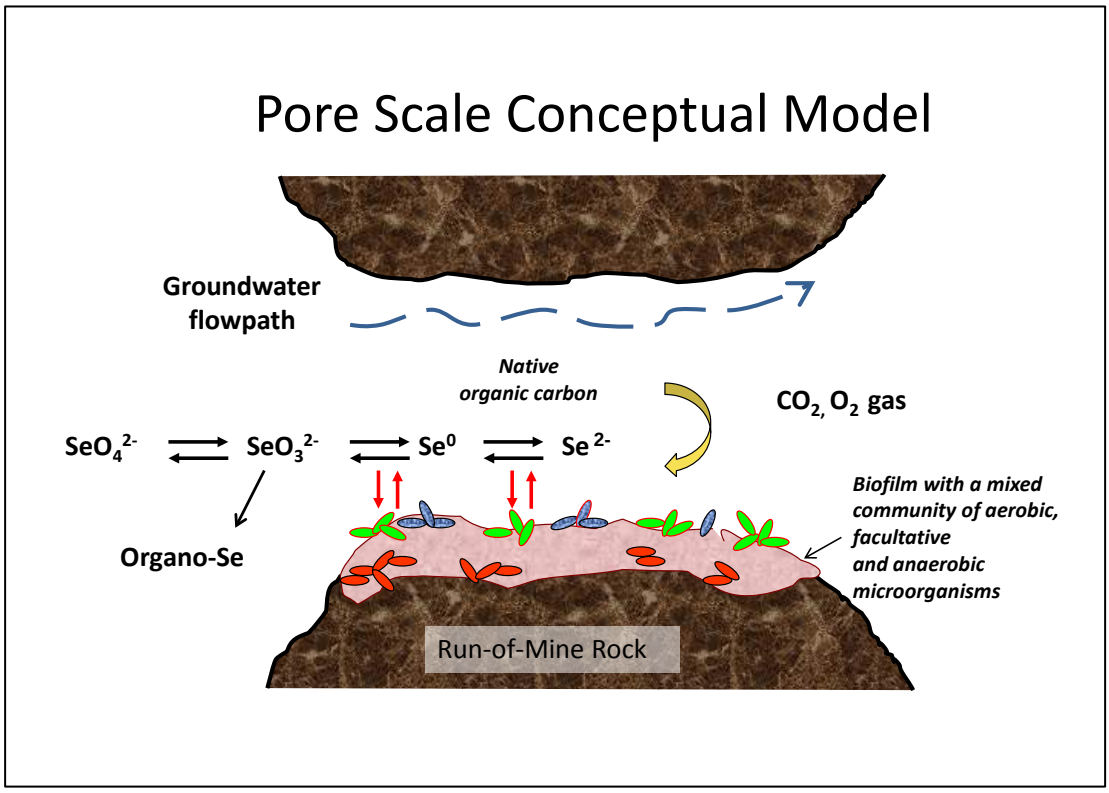
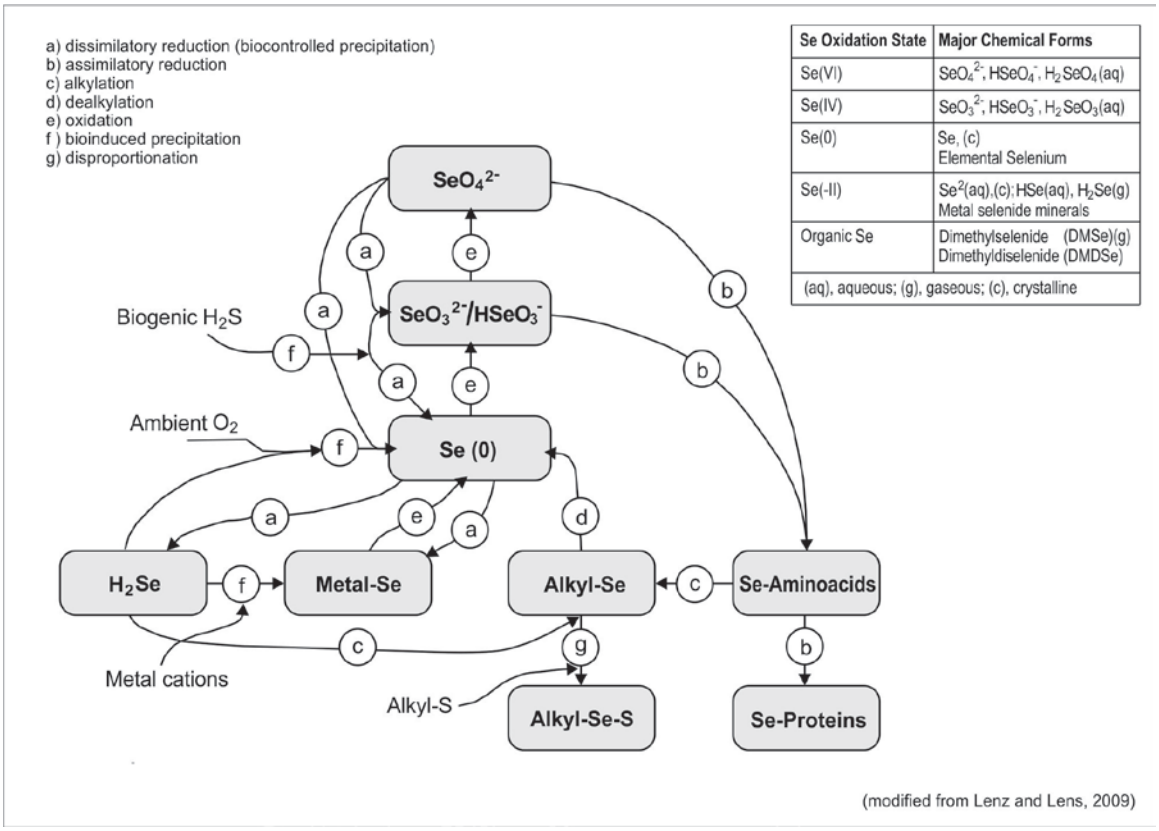



Figure 4. Conceptual model of Se reduction by mixed microbial consortia in groundwater and biofilm developed on mineral surfaces within the pore environment, as influenced by C and $O_2$ availability, $CO_2$ production, and moisture content.

## Se Geochemistry

Selenium is a chalcogen and is classified as a metalloid [11]. It has four stable oxidation states under ambient conditions, Se(VI), Se(IV), Se(0), and Se(-II) [35] and has six natural stable isotopes, dominated by $^{78}$Se and $^{80}$Se [36]. As shown in Figure 5, these redox states occur in multiple chemical forms, for simplicity, the chemical forms, rather than the general redox states, are used in this document. Selenium chemical behavior is similar to that of sulfur (S), for which it substitutes in both mineral and organic

compounds [37], and that of arsenic (As), with which it shares similar valence structures, atomic radii, and tendency to form negatively charged oxyanions in solution [5].




| Se Oxidation State | Major Chemical Forms |
|---|---|
| Se(VI) | $SeO_4^{2-}$, $HSeO_4^-$, $H_2SeO_4$(aq) |
| Se(IV) | $SeO_3^{2-}$, $HSeO_3^-$, $H_2SeO_3$(aq) |
| Se(0) | Se, (c)<br>Elemental Selenium |
| Se(-II) | $Se^2$(aq),(c); HSe(aq), $H_2Se$(g)<br>Metal selenide minerals |
| Organic Se | Dimethylselenide (DMSe)(g)<br>Dimethyldiselenide (DMDSe) |
| (aq), aqueous; (g), gaseous; (c), crystalline | |

Figure 5. Simplified biochemical Se cycle with a) dissimilatory reduction, b) assimilatory reduction, c) alkylation, d) dealkylation, e) oxidation, f) bioinduced precipitation and g) disproportionation, after [4]. Insert table summarizes chemical compounds by oxidation state, after [1].

Selenium occurs as a common trace element in sulfidic coal, shale, and volcanic deposits, and its release is associated with agricultural, mining, and coal combustion activities [4, 38]. Oxidation and dissolution of seleniferous $S^{2-}$ and $SO_4^{2-}$ minerals, as well as organo-Se compounds, releases mobile and potentially toxic forms of Se that may threaten down-gradient water quality and biological resources.

The stability of inorganic Se compounds under changing redox and pH conditions are shown at 1 atm pressure and T=25°C for the system Se-Fe-Ca-$H_2O$ in Figure 6. Selenium occurs in its most oxidized forms in natural water as the oxyanions $SeO_4^{2-}$, $SeO_3^{2-}$, or $HSeO_3^-$, whereas more reduced elemental selenium ($Se^0$) and metal $Se^{2-}$ minerals are relatively insoluble and slow to re-oxidize [32, 39, 40]. Under the neutral to slightly alkaline pH conditions observed in the S.E. Idaho Phosphate Resource Area, which were measured *in situ* between 6.5 to 7.8 (Table 4), dissolved Se(IV) may be present as either $SeO_3^{2-}$ or $HSeO_3^-$.

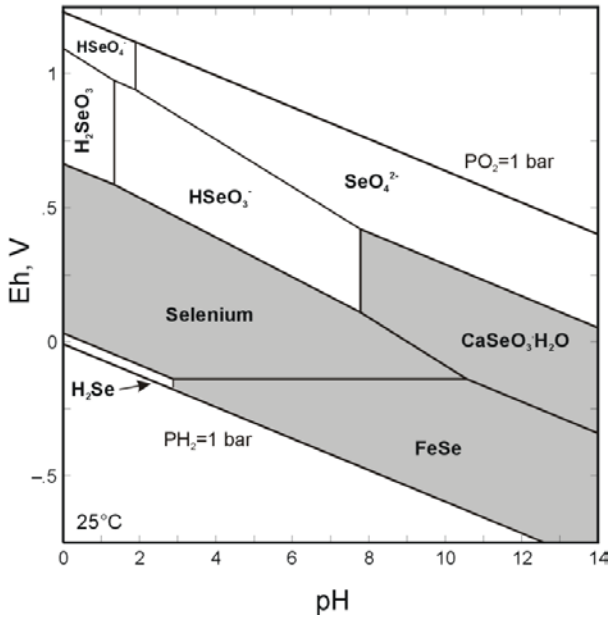



Figure 6. Eh-pH diagram for the system Se-Fe-Ca-$H_2O$, T= 25°C, p = 1 atm from [32].

Elemental Se has a large stability field under moderately reducing conditions that extends across a wide range of pH conditions in natural water. Above pH 8, where soluble calcium is available, a hydrated calcium selenite mineral is stable, as shown in

Figure 6. Other metal selenite minerals, such as mandarinoite ($Fe_2Se_3O_9 \cdot 6H_2O$), can also occur under oxidizing and metal-rich conditions, but are quite rare in the natural environment. Metal selenide minerals, in addition to the FeSe phase shown in Figure 6, such as penroseite ($NiSe_2$), also occur [23]. There is thus limited solubility control of $SeO_4^{2-}$, $SeO_3^{2-}$, and $HSeO_3^-$ under neutral to alkaline and oxidizing conditions in natural environments. Selenium speciation along multiple biotic and abiotic pathways can result in the co-existence of different Se species in any given environment.

A simplified speciation map is shown in Figure 5 after [4] , which illustrates the various biogeochemical pathways involved in Se transformation coupled with a table summarizing the common chemical forms of Se at each oxidation state.

In the following section, biogeochemical pathways controlling $SeO_4^{2-}$ and $SeO_3^{2-}$/ $HSeO_3^-$ reduction; $Se^0$ and $Se^{2-}$ precipitation; alkylation and formation of methylated Se compounds; and re-oxidation of reduced Se are described. The potential influence of Se sorption; iron (Fe) and manganese (Mn) biogeochemical cycling; and the organic C speciation and content of the Meade Peak shale are also discussed.

Selenate Reduction

Abiotic reduction of $SeO_4^{2-}$ to $SeO_3^{2-}$/$HSeO_3^-$ occurs very slowly within circumneutral pH and sub-surface temperatures, creating a kinetic barrier to abiotic reduction [41]. This is explained by a one electron reduction potential barrier imposed by an intermediate Se(V) valence state [42]. Because of this kinetic barrier, most reduction of $SeO_4^{2-}$ to $SeO_3^{2-}$ is thought to be biologically mediated [4, 11, 43-48].

Selenate reduction by green rust, an $Fe^{2+}/Fe^{3+}$ hydroxysulfate that occurs in suboxic sediments, is the best known abiotic mechanism of $SeO_4^{2-}$ reduction [49]. The use of nanoparticle suspensions of zerovalent Fe to reduce $SeO_4^{2-}$ to $Se^{2-}$ compounds has also been reported [50].

Many $SeO_4^{2-}$-transforming microbes are strict anaerobes, but others are able to tolerate limited $O_2$ exposure. The important influence of $O_2$ on microbial reduction of Se oxyanions has been identified in numerous studies [51, 52]. Although most Se reduction occurs under anaerobic or microaerophilic conditions, both obligate and facultative anaerobes are capable of Se reduction [44]. Growth is observed using a variety of electron donors, ranging from acetate and lactate to aromatic compounds [53].

Selenate reduction to $Se^0$ is energetically favorable, yielding 71 kcal per mole $SeO_4^{2-}$ reduced, when calculated using hydrogen as electron donor at standard state [4]. This yield is less than the energy produced by the reduction of $Fe^{3+}$ to $Fe^{2+}$ (-114 kcal), $Mn^{4+}$ to $Mn^{2+}$ (-106 kcal), and $NO_3^-$ to $N_2$ (-112 kcal), but greater than that obtained through arsenate reduction toarsenite (-45 kcal) or $SO_4^{2-}$to $S^{2-}$ (-19 kcal) reduction (Figure 5, reaction pathway a), [4].

Biological Selenate Reduction: Anaerobic dissimilatory reduction of $SeO_4^{2-}$ is accomplished by obligate or facultative anaerobes, using one of several specific $SeO_4^{2-}$ reductase enzymes [54, 55]. Reduction of $SeO_4^{2-}$ for detoxification purposes appears to occur using a specific $SeO_4^{2-}$ reductase in *Enterobacter cloacae* SLD1a1 [56], but otherwise is reported to involve non-substrate specific enzymes associated with $NO_3^-$ and nitrite ($NO_2^-$) [46, 57, 58] or $SO_4^{2-}$ reduction [59, 60].

Much of the research addressing $SeO_4^{2-}$ reduction mechanisms has focused on characterization of the expression of $SeO_4^{2-}$ reductase enzymes and potential inhibition of non-specific $SeO_4^{2-}$ reduction by $NO_3^-$ and $NO_2^-$ reductase enzymes under denitrifying conditions [61-64]. Although the first reports of $SeO_4^{2-}$ reduction associated the process with $SO_4^{2-}$ reduction [43], the energy yield of the $SeO_4^{2-}$ to $SeO_3^-$ and $SeO_4^{2-}$ to $Se^0$ couples is closer to that of denitrification. Selenium is thus typically reduced and removed from a microaerophilic environmental system (or a water treatment process) well before the anoxic conditions that support $SO_4^{2-}$-reduction develop. Nevertheless, $SO_4^{2-}$-reducing bacteria can reduce Se, although some mutual inhibition is reported. In this study, both $SeO_4^{2-}$ and $SeO_3^{2-}$ were shown to be removed from $SO_4^{2-}$-rich media by a biofilm-selected strain of *Desulfomicrobium*, with variation in end products controlled by the concentration of $SO_4^{2-}$. Under low $SO_4^{2-}$ growth conditions, $S^{2-}$ was formed, while $Se^0$ was formed under excess $SO_4^{2-}$ conditions. The $SeO_4^{2-}$ was reduced under $SO_4^{2-}$-reducing conditions by an unspecified reductase [65], while the $SeO_3^{2-}$ was reduced through abiotic reaction with $HS^-$ produced through $SO_4^{2-}$ reduction [59]. Lenz showed that $SeO_4^{2-}$ reduction to $Se^0$ was possible in a $SO_4^{2-}$-reducing bioreactor, with the extent of $SO_4^{2-}$reduction dependent on the $SeO_4^{2-}$ to $SO_4^{2-}$ ratio, suggesting inhibition of an unspecified common enzyme [66]. Because $SO_4^{2-}$-reducing conditions are likely to be less important in the microaerophilic backfills of the S.E. Idaho Phosphate Resource Area, the potential role of $SO_4^{2-}$-reducing organisms is not emphasized here.

Selenate Reductase Enzymes: The first identified Se reductase was the periplasmic $SeO_4^{2-}$ reductase (*Ser*ABCD) described in *Thauera selenatis.* This enzyme is

a member of the DMSO molybdoenzyme family Nar clade, which includes the $NO_3^-$, chlorate, perchlorate, dimethylsulfide, and dehydrogenase reductase enzymes. Synthesis of *Ser* (which only reduces $SeO_4^{2-}$ to $SeO_3^{2-}$) is regulated by *T. selenatis* in response to anaerobic conditions and the presence of elevated $SeO_4^{2-}$concentrations [67]. The $SeO_4^{2-}$ reductase isolated from *T. selenatis* consists of 3 subunits, *serA* (96 kDa), *serB* (40 kDa) and *serC* (23 kDa), with a molecular weight of 159 kDa. The $K_m$ for $SeO_4^{2-}$ is 16 μM and $V_{max}$ is 40 μmol $SeO_4^{2-}$ reduced/min for each mg of protein. Cofactor constituents include Mo, Fe, acid labile S, and a cytochrome b [54]. Santini and Stolz [67] proposed an electron transport chain for the reduction of $SeO_4^{2-}$ to $SeO_3^{2-}$, involving donation of electrons by the periplasmic cytochrome b to SerC, following receipt of electrons from a membrane-bound cytochrome-bc1 complex or from mobile electron carriers such as quinol. Different reductase characteristics have been described for *Sulfurospirillum barnesii*, wherein the $SeO_4^{2-}$ reductase is membrane bound and much broader in terms of specificity [41, 68], but characterization of this reductase is incomplete. It appears that unique reduction mechanisms result in different biomineralization locations within cells, with $Se^0$ reported within cytoplasm, periplasm, or as extracellular deposits depending on the organism.

Another well characterized $SeO_4^{2-}$ reductase has been described in *E. cloacae* SLD1a1. Like that of *T. selenatis*, this reductase contains Mo, heme, and non-heme Fe subunits, but it differs in that it is an insoluble membrane bound protein that relies on the global fumarate and nitrate reductase (fnr) regulatory system, as well as the *tatABC* membrane translocation pathway genes, and the menaquinone biosynethic pathway genes

*men*FDHBCE [69]. The fnr regulon controls gene expression in some facultative anaerobic bacteria, allowing them to up-regulate genes controlling the reduction of fumarate, $NO_3^-$, and other electron acceptors in response to declining $O_2$ concentrations [70].

A reductase with much broader specificity has been described for *S. barnesii*, [41, 68], and still another has been described for *Bacillus selenatarsenatis SF1* [71]. Recent work by Butler and others addresses the influence of different Se reduction pathways and reductase enzymes on biomineralization end-products and has shown that *T. selenatis* and *E. cloacae* SLD1a-1, which have periplasmic and membrane-bound $SeO_4^{2-}$ reductases, respectively, produce different reduced $Se^0$ mineral precipitates following unique $SeO_3^{2-}$ reduction pathways [72].

Selenate Reduction to Selenite/Biselenite: Most known $SeO_4^{2-}$-reducing microbes are *Bacteria*, although *Archaea* and *Eukarya* are known to reduce $SeO_4^{2-}$ as well [4]. Selenate-reducing microorganisms have been identified within the Crenarchaeota, low and high G+C gram positive bacteria, and much of the Proteobacteria [67, 73]. Metabolically and taxonomically diverse communities of $SeO_4^{2-}$-respiring organisms from the class *Gammaproteobacteria, Betaproteobacteria, Epsilonproteobacteria, Chrysiogenetes, Deferribacteres, Deltaproteobacteria,* and the phylum *Firmicutes* were identified in aquatic sediments from four freshwater environments [44].

Selenate Reduction to Elemental Se: Bacteria known to accomplish anaerobic dissimilatory $SeO_4^{2-}$reduction to $Se^0$ (Figure 5, reaction pathway a) include *T. selenatis*

[74], *Geospirillum* SeS-3 [68, 75], *S.barnesii* [46], *B. arsenicoselenatis* and *Selenihalanaerobacter* [76]. *Enterobacter homaechei* was also shown to reduce $SeO_4^{2-}$ to $Se^0$, although it is not clear whether this was a dissimilatory or a detoxification process [77]. The same is true of several members of the genus *Dechloromonas [78, 79]*. Some bacteria such as *S. barnesii* can reduce $SeO_4^{2-}$ to $Se^0$ while others like *T. selenatis* and *E. homaechei* can only reduce $SeO_4^{2-}$ to $SeO_3^{2-}$. Thus, achieving the precipitation of an insoluble $Se^0$ or $Se^{2-}$ mineral can require the participation of multiple members of the microbial community. In some environmental settings, $NO_3^-$ and $SO_4^{2-}$ were shown to inhibit $SeO_4^{2-}$ reduction to $Se^0$ [48], perhaps due to inhibition of $SeO_3^{2-}$ reduction by non-Se-specific reductase enzymes with greater specificity for $NO_3^-$ or $SO_4^{2-}$. However, this is not true in all settings; and $NO_3^-$ has been shown to be consumed concurrently with $SeO_4^{2-}$ reduction in mixed community microcosms.

It appears that unique $SeO_3^{2-}$ reduction mechanisms result from specific $SeO_4^{2-}$reductase enzyme expression and regulation, which yield characteristic localization of biomineralization, with $Se^0$ precipitates reported in cytoplasm, periplasm or extracellular deposits for different organisms [72]. The mechanism and location of $SeO_4^{2-}$ reduction thus influences potential biogeochemical pathways for subsequent $SeO_3^{2-}$ reduction.

Selenate Detoxification: Bacterial species that have been shown to reduce $SeO_4^{2-}$ for detoxification purposes (e.g., non-growth dependent reduction) include *E. cloacae* SLD1a-1 [80], *Anaeromyxobacter dehalogenans* [81] and *Desulfovibrio desulfuricans* [43]. Aerobic and microaerophilic non-respiratory reduction of both $SeO_4^{2-}$ and

$SeO_3^{2-}$oxyanions has also been reported for *Pseudomonas stutzeri* [82-85] and *Azospira oryzae* [86]. *Stenotrophomonas maltophilia* isolated from drainage pond sediment did not grow on Se oxyanions, $SO_4^{2-}$, or $NO_3^-$, but was able to reduce $SeO_4^{2-}$ to $Se^0$ under microaerophilic conditions upon reaching stationary phase; it also produced volatile $Se^{2-}$ compounds [52].

The molecular mechanisms of $SeO_4^{2-}$ detoxification are different from dissimilatory mechanisms. An earlier proposed detoxification mechanism involves maintenance of redox poise through removal of excess electrons by a membrane-bound metal reductase [41]. A more recent study of detoxification in *E. cloacae* [56, 87, 88] identified facultative regulation of $SeO_4^{2-}$ reduction by the global anaerobic regulatory gene fnr, which limits the expression of the $SeO_4^{2-}$ reductase enzyme to low $O_2$ conditions. Selenate reduction in *E. cloacae* is catalyzed by a unique Mo-dependent, cytoplasmic membrane-bound (periplasm-facing) enzyme that is distinct from the Ser reductase as well as the $NO_3^-$ reductase [89]. This reductase could only sustain very slow growth on $SeO_4^{2-}$ under $NO_3^-$-limited conditions [64].

Selenite Reduction

Although $SeO_3^{2-}$ and $HSeO_3^-$ are less mobile than $SeO_4^{2-}$, because of a higher tendency to sorb to mineral surfaces under neutral pH conditions, they are more toxic and can bioaccumulate more readily. Review of available literature suggests that factors influencing the $SeO_3^{2-}$ reduction biogeochemical pathways directly influence the ultimate stabilization of Se within mined by-products and thus have obvious bearing on its mobility and toxicity. Potential mineralization end points include formation of sorbed

complexes on mineral surfaces, precipitation of $Se^0$ or $Se^{2-}$ as minerals, or volatilization of methylated compounds.

Selenite reduction (Figure 5, reaction pathway a) is much less kinetically constrained than $SeO_4^{2-}$ reduction. Abiotic reduction of Se(IV) to Se(0) and/or Se(-II) thus occurs far more readily than Se(VI) reduction, through redox reactions with $Fe^{2+}$-containing minerals in mixed $Fe^{2+}/Fe^{3+}$ hydroxysulfate green rust [49], freshly precipitated Fe oxide [90], siderite [91], and $Fe^{2+}$ complexed on the surface of montmorillonite [92], clay [93], and calcite [94]. Aqueous $Se^{2-}$ and $SeO_3^{2-}$ have also been shown to undergo abiotic redox reactions with sulfide minerals [95, 96], resulting in the reductive formation of either $Se^0$ or $FeSe_x$ depending on the oxidation state of the precursor sulfide mineral [97]. Selenite was also shown to co-precipitate with biogenic sulfide within a $SO_4^{2-}$-reducing biofilm [59].

Mechanisms of Selenite Reduction: Microbial reduction of $SeO_3^{2-}$ to $Se^0$, and selenide minerals and methylated forms is also common [51,82, 98], in spite of the fact that the reduction of $SeO_3^{2-}$ is not kinetically constrained. Selenite reduction to $Se^0$ is energetically less favorable than $SeO_4^{2-}$ reduction (-65 kcal/mol, [4]) but offers greater potential energy yield than is offered by $SO_4^{2-}$ reduction [41]. No $SeO_3^{2-}$-specific reductase has been reported. The mechanisms of biological $SeO_3^{2-}$ reduction appear to be more diverse, involving a variety of reductase enzymes capable of transforming elements including $NO_3^-$, $NO_2^-$, tellurite, sulfite and $SO_4^{2-}$. Specific possibilities include the periplasmic $NO_2^-$ reductase or hydrogenase I [52] and the glutathione reductase [99].

A recent study in *E. coli* showed that $SeO_3^{2-}$ reduction was accelerated by several natural and synthetic quinone compounds that enhance electron transfer in promoting Se reduction both inside and outside microbial cells [100]. Another recent study showed that members of the genera *Enterobacter*, *Bacillus* and *Delftia* had a strong physiological capacity to adapt to high concentrations of $SeO_3^{2-}$ under both aerobic and anaerobic exposure conditions, with resulting changes in cell morphology, fatty acid composition, and intracellular precipitation of $Se^0$ [101].

Selenite Respiring Microbes: Bacterial species known to respire $SeO_3^{2-}$ include *Cupriavidus metallidurans* [102], *Bacillus selenitireducens* [103], *Shewanella oneidensis* [104], *Aeromonas salmonicida* [105], and *Rhodobacter sphaeroides* [106]. *C. metallidurans* has a unique capacity to accumulate both $SeO_4^{2-}$ and $SeO_3^{2-}$, but has only been shown to reduce $SeO_3^{2-}$, to $Se^0$ [107] and selenomethionine [102, 108]. In 2010, the complete genome for *C. metallidurans* CH34 was reported. It is a model organism for bioremediation with a capacity to withstand millimolar concentrations of over 20 different heavy metal ions, including Se and Cd [109].

Selenite Detoxification: Non-respiratory $SeO_3^{2-}$ reduction has been described for the bacterial species *Klebsiella pneumonia, Pseudomonas fluorescens, Enterobacter amigeneus* [77], and *Stenotrophomonas maltophilia* [52]. Algal species, including *Chlamydomonas reinhardtii* [110], *Spirulina platensis* [111], and *Chlorella vulgaris* [112] can accumulate and reduce $SeO_3^{2-}$.

Elemental Se and Selenide Precipitation

Precipitation of $Se^0$ results from the reduction of either $SeO_4^{2-}$ or $SeO_3^{2-}/HSeO_3^-$ (Figure 5). Selenide can be formed via reduction of either $SeO_3^{2-}/HSeO_3^-$ or $Se^0$ as reported for *B. selenitireducens* by Herbel et al. [103], or through biological transformation of alkylated Se (see Figure 5, reaction pathways a and d). Both $Se^0$ and $Se^{2-}$ minerals are relatively insoluble and readily precipitate as solid phases. Crystalline $Se^0$ has a reddish grey color, while biogenic $Se^0$ has a unique salmon red color and often occurs as framboids or nanospheres [76]. Selenide reacts with metal cations to form insoluble metal complexes (Figure 5, reaction pathway f), such as the mineral isomorphs dzarkenite/ferriselite ($FeSe_2$), or the mineral clausthalite (PbSe). Pearce et al. described the formation of $Se^0$ and $Se^{2-}$ minerals as a result of the microbial oxyanion reduction of $SeO_3^{2-}$ [113].

Organo-Se Compounds

The assimilation of Se in proteins, and formation of methylated Se compounds, may also influence Se cycling and the formation of stable Se compounds within the phosphate backfill environments in the S.E. Idaho Phosphate Resource Area. Microbial assimilation of Se in organic compounds such as selenocysteine (Figure 5, reaction pathway b) may occur in algal biomass-enriched zones. During assimilation into cells, both $SeO_4^{2-}$ and $SeO_3^{2-}$ can be transported across cell membranes via the $SO_4^{2-}$ ABC transporter using permease enzymes, but there is evidence for multiple uptake systems for the more toxic $SeO_3^{2-}$ compound [5, 11]. Selenium is incorporated into proteins in a manner that is analogous to that observed for S, but it has several unique properties that

result in the production of selenols (as opposed to thiols) and mixed thiol-selenol compounds [114].

Selenium can also be converted into volatile organic compounds, such as dimethylselenide and selenomethionine, by microbes along alkylation pathways as shown in Figure 5, reaction pathway c [11, 13, 115, 116]. Alkylation, the linking of alkyl groups including methyl compounds to Se, is reported to involve reaction of inorganic $SeO_3^{2-}$with S-adenosylmethionine (SAM), forming a series of reaction products including dimethyldiselenide, dimethylselenone, dimethylselenide, and trimethylselenonium [8]. This process increases the volatility of Se compounds and improves membrane transport and therefore microbial excretion. This process also decreases toxicity, but the increased membrane permeability raises potential for environmental accumulation by some species [117]. Methylation is a common detoxification mechanism [11] and both Se(0) and Se(IV) compounds can be methylated. Recent work by Ranjard et al. [118, 119] has identified a bacterial thiopurinemethyltransferase involved in Se biomethylation in freshwater environments. Rapid microalgal metabolism of $SeO_4^{2-}$ to volatile dimethylselenide in freshwater [120] and production of volatile DMSe and DMDSe species by the microfungus *Alternatia alternata* has been described [121]. Seleno-L-methionine was identified as the dominant organo-Se compound produced by the bacterial strain *C. metallidurans* CH34 upon exposure to $SeO_4^{2-}$ or $SeO_3^{2-}$ [108]. The majority of gaseous Se compounds that are stable under the neutral pH conditions typical of phosphate backfill environments are likely to be methylated compounds, since $H_2Se$ gas is only stable under strongly acidic and reducing conditions (Figure 3). Under certain

conditions, volatilization of Se may contribute to a decrease in soluble and bioavailable Se within mine waste facilities.

Several fungi and *Bacteria* are known to contribute to methylation and volatilization under dominantly aerobic and unsaturated conditions (18 to 70% of saturated water content) when sufficient C is provided and where $NO_3^-$ and heavy metal concentrations are low [8, 11]. Bacterial genera with members capable of producing methylated Se compounds include some members of the *Aeromonas, Flavobacterium, Pseudomonas,* and *Rhodocyclus.* Dealkylation (Figure 5, reaction pathway d) by methylotrophic and methanotrophic organisms has also been reported in anaerobic sediments [11].

Selenium Oxidation

Reduced $Se^{2-}$ hosted in sulfide minerals and organo-Se compounds is initially released by oxidation of mined materials. The potential for the reoxidation of reduced and immobile Se compounds is also important to predictions of the long-term effectiveness of *in situ* microbial reduction as a method of source control. Reduced Se often occurs as $Se^{2-}$ substituted in sulfide minerals [122], but also as $Se^0$ and discrete $Se^{2-}$ mineral phases. Selenium release associated with weathering of mine waste in the S.E. Idaho Phosphate Resource Area is thus commonly due to oxidation of pyrite and other sulfide minerals [123]. As a result, the release of Se follows comparable oxidation pathways to those described for sulfide oxidation, both abiotic and biotic. Under abiotic conditions, $O_2$ is the most powerful oxidant, but $Fe^{3+}$, $Mn^{4+}$, and $NO_3^-$ all have the potential to serve as oxidants in its absence. Sulfide and selenide minerals are known to be oxidized (Figure 5,

reaction pathway e) by the sulfide oxidizing bacteria *Acidiothiobacillus ferrooxidans* [124] and a *Leptothrix sp.* [39].

Selenium in the reduced oxidation states is slow to re-oxidize, although a range of rates are reported. Dowdle and Oremland reported rate constants for $Se^0$ oxidation that were four orders of magnitude lower than those for dissimilatory $SeO_4^{2-}$ reduction in organic-rich, anoxic sediments [39]. In that study, $Se^0$ was oxidized mostly to $SeO_3^{2-}$ with smaller quantities of $SeO_4^{2-}$ produced in live soil microcosms where $SeO_3^{2-}$ sorption limited further production of $SeO_4^{2-}$. However, Tokunaga reported rapid reoxidation of freshly precipitated nanoparticulate $Se^0$ in ponded sediments when exposed to $O_2$ [125]. Remobilization of almost half of colloidal biogenic $Se^0$, which can remain suspended in aerated aquatic systems, has also been demonstrated [126]. Only one bacterial species, *Bacillus megaterium*, has been singled out in the literature for its capacity to oxidize $Se^0$ to $SeO_3^{2-}$ [127]. Selenite is also reported to be slow to reoxidize abiotically in the presence of $O_2$ at neutral or alkaline pH and requires stronger oxidation by ultra-violet radiation or redox-active elements such as $Fe^{3+}$ [38] or Mn oxides [128] under more acidic conditions.

Adsorption of Se Species

Adsorption is the dominant abiotic control of Se solubility at neutral pH. A few $SeO_3^{2-}$ minerals are known, such as ferroselite, $Fe_2(OH)_4SeO_3$, and $CaSeO_3 \times H_2O$, as shown in Figure 6. These minerals are rare, however, and there are no known insoluble $SeO_4^{2-}$ minerals [1]. Although $SeO_4^{2-}$ sorption is likely to be limited under the neutral to alkaline conditions observed in backfilled phosphate mine waste, $SeO_3^{2-}$ sorption may be

an important attenuation mechanism if sufficient mineral substrate is available. Dissolved concentrations of $SeO_4^{2-}$ and $SeO_3^{2-}/HSeO_3^-$ are controlled abiotically under oxidizing conditions through sorption to metal oxides [129-133], apatite [134], carbonate [135], and clay [136-138] minerals. Adsorption of $SeO_3^{2-}/HSeO_3^-$ to Fe and Mn oxides, carbonate, apatite, and clay minerals is possible within the waste lithologies mined in the S.E. Idaho Phosphate Resource Area. Sorption of the oxyanionic species increases with the increased positive charge of protonated oxide and clay mineral surfaces under acid conditions. At neutral pH, $SeO_4^{2-}$ adsorption is much less efficient than the more significant attenuation of $SeO_3^{2-}/HSeO_3^-$ under mildly reducing conditions. Maximum $SeO_4^{2-}$ sorption requires a pH below 6. In one study, the midpoint of an adsorption isotherm for $SeO_4^{2-}$ to hydrous ferric oxide ranged from pH 5.5 to 6.7 for total Se concentrations between 0.1 and 10 µmol/L; in contrast, the mid-point for $SeO_3^{2-}$ sorption was reported between pH 8.8 and 9 for the same range in concentration [130]. Sorption of Se oxyanions has been described in coal mine environments [139] and modeling parameters have been developed for Se species within the constant capacitance [140] and triple layer models [141]. High concentrations of organic matter in soil also appear to limit the solubility and bioavailability of Se [10], although organic acids may compete for sorption sites on goethite [130]. Sulfate and $NO_3^-$ may also compete with Se oxyanions for sorption sites.

## Iron and Manganese Biogeochemistry

Biogeochemical cycling of Fe and Mn by microorganisms potentially influences

Se mobility within the backfilled phosphate environments in the S.E. Idaho Phosphate Resource Area. Iron and Mn-cycling microorganisms may drive the mineralization of complex hydrocarbon compounds present in the Meade Peak sediments and/or alter the availability of oxidized Fe and Mn mineral substrate capable of sorbing $SeO_3^{2-}$/$HSeO_3^-$. Iron has been shown to be important in abiotic Se sorption and precipitation of ferroselite ($FeSe_2$) [142]. It has been suggested that additional Fe should be added to S.E. Idaho Phosphate Resource Area waste to stabilize Se as an Fe-selenide compound in waste deposits [143]. Because of the high concentration of Mn in the Meade Peak sediments, reduction of Mn oxides by $Fe^{2+}$ (with subsequent cycling of both elements) may play an important role in this system [144].

Microbial oxidation of C coupled to $Fe^{3+}$ reduction is thought to mineralize much of the reduced organic matter in sedimentary and aquatic environments [145, 146]. Organisms capable of this metabolism likely influence the bioavailability of native C to the indigenous consortia of *Bacteria* that affect Se mobility in the Meade Peak sediments. They also influence the presence of reactive $Fe^{2+}$ species in the environment. The importance of Fe and Mn cycling in hydrocarbon degradation has been the subject of numerous investigations [145, 147-151], which are discussed further below and in Chapter 4. Of particular interest to the questions of *in situ* Se reduction using complex bitumen-derived native C in blasted rock within subsurface deposits is the potential for anaerobic, $NO_3^-$-dependent Fe oxidation [152, 153]. The potential role of $Fe^{2+}$/$Fe^{3+}$ and $Mn^{2+}$/$Mn^{3+}Mn^{4+}$ as electron shuttles is also interesting in context of $SeO_3^{2-}$ and $NO_3^-$ reduction within the backfill [151, 154]. Given the neutral pH of the phosphate mine

waste, Fe and Mn are likely to form stable oxide minerals, unless reduced through biological activity. Dynamic cycling of Fe and Mn coupled to hydrocarbon reduction creates reducing potential for the subsurface backfill deposit system which balances the flux of oxidants.

Belzile et al. [155] described a multi-scale chemical, biological, and physical process of Se attenuation. Reduced $SeO_3^{2-}$ is sorbed onto Fe-Mn oxyhydroxides, which are dissolved through biotic reduction under progressively reducing conditions developed during diagenesis. Biotic Fe- and Mn-reducing organisms promote mineralization of organic matter, thus driving Se sequestration as $Se^0$, seleniferous pyrite, and selenide minerals [155].

## Organic Geochemistry of the Meade Peak Shale

The organic C content of the phosphate overburden lithologies varies considerably. The dark brown-to-black colored Meade Peak shale sediments are highly carbonaceous, containing up to 15% C by weight [26]. These sediments were source rocks for evolved hydrocarbon, which migrated out of the Meade Peak member during Jurassic time, leaving behind immature kerogen and bitumen residues that reflect a complex thermal maturation history [156, 157]. Extractable, naturally occurring Meade Peak hydrocarbons are asphaltic in composition, ranging from simple alkane and alkene compounds to monocyclic and polycyclic aromatic hydrocarbons. Carbon species naturally present within the Meade Peak shale include toluene, benzene, naphthalene, phenanthrene, and dibenzothiophene (Chapter 3). Conversely, the Rex chert member is

variably argillaceous and oxidized with grey nodular chert concretions in a locally iron-oxide-bearing matrix; it is much less carbonaceous and contains far fewer aromatic compounds (Chapter 3).

Bioavailability of the C that occurs in the phosphate overburden is an important factor that will limit the extent of *in situ* Se reduction by native microbes. In other studies of organic-rich, metal-bearing shales, native *Microbacteria* spp. and *Pseudomonas* spp. have been shown to grow using primary C as the sole C and energy source [158]. Similarly, microbial growth on kerogen in shales has been documented [159, 160]. The ability of native organisms to grow using indigenous C is hypothesized to drive the reduction of Se and other metals in the S.E. Idaho Phosphate Resource Area backfills. In this backfill, application of $NO_3^-$- and $SeO_4^{2-}$-rich water appears to have triggered biological reduction of both, without addition of excess C or other modification (e.g., addition of Fe). Based on this observation, C is believed to be bioavailable and present in excess at other subsurface locations where Meade Peak sediments are placed as backfill.

## Microbial Degradation of Complex Hydrocarbon Compounds

The goal of this study is to evaluate the potential for native microorganisms to accomplish $SeO_4^{2-}$ reduction as a control on aqueous Se concentrations in backfilled phosphate overburden without amendment, that is to say, relying strictly on the C compounds available in the rock itself. To that end, published literature addressing aerobic and anaerobic subsurface degradation of complex hydrocarbons by bacteria was reviewed in developing a conceptual model for the investigation. There is an extensive

body of literature treating this subject, including several excellent reviews [144, 153, 161]. The identification of native monocyclic and polycyclic aromatic hydrocarbons compounds in analyses of the shale and chert overburden (Chapter 3), as well as identification of bacteria capable of degrading those compounds (Chapter 4), guided the following literature review.

Key physical and chemical factors controlling hydrocarbon degradation include: complexity of C compounds, dispersion or sorption of the hydrocarbon, concentration, temperature, availability of $O_2$ and nutrients, activity of water, and pH [145]. Based on the variable saturation and $O_2$ content measured in the backfilled mine pits, it is likely that bacterial mineralization of aromatic hydrocarbons present in the Meade Peak shale proceeds under both aerobic and anaerobic conditions at neutral to slightly alkaline pH. Optimal rates of overall mineralization may reflect both aerobic and anaerobic processes, under conditions ranging from 30 to 90% water saturation [145]. It is likely that both $NO_3^-$ (from blasting) and primary phosphate are present in the overburden, and diesel fuel used in ammonium nitrate-fuel oil (ANFO) compounds for blasting purposes in the less carbonaceous chert may also be used as a C source. Hydrocarbon degradation in the mixed and blasted waste rock may be the result of microbial respiration using $O_2$, or anaerobic processes involving reduction of $Fe^{3+}$, $Mn^{4+}$, $NO_3^-$, $SeO_4^{2-}$, $SeO_3^{2-}$, or $SO_4^{2-}$ [30]. Degradation of organic C under $Fe^{3+}$-, $Mn^{4+}$-, and $NO_3^-$-reducing conditions is of particular interest in these transitional redox environments. Because $SO_4^{2-}$ concentrations remain high in monitored, mine-affected water, $SO_4^{2-}$ reduction does not seem to be an important process within the backfills. It is possible that these metabolisms occur within

backfill micro-habitats, due to local development of suboxic conditions as a result of abiotic and biotic consumption of $O_2$.

A diverse group of microorganisms has been shown to be capable of anaerobic degradation of aromatic hydrocarbons [162]. A mixed benzene-degrading culture that used $NO_3^-$ as an electron acceptor has been described, which was comprised of several *Bacteria* including members of the genus *Dechloromonas [163]*. Degradation of aromatic hydrocarbons via aerobic pathways is energetically more than an order of magnitude more favorable than anaerobic processes [145], with comparable energy yields from $Fe^{3+}$ reduction (-78.7 kJ/e-) and denitrification (-72.2 kJ/$e^-$) [164]). Energy yield is particularly high for benzene and toluene degradation coupled to $NO_3^-$ or $Fe^{3+}$ reduction [153]. Recently published studies of anaerobic degradation of benzene [165-169], toluene [170], naphthalene [164], and phenanthrene [171] suggests that these processes are more common than previously recognized.

Anaerobic degradation of benzene likely involves hydroxylation, alkylation, or carboxylation to form toluene, hydroxybenzoate, or fumarate [167], with subsequent alkylbenzene degradation via co-A pathways [172] under either $SO_4^{2-}$- or $NO_3^-$-reducing conditions [153]. Anaerobic benzene degradation was first reported under denitrifying conditions [168], by *Dechloromonas aromatica*. Bacteria having greater than 98% identity to *D. aromatica* have also been isolated from phosphate backfill at both the Smoky Canyon and Dry Valley mines, and shown, in this study, to reduce $SeO_4^{2-}$.

Many subsurface bacteria survive using anaerobic or facultative metabolisms under low nutrient and temperature conditions and have low metabolic rates [173]. The

rates of mineralization for aromatic compounds are more directly influenced by hydrophobicity compared to rates of mineralization for the more soluble alkane compounds [145]. Denitrification plays an important role in the degradation of monocyclic aromatic benzene and toluene [153], and polycyclic aromatic naphthalene and phenanthrene [173]. An *in situ* study of mixed microaerophilic and anaerobic microbial communities in benzene-contaminated groundwater [174] indicated the presence of phylotypes highly similar to members of the aerobic (or denitrifying) genera *Pseudomonas, Polaromonas, Acidovorax,* and *Rhodoferax,* together with methanogenic organisms, suggesting biodegradation of benzene through paired aerobic and anaerobic metabolism. Anaerobic degradation of benzoate and hydroxybenzoate compounds under $SeO_4^{2-}$-reducing conditions by diverse members of the *Gamma proteobacteria* has been reported [53].

## Conceptual Model of Phosphate Backfill Se Biogeochemistry

Insight into biogechemical processes that influence Se cycling and mobility based on the preceding literature review allows definition of a conceptual biogeochemical model for *in situ* reduction of Se by native consortia using indigenous C within backfilled mine pit environments at both the mine facility and micro (pore) scales (Figures 3 and 4). Previous investigation has shown that lithology, C content, geochemistry (particularly, $NO_3^-$, Fe, and Mn), and mineralogy of overburden will influence Se biomineralization. Temperature, pH, Eh, and Se speciation clearly influence the biogeochemical transformation of Se. Availability of water influences biological productivity, $O_2$ availability, and both the release (e.g., vapor phase Se) and transport of Se (e.g., saturated

vs. unsaturated conditions). Oxygen availability is a particularly important factor influencing styles of microbial metabolism that affect both hydrocarbon degradation and Se speciation. These factors were evaluated through *in situ* monitoring of backfilled phosphate overburden, as described in Chapter 3, and used to identify experimental conditions for sample collection and storage, microbial isolation, and construction of microcosm reactors used in this study.

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# CHAPTER THREE

## SITE DESCRIPTIONS, SAMPLING METHODS AND EXPERIMENTAL DESIGN

Microbial communities within mine backfill deposits, and their ability to reduce selenium (Se), are directly affected by: 1) lithology and geochemistry of rock; 2) the flux of oxygen ($O_2$) and water into the subsurface; and 3) resulting groundwater geochemistry. To characterize and quantify these conditions in the S.E. Idaho Phosphate Resource Area, rock and groundwater was sampled and analyzed, together with *in situ* hydrogeochemical measurements of temperature, $O_2$, and carbon dioxide ($CO_2$), and moisture. Samples of rock and water, where possible, were collected at the Agrium Nu-West Dry Valley, J.R. Simplot Smoky Canyon, and Monsanto Enoch Valley mines (Figure 7).

Members of the Idaho Mining Association (IMA) Phosphate Working Group have conducted several studies of overburden hydrogeology and Se geochemistry since 1996 [1]. Sampling and analysis of overburden, groundwater, and subsurface conditions in addition to the Tetra Tech/MFG, Inc. work was conducted for this study. This chapter begins with a brief description of backfill at the three mine sites, as well as a summary of previous work characterizing the hydrogeochemistry of Se in these locations [2]. These include overburden sampling and analyses in 2005; drilling, in 2006, and characterization of overburden geochemistry, reported in 2008, conducted in association with this study [2], *in situ* monitoring of backfill hydrogeochemistry, 2007-2010 [2, 3], and groundwater sampling, this study and previous related investigations [4, 5].

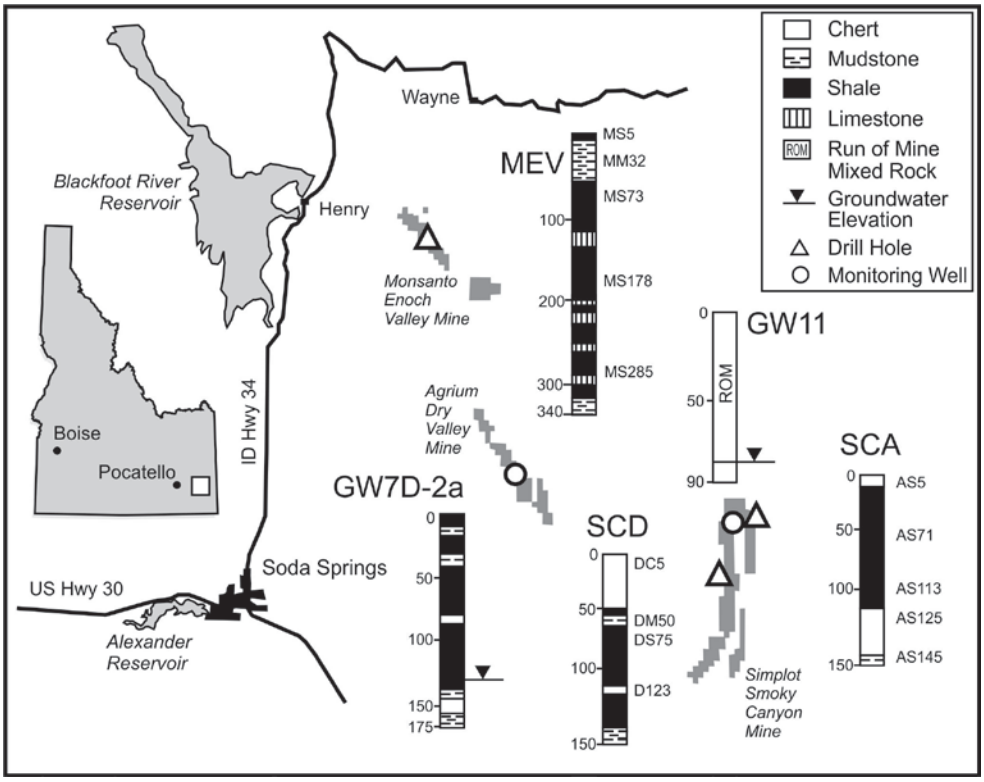



Figure 7. Location of 3 sampled drill holes and 2 monitoring wells in S.E. Idaho Phosphate Resource Area. Depth to exposed lithologies and water in each hole are illustrated. Rock sample locations are indicated to the right of each drill hole at the corresponding sampled depth; the ID reflects the sampling location and rock type, e.g. MS5 represents M for Monsanto's Enoch Valley drillhole MEV, S for Shale, and 5 for 5 feet of depth. Likewise, SCA represents Smoky Canyon drillhole A and SCD represents Smoky Canyon drill hole D. Groundwater monitoring wells are numbered as shown, GW7D-2a (Dry Valley) and GW11 (Smoky Canyon).

Technical reports cited above are publically available from the state of Idaho and/or federal land management agencies, including U.S. Forest Service and U.S. Bureau of Land Management. Some of the reports providing site descriptions cited above or discussed below are provided in Appendix A. Collectively, site conditions are summarized to define experimental design and material requirements for the biogeochemistry experiments presented in Chapters 4 and 5.

## Backfilled Mine Panels in S.E. Idaho Phosphate Resource Area

Phosphate ore is mined from regional, NNW-trending folds developed in the Phosphoria Formation within the Meade Peak overthrust. Mine pit (a.k.a. panel) backfills at the Dry Valley, Smoky Canyon, and Enoch Valley mines have been constructed using randomly placed run-of-mine (ROM) waste rock mined from the Meade Peak and Rex chert members of the Phosphoria Formation. At Enoch Valley, more limestone was mined than at both the Dry Valley and Smoky Canyon mines, where more chert was removed. Changes in backfill construction methods, water management, cap construction, and proximity to the regional groundwater table further distinguish backfill conditions at each site. Collectively, these three mines provide an excellent overview of field conditions in S.E. Idaho Phosphate Resource Area backfill.

### Agrium Dry Valley Mine

The management history, geology, hydrology, aqueous chemistry, $O_2$ and $CO_2$ concentrations, and temperature of backfill at Dry Valley were described by Tetra Tech/Maxim [6]. The B pit backfill at Agrium's Dry Valley Mine was constructed through plug-dumping (rather than end-dumping) by individual trucks on constructed lifts (benches) of fixed height, with random placement of shale, chert, mudstone, and limestone (in descending order of relative tonnage). The pit has a 165 acre footprint, extending nearly 9000 feet along strike and 800 feet across, as shown in Figure 8. Depth is approximately 200 feet below the local ground surface. Pit water was discharged seasonally in 2000 and 2001 into multiple ponds on the surface of the reclaimed B pit

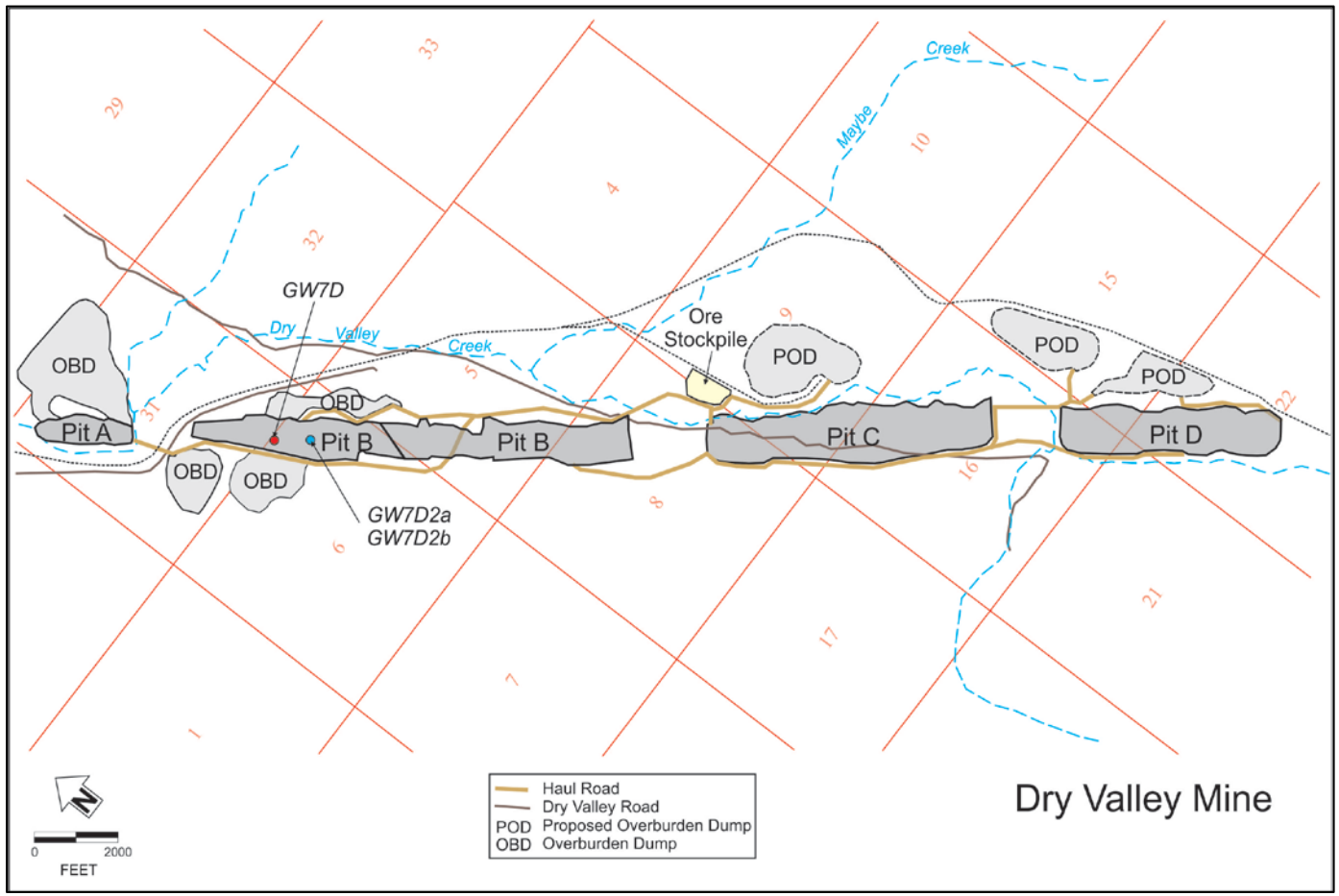



Figure 8. Map of Dry Valley Mine showing backfilled pits A to D and groundwater monitoring wells GW7D, GW7D2a/2b. After Tetra Tech, 2007, [1]

backfill during mining of the adjacent C panel (Figure 8). This resulted in temporary saturation of the backfill with $NO_3^-$- and $SeO_4^{2-}$-rich water, followed by draining to field capacity within the upper backfill [6]. A perched zone of saturation exists low in the backfill, which lies above the regional groundwater table in the underlying limestone bedrock (approximately 60 feet below ground surface). The limestone between the saturated backfill and the groundwater is relatively dry, reflecting the confined nature of the groundwater within the backfill. Results suggest that the methods of placement (e.g., constructed lifts rather than end dumping down long waste rock slopes) [2], as well as the temporary saturation of these materials [6] distinguish the physical hydrogeology and therefore the biogeochemistry of these backfills from other S.E. Idaho Phosphate Resource Area backfills.

The cross section shown in Figure 9 illustrates the monitoring wells installed at the Dry Valley Mine in 2003. Groundwater well GW7D was completed in deep backfill at the northern end of reclaimed pit B at the Dry Valley Mine. This well has been monitored for compliance purposes since 1998 and is located within the footprint of historic pit dewatering discharge ponds. A second well was drilled, GW7D2, approximately 500 feet to the north of the GW7D well. Two monitoring wells completed at the second location, one deep (GW7D2a, 180 feet, bottom of backfill) and one more shallow (GW7D2b, 140 feet, the approximate groundwater elevation), have been sampled since October 2002. Water quality data from GW7D and GW7D2a/2b are provided in Appendix A1, Tables A1-1 and Tables A1-2. Near-surface gas vapor points and lysimeters at depths of 5, 10, 20 and 30 feet were also installed (Figure 9). Descriptions

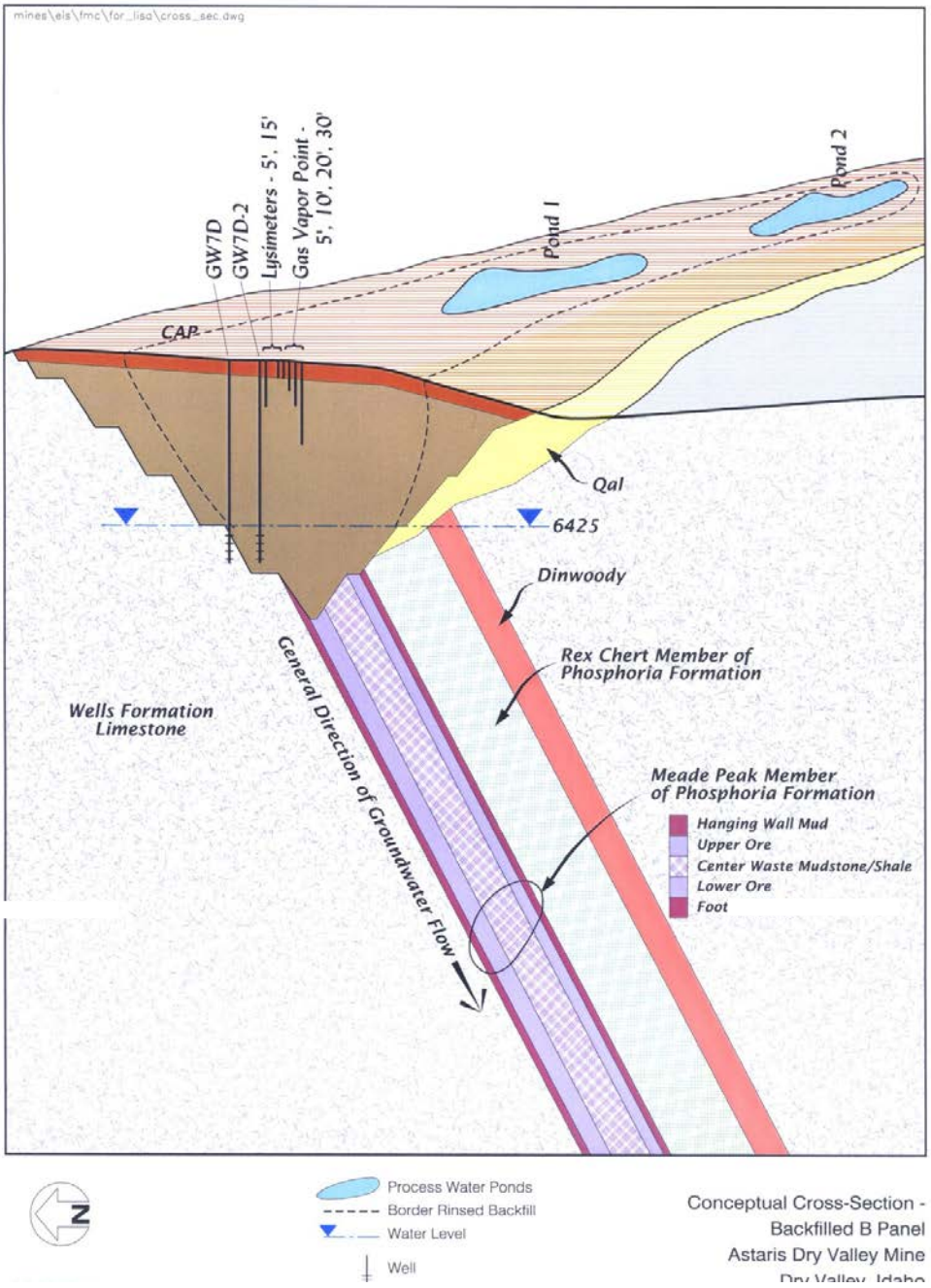



Figure 9. Dry Valley cross section showing monitoring installation, after [7].

of the subsurface monitoring installations, and data collected from them are provided in the Agrium Groundwater Validation study [6], portions of which are also provided in Appendix A1. These data show higher concentrations of total dissolved Se in near-surface (<10 feet) lysimeter samples (mean 0.22 mg/L, with values as high as 0.74 mg/L). Samples collected below a depth of 10 feet in backfill show $O_2$ depletion (concentration below detection limit (0.1 mg/L), with $CO_2$ enrichment up to 7% $CO_2$. Of particular interest are the low soluble concentrations of Se in the two deep wells (GW7D and GW7D2a) at the Dry Valley Mine, which are below the Idaho groundwater standard of 0.05 mg/L. These low Se values correspond with the absence of detectable dissolved $O_2$ and $O_2$ gas within backfilled sediments above the water table [6].

J.R. Simplot Smoky Canyon Mine

The hydrogeochemical conditions in backfill at the Smoky Canyon and Enoch Valley mines were described by Tetra Tech/MFG [2]. Three distinct portions of the overburden at the Smoky Canyon Mine have been incorporated into this study, which include the backfilled portions of panels A and D, and the external surface overburden dump located to the southeast of panel A (Figure 10). Most overburden at the Smoky Canyon Mine was placed by end-dumping mine wastes from individual trucks along angle of repose slopes from panel highwall benches (excavated benches that step back from the bottom of a pit or panel in order to create a stable slope on the margins of the pit) or from dump crests (the top of mine waste rock piles). Here, individual truckloads of overburden tend to be distributed along dump faces tens or hundreds of feet in length, where size fractionation occurs by sorting and materials are comingled more completely

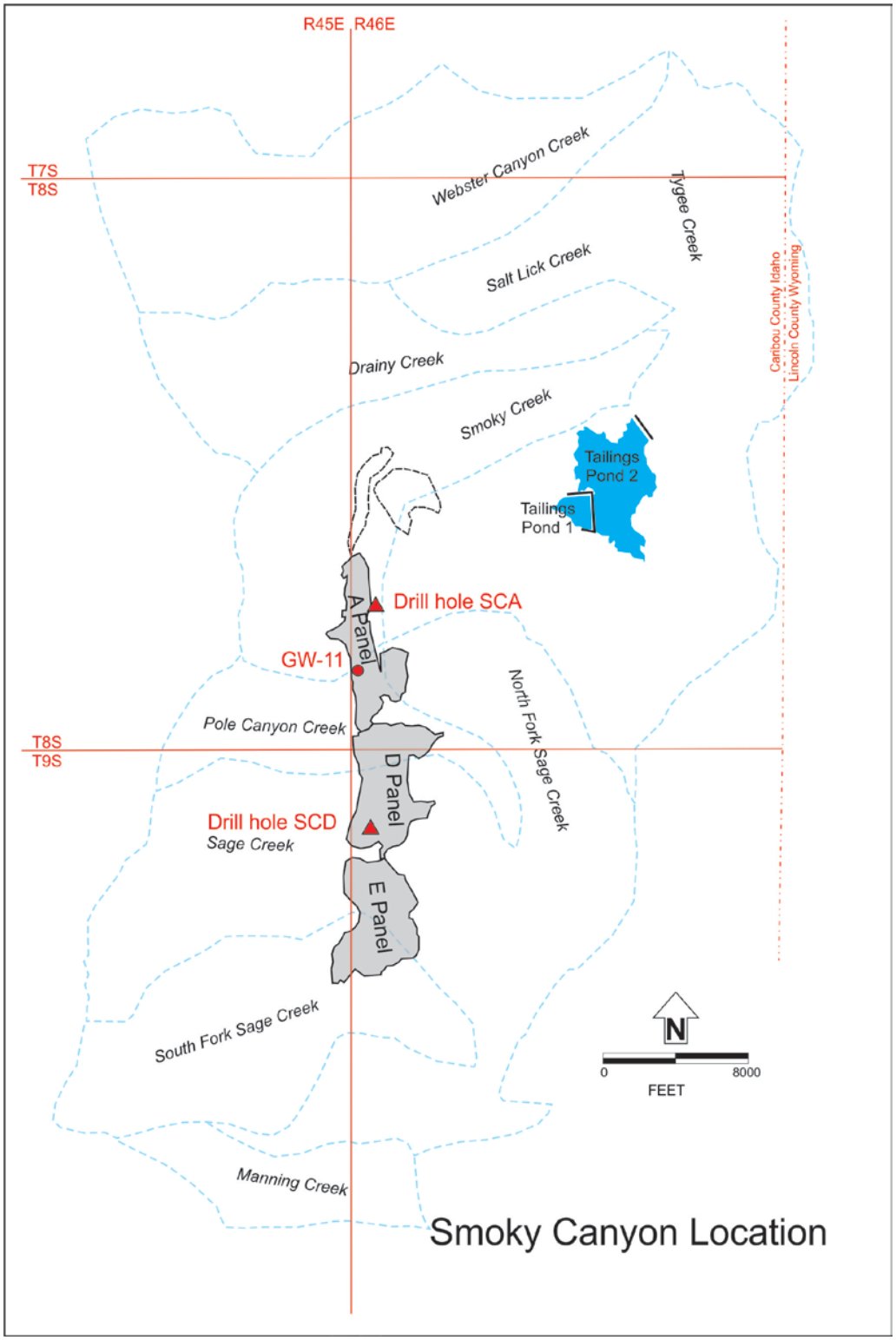



Figure 10. Map of Smoky Canyon Mine showing 2006 drilling locations relative to backfilled panels (pits) A, D and E.

than would occur where individual trucks dump isolated plugs of material that is less well sorted (Dry Valley).

Coarser layers provide conduits for enhanced gas exchange, relative to fine grained layers [8] and also serve as capillary breaks, elevating the saturation and water storage within overlying finer grained zones [9-11]. The D backfill panel extends over a footprint of 370 acres and contains approximately 64 million cubic yards of backfilled overburden. Depth is approximately 220 feet. It was constructed between 1993 and 1997, and the southern third of the facility was capped with chert, graded, and covered with topsoil for re-vegetation in 2004. The northern portion of the D panel was constructed and re-vegetated prior to initiation of chert capping as a best practice for Se management [2].

Panel A (and the associated, free standing external Panel A dump) were constructed beginning in 1984 and are significantly older than the D panel backfills. Panel A backfill is approximately 100 feet thick and extends over a footprint of 120 acres. The Panel A external dump covers some 80 acres and contains 7 million cubic yards of mixed overburden. The external dump was re-graded and covered with topsoil that varies in thickness (3 inches to 3 feet) prior to re-vegetation in 1996; it was not capped with inert rock [2]. The GW11 monitoring well at the Smoky Canyon Mine was completed in approximately 100 feet of backfill along the east side of the A panel, where it was collared (the point at which a well starts at the surface) in a maintenance area located adjacent to the main haul road where the grade was flat. Water ponding in this unreclaimed area resulted in episodic recharge in response to changes in precipitation.

The elevation of ground water was thus variable in GW11 and intermittently dropped below the maximum depth of the well, so that it was not possible to consistently collect water from this well. The well was accessible from 2001 to 2009, after which it was decommissioned to allow for construction of another lift of overburden in that location. Water quality data from GW11 reported in 2002 are provided in Appendix A1, Table A1-3. A description of the groundwater sampling protocol used at GW11 is also provided in Appendix A1.

Monsanto Enoch Valley Mine

The mined pit at the Enoch Valley Mine was backfilled between 2000 and 2002, and reclaimed in 2003. Backfill placed in the lower portion of the facility was end dumped down angle of repose slope distances longer than 50 feet; the upper backfill was constructed with a combination of plug dumping and dozing (pushing of rock with equipment) thereby constructing discrete lifts or tiered benches. This location has a higher proportion of limestone in the backfill than other studied locations. Seleniferous waste materials were isolated within the center of the facility, which does not extend above the original topography. The surface was re-contoured and capped with 4 feet of limestone, and overlain by 18-24 inches of alluvium and chert as topsoil. The backfill lies approximately 128 feet above the regional groundwater table; consequently, it was not possible to sample groundwater at the Enoch Valley Mine (Randy Vranes, Monsanto, personal communication, 2007 and 2011).

## Sampling and Analysis Methods

Mined overburden and groundwater from backfilled panels were sampled at multiple time points. Methods used to obtain data and materials for experiments are described below. This study also relied on sampling and analysis conducted by others [4-6], as well as work that was conducted at the same time as this study [2, 3].

### Overburden Sampling Program

The majority of rock sampling was completed in March 2005 (Appendix A-2) and March 2006 (Appendix A-3). Initial sampling (2005) was limited to archived drill samples from the Dry Valley Mine (hole GW7D2) and near-surface exposures of bedrock and overburden at the Smoky Canyon Mine. Subsequent drilling (2006) at the Smoky Canyon and Enoch Valley mines provided greater access for subsurface sample collection. The method and timing was largely dictated by access to equipment and drilling schedules, and an effort was made to avoid the most difficult weather and road conditions.

2005 Overburden Sampling: A total of 58 samples of overburden were collected in 2005 at the Dry Valley and Smoky Canyon mines (Figure 7). Specifically, 34 rock samples were collected from an archived core of unconsolidated backfill from the Dry Valley Mine groundwater monitoring well GW7D2; geochemical analyses were reported previously for select samples from this drilled hole, as described in Table A2-1 [6]. An additional 24 samples were collected at the Smoky Canyon Mine by excavating portions of the unreclaimed D & E panel backfills, as shown in the photo log provided in

Appendix A-2. Overburden was exposed and sampled in several locations within the limits of safe excavation practice in unconsolidated material (at a depth of 6 to 10 feet, depending upon topography).

Multiple samples of each rock type were collected to represent the range of mineralogical, textural, and geochemical variation in backfilled pits. A sampling protocol is provided in Appendix A-2. Approximately 4 kilograms (kg) of overburden were collected at each location; samples were air-dried and sieved. Due to formation of hard pan surface and aggregates during the air-drying process, samples were passed through a bench top soil flailer (Custom Laboratory Equipment, Orange City, FL) at the MSU Plant Growth Center prior to sieving. Particle gradation analyses were obtained using standard dry sieving methods (after ASTM C136, 2005) with 2 inch, 1 inch, and no. 10, 20, 40, 60, 80, 100 and 200 mesh screens on a sieve shaker. The relative clay and silt-sized fractions were calculated based on hydrometer measurements of the sub-200 mesh fraction for some of the samples. Results of the particle gradation analyses are provided as Table 2-1 and Figures A2-1 and A2-2.

Material was coned (mounded into a pile on a plastic sheet) and divided into quarter splits, and 100 grams (g) of each individual sample was archived. Sub-1/4 inch material was composited for subsequent experimental use and analyzed for total organic carbon (TOC) (APHA Standard Method 5310) and total element content (aqua regia digestion followed by multi-element Inductively Coupled Plasma (ICP) Mass Spectroscopy (MS)) for the shale and chert composites from both mines at ALS Chemex (Appendix A2). More detailed analysis of organic C speciation was completed for the

chert and shale composites from the Smoky Canyon Mine following EPA method 3350 with Gas Chromatography Mass Spectroscopy (GCMS) by EPA method 8270C at Energy Labs in Billings, MT (Appendix A2).

2006 Drilling, Geochemistry and *In Situ* Monitoring Program: This study was conducted in parallel with a broader regional study of overburden geochemistry conducted throughout the S.E. Idaho Phosphate Resource Area by the IMA Phosphate Working Group and its contractors (Appendix A3, [2]). Subsurface samples were collected in 2006 using sonic drilling methods, with control of gas exposure and aseptic technique (see below) for additional lithological, textural, geochemical, moisture, and microbial community characterization. Specific objectives for Tetra Tech/MFG (contractor to the IMA Phosphate Working Group) included characterizing the $O_2$ content within overburden deposits; estimating availability of organic carbon (C) as substrate for microbial activity; characterizing total and leachable Se content of samples; producing an acid-base account for overburden waste; assessing grain size distributions; and sampling for microbiological testing (this study). Four holes, drilled at the Smoky Canyon Mine (Smoky Canyon A dump (SCA) and D dump (SCD)), Enoch Valley Mine (Monsanto Enoch Valley (MEV)), and Rasmussen Ridge Luxor Mine (LUX), were situated to intercept maximum thickness of randomly deposited mine waste in deposits with a range of ages, topographical aspects, duration of weathering, and styles of construction/management. Due to limited resources, only three of these drill holes could be sampled for microbiological analyses; two backfill (SCD and MEV) and one external dump (SCA) locations were thus chosen for this microbial study.

A field sampling protocol was developed for this study in coordination with MFG to provide for rapid, in-field aseptic handling and sampling of all cores (Appendix A-3). Samples were collected and stored to preserve temperature, gas, moisture, and redox conditions to the extent possible. A total of 16 samples were collected for microbial community analysis based on depth and lithology following inspection under nitrogen ($N_2$) gas at the drill site (see photos Appendix A-3). As samples were collected from the core barrel, they were placed into Lexan® plastic bags, labeled and temperature was recorded. Heating of the core within the core barrel varied due to increased friction under rough drilling conditions. Only one sample was excluded from further evaluation based on a temperature that exceeded 37°C. Each interval was placed into the $N_2$-flooded and sanitized (10% bleach followed by 70% ethanol) glove box on sheets of fresh plastic.

The sample bag was opened under $N_2$ gas and the lithology was described in hand specimen for mineralogy, moisture, and clastic content, followed by subsequent laboratory analyses by Tetra Tech/MFG [2]. Sampled intervals were chosen to represent the three principal lithologies (chert, mudstone, and shale) at different depths within both backfilled and external dump overburden facilities. To collect a sample for microbial analysis, the internal core was exposed and sub-sampled using sterilized utensils. These sub-samples were composited, split into several containers for mineralogical, microbial, molecular, and geochemical analysis, and preserved under sterile gas headspace conditions, using filtered air and $N_2$ gas to create oxic and anoxic conditions, respectively. Containers were sealed to conserve moisture and stored in the dark at temperatures at or below the measured average subsurface temperature of 10°C. Samples

stored under aerobic conditions were aerated periodically under sterile conditions or maintained with a filtered port to allow for air exchange.

Samples selected for microbial study from drill holes at the Smoky Canyon Mine backfilled panels D (SCD) and A (SCA), and the Enoch Valley Mine (MEV) were also submitted by Tetra Tech for analysis of TOC (ASA Method 9 29-2.2.4 combustion IR); leachable organic C (EPA SW-846 followed by ASA method above); total Se (EPA SW-846 3050/6020) analyses; leachable Se (EPA SW-846 1312/6020); acid base accounting (EPA M600/2-78-054 1.3); moisture content (ASTM D2216); grain size (ASTM C136, 2005), and soil moisture retention [2].

The holes drilled by MFG were completed with 2-inch PVC well stem in a sand-packed annular space. Nine sections of high density nylon tubing extending to elevations proportional to 10% of the total depth for each hole were attached to the exterior of the PVC casing, for use in monitoring $O_2$ and $CO_2$ concentrations (see TetraTech 2008 report in Appendix A3 for completion details). Well stems were also equipped with a single thermistor for *in situ* temperature measurement. Gas and temperature measurements were made in June and December 2006, see [2] in Appendix A-3.

## Groundwater Monitoring and Sampling

Groundwater was collected at both the Smoky Canyon and Dry Valley mines, for measurement of *in situ* parameters (temperature, pH, Eh, Specific Conductivity (SC), and dissolved $O_2$) and geochemistry (multiple parameters). Native groundwater and live cultures were also collected for use in isolation, enrichment, and culturing studies.

Samples were collected periodically from the three wells (GW7D, GW7D2a, GW7D2b) at the Dry Valley Mine, to obtain live cultures, during site visits that occurred between 2004 and 2008. Samples of groundwater were also collected from well GW11 at the Smoky Canyon Mine, between 2002 and 2007.

Chemical analyses of groundwater were reported independently by the Dry Valley and Smoky Canyon Mines. Tables A1-1 through A1-3 in Appendix A1 summarize groundwater chemistry for wells GW7D, GW7D2a/ GW7D2b, and GW11, respectively. Each of these wells has been monitored over several years (1998 to present) for a suite of regulated parameters. Groundwater chemistry samples were collected following standard operating procedures at each mine site, which are consistent with best management practice for regulatory compliance. Generally, this involved low flow pumping to avoid disturbing sediment or degassing of samples, monitoring of physical and chemical parameters, and appropriate filtration and preservation of samples for analysis within required holding times. Data were managed by third-party contractors and disclosed in operational compliance reports [4, 5]. Monitoring continues at the Dry Valley Mine, but ended in 2007 at the Smoky Canyon Mine when the well head was buried as a result of ongoing backfill construction.

Groundwater collected from these monitoring projects allowed collection of samples for microbial cultivation and collection of sediment for molecular biology studies. Samples for microbial work were collected manually, using a new plastic disposable bailer, sealed in a plastic bag, for each sampling event. Each bailer was rinsed several times with groundwater from the well prior to collection of the sample for

analysis. The bailer was used to stir-up water low in the drill hole and to obtain turbid samples containing both sediment and water from each well. Samples were stored in sterile glass bottles, in the dark, with zero headspace at 4°C. A protocol for collection of groundwater microbial samples is provided in Appendix A1.

## Results – Backfill Hydrogeochemistry

### 2005 Overburden Sampling and Analysis

Overburden samples were collected at the Dry Valley and Smoky Canyon mines in 2005, as described in the field notes and photo log of this work provided in Appendix A-2. A total of 4 chert, 21 shale, 7 mudstone, and 2 limestone samples were collected from GW7D2 at the Dry Valley Mine. An additional 24 samples (5 chert, 12 shale, and 7 mudstone) were collected at the Smoky Canyon Mine, using methods described in Appendix A-2.

Gradation data (sorting of material by particle sizes) for all samples are summarized in Table A2-3 and Figures A2-1 and A2-2. Average gradation data for key rock types from both mines are shown in Figure 11, which indicate dominantly gravel and sandy materials with lesser amounts of silt and clay. As anticipated, gravel content is higher in chert than in shale, and mudstone samples contained higher concentrations of fine silt and clay. Chert samples collected from archived sonic cores were finer grained than those collected via excavation at the Smoky Canyon Mine. The overall lower percentage of fine sediments reported in this study (relative to those reported by Tetra

Tech/MFG for samples collected in 2006) likely suggests differences in sample preparation procedures and may reflect the presence of aggregates in these samples.

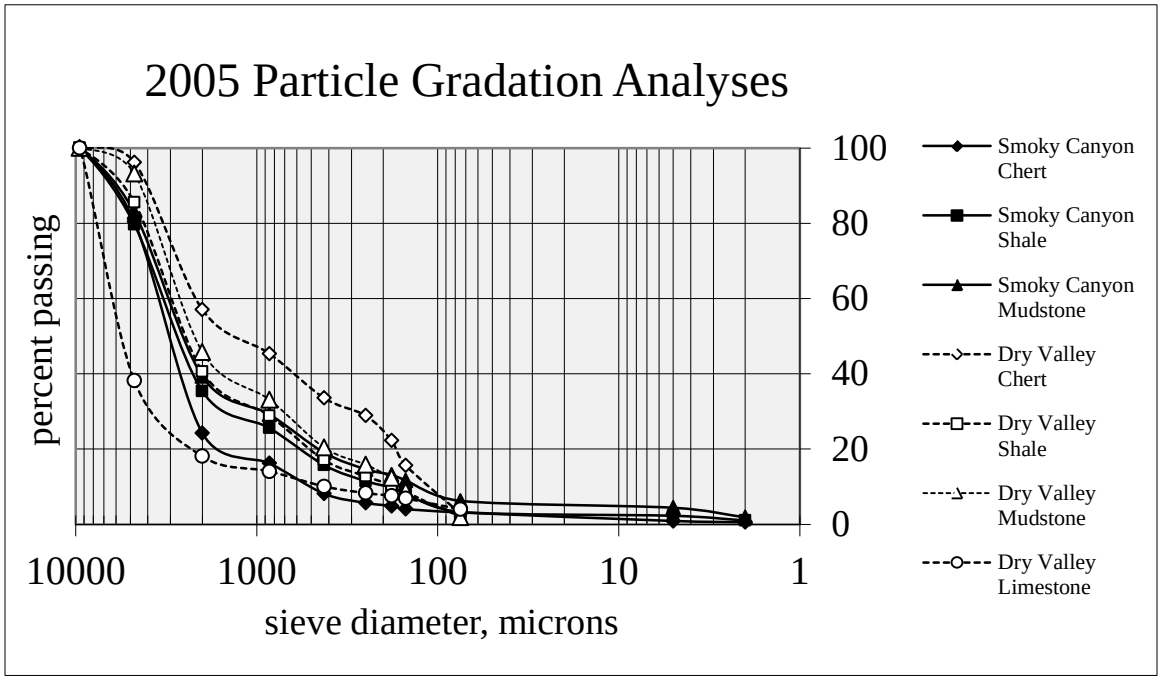



Figure 11. Average particle size distributions for rock samples from Dry Valley and Smoky Canyon mines.

Results of the total element analyses (multi-element ICP following aqua regia digestion) and TOC analyses run for composited chert, shale, and a mixed ROM composite (55% shale, 45% chert, and 10% mudstone) are summarized in Table 1. The data show that shale has higher organic C content than the chert, with values that are comparable between the two mine sites. Sulfur (S) and Se content measured by aqua regia extraction and ICPMS analysis (ALS Chemex) vary between lithology as well, with lower values observed in chert at both mine sites. Iron (Fe) and manganese (Mn) concentrations

extracted by aqua regia vary more subtly between lithology and are consistent between mine sites. Original lab reports for this work are provided in Appendix A2. Analyses of

Table 1. Overburden geochemistry for chert, shale, and run-of-mine rock from Dry Valley Mine and Smoky Canyon Mine D and E panels.

| **Dry Valley Mine - Well GW7D2A** | | | | **chert** | **shale** | **ROM [1]** |
|---|---|---|---|---|---|---|
| **No. of Samples** | | | | **4** | **21** | Composite |
| | **Method** | **Lab** | **Source** | | | |
| **Se, mg/kg** | Aqua Regia/ICPMS MEMS 41 | ALS Chemex | a | 15 | 86 | 56.8 |
| **Mn, mg/kg** | Aqua Regia/ICPMS MEMS 41 | ALS Chemex | a | 345 | 271 | 314 |
| **Fe, wt%** | Aqua Regia/ICPMS MEMS 41 | ALS Chemex | a | 1.61 | 1.68 | 1.65 |
| **S, wt%** | Aqua Regia/ICPMS MEMS 41 | ALS Chemex | a | 0.17 | 0.86 | 0.6 |
| **Soluble Se, mg/L** | EPA 1312/6020 | Energy Labs | b | 0.006 | 0.089 | 0.054 |
| **Soluble Mn, mg/L** | EPA 1312/6020 | Energy Labs | b | 0.228 | 0.082 | 0.137 |
| **Soluble Fe, mg/L** | EPA 1312/6020 | Energy Labs | b | 2.1 | 1 | 1.5 |
| **Soluble Nitrate, mg/L** | 3:1 DI bottle roll extract | MSU Soil Lab | a | 44.3 | 9.2 | nm |
| **Soluble Sulfate, mg/L** | 3:1 DI bottle roll extract | MSU Soil Lab | a | 15 | 413 | nm |
| **Leachable Organic Carbon, mg/L** | EPA method 5310 GCMS | Energy Labs | b | 16.8 | 72.1 | nm |
| **Total Organic Carbon, wt%** | Walkley Black | Energy Labs | b | 0.37 | 3.43 | nm |
| **Smoky Canyon Mine D and E panel excavation - Well GW11** | | | | **chert** | **shale** | **ROM [1]** |
| **No. of Samples** | | | | **15** | **12** | **1** |
| | **Method** | **Lab** | **Source** | | | |
| **Se, mg/kg** | Aqua Regia/ICPMS MEMS 41 | ALS Chemex | a | 8 | 44 | 28.9 |
| **Mn, mg/kg** | Aqua Regia/ICPMS MEMS 41 | ALS Chemex | a | 438 | 289 | 393 |
| **Fe, wt%** | Aqua Regia/ICPMS MEMS 41 | ALS Chemex | a | 1.15 | 1.32 | 1.31 |
| **S, wt%** | Aqua Regia/ICPMS MEMS 41 | ALS Chemex | a | 0.08 | 0.40 | 0.26 |
| **Soluble Se, mg/L** | saturated paste extract | Energy Labs | c | <0.01 | 0.22 | 0.13 |
| **Soluble Mn, mg/L** | saturated paste extract | Energy Labs | c | 0.15 | 0.10 | 0.12 |
| **Soluble Fe, mg/L** | saturated paste extract | Energy Labs | c | <0.1 | <0.1 | <0.1 |
| **Soluble Nitrate, mg/L** | saturated paste extract | Energy Labs | c | 1.66 | 1.75 | 1.45 |
| **Soluble Sulfate, mg/L** | saturated paste extract | Energy Labs | c | 8 | 232 | 135 |
| **Leachable Organic Carbon, mg/L** | 3:1 DI bottle roll extract Method 4500 infrared | MSU | c | 43.7 | 84.5 | nm |
| **Total Organic Carbon, wt%** | saturated paste extract | Energy Labs | c | 0.36 | 4.63 | <0.03 |

SOURCE: (a) This study, (b) TetraTech/Maxim and Geomatrix 2008, (c) Smoky Canyon B & C EIS. Summary of supporting data in Appendix A2.
nm = not measured.
[1]Composite of 15 chert, 12 shale, and 7 mudstone samples to create ROM rock sample, mixed in proportion to percent lithologic occurrence in run-of-mine placement – 55 shale: 35 chert: 10 mudstone.

texture, organic C, total S, and total element analyses of Cd, Mn, and Se in aqua regia extracts were reported for individual samples by Tetra Tech [6], as summarized in Tables A2-3 in Appendix A2. These results generally agree with those reported for this study in Table 1.

Further analysis of organic C content and speciation was conducted for the chert and shale composite from the Smoky Canyon Mine, as summarized in Table 2. The

Table 2. Methylene-chloride extractable compounds from Phosphoria Formation Meade Peak shale and Rex chert composites, Smoky Canyon Mine, S.E. Idaho.

| | **Shale** *mg/kg* | **Shale** *common compounds* | **Chert** *mg/kg* | **Chert** *common compounds* |
|---|---|---|---|---|
| **Alcohol** | *nd* | | 1.2 | hexadecanol |
| **Alkane** | 32.2 | decane, hexane | 9.7 | decane, eicosane |
| **Alkene** | 0.6 | octadecene | *nd* | octadecene |
| **Amide** | 7.7 | decanamide | 3.6 | decanamide |
| **Aldehyde** | 0.5 | octadecenal | 0.4 | dimethyl octenal |
| **Heterocyclic** | 0.3 | azetidine | 0.2 | tetrahydropyran |
| **Monocyclic aromatic** | 14.8 | phthalate, benzene, toluene | 1.9 | phthalate, benzene |
| **Diaromatic** | 9.9 | naphthalene | *bdl* | |
| **Polycyclic aromatic** | 6.0 | dibenzothiophene, phenanthrene | *bdl* | |
| **Solvent Extractable Organic Carbon** | 72.1 | | 16.8 | |
| **Non-Aromatic** | 41.4 | | 15.1 | |
| **Aromatic** | 30.7 | | 1.9 | |
| **Ratio Arom/total** | 43% | | 12% | |
| Summary of Energy Laboratories Report B08051823 provided in Appendix A2. | | | bdl - below detection limit | |

methylene chloride-extractable compounds shown were measured using GC-MS and reported in mg/L. These results demonstrate the greater organic C content of the shale, and show that it contains a higher concentration of aromatic hydrocarbons than the chert. Both chert and shale types contain alkane (e.g. decane), amide (decanamide), aldehyde

(octadecanal), and monocyclic aromatic (phthalate and benzene) compounds, but the shale also contains substantial amounts of naturally occurring polycyclic aromatic hydrocarbons (naphthalene, dibenzothiophene, and phenanthrene).

The 2005 overburden sampling program yielded representative bulk composites of shale, chert, and mudstone from the Dry Valley and Smoky Canyon mines with known composition (mineralogy, Se, Mn, Fe, S and organic C). These samples were not collected with the deliberate intent of minimizing microbial contamination between samples and were not preserved to protect the microbial community. Microbial cross-contamination could not be avoided in the sieving process. Rock samples collected in 2005 were thus not appropriate for microbial analysis. These rock samples were autoclaved (steam sterilized 45 minutes at 121°C (250°F) at a minimum of 15 psi), rested for 48 hours, and re-autoclaved to ensure sterilization prior to use as a substrate for rate experiments performed with live cultures collected through groundwater sampling. It is possible that steam sterilization altered the underlying mineralogy to some degree, but this was deemed unavoidable to maintain experimental control with available resources.

As all experiments were treated equally, the influence of this method was likely uniform throughout the study.

### 2006 Drilling, Microbial Geochemistry, and *In Situ* Monitoring Program

In this study, 15 samples (3 chert, 7 shale, 4 mudstone and 1 limestone) were collected from three of the 2006 sonic drill holes (SCA, SCD, and MEV) for analysis of

Table 3. Overburden samples, *in situ* moisture and $O_2$ content, and select solid phase geochemistry, after (Tetra Tech 2008).

| Location | Sample type | Depth | Lithology | Moisture Content | T | $O_2$ * | $CO_2$ * | TOC | Leachable TOC | Tot Se | Leachable Se | Source |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | feet | | unsaturated, wt% | °C | vol% | vol% | wt% | mg/L | mg/kg | mg/L | |
| **SCD backfill** | **DC5** | 3 | chert | 4.6 | nm | 19.0 | 0.7 | <0.1 | <1 | 3.36 | 0.0009 | b |
| *Smoky Canyon Mine* | **DM50** | 50 | mudstone | 5.1 | nm | 17.7 | 1.0 | 0.2 | <1 | 3.23 | 0.0001 | b |
| | **DS75** | 75 | shale | 15.8 | nm | 17.0 | 1.3 | 2.6 | 1 | 15.70 | 0.0006 | b |
| | **DC123** | 123 | chert | 4.7 | 11.9 | 13.8 | 1.5 | 0.5 | <1 | 3.74 | 0.0042 | b |
| **SCA external dump** | **AS5** | 5 | shale | 6.6 | nm | 19.3 | 0.8 | 4.2 | <1 | 106.00 | 0.0279 | b |
| *Smoky Canyon Mine* | **AS71** | 71 | shale | 15.4 | nm | 11.1 | 5.0 | 5.5 | 2 | 70.80 | 0.0389 | b |
| | **AS113** | 113 | shale | 11.5 | nm | 17.0 | 0.7 | 2.7 | 2 | 35.20 | 0.0053 | b |
| | **AC125** | 125 | chert | 4.3 | nm | 16.7 | 1.0 | 4.3 | 2 | 51.00 | 0.0342 | b |
| | **AM145** | 145 | mudstone | 14.5 | 8.5 | *nd* | *nd* | 0.2 | 1 | 8.59 | 0.0127 | b |
| **MEV backfill** | **MS5** | 5 | shale | 14.4 | nm | *nd* | *nd* | 1.0 | 2 | 7.82 | 0.0009 | b |
| *Enoch Valley Mine* | **MM32** | 32 | mudstone | 18.1 | nm | 2.2 | 6.3 | <0.1 | <1 | 2.31 | 0.0005 | b |
| | **MS73** | 73 | shale | 15.2 | nm | 0.6 | 8.8 | 4.4 | <1 | 63.90 | 0.0022 | b |
| | **MM178** | 178 | mudstone | 10.2 | nm | 0.5 | 9.7 | <0.1 | <1 | 4.28 | 0.0007 | b |
| | **ML 261** | 261 | limestone | 12.2 | nm | 0.6 | 7.7 | <0.1 | <1 | 1.36 | 0.0037 | b |
| | **MS285** | 285 | shale | 24.4 | 10.4 | 0.6 | 9.5 | 2.7 | 2 | 32.40 | 0.0037 | b |
| **Dry Valley** | | | **Date** | | | | | | | | | |
| | **Vapor point** | 5 | Jun-03 | unsat | 18.2 | 1.1 | 7.5 | nm | nm | nm | 0.1260 | a |
| | **Vapor point** | 10 | Jun-03 | unsat | 16.3 | 0.0 | 7.2 | nm | nm | nm | nm | a |
| | **Vapor point** | 20 | Jun-03 | unsat | 14.9 | 0.0 | 6.2 | nm | nm | nm | 0.0260 | a |
| | **Vapor point** | 30 | Jun-03 | unsat | 13.8 | 0.0 | 6.2 | nm | nm | nm | nm | a |
| | **GW7D2B** | 130 | Jun-03 | water table | 9.1 | 1.8 | 2.0 | nm | nm | nm | nm | a |
| | **GW7D2A** | 130 | Jun-03 | saturated | 12.6 | 0.6 mg/L | 2.6 | nm | nm | nm | nm | a |
| | **GW7D** | 112 | Jun-03 | saturated | 7.5 | 0.2 mg/L | 0.2 | nm | nm | nm | nm | a |

Source: a Tetra Tech/Maxim, 2007 b Tetra Tech/MFG, 2008 (Appendix A3) *measured by Tetra Tech/MFG 06/2006 nm – not measured nd – not detected

^

their microbial communities via enrichment culturing and isolation, and culture-independent (molecular) techniques. Multiple additional samples were collected and frozen for additional DNA extraction as needed (Table A3.1). These samples are listed in Table 3, with the results of the geochemical analyses and *in situ* moisture, gas, and temperature data that were subsequently collected from these locations by Tetra Tech and O'Kane Consultants.

*In situ* monitoring data reported for Smoky Canyon from soil suction/temperature sensors, soil moisture content sensors, and lysimeters installed at three *in situ* locations indicate variable moisture content resulting from textural differences between the topsoil and chert layers [3, 11]. Topsoil had elevated water content (18 to 20%), with chert values increasing from 10-12% up to 12-14% (depending upon depth) throughout four months of monitoring between October 2006 and January 2007. Water content in the deepest portion of monitored ROM overburden (at a depth of approximately 72 inches), at a location with relatively low net percolation, the volumetric water content ranged from 18% during the late winter and early spring to a low of 8% during thesummer and fall. Under higher net percolation conditions, the volumetric water content in the deepest sensor ranged from a maximum value of 30% to a low of 16% [12]. More steady state conditions, with less seasonal variation, would be expected with depth, except where changes in grain size and compaction affect capillarity and moisture retention. These data indicate the presence of dominantly unsaturated conditions below the cover at the monitored locations.

Groundwater Monitoring

Multiple analyses of groundwater were reported by Agrium and Simplot for the studied monitoring wells; these results are summarized in Table 4 and provided in greater detail in Tables A1-1 through A1-3 included in Appendix A1. These results show increased $SO_4^{2-}$, $SeO_4^{2-}$, and $O_2$ concentrations in GW11, and lower Fe and pH, relative to the groundwater at the Dry Valley Mine that was measured in wells GW7D, GW7D2a and GW7D2b. Soluble Mn and $NO_3^-$ concentrations were approximately the same.

## Discussion – Backfill Hydrogeochemistry

Moisture and gas concentrations varied substantially throughout backfilled overburden in the S.E. Idaho Phosphate Resource Area. Saturated conditions existed deep in the backfill at the Dry Valley Mine, within a confined zone above the regional aquifer. Moisture contents close to or exceeding predicted field capacity based on particle gradation (22-27%) were also identified at the Enoch Valley (24%) and Luxor (22.6%) mines low in the backfill, but above zones of unsaturated rock (with moisture contents ranging from 6 to 14%), suggesting that perched aquifers with suboxic characteristics may also exist within the lower backfill at those locations. Moisture contents for other samples from the four monitored holes studied by Tetra Tech range from 4 to 19% moisture, and are dominantly unsaturated, with some minor variation in water content up to field capacity in rare instances[2]. Higher moisture contents, though still unsaturated, tended to be in the mid-depth intervals. No water was collected from any of the drill holes, although wells were completed for potential future collection of groundwater.

Table 4. Summary of study area hydrogeochemistry.

| **Site History** | **Sample** | **Lithology** | **Well depth** | ***In Situ* Parameters[4]** | | | | | | **Aqueous Chemistry[6]** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *media type* | | | **GW depth** | **$O_2$** | **$O_2$** | **$CO_2$** | **T** | **Moisture** | **pH** | **$SeO_4^{2-}$** | **$SO_4^{2-}$** | **N as $NO_3^-$** | **Fe[5]** | **Mn[5]** |
| | *name* | *in drill hole* | **ft** | **ft** | mg/L | vol% | vol% | **°C** | | | **mg/L** | **mg/L** | **mg/L** | **mg/L** | **mg/L** |
| **Dry Valley Mine** | | | | | | | | | | | | | | | |
| wells in plug-dumped and capped backfill fully saturated in 2000, then drained to field capacity | | | | | | | | | | | | | | | |
| **GW7D** | **gw** | ROM/ mix | 172 | 134 | 0.35 | nm | nm | 7.0 | Saturated below 172 | 6.6 | 0.033 | 629 | 1.30 | 0.05 | 0.35 |
| **GW7D-2a** | **gw** | ROM/ mix | 180 | 136 | 0.20 | nm | nm | 11.0 | Saturated below 150 | 7.8 | 0.016 | 705 | 0.23 | 0.20 | 0.44 |
| saturated below 136 feet, screened below water table | | | | | | | | | | | | | | | |
| **GW7D-2b** | **gw** | ROM/ mix | 150 | 136 | 0.30 | nm | nm | 9.8 | Saturated below 150 | 7.6 | 0.001 | 834 | 0.03 | 12.35 | 1.77 |
| Screened at water table during completion | | | | | | | | | | | | | | | |
| **Smoky Canyon Mine** | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | |
| **GW11** | **gw** | ROM/shale | 85 | 80 | 5.50 | nm | nm | 7.0 | Saturated | 6.5 | 1.010 | 1666 | 5.58 | 0.004 | 0.44 |
| intermittent saturation | | | | | | | | | field capacity | | | | | | |
| **SCD Backfill** | | **Drillhole SCD** | | >t.d. | nm | 15-20 | 1-5 | 8.5 | 5-18% | Bottle roll chemistry extracts for backfill samples 2.75:1 dilution by mass | | | | | |
| | **DC5** | chert | 5 | nm | nm | nm | nm | nm | | 8.6 | 1.9 | 173 | 0.34 | 0.01 | <0.1 |
| | **DM50** | mudstone | 50 | nm | nm | nm | nm | nm | | 7 | 1.5 | 328 | 0.24 | 0.15 | 0. 12 |
| placed as mined, end dumped and capped | **DS75** | shale | 75 | nm | nm | nm | nm | nm | un-saturated | 7.2 | 1.3 | 230 | 2.68 | 0.07 | <0.1 |
| | **DC123** | chert | 123 | nm | nm | nm | nm | nm | | 8.1 | 1.7 | 784 | 0.53 | 0.04 | 0.01 |
| **SCA External Dump** | | **Drillhole SCA** | | >t.d. | nm | 15-20 | 0.5-2 | 12.0 | 5-18% | Bottle roll chemistry extracts for backfill samples 2.75:1 dilution by mass | | | | | |
| | **AS5** | shale | 5 | nm | nm | nm | nm | nm | | 6.9 | nd | nd | nd | nd | nd |
| Run-of-mine rock, | **AS71** | shale | 71 | nm | nm | nm | nm | nm | | 7 | nd | nd | nd | nd | nd |

Table 4. Summary of study area hydrogeochemistry, continued.

| **Site History** | **Sample** | **Lithology** | **Well depth** | ***In Situ* Parameters[4]** | | | | | | **Aqueous Chemistry[6]** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *media type* | | | **GW depth** | **$O_2$** | **$O_2$** | **$CO_2$** | **T** | **Moisture** | **pH** | **$SeO_4^{2-}$** | **$SO_4^{2-}$** | **N as $NO_3^-$** | **Fe[5]** | **Mn[5]** |
| | *name* | *in drill hole* | **ft** | **ft** | mg/L | vol% | vol% | **°C** | | | **mg/L** | **mg/L** | **mg/L** | **mg/L** | **mg/L** |
| end dumped and capped | | | | | | | | | | | | | | | |
| | **AS113** | shale | 113 | nm | nm | nm | nm | nm | un-saturated | 7.7 | 1.7 | 1519 | 0.24 | <0.01 | 0.01 |
| | **AC125** | chert | 125 | nm | nm | nm | nm | nm | | 7.4 | 1.6 | 1183 | 0.22 | <0.01 | 0.01 |
| | **AM145** | mudstone | 145 | nm | nm | nm | nm | nm | | 7.2 | 1.8 | 148 | 0.14 | 0.02 | 0.25 |
| **Enoch Valley Mine** | | | | | | | | | | | | | | | |
| **MEV backfill** | | **Drillhole MEV** | | >t.d. | nm | <0.5 below 60 ft | 5-10 | 10.4 | 10-25% | Bottle roll chemistry extracts for backfill samples 2.75:1 dilution by mass | | | | | |
| | **MS5** | shale | 5 | nm | nm | nm | nm | nm | | 7.7 | 1.7 | 631 | 0.09 | 0.05 | 0.007 |
| placed as mined, end dumped and capped | **MM32** | mudstone | 32 | nm | nm | nm | nm | nm | un-saturated | 8.6 | 1.7 | 797 | 0.12 | <0.01 | <0.1 |
| | **MS73** | shale | 73 | nm | nm | nm | nm | nm | | 7.7 | nd | nd | nd | nd | nd |
| | **MS178** | shale | 178 | nm | nm | nm | nm | nm | | 8.2 | 1.7 | 624 | 0.19 | 0.04 | 0.008 |
| | **MS285** | shale | 285 | nm | nm | [2]nm | nm | nm | | 7.2 | 1.7 | 2465 | 0.12 | 0.02 | <0.1 |

[1] from Table 3-1, p. 40, Maxim 2007
[2] from Table 3-3, p. 41, Maxim 2007.
[3] data reported by Simplot for sample collected 10/2003.
[4] from MFG Preliminary Geochemical Characterization of Overburden except Dry Valley data, reported by Maxim, 2007, Appendix F. Values were reported for selected intervals that do not correspond with samples used for this study. For this reason, overall conditions observed in wells are summarized for each well rather than for discrete intervals.
[5] dissolved, filtered 0.45 um, analysis by ICP.
[6]Chemistry reported for rock samples for bottle roll extracts.
nm = not measured; gw = groundwater; <t.d. depth to water greater than total depth of well

Oxygen content varied from atmospheric to non-detectable levels depending on location. Oxygen was measured at generally atmospheric (or slightly lower levels) throughout the overburden at the Smoky Canyon Mine in both SCA and SCD, but was less than 0.2% volume within 10 feet of the overburden surface at the Dry Valley, Enoch Valley, and Luxor mines backfills. This volume is inferred to be due to differences in the method of dump construction, with end-dumped materials more open to barometric pumping and ongoing gas exchange with the atmosphere than dumps constructed in discrete lifts [2]. Dumps that were built in lifts appear to have more limited exchange of $O_2$ with the atmosphere, and any $O_2$ introduced during backfill construction has been depleted, presumably through biological or chemical oxidation processes. Temperature at depth in the monitored wells ranged from 8.5 to 11.9°C, which is typical of conditions in the shallow subsurface, with minor seasonal variation.

Geochemical analysis of Se leachability indicated generally low rates of Se release (compared to whole rock Se content) in all of the samples, although there was some variability that could be lithology or moisture dependent. Selenium leachability does not appear to be $O_2$ dependent (e.g., greater at the Smoky Canyon Mine than at other locations) in the relatively dry materials of the monitored sites. Apparent differences in Se leachability under unsaturated and saturated conditions may also be related to seasonal changes in groundwater flow. They may also reflect microscale changes in redox conditions resulting from biological activity.

The Tetra Tech study provides excellent geochemical background data for this investigation [2]. Acid-base accounting data, which are not summarized here, indicate

low levels of sulfide and abundant carbonate mineralization, with overall net neutralizing conditions throughout the backfill. Although total C varied between lithologies in all three drill holes, the dissolved organic C concentrations were stoichiometrically adequate to support microbial activity based on observed electron acceptor concentrations within the sampled backfill and dump [2]. The trace element and C content of chert and shale differ from one another, but are relatively consistent when compared between locations at the Dry Valley, Smoky Canyon, and Enoch Valley Mines. Key differences between the lithologies include total Se, S, Fe and organic C content (especially, aromatic C content). Backfill seems to have elevated concentrations of both Mn and $NO_3^-$, with some variability.

Groundwater chemistry described at these sites varied with $O_2$ availability in the backfill, with higher concentrations of $SO_4^{2-}$ and $SeO_4^{2-}$ (and lower pH and concentrations of Fe) present in water with detectable $O_2$. Concentrations of $SO_4^{2-}$ and $SeO_4^{2-}$ were higher, and dissolve Fe was lower, at Smoky Canyon in monitoring well GW11 than at Dry Valley, in GW7D or GW7D2a/2b, where $O_2$ levels were lower.

## *In Situ* Conditions Considered in Experimental Designs

The variation in lithology (including mineralogy, geochemistry, and organic C speciation), moisture content, and $O_2$ is potentially significant for Se reduction, as described in the conceptual models presented in Chapter 2. Shale, chert, and mudstone were placed randomly as backfill into mined-out panels, and contain variable amounts of total and soluble Se, S, Fe, Mn, and $NO_3^-$. Sulfate measured in groundwater reflects the

ongoing process of sulfide oxidation within these neutral to alkaline deposits. Oxygen ranged from atmospheric to below detection within backfill, apparently controlled by changes in gas flux at the facility scale and driven by differences in backfill placement methods. Moisture content varied considerably, from the as-mined values of 2 to 4% in coarse cherts to near field capacity in shales, and saturated conditions existed where panels extended below the groundwater table. In fact, the only consistencies between the facilities were the random variation of mixed waste rock mined from the same stratigraphic section of the Meade Peak member of the Phosphoria Formation and the temperatures, which were consistently between 8 and 12°C.

In spite of the complex setting, and the subtle effects of moisture, lithology, and $O_2$ controls on *in situ* Se biogeochemistry, the combination of conditions developed at the Dry Valley Mine has been sufficient to support *in situ* reduction of soluble Se and $NO_3^-$ for more than ten years. This study explores the biogeochemical processes that influence the rate and extent of this reduction. The potential to develop similarly reducing conditions through intentional design at other mine sites, once the required conditions have been adequately defined, is high. Although hydrocarbon degradation is likely to be slow in cold, subsurface environments, the large mass of available C and the slow flux of water within the backfill environments (with residence time estimated on the order of years) suggests that a sustainable process of C mineralization coupled to anaerobic reduction of $NO_3^-$, $Fe^{3+}$, and/or $Mn^{4+}$ could support long-term *in situ* $SeO_4^{2-}$ reduction. Demonstration of natural attenuation as a means of operational source control of Se within phosphate backfill requires documentation of biological processes that promote Se

reduction, within the complex biogeochemical environment of backfilled phosphate overburden. The experiments described in the following chapters were designed to integrate the *in situ* characterization results with a conceptual understanding of Se reduction at both the micro and facility scales. Integration of the *in situ* data with the conceptual models described in Chapter 2 offers a framework, described below, for experimental study of Se reduction within backfilled phosphate overburden in the S.E. Idaho Phosphate Resource Area.

*Samples.* To address the hypothesis that similar conditions for Se reduction could be developed throughout the S.E. Idaho Phosphate Resource Area, rock and groundwater samples were collected for study at three mine sites located (approximately) along a 30 mile transect from NW to SE through the region.

*Lithology and Oxygen as Key Variables.* Lithology, C content, trace element geochemistry, and mineralogy of substrate within backfilled mine waste varies, due to random placement of ROM waste rock (55% shale, 35% chert, and 10% mudstone) during mine backfill operations. Rock that is randomly backfilled into mined pits weathers as moisture infiltrates from the surface, transporting $O_2$ that is progressively consumed through oxidation of reduced mineral phases and aerobic metabolic processes. Changes in pore size within variably graded rock influence gas exchange and the potential for preferential flow. Availability of $O_2$ is determined by the balance between the relative rate of recharge and consumption processes, and therefore, $O_2$ availability and moisture content vary within the randomly placed backfill. Shale, with its finer grained texture and higher organic content was hypothesized to support more Se-reducing

bacteria (SeRB) and faster Se reduction than chert. Sampling and experimental protocols for estimating total numbers of SeRB, and for batch reactors, therefore addressed lithology and $O_2$ availability as primary variables.

*Native Rock and Groundwater Substrates*. Selenium, along with $SO_4^{2-}$, is mobilized through oxidation of primary (depositional) sulfide and selenide minerals. Secondary (alteration) Se-hosted minerals and soluble Se oxyanions contribute Se to solution via dissolution and/or desorption from exposed mineral surfaces. Phosphate from phosphorite mineralization may also be soluble, along with Fe and Mn. As these elements have potential to inhibit or support Se reduction by microbes, concentration changes in these elements were monitored as dependent variables in rate experiments. Experiments that address the number and identity of native Se-reducing organisms, under both aerobic and anaerobic conditions, and the rate of Se reduction, were therefore completed using native rock and groundwater to provide representative $SO_4^{2-}$, $NO_3^-$, $Fe^{3+}$, $Mn^{4+}$, and $PO_4^{3-}$ conditions.

*Saturated Experimental Focus*. Selenium transport from backfill into groundwater conceptually occurs most significantly under saturated conditions, when $SeO_4^{2-}$ is transported in groundwater from oxidized surface sediments into more reduced and compacted sediments within the backfill. As water moves deeper, $O_2$ is depleted and denitrifying conditions begin to dominate. Studies of Se reduction rates were therefore conducted under saturated, microaerophilic conditions.

*Electron Donors and Acceptors*. Hydrocarbon degradation pathways would also be expected to shift from aerobic to denitrifying and anaerobic pathways, with

progressively more involvement of Fe and Mn elemental cycling under anaerobic conditions. Under these hypothesized conditions, redox potential is lower, enhancing the stability of reduced Se phases. Simpler forms of C (such as decane or hexane) formed by degradation of more complex aromatic hydrocarbon compounds would continue to be produced to support $SeO_4^{2-}$ reduction. Transition from aerobic to microaerophilic or anoxic conditions implies the existence of a mixed community of microorganisms capable of aerobic, facultative, or obligate anaerobic metabolisms, using available C compounds as electron donors coupled with the reduction of a variety of possible electron acceptors ($O_2$, $Mn^{4+}$, $NO_3^-$, $Fe^{3+}$, $SeO_4^{2-}$, $SeO_3^{2-}$ or $SO_4^{2-}$). Changes in the microbial community, in response to changing $O_2$, $Mn^{4+}$, $NO_3^-$, $Fe^{3+}$, and $SO_4^{2-}$ concentrations measured in batch reactors, were described to evaluate this possibility.

*Ca-$HCO_3$-$SO_4^{2-}$ Groundwater Geochemistry.* The geochemistry of the phosphate wastes is dominantly alkaline, with Ca-$HCO_3$-$SO_4^{2-}$ rich groundwater, although sulfide oxidation within the sediments presumably generates local acidity at the pore scale, which is subsequently neutralized by available carbonate. The pH was allowed to vary independently in the experiments presented here. It was documented in bottle roll extracts used to develop media for the enumeration and isolation of microorganisms, as well as batch reactors.

*Temperature.* Temperatures ranged from an average of 10 °C in the subsurface to 25 °C or more, seasonally, at the land surface. Temperatures of 10 °C and 25 °C were imposed in rate experiments, to evaluate the influence of this range of temperatures on $SeO_4^{2-}$ reduction.

*Experimental conditions.* The experimental conditions imposed in the microbial isolations, enumeration studies, and rate experiments (Table 5) were designed to account for the observed variation in lithology, $O_2$, moisture content, pH, soluble $SO_4^{2-}$, $NO^{3-}$, Fe, and Mn concentrations.

All three lithotypes (chert, shale and mudstone) were used for these studies. Carbon was added to these experiments to ensure that the maximum possible number of $SeO_4^{2-}$-reducing organisms was represented. Enrichments were prepared under the

Table 5. Experimental designs based on subsurface backfill conditions.

| **Experiment** | **Lithology** | **Carbon** | **$O_2$** | **T** | **light** | **moisture** |
|---|---|---|---|---|---|---|
| Rock samples | chert, shale, mudstone | none added | $O_2$, $N_2$ | <10°C | dark | See table 3 |
| Most probable number | chert, shale, mudstone | 2 mM cocktail of lactate-acetate-pyruvate -$SeO_4$ | $O_2$, $N_2$ | 10°C | dark | saturated |
| Enrichments | chert, shale, mudstone | 2 mM cocktail of lactate-acetate-pyruvate -$SeO_4$ | $N_2$ | 10°C 25°C | dark | unsaturated, saturated |
| Rate Experiments | chert, shale, ROM | native only | $N_2$ | 10°C, 25°C | dark | saturated |

conditions described in Table 5, with a cocktail of simple C compounds including lactate, acetate, and pyruvate to stimulate growth for subsequent molecular studies. The most probable number (MPN) enumeration studies were performed under both aerobic and anaerobic conditions, at the field relevant temperature of 10°C and in the dark. The results of enumeration studies indicated that little, if any, $SeO_4^{2-}$ reduction occurred under aerobic conditions, and showed that the number of $SeO_4^{2-}$-reducing organisms was lower in unsaturated samples of chert and mudstone than in shale. Based on these MPN

results, subsequent rate experiments focused on anaerobic conditions in chert, shale, and ROM rock.

An understanding of the rate at which native organisms in the different lithologies can reduce $SeO_4^{2-}$ under microaerophilic to anaerobic conditions under controlled temperature conditions is essential to the design of pilot scale facilities with sufficient residence time to support *in situ* reduction as an operational method of Se source control. To address the hypothesis that Se reduction would proceed most rapidly, efficiently, and permanently in shale rather than chert or mixed waste under field temperatures, rate experiments were conducted for individual lithotypes under controlled temperature; known total Fe, Mn, $NO_3^-$, $SO_4^{2-}$, and $PO_4^3$; and variable $O_2$, pH, and C availability conditions in closed systems.

Measurements of C use and changes in biomass, as well as changes in pH and concentrations of soluble potential competitive electron acceptors, were used to describe the process and capacity for microbial reduction of soluble Se. Similarly, measurements of Se speciation and biomineralization products during the reduction process were made.

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# CHAPTER FOUR

## SUBSURFACE MICROBIAL SELENIUM REDUCTION BY NATIVE CONSORTIA IN PHOSPHATE MINE WASTE, SE IDAHO

### Contribution of Authors and Co-Authors

Manuscript in Chapter 4

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Contributions: principal author.

Co-author: Jared J. Bozeman

Contributions: lab assistant. Developed clone libraries.

Co-author: Susan E. Childers, PhD

Contributions: co-major advisor on microbial ecology, microbiology. Contributed a number of isolates, critical review of molecular work.

Manuscript Information Page

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# Subsurface microbial selenium reduction by native consortia in phosphate mine waste, S.E. Idaho

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## Abstract

A consortium of native microbes with potential for $SeO_4^{2-}$ reduction in subsurface mine waste at several S.E. Idaho phosphate mines were identified and enumerated under a range of field-relevant oxygen ($O_2$), moisture, and lithology conditions. Samples of groundwater and unsaturated sediments collected from the subsurface were used to isolate Se-tolerant and Se-reducing microorganisms from the overburden backfill. A most probable number (MPN) method was used to estimate the number of selenium-reducing bacteria (SeRB) in groundwater, chert, shale, and mudstone samples, and both cultivation and molecular methods were used to identify bacteria present in the most dilute positive MPN cultures. Bacterial clone libraries were developed for the two samples of shale with the highest estimated numbers of SeRB, and changes in microbial diversity as a function of lithotype and moisture conditions were compared using denaturing gradient gel electrophoresis (DGGE). The most favorable conditions for Se reduction appear to be in saturated or moist conditions (close to field capacity) where sufficient soluble Se and organic carbon is available to support higher numbers of SeRB. Molecular analysis of community structure in saturated and unsaturated sediments show 16S rRNA sequences with high similarity to known anaerobic and aerobic hydrocarbon-degrading genera *Polaromonas* and *Rhodoferax*. SeRB reduce $SeO_4^{2-}$ using complex naturally-occurring hydrocarbon compounds and potentially other electron donors under $Fe^{3+}$, $Mn^{4+}$ and $NO_3^-$ reducing conditions. It is proposed that degradation of complex shale hydrocarbons by aerobic and facultative anaerobic members of the community decreases available $O_2$, thus creating conditions favorable for $SeO_4^{2-}$ reduction by SeRB with high similiarity to the genera *Dechloromonas*, *Stenotrophomona*, *Anaeromyxobacter* and other hetrotrophic SeRB. This characterization of the indigenous $SeO_4^{2-}$-reducing community can be used to evaluate design options for *in situ* microbial source control of Se at phosphate mine operations.

## Introduction

Control of selenium (Se) concentrations in mine drainage is a concern for phosphate producers at several mine sites in the S.E. Idaho Phosphate Resource Area (Figure 12). Selenium is a naturally-occurring metalloid that is biologically essential in small doses, but toxic at higher concentrations [1]. The Rex chert and Meade Peak shale and mudstone members of the Permian Phosphoria Formation are mined as overburden

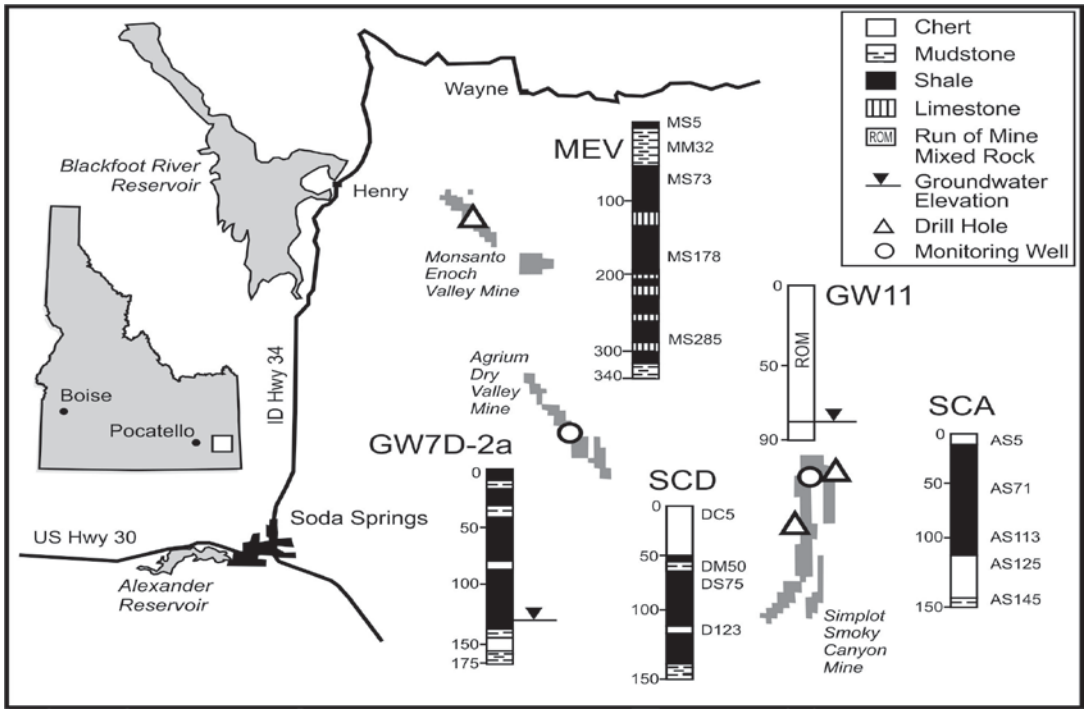



Figure 12. Location of 3 sampled drill holes and 2 monitoring wells in the S.E. Idaho Phosphate Resource Area. Depth in feet to exposed lithologies and water in each hole are illustrated. Rock sample locations are indicated to the right of each drill hole at the corresponding sampled depth; the ID reflects the sampling location and rock type, e.g. MS5 represents M for Monsanto's Enoch Valley drillhole MEV, S for Shale, and 5 for 5 feet of depth. Likewise, SCA represents Smoky Canyon drillhole A and SCD represents Smoky Canyon drill hole D. Groundwater monitoring wells are numbered as shown, GW7D-2a (Dry Valley) and GW11 (Smoky Canyon).

waste during phosphate extraction. Selenium associated with host minerals is released by oxidation and leaching of phosphatic mine overburden and persists as soluble selenate ($SeO_4^{2-}$) and biselenite-selenite ($SeO_3^{2-}$-$HSeO_3^-$) species under the neutral to alkaline conditions that characterize the near-surface geochemical environment [2]. Soluble Se concentration in these settings varies, depending on site-specific moisture content, oxygen ($O_2$) availability and biogeochemistry of overburden lithologies (Table 6) [3].

Groundwater monitoring in backfilled sediments at two mine locations, Smoky Canyon (GW11) and Dry Valley (GW7D2a), exhibited a 50-fold difference in soluble Se concentrations (Table 6) despite the fact that both wells were completed in mixed Phosphoria shale (55%), chert (35%) and mudstone (10%) waste produced using similar mining methods. The average Se concentration was highest in the shale, where Se substituted for sulfur (S) in sulfide minerals in unweathered rock and occurred as sorbed oxyanions or elemental selenium ($Se^0$) in weathered material. USGS reports Se values ranging from 1 to 1040 mg/kg for the Meade Peak member of the Phosphoria Formation, with an average value of 28 mg/kg in altered rock, and 82 mg/kg in unweathered shale [4]. In contrast, the Rex chert contains between <0.2 and 138 mg/kg Se, with an average value of 7 mg/kg [5]. Carbon content and speciation also varies between lithologies. Although some rock is placed in external dumps, most overburden is randomly placed as internal backfill into mined-out panels (or pits), creating complex subsurface hydrogeochemical environments that vary in moisture and $O_2$ content (Table 6, after TetraTech, 2008). Abiotic mechanisms known to control concentrations of Se oxyanions are limited in the neutral pH to alkaline pH range relevant in these settings. Mineral

Table 6. Summary of background conditions in S.E. Idaho phosphate overburden, *in situ* groundwater and rock geochemistry

| | | | | ***in situ* conditions** | | | | **Groundwater Chemistry** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Location** | **Sample** | **Depth feet** | **Rock Type** | **T ˚C** | **Moisture Content** | ***in situ* $O_2$ mg/L** | **pH** | **$NO_3^-$ mg/L** | **Dissolved Se µg/L** | **$SO_4^{2-}$ mg/L** | **DOC mg/L** | **Total Dissolved Fe mg/L** | **Total Dissolved Mn mg/L** |
| *Dry Valley Mine* | | | | | | | | | | | | | |
| **GW7D-2a, 6/12/2007** | **GW** | **180** | ROM | 9.8 | Saturated | 0.20 | 7.8 | 0.3 | 0.021 | 710 | 9.98 | 0.2 | 0.47 |
| *Smoky Canyon Mine* | | | | | | | | | | | | | |
| **GW11, 6/1/2007** | **GW** | **90** | ROM | 7 | Saturated | 5.50 | 6.5 | 5.60 | 1.010 | 1666 | nd | 0.004 | 0.44 |
| | | | | ***In Situ* Conditions** | | | | | **Rock Geochemistry** | | | | |
| **Location** | **Sample** | **Depth, feet** | **Rock Type** | **T°C** | **Moisture Content wt%** | **$O_2$ vol%** | **$CO_2$ vol%** | **Tot Se mg/kg** | **Leach Se µg/L** | **S wt %** | **DOC mg/L** | **TOC %** | |
| **SCD backfill** | | | | | | | | | | | | | |
| *Smoky Canyon Mine* | **DC5** | **5** | chert | nd | 4.6 | atm | atm | 3.4 | 0.9 | <0.01 | nd | <0.1 | |
| | **DM50** | **50** | mud | nd | 13.0 | 17.7 | 1 | 3.2 | 0.1 | 0.06 | <1 | 0.2 | |
| | **DS75** | **75** | shale | nd | 15.8 | 17.4 | 1.1 | 31.8 | 1.1 | 0.42 | 1 | 3.2 | |
| | **DC123** | **123** | chert | 11.9 | 4.7 | 14.8 | 2.1 | 3.7 | 4.2 | 0.10 | <1 | 0.5 | |
| SCA external dump | | | | | | | | | | | | | |
| *Smoky Canyon Mine* | **AS5** | **5** | shale | nd | 6.6 | atm | atm | 70.8 | 38.9 | 0.58 | 2.0 | 5.5 | |
| | **AS71** | **71** | shale | nd | 15.4 | 14.1 | 9.8 | 35.2 | 5.3 | 0.45 | 2 | 2.7 | |
| | **AS113** | **113** | shale | nd | 11.5 | 18.3 | 0.9 | 51 | 34.2 | 0.54 | 2 | 4.3 | |
| | **AC125** | **125** | chert | nd | 4.3 | 18.2 | 1.4 | 8.6 | 12.7 | 0.06 | 1.0 | 0.5 | |
| | **AM145** | **145** | mud | 8.5 | 14.5 | nd | nd | 1.3 | 0.6 | <0.01 | 1 | 0.1 | |
| MEV backfill | | | | | | | | | | | | | |
| *Enoch Valley Mine* | **MS5** | **5** | shale | nd | 15 | atm | atm | 7.8 | 0.9 | 0.06 | 2.0 | 1.0 | |
| | **MM32** | **32** | mud | nd | 18.0 | 9.2 | 6.6 | 2.3 | 0.5 | nd | nd | nd | |
| | **MS73** | **73** | shale | nd | 15.2 | 0.5 | 9.4 | 63.9 | 2.2 | 0.64 | nd | 4.4 | |
| | **MM178** | **178** | mud | nd | 10.2 | 0.5 | 9.6 | 4.3 | 0.7 | nd | nd | nd | |
| | **MS285** | **285** | shale | 10.4 | 24.4 | 0.5 | 9.8 | 32.4 | 3.7 | 0.38 | 2 | 2.7 | |

nd =not detected atm = atmospheric

ROM is run-of-mine mixture of lithologies incl. shale, chert, and mudstone. Sample ID based on drill hole, material type and depth e.g., SCD chert 5 ft = DC5.

Groundwater chemistry as reported by TetraTech 2007, $O_2$, $CO_2$ (vol%) measured in situ Aug 2006[3], Total S% by LECO, Sobek 1978

Total Selenium by method SW-846 3050/6020, Leachable Selenium by method SW-846 1312/6020 [3]

Total Organic Carbon (TOC) by method ASA9-29-2.2.4 [3]

Dissolved Organic Carbon (DOC) by SW-846 followed by ASA 9-29-2.2.4 [3]

solubility is unlikely to control dissolved Se concentrations, and sorption onto metal oxide, carbonate, and clay minerals is likely to be inefficient [6, 7]. Neither mechanism could be shown to fully explain the differences observed between the two wells, suggesting that biotic mechanisms may control soluble Se concentrations.

Abiotic reduction of Se(VI), the oxyanion $SeO_4^{2-}$, to Se(IV), which occurs as either $HSeO_3^-$ or $SeO_3^{2-}$ depending upon pH, is kinetically limited and proceeds very slowly except when catalyzed by green rust [8, 9] or Se-reducing microorganisms (SeRB)[10]. SeRB are phylogenetically diverse and include many *Bacteria* and some *Archaea* and *Eukarya* that can detoxify or respire $SeO_4^{2-}$ or $SeO_3^{2-}$. Substantial energy can be gained through respiratory reduction of $SeO_4^{2-}$ to $SeO_3^{2-}$ by strict and facultative anaerobes [11], including *Thauera selenatis* [12], *Sulfurospirillum barnesii* [13], *Pelobacter seleniigenes [14]*, *Selenihalanaerobacter shriftii* [15], *Citrobacter* sp. strain JSA [16], and several *Bacillus* species[17]. Selenium reduction along inducible respiratory pathways is not always growth-dependent [18, 19]. A few bacterial genera capable of aerobic $SeO_4^{2-}$ reduction have also been identified [20, 21]. Reduction of $SeO_4^{2-}$ for detoxification purposes appears to occur using a specific of $SeO_4^{2-}$ reductase in *E. cloacae* SLD1a1 [22], but otherwise involves non-substrate specific enzymes associated with $NO_3^-$ and $NO_2^-$ [19] or $SO_4^{2-}$ reduction[23, 24]. While $SeO_3^{2-}$ reduction to Se(0) or Se(-II) is less energetically favorable than $SeO_4^{2-}$ reduction, this strategy is used for detoxification by a variety of organisms.

Some organisms can reduce $SeO_4^{2-}$ fully to Se(0) or Se(-II), while others will only reduce $SeO_4^{2-}$ to $SeO_3^{2-}$ or $SeO_3^{2-}$ to Se(-II). Complete reduction of soluble oxyanions to

insoluble $Se^0$ or $Se^{2-}$ minerals can thus depend on community-level interactions between multiple organisms [25].

Previous work on microbial Se transformations in phosphate mine wastes from the Smoky Canyon Mine identified a variety of $SeO_4^{2-}$-reducing microorganisms using cultivation and molecular methods [26]. Using near surface samples from the Smoky Canyon Mine, the microbial community was evaluated along with its potential to influence Se speciation in response to the application of iron (Fe), compost, and whey amendments as potential bioremediation treatments. A number of isolates were identified, based on their ability to reduce $SeO_4^{2-}$, and were found to belong primarily to the *Enterobacteriaceae* family, although other gamma- and betaproteobacteria were found, including members of the *Aeromonadaceae, Comamonadaceae, Oxalobacteraceae,* and *Rhodocyclaceae* families. Knotek-Smith, et al. [26] concluded, based on amended column experiments, that Fe amendment to promote the precipitation of insoluble Fe-selenide minerals is the preferred strategy for remediation of $SeO_4^{2-}$ in phosphate waste [27]. Of common interest to the present study was the identification of $SeO_4^{2-}$ reduction by members of the *Rhodoferax* and *Rahnella* genera in laboratory experiments, and the conclusion that further study of microbial populations in environmental samples was needed to resolve questions about $SeO_4^{2-}$ mobility in a mine waste setting [26].

The objectives of the present study were to (1) characterize the indigenous microbial population involved in the Se reduction observed in backfill at the Dry Valley Mine, and (2) to test for $SeO_4^{2-}$ -reducing organisms in subsurface mine waste samples collected from the nearby Smoky Canyon and Enoch Valley phosphate mines, under a

range of conditions identified *in situ*. Here, samples of turbid groundwater and sediment from $SeO_4^{2-}$-reducing enrichments were used to isolate and enumerate Se-tolerant and Se-reducing microorganisms from groundwater, chert, shale, and mudstone samples. Mixed communities of *Bacteria* in waste rock were described using bacterial clone libraries and denaturing gel gradient electrophoresis (DGGE). Together with data characterizing Se reduction rates in specific lithotypes under controlled $O_2$ and temperature conditions, and aqueous and mineral Se speciation data (Chapter 5), this characterization of the indigenous $SeO_4^{2-}$ -reducing community can be used to evaluate options for *in situ* microbial source control of Se at phosphate mine operations.

## Materials and Methods

### Sample Collection and Preservation

Multiple samples of groundwater and overburden were collected for the isolation of SeRB and for the characterization of microbial community diversity using molecular methods. Sampling locations shown in Figure 12 included two groundwater monitoring wells, and multiple depths within three drill holes completed in mined overburden. Groundwater samples were collected from a well completed in saturated backfill at the base of backfilled pit B at the Dry Valley Mine (GW7D2a) and from a well completed in partially (and intermittently) saturated backfill at the Smoky Canyon Mine (GW11). Each well was sampled for major and trace element chemistry, and select results are presented for key elements in Table 6. Water was bailed manually using a new, disposable plastic bailer at each site, weighted to facilitate sediment recovery during sampling. The bailer

was rinsed with groundwater repeatedly prior to sample collection. Samples were transferred immediately into sterile glass or polypropylene bottles and stored at 10°C in the dark under absence of headspace. Groundwater pH, dissolved oxygen (DO) content, and temperature were recorded at the time of sampling. DO was measured using a YSI probe at the Dry Valley Mine, and initially with a Hach kit at the Smoky Canyon Mine and later using an $O_2$ probe. Sediments associated with the groundwater samples were concentrated by centrifugation at 13000xg for 10 minutes and stored saturated at or below 10°C until use.

Overburden rock samples were collected from existing mine backfill and rock dump facilities at the Smoky Canyon Mine (drill holes SCA and SCD) and Enoch Valley Mine (drill hole MEV) using a sonic drill. A total of 14 samples were selected to represent the range of lithologies encountered within the three drill holes. During the drilling process, samples were quickly preserved to limit (to the extent possible) any changes in temperature, gas, moisture, and redox conditions resulting from exposure of rocks to surface conditions. As samples were removed from the core barrel, they were placed into Lexan® plastics and labeled. The temperature of core samples was measured to avoid collection of overheated (above 37°C) samples as a result of friction between the core barrel and the rock during the drilling process. Each interval was placed on sheets of fresh plastic within a nitrogen ($N_2$)-flooded and sanitized (10% bleach and 70% ethanol) glove box. Once in the glove box, the sample bag was opened and the mineralogy, moisture, and clastic content were described qualitatively. Afterwards, the internal core was exposed and sub-sampled using sterilized utensils. The sub-samples were

composited, split into several sterile containers for mineralogical, microbial, molecular, and hydrogeochemical analysis, and preserved under both aerobic and anaerobic (20x pore volume flushed $N_2$ headspace) conditions. Containers were sealed to conserve moisture and stored in the dark at temperatures at or below the measured average subsurface temperature of 10°C. Samples stored under aerobic conditions were aerated periodically or maintained with a 0.22 µm filtered port to allow for atmospheric exchange.

Samples of rock collected for this study were analyzed independently to determine total and leachable organic carbon, Se and S, and moisture content by TetraTech, Inc., an independent contractor who managed the drilling program (summarized in Table 6). Total Se was extracted following EPA method 3050 and leachable Se was extracted using EPA method 1312, followed by Inductively Coupled Plasma- Mass Spectrometry (ICP-MS) analysis using method EPA 6020. Leachable and total organic carbon (TOC) were determined for rock samples using method SW-846 with ASA 9-29-2.2[28]. Total S was measured by LECO furnace with S speciation determined using the modified Sobek method (M600/2-78-054 1.3 [29]. Moisture content was determined by measuring sample weight before and after drying in a 34°C oven [28]. Temperature, $O_2$, and carbon dioxide ($CO_2$) concentrations were also measured *in situ* at multiple depths within the drill holes using thermocouples and plastic tubing taped to the outside of the well casing and completed at select depths, as described in the installation report provided in Appendix A3 [3].

Se Reduction by Native Microbes**:** Batch experiments were conducted to verify biotic $SeO_4^{2-}$ reduction in mixed mine overburden. Batch reactors were constructed in triplicate using 30 g of steam-autoclaved rock (mixed rock comprised of 45% shale, 35% chert, and 10% mudstone) and 30 mL of sterile deionized water in 250 mL glass serum bottles. The grain size distribution of these lithologies is described in Figure 11. Rock used in these experiments represented a composite of each lithotype. Hydrocarbon species were extracted from representative samples of shale and chert collected at the Smoky Canyon Mine using methylene chloride, followed by Gas Chromatography-Mass Spectrometry (GC-MS) analysis, to identify the native hydrocarbon compounds that were present in the run-of-mine (ROM) waste. Each reactor was mixed on a shaker table open to the atmosphere for 12 hours to dissolve salts under the aerobic conditions expected to exist within near surface portions of mine waste. Reactors were inoculated with 25.3 mL turbid groundwater (live and autoclaved control), sealed, and residual $O_2$ removed by flushing bottles with ultrapure $N_2$ gas through a 0.2 µm sterile filter. Each reactor was spiked with 10 mg/L Se as $SeO_4^{2-}$ (based on maximum field measured concentrations) and incubated at room temperature (approximately 25°C) and 10°C under dark conditions. No external carbon source was added. Samples of mixed water and sediment were collected immediately using a $N_2$-purged syringe, and every 12 hours for 10 days, and centrifuged at 13,000x g to remove solids. The supernatant was removed and diluted 1:500 with an acid mix comprised of 1.0% $HNO_3$, 0.5% HCl prior to analysis of total Se, Fe, and Mn using an Agilent 7500ce ICP-MS with the hydrogen ($H_2$)-gas collision cell following EPA method 200.8 [30]. Samples were removed from the reactor for $O_2$

measurement using a Hach AQ4 dissolved $O_2$ meter (Loveland, CO) and pH measurement using an Acumet AB15 pH meter with a probe model no. 13-620-AP (Cole Parmer).

Enrichments and Cultivation: Enrichment cultures were prepared to obtain SeRB from rock samples. Isolations of SeRB were also performed using the most dilute positive most probable number (MPN) cultures (see method, Appendix B). Enrichment and isolation methods are summarized in Appendix C1. Media consisted of filter sterilized groundwater containing 0.01% yeast extract to provide trace vitamins and nutrients and 0.2 to 20 mM $SeO_4^{2-}$. Carbon sources included native carbon extracted from rock, or lactate, acetate, and/or pyruvate individually or combined, or a cocktail containing all of the above, at total concentrations that were in approximate molar proportion to the amount of added $SeO_4^{2-}$. The native carbon that was present in the rock varied, both in chemistry and concentration (Table 7), and its extractability in deionized water differed between lithotypes. A mixture of shale, chert, and mudstone was thus used to make media in a ratio based on the relative proportion of shale:chert:mudstone lithotypes typical of mined overburden (55:35:10). The concentration of carbon that could be extracted from rock samples varied, unlike that of lactate, acetate, and pyruvate, which was used in constant molar proportion. Enrichments were prepared in a glove box with a headspace of 2%$H_2$/98%$N_2$ and kept in sealed bottles under a $N_2$ headspace. Enrichments for autotrophic SeRB were similarly prepared except half of the $N_2$ headspace was replaced with a 1:1 mixture of $H_2$-$CO_2$ and no exogenous carbon sources or yeast extract were added. Enrichments were incubated for 6 to 8 weeks at 10°C or until

a red precipitate was noted visually. The cultures were sampled periodically for changes in turbidity and color.

Table 7. MPN solution chemistry (in bottle roll extracts)

| Parameter | Method | Detection Limit | Average Chert | Average Shale | Average Mudstone |
|---|---|---|---|---|---|
| pH, standard units | EPA 150.1 | 0.1 | 8.4 | 7.7 | 8.0 |
| $NO_3^-$, mg/L | EPA Method 6010c | 0.5 | 1.7 | 1.8 | 0.9 |
| Total N, mg/L | Standard Method 4500, Shimadzu | 0.1 | 34.7 | 15.1 | 12.0 |
| $SO_4^{2-}$, mg/L | EPA300.0 | 1 | 8.0 | 232.0 | 18.0 |
| DOC, mg/L | Standard Method 5310 Shimadzu infrared | 0.1 | 84.5 | 96.9 | 77.7 |
| Volatile Hydrocarbons in Aqueous Phase, relative% | Head Space-Solid Phase MicroExtraction Gas Chromatography (qualitative only) | Alkanes | 64 | 87 | nm |
| | | Alkenes | nd | 2.8 | nm |
| | | Aromatic | 15 | 5.3 | nm |
| | | Cyclic | 21 | 5.6 | nm |
| Total dissolved Fe, mg/L | EPA 200.8 | 0.01 | 36.1 | 1.8 | 36.1 |
| Total dissolved Mn, mg/L | EPA 200.8 | 0.01 | 3.2 | 0.0 | 0.9 |
| Dissolved $SeO_4^{2-}$, µg/L | EPA7131-A GFAA-hydride | 2 | 1751 | 1609 | 1655 |
| Dissolved $SeO_3^{2-}$, µg/L | EPA7131-A GFAA-hydride | 2 | 436 | 386 | 425 |

nd= not detected nm=not measured
Averages taken from Tables B.1 and B.2, Appendix B1.

Samples of turbid groundwater and MPN-diluted rock samples were directly plated onto solid media to obtain SeRB. Agar (3% by weight) was added to the enrichment media described above, and plates were poured and allowed to solidify in the glove box. Groundwater samples (0.1 ml) were spread onto solid media and plates were kept anaerobic using an Anaeropak™ system under a 21% $CO_2$ atmosphere (Fisher Scientific). Samples from drill holes MEV, SCA, and SCD were serially diluted to $10^{-4}$, $10^{-5}$ and $10^{-6}$ in 5 mM carbon-specific filter-sterilized groundwater media (e.g., native

carbon or lactate or acetate or pyruvate) and 0.1ml of each dilution was plated onto solid media in the glove box. Colonies showing unique morphologies were isolated by streaking onto solid media until pure. Pure cultures were then maintained in aqueous medium comprised of filter-sterilized site specific groundwater containing 0.03 mM native carbon (extracted from ROM rock using deionized water in a bottle roll for digestion), to which 0.01% yeast extract, 2 mM $SeO_4^{2-}$, and 2 mM each of lactate, pyruvate, and acetate were added to create the enrichment cocktail. Notes and photographs describing the enrichment process are provided in Appendix C1.

Enumeration of $SeO_4^{2-}$-Reducing Microorganisms: An MPN approach [31] was used to estimate the number of SeRB in each sample. Samples of chert, mudstone, shale, and groundwater were serially diluted with a rock extract solution containing 0.2 mM each of acetate, pyruvate, and lactate, and a field-relevant concentration of 0.1 mM $SeO_4^{2-}$. The bottle roll rock extract solution was prepared for each rock sample using a gyrator-shaken sample of rock and water (water: rock mass ratio of 2.65:1), covered but open to the atmosphere. After 12 hours, solids were allowed to settle and fines in suspension were pelleted by centrifugation at 13,000xg for 10 minutes. The rock extract was then filter-sterilized using a 0.22 µm filter, the pH was recorded, and sulfate ($SO_4^{2-}$), total dissolved organic carbon (DOC), $NO_3^-$, $SeO_4^{2-}$, and $SeO_3^{2-}$, were analyzed (Table 7). Ion chromatography was used to measure anion concentrations using a Dionex model DX500 equipped with an IonPac AS-9-HC (4 x 250 mm) anion column and a CD 20 detector. Samples (25µL) were injected into an 11 mM $Na_2CO_3$ mobile phase flowing at 0.9 mL/min, both at full strength to measure low concentrations of most ions and diluted

25X with deionized water to measure higher concentrations of $SO_4^{2-}$. Solvent extractable organic carbon was extracted using methylene chloride by EPA method 3550B and analyzed by GC-MS following EPA Method 8270C [32]. Dissolved organic carbon was measured by Shimadzu infrared, following standard method 5310. Dilutions were performed following a standard test protocol with 10-fold dilutions carried out to $10^{-8}$ dilution. The resulting solutions were then divided into aliquot tubes, which contained 10 ml each. MPN cultures were prepared both aerobically in capped test tubes, and anaerobically in sealed serum bottles, with a 50% $N_2$, 25% $H_2$ and 25% $CO_2$ headspace, and were kept at 10°C. The chemical analyses of bottle roll extracts (pH, $NO_3^-$, total N, $SO_4^{2-}$, DOC, total dissolved Fe and Mn, $SeO_4^{2-}$, and $SeO_3^{2-}$) are provided in Table 7.

Due to the strong humic content of the phosphatic shales, and dark color of the rock, it was necessary to replace colorimetric indicators of reduction in MPN tubes with quantitative measurements of total dissolved Se. Samples (1 ml) were removed from each culture and centrifuged at 13,000x g to remove solids. The supernatant was diluted 1:500 with an acid mix comprised of 1.0% $HNO_3$, 0.5% HCl prior to analysis of total Se using an Agilent 7500ce ICP-MS with the hydrogen-gas collision cell following EPA method 200.8 [30]. Tubes were scored positive if they showed a minimum of 10% reduction in soluble Se concentration. ICP-MS analyses of total Se collected for the approximately 2,600 MPN tubes (16 samples for two $O_2$ treatments, in duplicate, with 40 tubes per experiment) are listed in Table B.1.4 of Appendix B. The original ICP-MS data are provided on the accompanying CD.

DNA Extractions and PCR**.** Nucleic acids were extracted from enrichments, soil, and groundwater sediment samples. Attempts at direct extraction of DNA from MPN cultures were initially unsuccessful, likely due to the low biomass in the limited culture volume. Therefore, samples from MPN tubes were transferred to fresh media using methods described above to obtain a sufficient volume of biomass for extractions. Nucleic acids were extracted using the Power Soil DNA Isolation Kit ™ (Mo-Bio Laboratories, Carlsbad, CA).

The manufacturer's method was modified by first incubating samples in 20% SDS at 70°C for 1 hour, then vortexing for 20 minutes to breakup any biofilm and detach organisms. After mixing, the entire sample was used for the extraction of nucleic acids as detailed in the protocol provided by the manufacturer. PCR was performed on extracted DNA to amplify 16S rRNA genes using a nested approach to optimize yield [33]. Initially, 10 cycles were run using primers 1070F (5'-ATG GCT GTC GTC AGC T-3') and 1392R (5' ACG GGC GGT GTG TAC-3') [34]. The products from the initial PCR were diluted 1:10 and used as template in a 30 cycle PCR using 1070F and 1392GC. Reactions (50 µl) contained template (2 to 50 ng DNA), 0.2 mM each primer, and PCR mastermix (Promega, Madison, WI, 25 µL), and PCR was performed using an Eppendorf Mastercycler Gradient thermocycler. Conditions were the same for both PCRs and included denaturation at 94°C for 10 min, followed by 10 or 30 cycles of 94°C for 45 sec, 50°C for 45 sec, 72°C for 45 sec, and a final extension at 72°C for 7 min. PCR amplicons were separated on a 0.8% agarose gel and visualized by staining with ethidium bromide.

Colony PCR was used to amplify 16S rRNA genes from isolated SeRB. The resulting PCR products were screened for redundancy using the restriction fragment length polymorphism (RFLP) procedure. Colonies were picked using sterile pipet tips and suspended in nuclease-free deionized water (10 µl). Suspended cells (2 µL) were used as template in a 30 cycle PCR using 334F (5'-CCA GAC TCC TAC GGG AGG CAG C-3') and 926R (5' CCG ICI ATT IIT TTI AGT TT-3') [35]. Reactions (50 µl) contained template (2µl), 0.2 µM primers, and PCR mastermix (Promega) and PCR was performed using a Techne TC-312 thermocycler. PCR conditions were 10 min at 94°C, followed by 30 cycles of denaturation at 94°C for 45 sec, annealing at 50°C for 45 sec, and extension at 72°C for 45 sec. The final extension period was 5 minutes at 72°C. The amplicons were digested for 3 hours at 37°C in a 20 µL volume that contained 10 µL PCR product, 1X reaction buffer and 20U *HaeIII*. Fragments were separated in a 3.5% agarose gel, and digestion patterns were visualized and grouped based on similarity. The PCR amplicon from several colonies representative of each RFLP group were used to obtain 16S rRNA gene sequence information.

DGGE and Sequencing**:** PCR products were separated by electrophoresis in 8-12% acrylamide gels containing a 50-60% urea-formamide gradient at 70 V and 60°C for 20 hours. Gel electrophoresis was performed using a DGGE-2401 system manufactured by CBS Scientific®, based on the general method described by Muyzer et al. [36]. Gels were stained with SYBR Gold (Life Technologies Invitrogen ™) and visualized using UV light. A ladder of PCR amplified products from known isolates was prepared and used to compare with samples. The ladder included PCR amplicons from isolates with

>99% similarity to members of the *Actinobacterium, Pseudomonas, Rhodoferax, Dechloromonas, Dechloromonas, Brevundimonas, Rahnella, Sphingomonas,* and *Cellulomonas genera*. Individual bands of interest not represented in the ladder were excised from the gel, resuspended in 15 µL of nuclease-free water, and allowed to diffuse from the gel overnight at 60°C. Numerous bands were also cut from the gel to confirm ladder identification. Samples were mixed and acrylamide was pelleted by centrifugation. The resulting supernatant was removed, diluted 1:10 in nuclease free water, and used as template in a 30 cycle PCR using primers 1070F and 1392R as described above. PCR products were purified using a Wizard SV Gel and PCR Cleanup System (Promega) and quantified using a Qbit fluorimeter (Life Technologies Invitrogen™). Samples were submitted to the Idaho State University Molecular Research Core Facility (ISU MRCF, www.isu.edu/bios/MRCF ) for sequencing using an Applied Biosystems® 3130XL Genetic Analyzer. The Basic Local Alignment Search Tool (BLAST, NCBI,http://blast.ncbi.nlm.nih.gov) was used to query resulting sequences against a nonredundant database [37]. Images of a number of the gels used for the project are provided in Appendix C4.

Clone Libraries: Clone libraries were constructed for primary enrichments of rock samples AS71 and AS113, which hosted the greatest number of $SeO_4^{2-}$-reducing organisms as estimated by MPN. 16S rRNA gene fragments were amplified by PCR using bacterial primers 1070F/1392R or archaeal primers Arc 21F (Integrated DNA Technologies, Mfg ID 41725659) and 958R (Integrated DNA Technologies, Mfg ID 41725669) [35]. The bacterial primers were chosen to be consistent with those used for

DGGE in the study. PCR conditions were 94°C for 5 min followed by 30 cycles of 94°C for 45 sec, 55°C for 45 sec, 72°C for 45 sec, and a final extension step at 72°C for 1 minute. PCR products were cloned as described in the Invitrogen TOPO Cloning kit ® protocol, cloned products were transformed into chemically competent *E. coli* cells, and transformants were plated onto solid media and screened by blue-white selection. White colonies were transferred to liquid media (LB), grown overnight, and plasmid DNA was extracted using a QIAprep® Miniprep kit (Qiagen, Valencia, CA). The concentration of plasmid DNA was quantified using the Invitrogen QBit® fluorometer BR dsDNA (broad range double stranded DNA) assay. Cloning methods are summarized in Appendix C3.1.

Plasmid DNA samples were shipped to the ISU MRCF for DNA sequencing.Sequencing results were screened to eliminate samples that were not comprised exclusively of unambiguous bases or greater than 300 base pairs in length. Sequences were aligned using ClustalX, organized into a tree using the program ARB, and compared using Unifrac. Diversity within each library was analyzed using the program DOTUR, a program for defining operational taxonomic units and species richness (Schloss, 2005). Sequences were evaluated using BLAST to identify the closest relative. Further analysis of sequences allowed correction of antisense sequences resulting from inversion of the plasmid insert during the initial ligation, using the program Reverse Complement (Java Boutique, http://javaboutique.internet.com/revcomp/). Bioinformatics data and analyses are summarized in Appendix C3.2.

# Results

## Sampling and *In Situ* Subsurface Characterization

Sixteen samples were collected from the Smoky Canyon, Dry Valley, and Enoch Valley mines, including two samples of groundwater and fourteen samples of sediment from drill cores of unsaturated and unconsolidated overburden. Groundwater was collected from mixed lithology backfill deposits at the Dry Valley (GW7D2a) and Smoky Canyon (GW11) mines; no groundwater was available from the Enoch Valley Mine. Samples collected from drill cores were chosen to represent the range of observed lithology and moisture conditions observed with depth in holes drilled into the randomly backfilled overburden deposits (Figure 12). Geochemistry (summarized in Table 6) and particle size data (not reported) were measured for the same samples [3].

Table 6 summarizes *in situ* conditions in the groundwater monitoring wells completed in backfilled overburden (GW7D2a, GW11), with conditions at sampled depths in drill holes MEV, SCA, and SCD. Measured pH in groundwater ranged from 6.5 to 7.8 and temperature ranged from 7 to 10°C. The concentration of dissolved Se in Dry Valley well GW7D2a was 0.021 mg/L, considerably below the Idaho groundwater standard of 0.050 mg/L, in contrast to the higher Se concentration of 1 mg/L measured in the Smoky Canyon well GW11. Dissolved oxygen (DO) is non-detectable in groundwater at Dry Valley, in contrast with 5.5 mg/L DO at Smoky Canyon. The highest $SO_4^{2-}$ concentration, 1666 mg/L, correlated with higher DO levels in GW11, and indicates a higher rate of sulfide oxidation relative to GW7D and GW7D2 at the Dry Valley Mine

(See data Chapter 3). $NO_3^-$ in groundwater, likely resulting from blasting residues, ranged from 0.3 to 5.6 mg/L. Levels of soluble Fe (0.004 to 0.2 mg/L) and Mn (0.44 to 0.47 mg/L) were comparable in groundwater monitored at both the Dry Valley and Smoky Canyon Mines. No comparison could be made with the Enoch Valley Mine, where groundwater water quality was not reported.

Table 6 also shows *in situ* conditions and characteristics of rock samples collected from drill holes MEV, SCA, and SCD. Drill hole temperatures ranged from 8 to 12°C. The $O_2$ concentration was close to atmospheric in both SCA and SCD, but was not detectable below 32 feet in MEV or 10 feet at Dry Valley, apparently reflecting differences in the way rock was dumped during facility construction [3]. Carbon dioxide concentrations ranged from 6.6 to 9.6 volume % in MEV to only 4 volume % in the SCA and SCD holes. Total and dissolved Se, as well as TOC and DOC, varied with lithotype and were consistent with the values reported by the USGS for these sediments [4, 5, 38]. Carbon speciation varied between lithotype in the Phosphoria overburden as well, with shale containing more carbon and higher concentrations of aromatic hydrocarbons, such as benzene, phenanthrene, toluene, and dibenzothiophene relative to chert (Table 8).

Potential for *in situ*
Biological Se Reduction

Potential for *in situ* biological reduction of Se was confirmed through comparisons of killed controls with $SeO_4^{2-}$ reduction in live batch reactors of sterile mixed waste rock inoculated with site specific groundwater. Reduction of $SeO_4^{2-}$ to

Table 8 GC-MS analysis of methylene chloride extracted solid phase carbon in overburden samples from Phosphoria Formation. chert and shale.

| | **Shale** | | **Chert** | |
|---|---|---|---|---|
| | *mg/kg* | *common compounds* | *mg/kg* | *common compounds* |
| **Solvent Extractable Organic Carbon** | 72.1 | | 16.8 | |
| **Non-Aromatic** | 41.4 | | 15.1 | |
| **Aromatic** | 30.7 | | 1.9 | |
| **Ratio Aromatic/Total** | 43% | | 12% | |
| **Alcohol** | *no data* | | 1.2 | hexadecanol |
| **Alkane** | 32.2 | decane, hexane | 9.7 | decane, eicosane |
| **Alkene** | 0.6 | Octadecene | *no data* | octadecene |
| **Amide** | 7.7 | Decanamide | 3.6 | decanamide |
| **Aldehyde** | 0.5 | Octadecenal | 0.4 | dimethyl octenal |
| **Heterocyclic** | 0.3 | Azetidine | 0.2 | tetrahydropyran |
| **Monocyclic aromatic** | 14.8 | phthalate, benzene, toluene | 1.9 | phthalate, benzene |
| **Dicyclic aromatic** | 9.9 | naphthalene | *no data* | |
| **Polyaromatic** | 6.0 | dibenzothiophene, phenanthrene | *no data* | |

$SeO_3^{2-}$ and removal of Se from the aqueous phase began in the mixed overburden rate reactors once $O_2$ was consumed to a concentration below 0.3 mg/L, following an initial lag of approximately 80 hours (Figure 13). Nitrate was also consumed during this phase, although its complete removal was not required for $SeO_4^{2-}$ reduction to proceed. The reduction of $SeO_4^{2-}$ to $SeO_3^{2-}$ and its ultimate removal from solution was associated with an increase in soluble Fe (to an upper limit of 110 mg/L) and Mn (to 4 mg/L), presumably due to concurrent microbial Fe and Mn reduction. The cause of the loss of soluble Fe at 272 hours is not known, but it may be related to the formation of the insoluble mineral ferroselite, $FeSe_2$, which was identified in post reduction mineralogy studies (see chapter 5). Essentially no change was observed in the concentration of $PO_4^{3-}$,

which remained below detection (1 mg/L). The concentration of $SO_4^{2-}$ initially increased from 1035 mg/L to 1390 mg/L and remained constant during the Se reduction process. Se

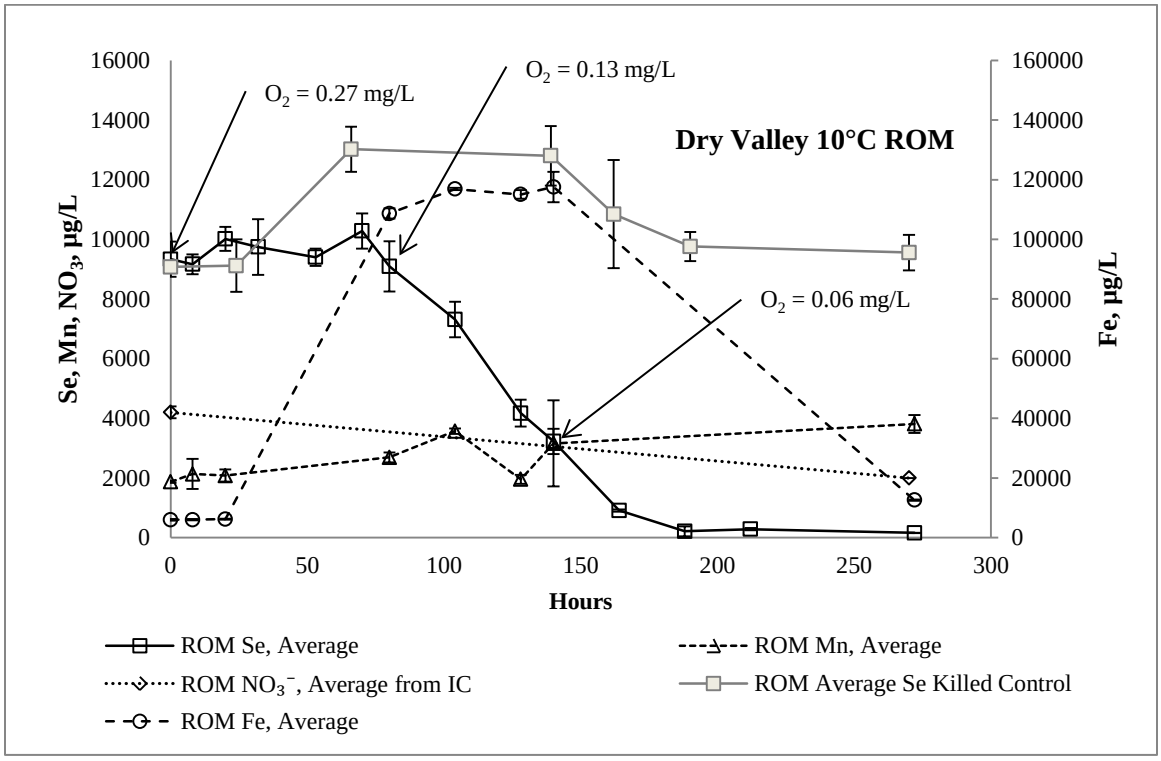



Figure 13. Dissolved Se, Mn, Fe, and $NO_3^-$concentrations in mixed overburden rate reactor, Dry Valley Mine at 10°C. pH varied from 6.6-6.8 under confined headspace throughout the experiment. ROM is mixed run-of-mine waste rock. Error bars represent +/- standard deviation for triplicate reactors,

speciation data obtained through ion chromatography indicated detectable $SeO_3^{2-}$ mid-way through the reduction process (at 128 hours) as $SeO_4^{2-}$ was removed from solution (Appendices D1 and D2).

Isolation and Identification of SeRB

Direct plating of groundwater and serially diluted rock samples was used to isolate potential SeRB. Colonies exhibited variable morphology and ranged in color from

clear or white to light orange, red and deep brick red. Colonies that exhibited deep orange to red coloration suggested $SeO_4^{2-}$ reduction to $Se^0$ (see photos Appendix C1). Many of the apparent SeRB were mixed cultures and were observed to be closely associated with, and difficult to separate from, non-$SeO_4^{2-}$ reducing organisms based on microscopy and sequencing results. The bacteria listed in Figure 14 were isolated from the mixed

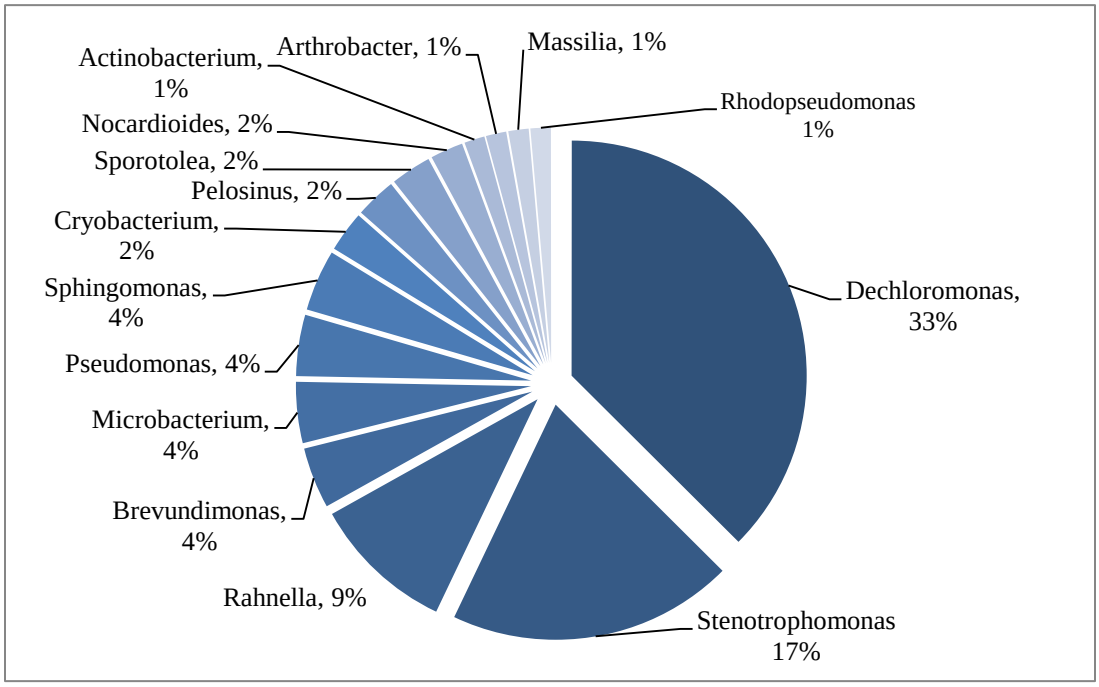



Figure 14. Genera identifications obtained from S.E. Idaho groundwater and rock (percentages reflect frequency of detection in the isolate pool), (n=80).

groundwater and rock cultures while attempting to identify $SeO_4^{2-}$ reducers. Hundreds of colonies were screened in this process and eighty were chosen for identification by sequence analyses of 16S rRNA genes based on visual production of red elemental Se. Growth of isolates was slow at field relevant temperatures of 10°C, but was observed on all carbon substrates tested at various concentrations, and some isolates reduced as much as 10 mM $SeO_4^{2-}$.

Figure 14 shows the diversity of microorganisms that were isolated. A list is provided as Table C.1.1 in Appendix C1. The majority of phylotypes associated with organisms isolated from samples of groundwater were highly similiar to the genus *Dechloromonas;* these phylotypes comprised more than 33% of the bacterial population. Most sequences obtained for these isolates were >97% identical to members of the *Dechloromonas* genus (based on an average read length of 584 bases), but several sequences were > 96% similar to *D. hortensis, D. denitrificans or Dechloromonas* sp. A-34 (S. Childers, unpublished). Another 17% of the isolate phylotypes were >99% similar to the bacterial genus *Stenotrophomonas* Additional organisms isolated from groundwater were >97% similar to members of the *Rahnella, Brevundimonas, Microbacterium, Pseudomonas, Sphingomonas, Pelosinus, Sporotolea,* and *Nocardioides* genera, based on sequences that ranged in length from 240 to 628 base pairs. A list of the genus level identifications for the microbes isolated during this study is provided in Appendix C1; corresponding sequences are provided in Appendix C2.

Organisms most similar to members of the *Rhodoferax* were particularly challenging to isolate, due to their frequent occurrence in co-cultures with organisms that identified closely with members of the *Cellulomonas* and *Actinobacterium* genera. The organisms producing sequences that identified highly with the genera *Massilia, Sporotolea, Arthrobacter, Actinobacterium, Rhodopseudomonas, Oleomonas,* and *Sporosarcina* were less commonly isolated.

Enumeration of SeRB

A MPN approach [31] was used to estimate the number of SeRB present in groundwater and in the individual lithology specific samples. The results of ICP-MS analyses of total Se used for the MPN analysis are summarized in Table B1.3; the data source files are listed in Table B1.5 and provided electronically.

Table 9 shows that the estimated number of SeRB was greatest in MPN tubes prepared and maintained under anaerobic conditions. Although aerobic tubes showed growth as evidenced by visible turbidity, little to no $SeO_4^{2-}$ reduction was evident. Sediments from Dry Valley GW7D groundwater samples incubated under anaerobic conditions averaged $4.6 \times 10^6$ SeRB per gram of rock. Of the various rock lithotypes, higher numbers of SeRB were associated with shales than with cherts or mudstones, and more SeRB were present in the external Smoky Canyon panel A rock dump than in the backfilled overburden in Smoky Canyon panel D or at the Enoch Valley Mine (Table 9). The shale samples AS71 and AS113 had the highest number of estimated $SeO_4^{2-}$ reducers, with values ranging between $10^5$ and $10^6$ organisms per gram of sediment. In contrast, fewer than $10^3$ SeRB were present in mudstone or chert.

Table 9. MPN results and dominant bands cut from DGGE for most dilute positive MPN cultures.

| **Location** | **Sample type** | **Lithology** | **MPN estimated No. $SeO_4^{2-}$ Reducers per gram[+]** | | ***Bacteria* with greatest similarity to 16S rRNA sequences for DGGE dominant bands for MPN samples** |
|---|---|---|---|---|---|
| | | | **Anaerobic** | **Aerobic** | |
| **GW7D-2a** | **Groundwater** | ROM mix | **$4.67*10^6$** | **846** | *Pseudomonas, Rhodoferax, Dechloromonas* spp. |
| *Dry Valley Mine* | | | | | |
| **GW11** | **Groundwater** | ROM shale | **$1.67*10^4$** | **$1.1*10^4$** | *Pseudomonas, Rhodoferax, Dechloromonas* spp. |
| *Smoky Canyon Mine* | | | | | |
| **SCD backfill** | **ROM rock** | | | | |
| *Smoky Canyon Mine* | DC5 | chert | **360** | **15.5** | *Pseudomonas, Dechloromonas* sp.*Commamonas, Actinobacterium, Pelosinus, Sphingomonas* |
| | DM50 | mudstone | **188** | **3.25** | nd |
| | DS75 | shale | **$1.4*10^3$** | **18** | *Dechloromonas, Polaromonas* |
| | DC123 | chert | **271** | **1** | nd |
| **SCA external dump** | **ROM rock** | | | | |
| *Smoky Canyon Mine* | AS5 | shale | **$1.7*10^4$** | **6.5** | *Commamonas, Pseudomonas, Rhodoferax, Dechloromonas* spp., *Polaromonas, Pelosinus* |
| | AS71 | shale | **$5.2*10^6$** | **1** | *Pseudomonas, Rhodoferax, Dechloromonas* spp., *Polaromonas* |
| | AS113 | shale | **$3.2*10^5$** | **1** | *Pseudomonas, Rhodoferax, Dechloromonas* spp, *actinobacteria, Cellulomonas* |
| | AC125 | chert | **57** | **8.9** | nd |
| | AM145 | mudstone | **$1.7*10^3$** | **77** | *Rhodoferax, Pelosinus, Pseudomonas* |
| **MEV backfill** | **ROM rock** | | | | |
| *Enoch Valley Mine* | MS5 | shale | **$4.3*10^3$** | **19** | *Pseudomonas, Rhodoferax* |
| | MM32 | mudstone | **309** | **3** | *Pseudomonas, Rhodoferax, Dechloromonas* sp. *Pelosinus, actinobacteria* |
| | MS73 | shale | **$1.2*10^4$** | **220** | *Pseudomonas, Rhodoferax, Pelosinus* |
| | MM178 | mudstone | **238** | **21.9** | nd |
| | MS285 | shale | **$1.6*10^5$** | **229** | *Rhodoferax, Dechloromonas* spp. *Sphingomonas, actinobacteria* |

reported MPN values are an average of two replicates , [+]MPN data provided in Appendix B, Table B1-3
ROM = Run-of-mine, nd = not detected

SeRB Community Diversity in
Saturated and Unsaturated Sediments

Community diversity was measured using clone libraries, DGGE, and 454 pyrosequencing of the anaerobic MPN dilutions for samples of the lithotypes with the highest numbest of estimated $SeO_4^{2-}$ reducing organisms. The most dilute positive MPN enrichments for select shale samples were compared with single samples of mudstone and chert using DGGE. Figure 15 shows a moderate level of diversity with 5-10 bands evident for each sample; chert had greater diversity than shales as indicated by the greater number of bands in the upper gel. The shale samples AS71 and AS113, which had the greatest number of SeRB in MPN estimates, showed somewhat less diversity and generally consistent community characteristics when compared with other shales or mudstone.

Comparative sequence analysis of excised DGGE bands (see Figure B1.1 and Table B1.4, Appendix B1), yielded the limited number of phylotypes shown in Table 9. Comparison of samples in Figure 15 with a ladder (left) constructed using DNA from microbes isolated during this study suggests that the majority of rock samples contain phylotypes that are strongly similar to members of the genera *Pseudomonas* (>99%, 260-280 bp) and *Rhodoferax* (>99%, 250-310 bp). Faint bands that align with one or more of the three *Dechloromonas* isolates are evident in shale samples, whereas the most dilute chert and mudstone MPN samples show bands that align with the amplicon for the isolate *Dechloromonas* sp. A-34 only. The identity of microorganisms represented by these bands could not be confirmed because they were too faint to cut. Phylotypes listed in italics in Figure 15, which were not included in the isolate ladder, were identified using

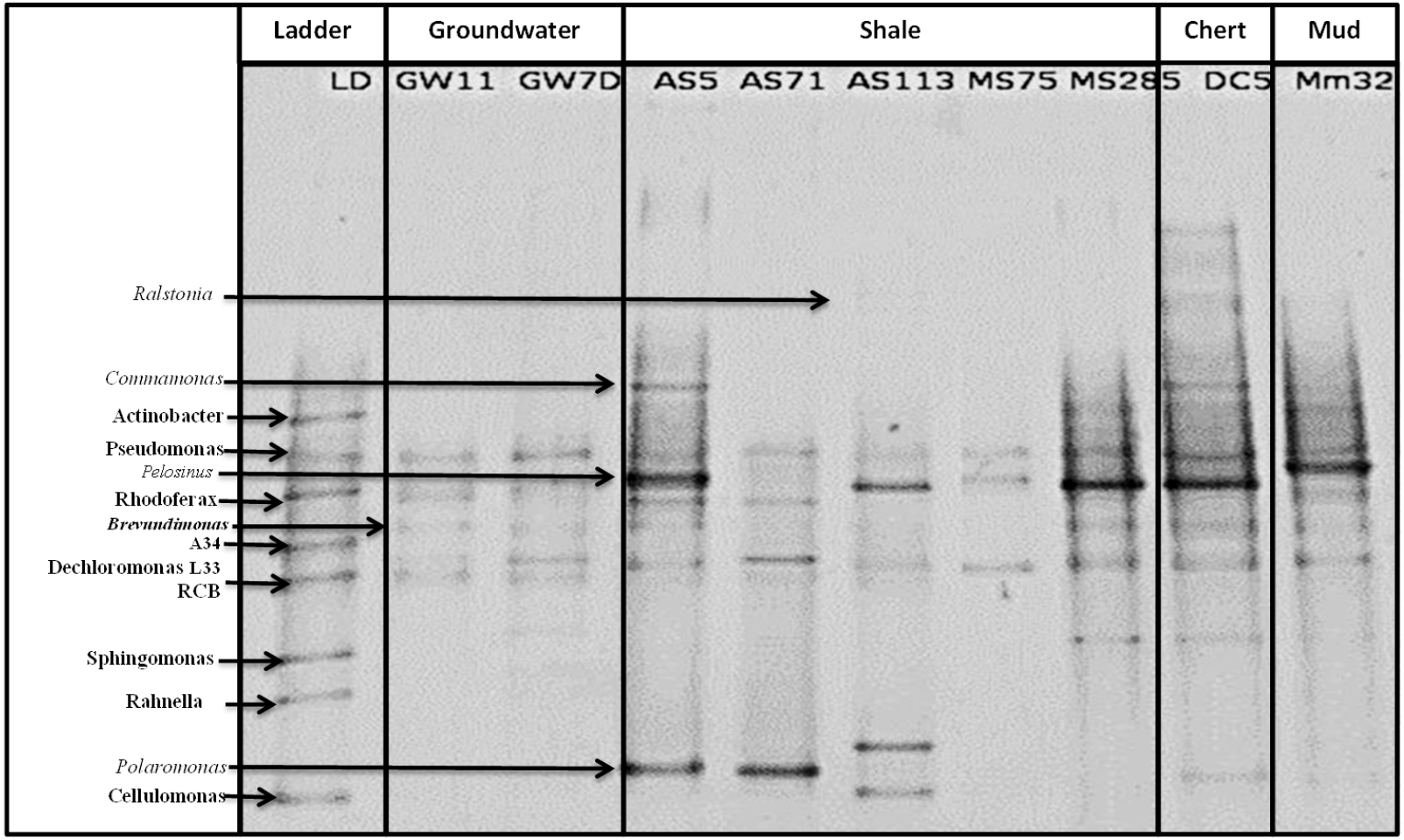



Figure 15. DGGE profiles comparing isolate ladder with groundwater and waste rock samples from Smoky Canyon, Dry Valley, and Enoch Valley mines, S.E. Idaho.

bands cut from gels where visualization and cutting of the bands was possible. While certain bands may have visually aligned, this does not necessarily guarantee that the bands came from the same population. To confirm that the ladder was servicing its purpose, a number of bands aligning with the isolate ladder were cut and submitted for sequence confirmation.

Groundwater samples from backfills at the Smoky Canyon and Dry Valley mines were also compared with one another, the sediment samples, and the isolate ladder in Figure 15. DNA was extracted directly from the biomass collected from these groundwater samples. Groundwater DNA samples yielded bands that were confirmed by comparative sequence analysis and also aligned with the ladder isolates of *Rhodoferax* (>99%), *Dechloromonas* A-34 (>98%), *D. hortensis L-33* (>96%), and *D. aromatica* RCB (>99%). Other bands in samples of groundwater from GW7D2a that were cut, extracted and sequenced had strong similarity to phylotypes associated with the genera *Brevundimonas, Rhodoferax* (>97%), *Polaromonas* (>99%) and *Acidovorax* (>98%).

Shale samples AS71 and AS113 had high estimated numbers of SeRB and contained phylotopes that were > 97% similar to known genera. Phylotypes sequenced from gel bands were closely related to several *Rhodoferax* species, including *R. fermentans[39], Rhodoferax sp.* AsD (>98%), and *R. ferrireducens* T118 (>98%, [40]). Members of the *Polaromonas* (>98%) and *Pseudomonas* (>99%) genera were also identified. Though bands suggestive of several *Dechloromonas* species were apparent based on ladder alignment, these phylotypes could not be confirmed because bands were too faint to be excised and/or did not amplify successfully from excised bands.

Comparable diversity patterns are evident between shale samples (AS5, MS 5, MS73, and MS 285) in Figure 15, and samples also contained phylotypes highly similar to representatives of the genera *Pseudomonas*, *Rhodoferax*, and *Pelosinus*. Supporting data are provided for isolates (Appendix C1), DNA sequences (Appendix C2), clone libraries (Appendix C3), and DGGE images (Appendix C4).

Clone Libraries

Clone libraries were constructed for both *Archaea* and *Bacteria* using primary enrichments of shale samples AS 71 and AS113 (Appendix C3). Bacterial clone libraries constructed for samples AS71 and AS113 contained 72 and 46 clones, respectively, as listed in Tables C3-1 and C3-2, respectively. Species richness and rarefaction curves from DOTUR (Appendix C3, Figures C3.1-3) indicate that clone sample diversity approached actual diversity. The bacterial library for AS71 had 30 OTUs (operational taxonomic units), out of 72 sequences at the 95% confidence level. The bacterial library for AS113 was less diverse, with 7 OTUs (out of 46 sequences) at the 95% confidence level. The two clone libraries represent statistically different populations based on a Unifrac analysis correlation coefficient of $0.001 < p < 0.01$. Sequence length was typically between 380 and 320 bases in length, allowing genus level identification. Figure 16a shows the bacterial phylotypes occurring at a frequency of 2% or greater in the AS71 library. This sample had the highest number of SeRB based on the MPN results. Nearly two-thirds of the clones had sequences that best matched members of hydrocarbon-degrading, Fe-reducing genera including *Anaeromyxobacter*, *Pelobacter*, *Polaromonas*, *Pelosinus*, *Geobacter*, and *Variovorax*. Phylotypes strongly similar to

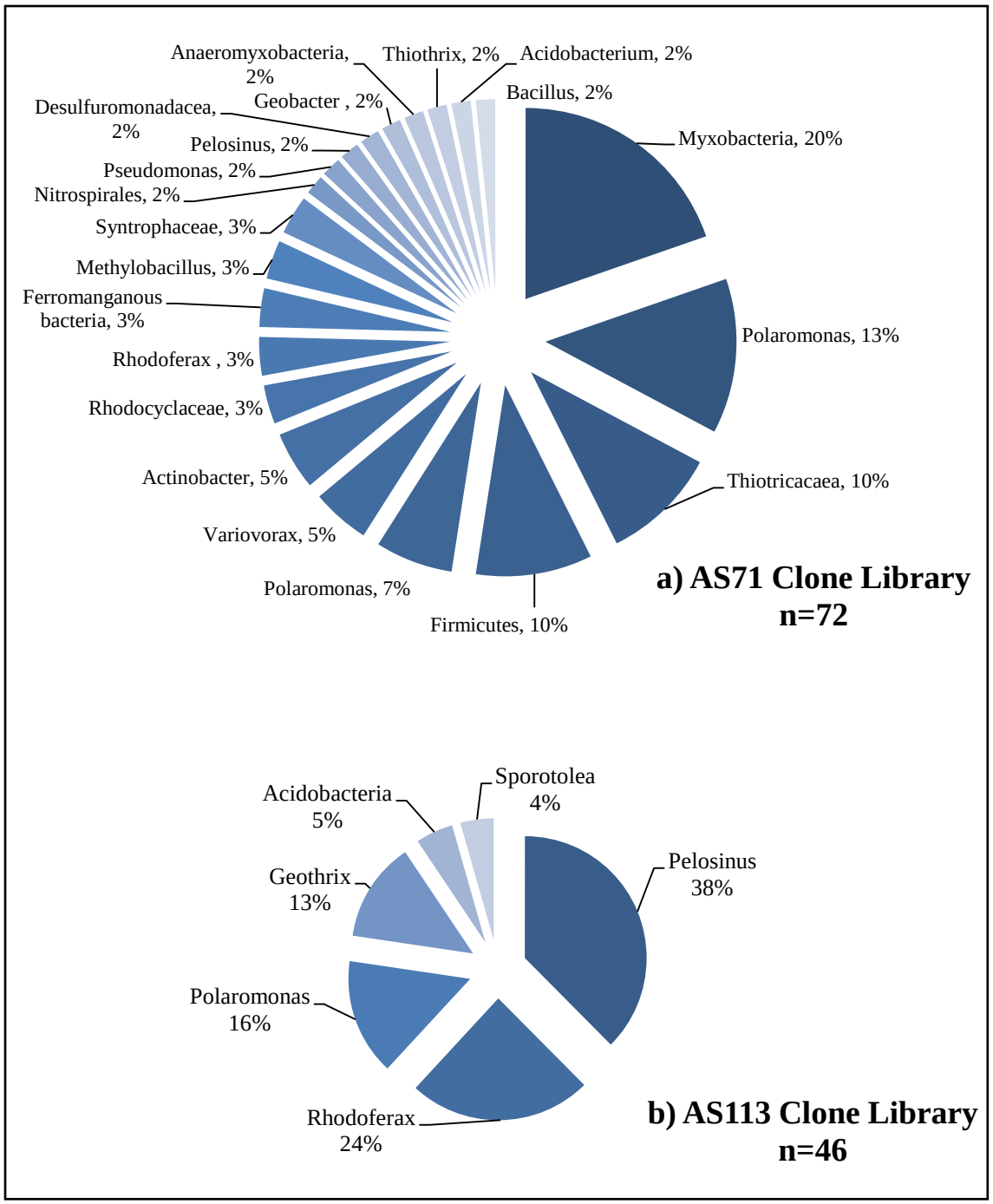



Figure 16. Bacterial clone libraries for overburden samples (a) AS71 and (b) AS113.

Fe- and S-oxidizing members of the *Acidoferrobacter* genus, and the Fe- and S-reducing genera *Desulfuromonas* and *Magnetobacter*, represent another 20% of observed diversity.

Less diversity is evident in the AS113 clone library (Figure 16b), with only 7 principal phylotypes representing the community. Most clones closely (> 97% identity) matched the phylotopes of Fe-reducing, hydrocarbon-degrading genera, including *Pelosinus* (38% of the diversity), *Rhodoferax* (24%), *Polaromonas* (16%), *Geothrix* (13%), *Acidobacteria* (5%), *Sporotalea* (4%) and *Anaeromyxobacter* (2%). The AS113 community is more limited than the AS71 community, but the metabolisms of the microorganisms represented in AS113 are consistent with the dominantly hydrocarbon-oxidizing, Fe-reducing community identified for AS71.

Of the phylotypes identified in the clone libraries, only *Anaeromyxobacter* is known to be a $SeO_4^{2-}$ reducing organism. Interestingly, no *Stenotrophomonas-like phylotypes* were identified in the libraries, consistent with the results of the DGGE molecular work, but not the isolation work. *Dechloromonas* phylotypes were also not identified in the clone libraries.

Results for the *Archaeal* library for AS113, which contained 35 clones, are provided in Appendix C3. The rarefaction curve is also provided as Figure C3.1 in Appendix C3. Creation of an *Archaeal* library for AS71 was unsuccessful due to low DNA yield.

### Community Diversity in Saturated and Unsaturated Overburden

Lithotype and moisture content are hypothesized to influence the microbial community composition and capacity for $SeO_4^{2-}$ reduction. DGGE profiles were used to test this hypothesis by comparing changes in community diversity between lithotypes and groundwater samples from saturated backfill (Figure 14). This approach was taken to complement the isolation work by identifying microorganisms that were not readily cultivated. The DGGE banding patterns in the two groundwater samples were nearly identical and reflected less diversity than the unsaturated rock samples. There was considerable similarity between identified communities, however, and bands representing the *Pseudomonas, Rhodoferax,* and *Dechloromonas* genera were observed in both saturated and unsaturated sediments. DGGE patterns of the shale samples show a relatively consistent community in that lithotype, whereas the DGGE patterns for the mudstone and chert samples differ somewhat from the shales and from each other.

## Discussion

Culture dependent and independent techniques were used to characterize the $SeO_4^{2-}$ reducing microbial populations present in backfill at three phosphate mine sites in S.E. Idaho. The study was undertaken because physical and geochemical monitoring data indicate there are important differences between the overburden deposits at each location, which may influence the release of Se to the surrounding environment.

Subsurface Selenium Biogeochemistry
Supports Potential for Se Reduction

Groundwater chemistry at the Smoky Canyon Mine (GW11), where SeRB numbers were lower, showed lower pH values and higher $SeO_4^{2-}$, $SO_4^{2-}$, $NO_3^-$, and $O_2$ concentrations compared to conditions at the Dry Valley mine, where $SeO_4^{2-}$ concentrations are below the Idaho groundwater standard of 0.050 mg/L. These data reflect the higher concentration of $O_2$ at the Smoky Canyon Mine, where increased $SO_4^{2-}$ was measured, and decreased biological reduction of $SeO_4^{2-}$ and $NO_3^-$ was indicated by the relatively higher concentrations of Se and lower numbers of SeRB. The probable importance of microbial reduction of $SeO_4^{2-}$ in the Dry Valley Mine sediments under suboxic conditions is demonstrated by the comparison of live and killed cultures in batch reactors, where concurrent $NO_3^-$, $Fe^{3+}$ and $Mn^{4+}$ reduction is evident (Figure 13). The Smoky Canyon monitoring well GW11 is intermittently saturated with higher concentrations of $O_2$, while groundwater in the Dry Valley backfill, where concentrations of $SO_4^{2-}$, $SeO_4^{2-}$, and $NO_3^-$ are lower, demonstrates more consistently microaerophilic to anoxic conditions. This condition may be due to prior saturation during pit water discharge onto backfill in 1999 and 2000, but conditions have remained sub- to anoxic even though much of the upper backfill has drained to field capacity moisture content since that time [2]. Elevated concentrations of $SO_4^{2-}$ at both locations suggest that while conditions are moderately reducing, they are too oxidizing to support significant $SO_4^{2-}$ reduction. Iron concentrations in both groundwater wells (<0.2 mg/L) are low relative to higher manganese concentrations (<0.5 mg/L).

Rock sampled from the Smoky Canyon backfill D and external A dumps has variable water content that is consistently below reported moisture retention capacities (Table 6) [3]. At the Enoch Valley Mine site, gas and moisture conditions fall between the partially aerobic, dominantly unsaturated conditions at the Smoky Canyon Mine and the suboxic, more saturated conditions at Dry Valley backfills. Core samples from the Enoch Valley Mine are also unsaturated, but generally have higher water content than the Smoky Canyon Mine samples. Oxygen is not detected in samples collected from below 30 feet of depth at the Enoch Valley Mine, in spite of the unsaturated character of these deposits, reflecting the distinct manner in which the rock was dumped in individual lifts during facility construction. The locations sampled in this study thus offered a representative range of conditions in which to study changes in the SeRB microbial community within mined overburden under field conditions. The *in situ* monitoring data in Table 6 suggest that observed differences in $SeO_4^{2-}$ concentrations between the Dry Valley and Smoky Canyon mines are more likely the result of variations in $O_2$ levels, moisture content, and lithology (e.g., Se, N and C content and material texture) than differences in temperature or pH, which show little difference between locations. These variables, which can be influenced by mine facility design, were used to further explore changes in microbial community numbers and diversity.

A comparison of the estimated number of SeRB with the physical and chemical parameters of the samples shows that the greatest numbers of SeRB occur in shale or groundwater, where soluble Se and DOC contents are elevated and samples are saturated. Interestingly, measurements of $O_2$ within the drill hole annulus and/or bulk moisture

contents shown in Table 6 (e.g., at the macro scale) do not correspond directly with SeRB numbers. This is not surprising due to the fine-grained and tightly compacted nature of the hydrocarbon-rich shales, which have a greater capacity to develop anoxic conditions as a result of aerobic hydrocarbon metabolism within partially saturated pore spaces that would not be evident at the macro scale. Significant populations of SeRB were not evident in MPN tubes under aerobic conditions or in samples of chert or mudstone with lower moisture or water soluble organic carbon content, nor were they present in near-surface shales with either low moisture content or low total Se concentrations. As might be expected, greater numbers of SeRB colonize shales where the total Se concentration is higher. For several samples where the SeRB were present in elevated numbers relative to other samples (AS71, DS75, MS5, MS73, and MS285), the ratio of soluble to total Se was (0.03-0.15) reflecting possible microbial influences on net Se solubility.

Identity of SeRB

The phylotypes associated with the SeRB isolated from the majority of $SeO_4^{2-}$ reducing enrichments were more than 99% identical to members of the *Dechloromonas* genus [41]. The *Dechloromonas* OTU's were most similar to *D. aromatica* [42], *D. denitrificans* [43], *D. hortensis* [44],and *Dechloromonas*A-34, a novel organism that was isolated from the Smoky Canyon Mine shales (Childers, unpublished). All of the *Dechloromonas* isolates obtained in this study could respire $SeO_4^{2-}$ (Childers, unpublished) and reduced soluble $SeO_4^{2-}$ to $Se^0$. Dechloromonads are rod-shaped, gram-negative, non-spore forming, strictly-respiring facultative anaerobes that can couple the oxidation of short chain volatile fatty acids and simple dicarboxylic acids to the reduction

of (per)chlorate, $O_2$, and in some strains, $NO_3^-$ [45]. Until now, no dechloromonads have been shown to respire $SeO_4^{2-}$, although some species can respire $SO_4^{2-}$ and $NO_3^-$. The $SeO_4^{2-}$-reducing *Decholoromonas* isolates obtained in this study did not grow with chlorate or perchlorate [hereafter referred to as (per)chlorate], indicating they cannot respire (per)chlorate and are physiologically distinct from all known *Dechloromonas* species isolated to date (Childers, in preparation). The majority of $SeO_4^{2-}$-reducing *Dechloromonas* isolates obtained in this study are most closely related to *D. aromatica* RCB which grows anaerobically by coupling the oxidation of benzene to nitrate or (per)chlorate, and has been shown to oxidize toluene, ethylbenzene, and xylene isomers using nitrate, (per)chlorate or $O_2$ as electron acceptors [42, 46]. Similar aromatic hydrocarbon compounds, such as benzene, toluene, and anthracene were identified in methylene chloride extractions of shales from the Phosphoria Formation (Table 7) and may provide a substrate for the $SeO_4^{2-}$ reducing dechloromonads present in these sediments.

Organisms from the *Dechloromonas* genus were readily isolated and their 16S rRNA genes amplified from groundwater, and they were detected frequently in culture enrichments. They could not be isolated from the most dilute positive MPN cultures, were not identified in clone libraries, and occurred only as faint bands in DGGE analyses of rock samples, however. This suggests that they may be present in very low numbers in unsaturated rock, or alternatively, that the enrichment and cultivation methods select for these organisms and misrepresent their relative dominance in the enrichment community (Figure 14). It also appears that these dechloromonads thrive under saturated conditions,

as they seem to be more readily isolated from saturated sediment samples collected from groundwater monitoring wells, but were not enriched from samples from unsaturated environments.

The second most frequently isolated SeRB was typically more than 99% identical to a *Stenotrophomonas* member, specifically *S. maltophilia*, and was also only found associated with groundwater rather than unsaturated shale. *S. maltophilia* can reduce $SeO_4^{2-}$ and $SeO_3^{2-}$ to $Se^0$ during stationary phase under microaerophilic conditions; cells are thus not dependent on $SeO_4^{2-}$ or $SeO_3^{2-}$ for growth [47, 48]. *S. maltophilia* has also been shown to reduce $SeO_4^{2-}$ in a mixed community of SeRB in coal mine tailing pond sediments [25], and was identified as a member of a benzene-degrading microbial consortium [49]. Interestingly, no *Stenotrophomonas* species were isolated from, or identified in the DGGE analysis of the most dilute positive MPNs. Members of this genus were also not identified in the clone libraries suggesting that it, too, may not be numerically important in the rock samples.

None of the other isolates obtained during this study exhibited $SeO_4^{2-}$ reduction independently, under the conditions used in this study. For example, efforts to isolate individual SeRB from the most-dilute positive MPN culture for the AS71 and AS113 samples were unsuccessful (e.g., individual phylotypes similar to organisms known to be capable of reducing $SeO_4^{2-}$ to $Se^0$), yet members of the *Rhodoferax* and *Cellulomonas* genera and *Actinobacteria* were identified from these samples through cultivation and/or confirmed through comparison with known isolates using DGGE. For this reason, there seems to be additional potential for Se reduction at the community level which could not

be demonstrated here, potentially involving different organisms handling individual steps of the reduction from the $SeO_4^{2-}$ to the $Se^0$ form. Several *Rahnella* spp. were isolated and although these isolates did not demonstrate $SeO_4^{2-}$ reduction, members of the *Rahnella* genus have been reported to reduce $SeO_4^{2-}$ in sediments from the Nile Delta [50]. *Rahnella* may play a broader community role, in that several members of this genus facilitate phytoremediation of Cd, Pb, Zn, and U [51] and have potential to dissolve hydroxyapatite, a common phosphate mineral [52, 53]. Likewise, the *Rhodoferax* isolates from this study did not reduce $SeO_4^{2-}$ although *R. fermentans* was identified as a $SeO_4^{2-}$-reducing microorganism in previous work at Smoky Canyon[26].

A mixed culture of one *Rhodoferax* and one *Cellulomonas* isolated from the most dilute AS113 MPN culture did show weak, late growth stage reduction of $SeO_4^{2-}$ to $Se^0$, but the $SeO_4^{2-}$ reduction was weak compared to the $SeO_4^{2-}$-reducing capacity of the original MPN culture from which the microorganisms were isolated. The potential capacity of *Rhodoferax* and/or *Rahnella* spp. to reduce $SeO_4^{2-}$ to $SeO_3^{2-}$, coupled with reduction to insoluble forms by other isolated $SeO_3^{2-}$-reducing genera such as *Cellulomonas* or *Pseudomonas,* should be further investigated to explain the inability to isolate an individual organism capable of reducing $SeO_4^{2-}$ to $Se^0$ in the most dilute MPN cultures. *Anaeromyxobacter* is another genus with members that are capable of community level Se reduction. Members of this genus are known to reduce $SeO_4^{2-}$ [54], and this genus was identified in clone libraries for both shale samples in this study, but was not isolated or identified in DGGE work.

The highest numbers of SeRB were associated with hydrocarbon-rich shales, which suggests that SeRB may be capable of coupling the reduction of $SeO_4^{2-}$ directly to the oxidation of complex hydrocarbons. Isolates grew on carbon extracted from the rock, which contained a mix of aliphatic and aromatic compounds of varying complexity, but each isolate has not been tested for growth independently on the individual compounds present in the extracts. However, none of the *Dechloromonas* isolates obtained as part of this study showed an ability to couple $SeO_4^{2-}$ reduction with the oxidation of benzoate.

Selenate-reducing isolates with strong similarity to the genera *Dechloromonas, Stenotrophomonas,*or *Rahnella* could not be isolated from the most-dilute positive MPN tubes of rock samples or identified in the clone libraries, indicating that these organisms are present in low numbers in samples of unsaturated rock but were selected for by the enrichment methods used in this study. The opposite is true for the groundwater samples, as all of the *Dechloromonas, Stenotrophomonas* and *Rahnella* isolates were obtained from groundwater sources. The DOTUR curves (Appendix A3) indicate that the clone libraries, while small, reasonably represent the observed variation in the bacterial community, but the 16S rRNA gene sequences for the $SeO_4^{2-}$-reducing isolates are absent in the clone libraries. This supports the conclusion that the Se-reducing organisms are rare members of the overall microbial community, and that the reduction of Se is a relatively minor part of the overall biogeochemical activity within the backfills.

A previous study reporting on the isolation of SeRB within phosphate mine wastes in SE Idaho yielded results different from this study [27]. In the earlier study, no *Dechloromonas* representatives were obtained from phosphate overburden nor were any

identified in the DGGE analyses, although uncultivated *Rhodocyclaceae* were reported by Knotek-Smith [55]. Archived samples of overburden used in this previous study were obtained and several members of the *Dechloromonas* genus were successfully isolated from the material, indicating that fundamental differences in the techniques used to isolate SeRB are likely the reason for the discrepancy.

Community Characteristics and Diversity

Results of this study provide a broader understanding of the microbial ecology of these complex overburden deposits. Many of the sequences obtained for microbes isolated from groundwater or enriched rock samples were not SeRB. Phylotypes with a high degree of similarity to denitrifying and hydrocarbon-oxidizing, and $Fe^{3+}$or $Mn^{4+}$-reducing organisms appear to dominate the microbial population of these variably saturated, phosphate overburden sediments. In the clone libraries, the only known $SeO_4^{2-}$-reducing genus that was detected was *Anaeromyxobacter;* the $SeO_3^{2-}$-reducing genus *Geobacter* was also identified.

Sequences related to S-oxidizing and methanotrophic organisms were also detected in clone libraries. Conversely, phylotypes with high similarity to known $SeO_4^{2-}$-reducing organisms were more frequently identified in isolates and DGGE-isolated bands than in clone libraries, and were more common in saturated sediments and in unsaturated samples of shale. It is therefore likely that these phylotypes (and the organisms they represent) occur in relatively low abundance within the overall microbial community.

Microbial phylotypes identified in this study indicate the presence of a mixture of aerobes and anaerobes, many closely related to microorganisms that can degrade

hydrocarbons. Degradation of aromatic hydrocarbons, such as benzene, naphthalene, phenanthrene, and other naturally occurring hydrocarbon compounds present in the Meade Peak shale sediments, is most likely to be most efficient under aerobic conditions, but can be stimulated by $NO_3^-$ and $Fe^{3+}$ or $Mn^{4+}$ reduction under anaerobic conditions. Demand for $O_2$ during such degradation can be sufficient to create locally anaerobic zones within soils [56], and may explain the development of microscale anaerobiosis in shales located within relatively aerobic waste facilities.

Several phylotypes similar to genera that are capable of aerobic or facultative degradation of hydrocarbons were identified. *Sphingomonas* [57], as well as *Anaeromyxobacter* and *Brevundimonas* [58], are heterotrophs and contain species capable of polycyclic aromatic hydrocarbon degradation. Members of the genera *Pseudomonas, Nocardioides,* and *Rhodoccoccus* are similar to the phylotypes identified in this study are also known to degrade aromatic carbon aerobically [59, 60]. Of particular interest is that *Polaromonas*, a genus of aerobes in the *Commamonadacae* family, have high metal tolerance and the capacity for degrading a variety of hydrocarbons, ranging from alkanes to aromatic compounds, under a wide range of metal and salinity exposures [61]. Phylotypes identified in this study were highly similar (>99%) to a *Polaromonas* sp. isolated from a naphthalene contaminated soil sample [62, 63].

Members of the genus *Anaeromyxobacter* are known to oxidize reduced humic acids [6] and can concurrently reduce Se and Fe [64]. Some *Variovorax* spp. can degrade polycyclic aromatic hydrocarbons under aerobic or $NO_3^-$ reducing conditions at low

temperature [65]. Members of the *Acidovorax* and *Pseudomonas* genera were associated with aerobic benzene-degrading communities in a study of $O_2$ depleted groundwater [56] and *Pseudorhodoferax* spp. are also known hydrocarbon-degrading members of the *Comamonadaceae* family [66]. Of additional interest is the capacity of a comamonad, *Rhodoferax ferrireducens,* to degrade benzoate under both aerobic and anaerobic conditions, as well as its ability to reduce $Fe^{3+}$ and $NO_3^-$ [67].

Hydrocarbon degradation is also fueled by Fe cycling in suboxic environments [68, 69]. Microaerophilic $Fe^{2+}$oxidation, and anaerobic, $NO_3^-$-dependent re-oxidation of $Fe^{2+}$, are important contributors to Fe-cycling in carbon-rich sediments [70-72]. Several of the *Bacteria* isolated from these phosphate overburden communities are strongly similar to organisms shown in published studies to couple hydrocarbon degradation to Fe- redox cycling and they are thus inferred to do so in these deposits. For example, *Geobacter metallireducens* was one of the first anaerobes shown to oxidize organic compounds using $Fe^{3+}$ as an electron acceptor [73], and perchlorate and $NO_3^-$ dependent re-oxidation of $Fe^{2+}$ by two species of *Dechloromonas* [42] was shown to occur in anaerobic settings. Abiotic oxidation of $Fe^{2+}$ by $Mn^{4+}$ may also support this process [68], by resupplying $Fe^{3+}$ for reduction by organisms similar to *R. ferrireducens,* a facultative $Fe^{3+}$-reducer highly similar to the phylotype identified in these sediments [40]. Other organisms similar to phylotypes identified in this study with capacity to reduce $Fe^{3+}$ and $Mn^{4+}$ include members of *Desulfuromonas* [74], G*eobacter* [75, 76], *Rahnella* and *Anaeromyxobacter.* Members of the genus *Pelosinus* are also known to reduce $Fe^{3+}$ and

humic compounds [77, 78]. Members of the *Acidobacteria* and *Acidovorax* genera identified in the backfill are additional potential contributors to $Fe^{2+}$ oxidation [79].

The community analysis suggested additional cycling of S, N, and methane. Both S-oxidizing and -reducing genera were identified in clone libraries, including *Acidoferrobacteria, Desulfuromonas,* and Candidatus *Magnetobacteria*. Methanotrophic genera known to be capable of dentrification (*Acidothermus*) and oxidation of organic compounds (*Methylobacter/Methylotenera*) were also identified in the clone libraries. These *Bacteria* are potentially important members of the community in sulfide-bearing sediments which exhibit elevated levels of $NO_3^-$ and $SO_4^{2-}$ due to $NO_3^-$ compounds used in blasting and the oxidation of sulfide minerals in the mining environment.

Communities with similar microbial diversity and ecology have been described in other low temperature subsurface, hydrocarbon-influenced environments. A consortium comprised of *Acidovorax, Pseudomonas, Sphingomonas,* and *Variovorax* species was reported under aerobic and nitrate-reducing conditions in soils where naphthalene, phenanthrene, and fluorene were degraded [65]. Phylotypes with a high degree of similarity to *Polaromonas* spp. were identified with *R. ferrireducens* in a mixed consortium of aerobic and anaerobic benzene-degrading organisms in a groundwater setting [80]. *Polaromonas* spp. were also present with *Acidobacterium* and *Sphingomonas* organisms in a benzene-degrading soil consortium [81]. In an $O_2$-depleted benzene contaminated groundwater, *Acidovorax*, *Pseudomonas*, and *Rhodococcus* spp. varied in relative abundance based on changes in $O_2$ availability [56]. An *Acidovorax* sp. was also identified as a member of a benzene-oxidizing, chlorate-reducing consortium

with *Mesorhizobia* and *Dechloromonas* spp. [49]. These studies support the probable role of representatives of the *Acidovorax, Polaromonas, Sphingomonas,* and *Variovorax* genera in hydrocarbon degradation under mixed aerobic/anaerobic conditions in subsurface backfills in S.E. Idaho.

## Summary

The diverse community of microorganisms identified in phosphate overburden at three mine sites located across the S.E. Idaho Phosphate Resource area can work together to reduce $SeO_4^{2-}$ under $Fe^{3+}$, $Mn^{4+}$, and $NO_3^-$-reducing conditions, using available native hydrocarbon and other available electron donors. With variable $O_2$ and moisture conditions within the mined overburden, there are opportunities for both aerobic and anaerobic degradation of complex shale hydrocarbons, as suggested by the diversity of organisms identified. Degradation of complex shale hydrocarbons by aerobic members of the community may decrease available $O_2$, thus creating conditions favorable for $SeO_4^{2-}$ reduction by species of *Dechloromonas* and *Stenotrophomonas,* and perhaps other SeRB such as *Cellulomonas* and *Rahnella* spp. The most favorable conditions appear to be in saturated or moist environments (close to field capacity) where sufficient soluble Se and organic C are available to support growth of SeRB. Opportunities to extend this understanding of biogeochemistry in subsurface phosphate overburden deposits include further evaluation of community level heterotrophic $SeO_4^{2-}$ reduction and evaluation of which native hydrocarbon compounds are being consumed by SeRB in removing Se from solution, as well as controlled studies of how the relative availability of $NO_3^-$ and $O_2$

affects the reliability of SeRB activity. Field scale monitoring of biogeochemistry and microbial community response to changes in $O_2$ availability that result from operational changes in mine rock placement and water management is needed to demonstrate how *in situ* microbial reduction of Se performs at the field scale. Based on the results of this study, an effective operational application of *in situ* microbial source control of Se in backfilled phosphate overburden will need to consider the influence of $O_2$ and $NO_3^-$ concentration, lithology, and water availability on the activity of SeRB, as well as the influence of $Fe^{2+}$ on the native hydrocarbon degrading community.

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# CHAPTER FIVE

## KINETICS OF SELENATE REDUCTION BY NATIVE MICROBES IN SATURATED PHOSPHATE MINE WASTE

### Contribution of Authors and Co-Authors

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Contributions: Principal investigator, led field work, designed and conducted all experiments and analyses.

Co-Author: Jared J. Bozeman

Contributions: laboratory assistant with all aspects of the project, data reduction, principal investigator as undergraduate scholar responsible for clone library construction and analysis.

Co-Author: Brandy D. Stewart

Contributions: Mineralogical analyses, using XRD, XANES, and S-XRD. Data reduction and interpretation.

Co-Author: Robin Gerlach

Contributions: Advisor, analytical and organic chemistry, chemical engineering. Supervised laboratory research and analyses.

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Contributions: Major Advisor, Biological and Chemical Engineering. Supervised laboratory research and analyses.

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# KINETICS OF SELENATE REDUCTION BY NATIVE MICROBES IN SATURATED PHOSPHATE MINE WASTE, S.E. IDAHO

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## ABSTRACT

Selenate ($SeO_4^{2-}$) reduction by native microbes, using naturally-occurring carbon in chert and shale phosphate overburden, has been studied under temperature, oxygen, and lithological conditions representative of subsurface backfills in S.E. Idaho Phosphate Resource Area. Selenate reduction is a biotic process, wherein $SeO_4^{2-}$ is reduced when trace oxygen ($O_2$) and nitrate ($NO_3^-$) in saturated, microaerophilic sediments is consumed by aerobic and denitrifying microbial activity. The rate and biogeochemical reduction pathways are lithology and temperature dependent. Near-complete reduction of $SeO_4^{2-}$ to selenite ($SeO_3^{2-}$) and elemental Se ($Se^0$) occurred more rapidly in chert, while $SeO_4^{2-}$ was reduced to selenomethionine, $Se^0$ and selenide minerals more slowly in shale. Concurrent hydrocarbon oxidation coupled to $Fe^{3+}$, $Mn^{4+}$, and $NO_3^-$ reduction was observed, with less than 40% of the available dissolved organic carbon used during the reduction process. Shifts in the community of microbes were observed, from an initial consortium comprised of the genera *Dechloromonas, Rhodoferax, Brevundimonas,* and *Sphingomonas* to one containing additional phylotypes associated with the genera *Ralstonia* and *Rahnella*. These results explain field-scale observations of differential Se reduction at different mine site locations due to changes in moisture and oxygen availability, and suggest that design of facilities to achieve *in situ* Se stabilization using native microbes and carbon is possible through management of water, rock, and gas flux.

## Introduction

Selenium (Se) release from mine waste may impact water quality and has potential to threaten aquatic ecosystems [1]. Mobility and toxicity depend upon Se speciation in response to complex biogeochemically-driven redox processes [2, 3]. Mine waste deposits host diverse communities of microbes known to influence metal, sulfur (S)[4], and nitrogen (N) geochemistry [5]. Biofilms developed on mineral surfaces within these deposits afford mixed microbial communities opportunities to manage water, carbon (C), nutrient, and oxygen ($O_2$) budgets [6-10]. The low solubility of Se under moderately reducing conditions has raised broad interest in microbial reduction of Se for bioremediation purposes [11, 12], but intentional development of biogeochemical conditions that foster Se reduction and attenuation within engineered mine waste facilities remains relatively unexplored. This study explores the biogeochemistry, rate, and products of Se reduction by a native consortium of microbes, under saturated microaerophilic conditions, using naturally available C in mined phosphate overburden facilities in S.E. Idaho Phosphate Resource Area. Improved understanding of the microbial ecology of *in situ* Se reduction can guide future efforts to design facilities for *in situ* Se source control.

Selenium exists in four major oxidation states, Se(VI), Se(IV), Se(0), and Se(-II) [13]. It substitutes for S in a variety of minerals, such as pyrite ($Fe(Se^{-II}, S^{-II})_2$ ) and gypsum, $Ca(Se^{VI}O_4, SO_4)\cdot 2H_2O$, which have potential to release Se when oxidized or leached [14]. Reduced Se minerals can be oxidized through biotic or abiotic processes [15-17]. Under acidic conditions, Se oxyanions are readily sorbed to highly protonated

surfaces of iron (Fe) and manganese (Mn) oxides [18-23], aluminum oxides [24], and clays [25, 26]. At neutral pH, selenate ($SeO_4^{2-}$) is poorly attenuated and sorption is only efficient for selenite ($SeO_3^{2-}$) [27, 28]. Alkaline and oxidized mine waste thus release $SeO_4^{2-}$ oxyanions that persist in solution and may bioaccumulate [1].

Kinetic barriers to abiotic $SeO_4^{2-}$ reduction create opportunities for its microbial respiration [29], while other organisms are known to reduce or methylate $SeO_3^{2-}$ for detoxification purposes [30]. Microbial reduction of soluble and potentially toxic $SeO_4^{2-}$ and $SeO_3^{2-}$ to the less soluble $Se^0$ and $Se^{2-}$ minerals limits Se mobility and bioavailability [31]. Organo-Se compounds, including selenocysteine (SeC) and selenomethionine (SeM), result from Se assimilation [32], and a variety of methylated compounds are produced via detoxification pathways [33, 34]. Facultative or anaerobic Se-reducing microbes range from mesophilic to extremophilic *Bacteria* and *Archaea* that are adapted to extremes of pH, salinity, or temperature [35, 36]. Some can reduce $SeO_4^{2-}$ fully to $Se^0$, while others are capable only of reducing $SeO_4^{2-}$ to $SeO_3^{2-}$ or $SeO_3^{2-}$ to $Se^{2-}$. Reduction of soluble oxyanions to the most reduced Se(0) or Se(-II) oxidation states can thus depend on community-level interactions between multiple organisms [37]. The ecology of microbial communities that reduce Se in mine waste is likely to be influenced by $O_2$ and C availability, as well as cycling of N, S, Fe, and Mn.

Groundwater monitored in backfilled sediments at two S.E. Idaho mines that produce phosphate from the Permian Phosphoria Formation (Figure 17), Dry Valley Mine (well GW7D2a) and Smoky Canyon Mine (well GW11), have significantly different soluble Se concentrations. This is despite the fact that both wells were completed in mixed

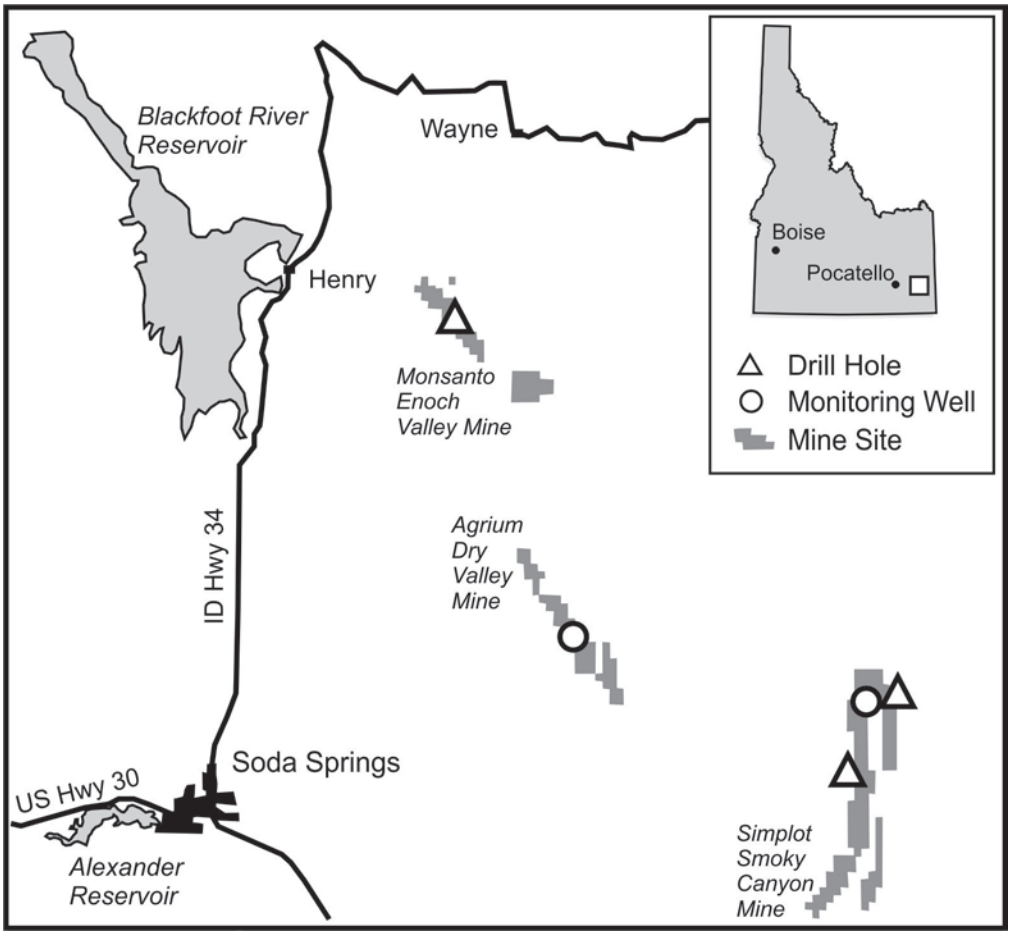



Figure 17. Map showing drill hole and monitoring well sampling locations at the Agrium Dry Valley and Simplot Smoky Canyon Mines, S.E. Idaho.

overburden that was mined from the same geologic formation using similar methods [38]. Concentrations of Se in Ca-$HCO_3$-$SO_4$ groundwater at the Dry Valley Mine, where seleniferous rock is saturated in deep backfill, have remained at or below the Idaho groundwater standard of 0.05 mg/L for more than 10 years, suggesting an ongoing process of microbial Se reduction. This is in contrast to concentrations as high as 1 mg/L measured in variably saturated, aerated backfill at the Smoky Canyon Mine. Highly carbonaceous, Se-enriched black shale and a clay-rich, Fe-oxide bearing chert are the dominant overburden lithotypes mined in the S.E. Idaho Phosphate Resource Area, with

lesser amounts of mudstone. The relative percentage of each lithology varies within randomly placed backfilled overburden.

## Objectives

In Chapter 4, a consortium of native microbes with potential for $SeO_4^{2-}$ reduction in subsurface mine waste at three S.E. Idaho phosphate mines was identified and enumerated under a range of representative $O_2$, moisture, and lithology conditions using samples of saturated and unsaturated overburden. A most probable number (MPN) method was used to estimate the number of $SeO_4^{2-}$-reducing bacteria (SeRB) in groundwater, chert, shale, and mudstone samples, and changes in microbial diversity as a function of lithotype and moisture conditions were compared using clone libraries and denaturing gradient gel electrophoresis (DGGE). The most favorable conditions for Se reduction appear to be in shale, under saturated or moist conditions (close to field capacity) where sufficient soluble Se and organic C is available to support higher numbers of SeRB.

Results reported in Chapter 4 for Se reduction in mixed overburden suggest that variable $O_2$ and moisture conditions within the mined overburden create opportunities for both aerobic and anaerobic degradation of complex shale hydrocarbon, as reflected by the diversity of identified hydrocarbon-degrading organisms. Results also indicate that SeRB reduce $SeO_4^{2-}$ using naturally-occurring C compounds under $Fe^{3+}$, $Mn^{4+}$, and $NO_3^-$ reducing conditions. It is proposed that degradation of complex shale hydrocarbons by aerobic members of the community decreases available $O_2$, thus creating conditions

favorable for $SeO_4^{2-}$ reduction by the facultative anaerobes identified in Chapter 4 including *Dechloromonas* spp. and *Stenotrophomonas* spp., and perhaps other heterotrophic SeRB.

To explain the observed differences in soluble Se concentrations at the Dry Valley and Smoky Canyon mines, we investigated the rate and extent of Se reduction in saturated batch reactors, under variable temperature and lithological conditions with limited $O_2$. These experiments address the hypotheses that (1) Se reduction observed in the sediments at the Dry Valley Mine is microbial and not abiotic; (2) reduction is most efficient at elevated temperature after $O_2$ is depleted; (3) changes in pH, dissolved organic carbon (DOC), and dissolved $NO_3^-$, $SO_4^{2-}$, total Mn, and total Fe in chert and shale mine waste are associated with the observed reduction, and (4) creating moist and microaerophilic conditions in sediments will promote Se reduction by native microbes using naturally available C and potentially, other available electron donors.

## Experimental

Samples of overburden and groundwater were collected from the Dry Valley and Smoky Canyon mines (Figure 17) and analyzed to describe the mineralogy, metal chemistry, soluble constituents, and C speciation of the materials used to construct reactors (supplement, Tables S1 to S4). Autoclaved samples of the dominant chert and shale lithologies collected from backfill were inoculated with live groundwater cultures from the Dry Valley and Smoky Canyon mines in saturated batch reactors. The rate and extent of $SeO_4^{2-}$ reduction (using naturally-occurring hydrocarbon compounds and other available electron donors) were measured experimentally under the temperature and

lithologic substrate conditions observed during *in situ* monitoring. Changes in dissolved $O_2$ and DOC, pH, and soluble $NO_3^-$, $SO_4^{2-}$, total Fe, and total Mn were measured at key points during the reduction process, including lag, initial reduction, mid-reduction, and post-reduction phases. Changes in Se mineralogy resulting from the monitored reduction process were studied using synchrotron x-ray diffraction (S-XRD) and bulk x-ray adsorption near edge spectroscopy (XANES). The response of the microbial community associated with $SeO_4^{2-}$ reduction was characterized using DGGE.

Saturated Batch Reactor Rate Experiments.

Batch reactors were constructed in triplicate using 30 g of sterilized sub-2 mm shale and chert with 30 mL of sterile deionized water in 250 mL glass serum bottles. The grain size of the sub-2 mm material is described in Appendix A-2. Rock was autoclaved (steam sterilized 45 min at 121°C at a minimum of 15 psi), rested for 48 hours, and re-autoclaved to kill spore-forming organisms prior to reactor construction. A sub-sample of live groundwater was also autoclaved for use in the sterile control experiment. Saturated sediment was stirred in the presence of $O_2$ for 12 hours to dissolve any existing oxidation products present in the waste rock, which result from weathering under the aerobic conditions known to exist within near surface portions of mine waste. The saturated sediment was then then inoculated with a consortium of native microbes through the addition of turbid site groundwater to bring the reactor to full volume (using fresh and autoclaved groundwater to create a live and killed control reactor, respectively). The sealed bottles were degassed with 0.2 µm filter-sterilized ultra-pure $N_2$ gas for 1 hour to remove most of the $O_2$. Each reactor was spiked with $Na_2SeO_4$ stock solution to a target

concentration of 10 mg/L Se as $SeO_4^{2-}$ and incubated at room temperature (between 20 and 25°C) and 10°C under dark conditions. The actual starting concentration varied in response to Se release from the rock in each reactor. No C was added as electron donor to the hydrocarbon that was naturally present in the rock and groundwater. Samples of mixed water and sediment were collected immediately using a $N_2$ purged syringe, and every 12 hours until Se reduction was complete (10 to 14 days). Oxygen and pH were measured in the aqueous phase at the beginning, middle, and end of each experiment, using a Hach AQ4 dissolved $O_2$ meter (Loveland, CO) and an Acumet AB15 pH meter with a probe model no. 13-620-AP (Cole Parmer).

Samples were centrifuged at 13,000x g to remove solids. The supernatant and the solids were separated and frozen for subsequent analysis including total Se, Fe, and Mn by Inductively Coupled Plasma (ICP) Mass Spectroscopy (MS); $NO_3^-$, $SO_4^{2-}$, $PO_4^{3-}$, $SeO_4^{2-}$, and $SeO_3^{2-}$ by ion chromatography (IC); and analysis of $SeO_4^{2-}$ and $SeO_3^{2-}$ at lower detection limits, together with SeC and SeM, by High Performance Liquid Chromatography (HPLC-ICP-MS). Protein content was measured at select time steps to track changes in biomass, using the Coomassie method [39]. Aqueous samples were also collected following centrifugation to remove solids and were preserved in glass at pH < 2 using phosphoric acid for analysis of DOC and total N. Additionally, samples of sediment were collected at multiple time steps for DNA extraction and mineralogical analysis.

ICP-MS Analysis of Total Se, Fe, and Mn Concentrations: Samples were diluted 1:500 in 1% $HNO_3$ and 0.5% HCl for measurement of total Se, Fe, and Mn by direct

injection ICP-MS (Agilent 7500ce ) following EPA method 200.8 [40]. Total Se was determined with the hydrogen-gas collision cell, to minimize analytical interferences, while Fe and Mn were measured directly without the collision cell. Limits of detection varied slightly within the analytical runs, but were generally 2 µg/L for Se, 1 µg/L for Fe, and 1 µg/L for Mn.

IC Analysis of $NO_3^-$, $SO_4^{2-}$, $PO_4^{3-}$, $SeO_4^{2-}$, $SeO_3^{2}$: Anion concentrations were measured by IC using a Dionex instrument with an IonPac AS-9-HC (4 x 250 mM) anion column and a CD 20 detector. Samples (25 µL) were injected into an 11 mM $Na_2CO_3$ mobile phase flowing at 0.9 mL/min, at full strength to measure low concentrations of $SeO_4^{2-}$ and $SeO_3^{2-}$, and diluted 25X with deionized water to measure higher concentrations of $SO_4^{2-}$ and $NO_3^-$.

Se Speciation by HPLC-ICP-MS. Selenium was speciated using an Agilent HPLC and a Hamilton PRPX-100 PEEK anion column with a pH 4.8, 5 mM ammonium citrate mobile phase modified to 2% methanol by weight [41]. The mobile phase was delivered isocratically at 1 ml/min for analysis using an Agilent 7500ce ICP-MS to quantify $^{78}Se$ using time resolved analysis with a plasma argon (Ar) flow of 15 L/min.

DOC and Total N Analyses: DOC and total N were measured using a Shimadzu TOC-V CSN Carbon/Nitrogen Analyzer (Kyoto, Japan). DOC was measured by standard method 5310 (non-dispersive infrared $CO_2$ detector) following acidification and two minutes of sparging with zero air (air with less than 0.1 ppm hydrocarbon). Total N was measured using standard method 4500 [42] following treatment with ozone.

Protein Assays: Protein was extracted following 0.5 N NaOH digestion for 10 min at 90°C, followed by neutralization with HCl. Digested samples were assayed using the Pierce Plus Coomassie protein assay method (Pierce no. 23236). Concentrations were measured by absorption at 630 nm using a Biotek Synergy HT multidata microplate reader. Replicate protein assays were attempted using the Pierce Compat-Able Protein assay (Pierce No. 23215) to remove interferences. For comparison, protein measurements were also made using a Qbit fluorimeter with the NanoOrange® Protein Quantitation kit.

XANES and S-XRD of Se Minerals: Se x-ray absorption spectra (XAS) were collected on beamline 4-1 at the Stanford Synchrotron Radiation Laboratory using published methods [43]. Samples were deposited on membranes and sealed with Kapton polyimide film to minimize oxidation. A liquid $N_2$-cooled Si (220) monochromator was used for energy selection, and higher order harmonics were rejected by detuning 30%. Fluorescence spectra were collected with a Lytle detector for Se. Extended X-ray fine structure (EXAFS) and XANES spectral scans were averaged, with the pre- and post-edge subtracted using SixPACK (Webb, 2005). Synchrotron-based XRD data were collected at SRL on beamline 11-3 and calibrated with lanthanum hexaboride and used to confirm the presence of species identified based on near-edge energy thresholds.

DNA, PCR, DGGE, and Sequencing: Nucleic acids were extracted from 1 g sediment samples taken from enrichment microcosms, or frozen samples of soil and groundwater, using the Power Soil DNA Isolation Kit ™ (MoBio). The method was modified by first incubating 1 g of sediment in 20% SDS at 70$^{o}$C for 1 hour, followed by 20 minutes in a vortex mixer. After vortexing, the entire sample was used for the

extraction of nucleic acids as detailed in the protocol provided by the manufacturer. PCR was performed on extracts to amplify 16S rRNA genes using a nested approach. Initially, 10 cycles were run using primers 1070F (5'-ATG GCT GTC GTC AGC T-3') and 1392R (5' ACG GGC GGT GTG TAC-3') [44]. The products from the initial PCR were diluted 1:10 and used as template in a 30 cycle PCR using 1070F and 1392R with a 40 base GC clamp. Reactions (50 µl) contained template (2 µl), 10 mM primers (1 µL ea), PCR mastermix (Promega, 25 µL) and were run in an Eppendorf Mastercycler Gradient thermocycler. Conditions included denaturation at 94°C for 10 min, followed by 10 or 30 cycles of 94°C for 45sec, 50°C for 45 sec, 72°C for 45 sec, and a final extension at 72°C for 7 min. Samples of PCR amplicons were visualized by ethidium bromide staining on 0.8% agarose gel.

PCR products were separated by electrophoresis in 8-12% acrylamide gels containing a 50-60% urea-formamide gradient at 70 V and 60°C for 20 hours. Gel electrophoresis was run using a DGGE-2401 system manufactured by CBS Scientific®, based on the general method described by Muyzer et al. [45]. Gels were stained with SYBR Gold and visualized under UV light. Samples were compared with a ladder comprised of DNA from known isolates, and individual bands of interest not present in the ladder were excised from the gel, resuspended in 15 µL of nuclease-free water, and allowed to diffuse from the gel overnight at 60°C. Samples were mixed and acrylamide was pelleted by centrifugation. The resulting supernatant was removed, diluted 1:10 in nuclease free water, and used as template in a 30 cycle PCR using primers 1070F and 1392R as described above. PCR products were purified using a Wizard SV Gel and PCR

Cleanup System (Promega) and quantified using a Qbit fluorimeter (Invitrogen). Samples were submitted to the Molecular Research Core Facility at Idaho State University for sequencing using an Applied Biosystems® 3130XL Genetic Analyzer. BLAST was used to query resulting sequences against the GenBank database [46].

## Results and Discussion

Changes in Se, $NO_3^-$, Fe, Mn, and $SO_4^{2-}$ concentrations, and in the associated microbial community, as well as the minerals produced as a result of Se reduction, are described below. These experiments were conducted in suboxic, saturated chert and shale batch reactors at two temperatures using samples from two mine sites.

### Se Reduction in Batch Reactors

Figure 18a and b show results of saturated batch Se reduction rate experiments run at the field relevant temperature of 10°C and room temperature (between 20 and 25°C), using rock and groundwater from the Dry Valley and Smoky Canyon mines. Supporting data are provided as supplemental information in Appendix D, as Tables D1.1 and D1.2 for Dry Valley and D2.1 and D2.2 for Smoky Canyon; analytical data supporting these tables are provided on the accompanying CD. Rate experiments were also run using mixed shale, chert, and mudstone (run-of-mine) samples from both mine sites, which show results that reflect the combined influence of the dominant chert and

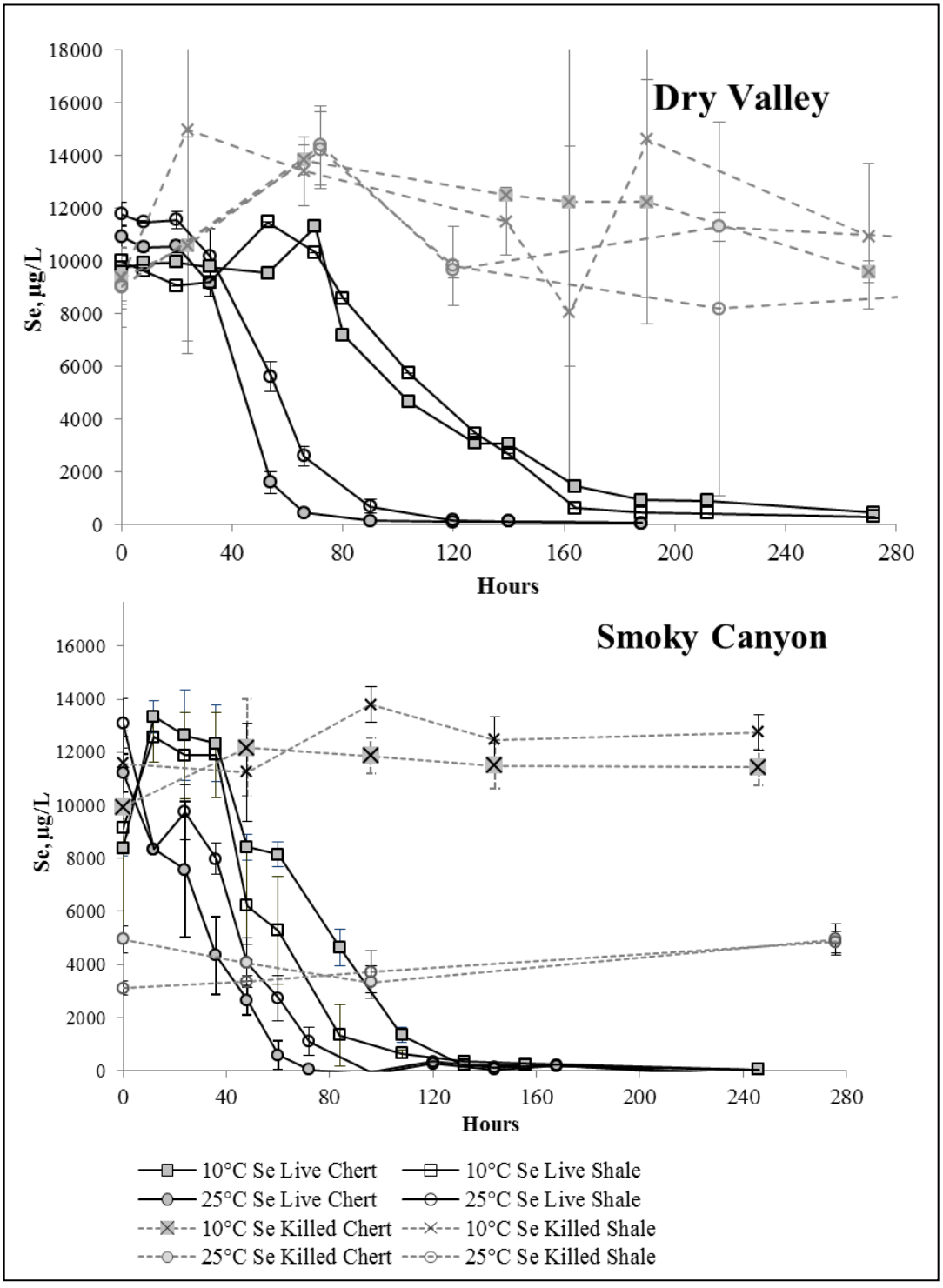



Figure 18. Comparison of Se concentrations in saturated rate experiments for two temperatures and lithologies for the a)Dry Valley and b)Smoky Canyon Mines. Values are averages (n=3), and error bars represent ± 1 standard deviation.

shale lithologies; these results are presented in Chapter 4, with supporting data presented in Appendix D.

Figure 18a shows total dissolved Se measured in live and killed control experiments for both shale and chert from the Dry Valley Mine. Results are given as the average, +/- the standard deviation, of three experimental replicates. Comparable trends in reduction are shown for shale and chert from the Smoky Canyon Mine in Figure 18b. Comparison with killed controls for these experiments shows that reduction did not occur under abiotic conditions in the reactors. In fact, dissolved Se concentrations increased in killed controls initially, above the spiked Se concentration of 10 mg/L, presumably due to abiotic oxidation of reduced Se-bearing sulfide minerals by residual $O_2$ or, following $O_2$ depletion, by $NO_3^-$. Continued dissolution of Se from mineral surfaces following closure and degassing of the reactors is also a possibility. This was less pronounced in live reactors, presumably due to biotic consumption of the available $O_2$ and $NO_3^-$.

Following a temperature-dependent lag phase, relatively rapid, near-complete reduction of soluble $SeO_4^{2-}$ was observed at the rates shown in Table 10. These rates were calculated by linear best fit to the steepest part of the reduction curve for each microcosm, using the data between the inflection point and the point at which concentrations become asymptotic at low concentration. In most cases, Se concentration was observed to increase slightly prior to the onset of reduction. There was a shorter lag time prior to initiation of Se reduction in the 10°C microcosms in samples from the Smoky Canyon Mine compared to those from the Dry Valley Mine (Figure 18). In samples from the Dry Valley Mine, Se reduction began sooner and proceeded more

Table 10. Dry Valley and Smoky Canyon mines, Se reduction rates.

| Rate of Se Reduction (Average (N=3) ± std dev, µg $SeO_4^{2-}$ reduced /g rock hr) | | |
|---|---|---|
| Mine Reactor °C | Chert | Shale |
| Dry Valley 10°C | 6.7 ± 1.6 | 3.0 ± 0.17 |
| Dry Valley 25°C | 10.3 ± 0.3 | 6.3 ± 1.09 |
| Smoky Canyon 10°C | 4.3 ± 1 | 4.3 ± 0.7 |
| Smoky Canyon 25°C | 6.0 ± 1.4 | 5.9 ± 0.5 |

rapidly in the chert than in shale regardless of incubation temperature. This is in contrast to the Smoky Canyon Mine where the rate of Se reduction in chert was very similar to that measure in shale. Unfortunately, monitoring well GW11, which produced the groundwater used to inoculate the Smoky Canyon reactor, was buried due to subsequent mine development, and it was not possible to repeat the experiment to verify the similarity in rate between lithotypes. It is likely that the more rapid Se removal in the experiments using chert from the Dry Valley Mine resulted from rapid sorption of $SeO_3^{2-}$ onto clay and/or Fe oxide mineral surfaces within the fine-grained, muddy matrix of the chert. This hypothesis is supported by mineralogical analyses reported below.

Se Speciation: Measurable concentrations of $SeO_3^{2-}$ were detected by IC (Tables D2-1 and D2-2, Appendix D2) mid-reduction in all reactors, except the 25°C chert reactor, where reduction proceeded very quickly. Select Dry Valley Mine samples were re-analyzed by HPLC-ICP-MS to more accurately measure $SeO_4^{2-}$, $SeO_3^{2-}$, SeC ($C_3H_7NO_2Se$), and SeM ($C_5H_{11}NO_2Se$) (Table 11). Unfortunately, the archived Smoky Canyon samples could not be re-analyzed with this method as they were beyond their holding time for volatile organo-Se compounds by the time the HPLC-ICP-MS analytical

capability became available, and the experiment could not be repeated following burial of the GW-11 well. The HPLC-ICP-MS results (Table 11) show that reduction of $SeO_4^{2-}$ to $SeO_3^{2-}$ was slower in shale than in chert, and was slower at 10°C than 25°C. Selenite was, not surprisingly, more efficiently removed from solution at higher temperatures, regardless of lithology. Measured SeC, an amino acid, was evident during Se reduction; this was most evident in the 10°C reactors. An increase in concentration of SeC reflects an assimilation of Se by bacteria, which appeared to increase under colder temperatures. The reason for this difference is not clear, but these data suggest that intracellular reduction may be favored at low temperatures. The measurement of SeC in supernatant solutions reflects the release of intracellular SeC, perhaps the result of detoxification or cell decomposition. Selenomethionine was observed at only one time step in one of the 1°C shale reactors, mid to late in reduction, along with unidentified organo-Se peaks that did not match standards included in our method. Chasteen and Bentley suggest that such results may indicate the methylation of Se and attributed accumulation of alkylation products to a toxicity response [34]. Such toxicity has been associated with limited capacity for Se reduction within a closed system [47].

The total mass of Se detected by HPLC-ICP-MS analyses from the Dry Valley Mine (Table 11) agrees reasonably well with the total Se analyses by ICP-MS (Figures 18 and 20, and Appendix D), with the dominant measured species being $SeO_4^{2-}$. Selenite was measured as a transient reduction product at much lower concentrations than $SeO_4^{2-}$; it is likely to have lower solubility due to its greater potential for sorption. These

Table 11. HPLC-ICP-MS data showing Se speciation for Dry Valley Mine chert and shale reactors at key time steps

| T°C | Lithology | Time, hours | Se-$SeO_4^{2-}$, mg/L | Se-$SeO_3^{2-}$, mg/L | Se-SeC, mg/L | Se- SeM, mg/L |
|---|---|---|---|---|---|---|
| 10 | Chert | 0 | 9.56 | b.d. | 0.045 | b.d. |
| 10 | Chert | 53 | 8.06 | b.d. | 0.048 | b.d. |
| S | Chert | 104 | 1.16 | 0.009 | 0.050 | b.d. |
| 10 | Chert | 128 | 1.46 | 0.001 | 0.041 | b.d. |
| 10 | Chert | 272 | b.d. | b.d. | 0.042 | b.d. |
| | | | | | | |
| 25 | Chert | 0 | 5.84 | b.d. | 0.022 | b.d. |
| 25 | Chert | 20 | 9.08 | b.d. | 0.014 | b.d. |
| 25 | Chert | 66 | b.d. | 0.007 | 0.019 | b.d. |
| 25 | Chert | 90 | b.d. | b.d. | 0.023 | b.d. |
| 25 | Chert | 120 | b.d. | b.d. | 0.013 | b.d. |
| 25 | Chert | 188 | b.d. | b.d. | 0.013 | b.d. |
| | | | | | | |
| 10 | Shale | 0 | 7.64 | b.d. | 0.051 | b.d. |
| 10 | Shale | 53 | 7.20 | b.d. | 0.049 | b.d. |
| 10 | Shale | 104 | 5.02 | 0.004 | 0.060 | b.d. |
| 10 | Shale | 128 | 2.51 | 0.014 | 0.046 | 0.023 |
| 10 | Shale | 272 | bd | 0.004 | 0.042 | b.d. |
| | | | | | | |
| 25 | Shale | 0 | 6.87 | b.d. | 0.022 | b.d. |
| 25 | Shale | 20 | 8.64 | b.d. | 0.015 | b.d. |
| 25 | Shale | 60 | 1.94 | b.d. | 0.016 | b.d. |
| 25 | Shale | 90 | b.d. | 0.001 | 0.017 | b.d. |
| 25 | Shale | 120 | b.d. | b.d. | 0.017 | b.d. |
| 25 | Shale | 188 | b.d. | b.d. | 0.016 | b.d. |

Detection limits Se-$SeO_4^{2-}$ =0.001, Se-$SeO_3^{2-}$ = 0.001, Se- SeM= 0.001
b.d. = below detection.

findings point to temperature-dependent shifts in metabolic pathway, but are not completely conclusive. Unfortunately, due to potential loss of volatile compounds during freezing and thawing of stored samples, it was not possible to confidently reproduce these results with archived reactor samples. Analyses of organo-Se compounds should be repeated with fresh samples to confirm the differential production of organo-Se compounds between lithology and temperature treatments.

Major Ion Chemistry During Se Reduction. Concentrations of $SO_4^{2-}$ and $PO_4^{3-}$, were measured, as well as pH and dissolved $O_2$, for samples collected at key time steps (lag, inflection, reduction, end of reduction), and are summarized in Table D2.1 of Appendix D2.

Oxygen was initially sparged to low levels with $N_2$ gas (<1 mg/L), and any residual was subsequently consumed (biotically and abiotically), based on declining concentrations observed in both live and killed control reactors (Figure 19). A temperature-dependent lag time of varying duration was observed in live reactors, which corresponds with consumption of $O_2$ and some $NO_3^-$ after $SeO_4^{2-}$ reduction was observed. Consumption of $O_2$ prior to denitrification, followed by metal/metalloid reduction, is typical in microaerophilic induction of anaerobic metabolism by facultative microbes [48]. Nitrate concentrations decreased in all reactors following depletion of $O_2$ concentrations to < 0.3 mg/L and initiation of $SeO_4^{2-}$ reduction, while $SO_4^{2-}$ was constant or increased slightly. Unfortunately, because $O_2$ and $NO_3^-$ were not measured for all time steps, it is not possible to separate the influence of the two potential inhibitors to $SeO_4^{2-}$ reduction. Measured pH remained relatively constant in live reactors, but dropped (as much as 0.5 pH units) in killed controls, perhaps reflecting generation of acid by oxidation. Surprisingly, for rock mined from phosphate deposits, soluble $PO_4^{3-}$ was low and detected infrequently (1 mg/L detection limit). Redox-sensitive parameters including $NO_3^-$, Fe, Mn and Se are compared by temperature for laboratory kinetic experiments

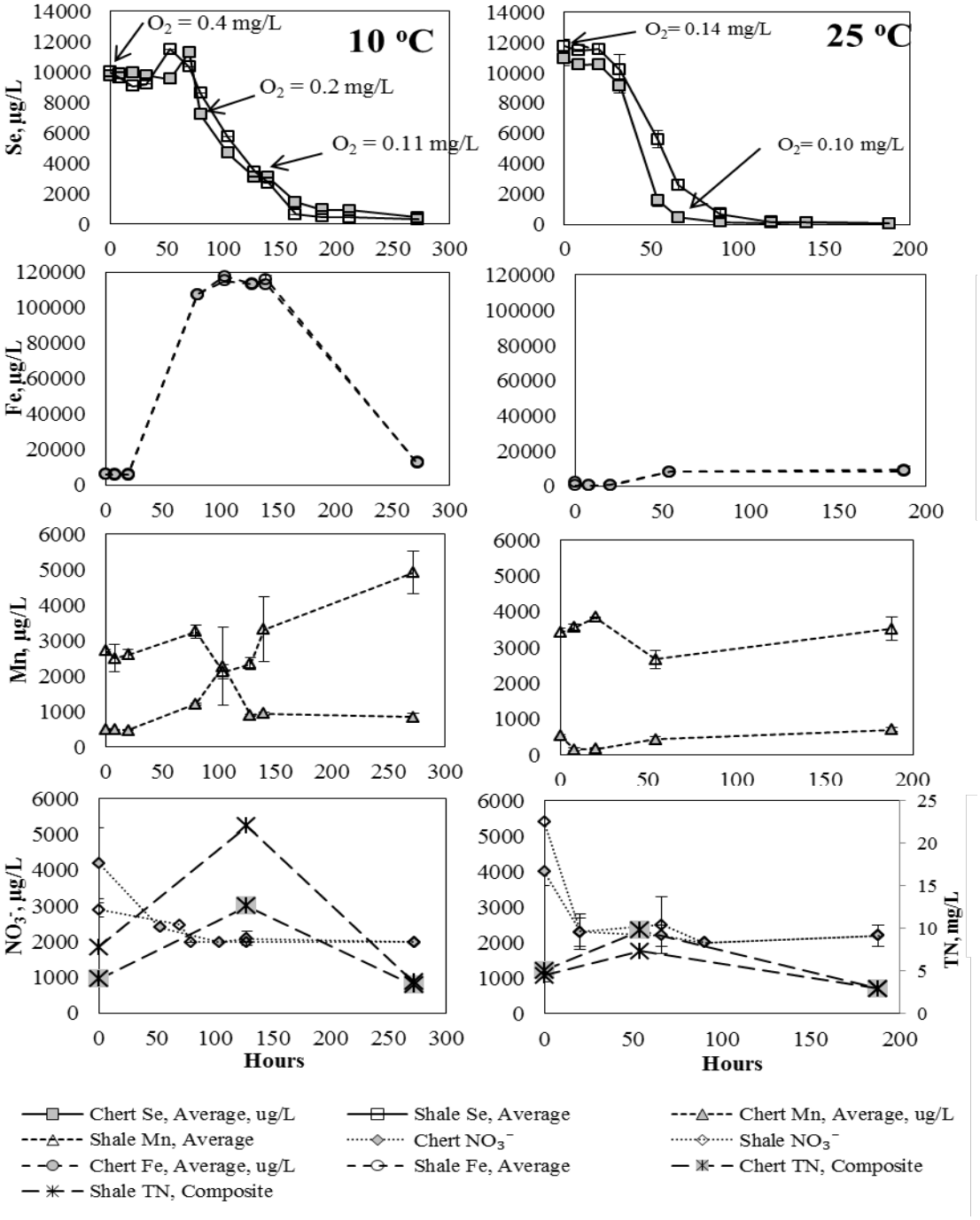



Figure 19. Saturated rate experiments for rock samples from the Dry Valley Mine: Se, Fe, Mn, $NO_3^-$, and TN concentrations for chert and shale at 10°C (left) and 25°C (right). Values are averages or composites (see legend) of n=3 replicates. Error bars = ±1 standard deviation. $O_2$ concentrations shown for chert only; see Appendix D2.1 for all $O_2$ data. Experiments were conducted until Se reduction was complete, thus the length of the 10 and 25°C experiments varied.

using Dry Valley Mine samples in Figure 19 and Smoky Canyon Mine samples in Figure 20.

Iron and Manganese During Se Reduction

Observed increases in Fe, and to a lesser extent, Mn concentration during Se reduction in the Dry Valley and Smoky Canyon mine reactors (Figures 19 and 20) support the hypothesis that Fe and Mn reduction is coupled to hydrocarbon degradation by members of the native microbial community. Concentrations of dissolved Fe shown in Figure 19a, which at pH 7 is most likely $Fe^{2+}$, rise as $O_2$ and $NO_3^-$ concentrations drop within the reactors. The dissolved Fe maintains a constant concentration around 120 mg/L throughout the period of Se reduction in the Dry Valley reactor, and then appears to drop at the end of the Se reduction process. The cause and timing of this change is unclear from available data, but this may reflect precipitation of $FeSe_2$ as selenide is produced, consistent with mineralogy analyses presented below. As $SO_4^{2-}$ concentrations were constant in the reactors (see values reported in Appendix D2), it is unlikely that precipitation of pyrite ($FeS_2$) occurred, and no iron sulfide was identified in mineralogical analyses. A similar increase in dissolved Fe concentration was observed in the Dry Valley Mine 25°C experiment; although the resulting dissolved Fe concentration was considerably lower than that measured in the 10°C microcosm. The lower concentration may reflect a shift in microbial community structure at different incubation temperatures. No subsequent decline in Fe concentration was observed at 25°C, but the experiment was shorter in duration, having been terminated at 180 hours following reduction of the

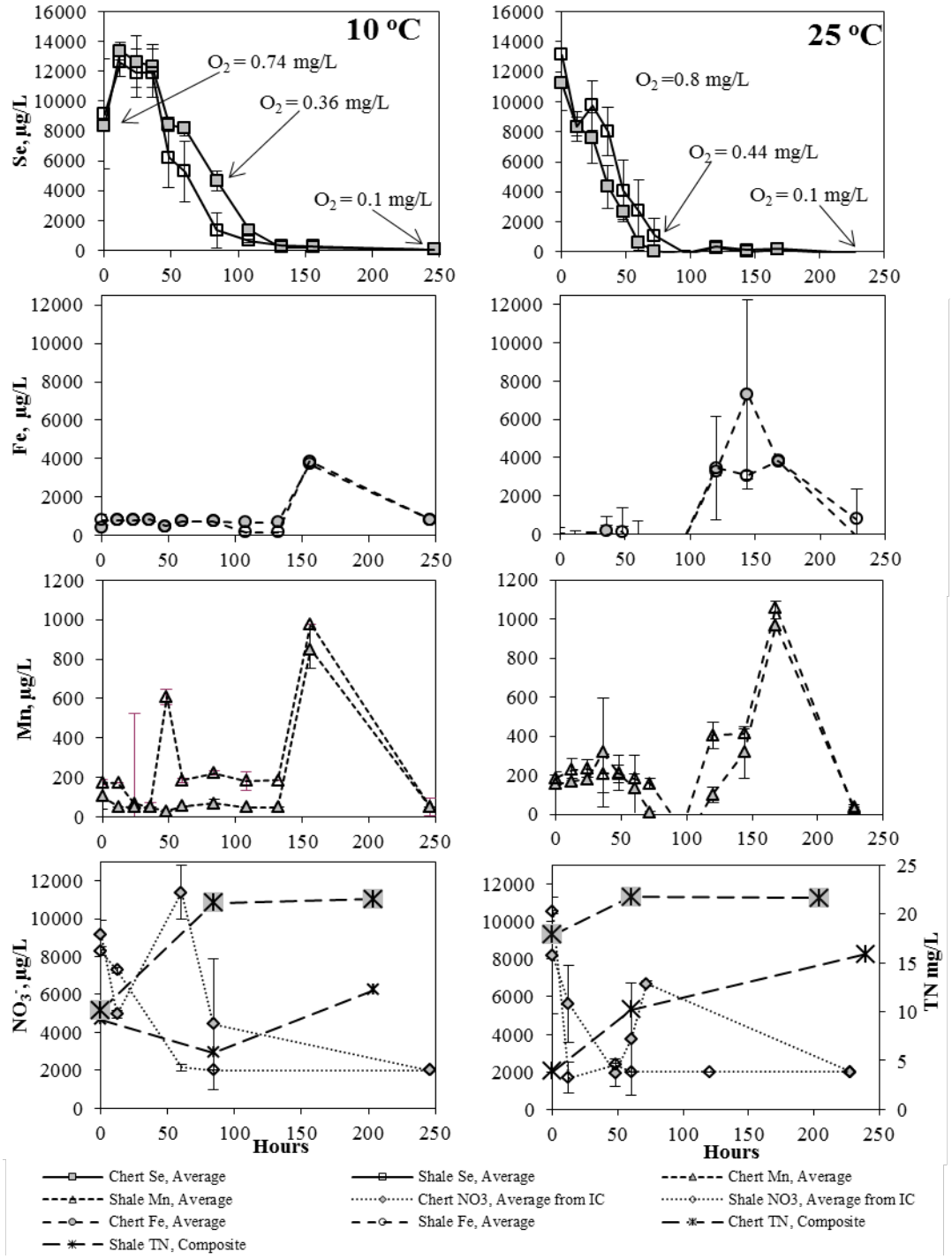



Figure 20. Saturated rate experiments for rock samples from the Smoky Canyon Mine: Se, Fe, Mn, $NO_3^-$, and TN concentrations for chert and shale at 10°C (left) and 25°C (right). Values are averages or composites (see legend) of n=3 replicates. Error bars = ±1 standard deviation. $O_2$ concentrations shown for chert only; see Appendix D2.1 for all $O_2$ data. Experiments were conducted until Se reduction was complete, thus the length of the 10 and 25°C experiments varied.

dissolved $SeO_4^{2-}$. If the experiment had been carried out to 250 hours, the same drop in dissolved Fe concentration might have been observed.

The observed increase in dissolved Fe is potentially due to the concurrent biological reduction of Fe. Genera known for Fe reduction capability, such as *Rhodoferax,* have been consistently identified with the hydrocarbon-degrading and heterotrophic $SeO_4^-$ reducing bacteria isolated during this study (Chapter 4). Interesting, members of *Rhodoferax* do not themselves have the demonstrated capability to reduce Se (this study; also, [49, 50]). Iron reduction is commonly associated with hydrocarbon degradation in suboxic environments, however [51, 52]. It is also possible that some of the observed Fe release results from redox reaction of the $SeO_3^{2-}/HSeO_3^-$ with primary $FeS_2$, especially in the Meade Peak shale, resulting in the formation of $Se^0$ and the release of $SO_4^{2-}$ and $Fe^{2+}$ similar to results reported by Kang et al. [53] and Naveau et al. [54].

Although the data for Mn show a less dramatic increase in concentration than the Fe, a substantial in concentration was observed in both the chert and shale reactors, once $O_2$ was depleted, especially in the Smoky Canyon Mine data. There were larger differences in the dissolved Mn concentrations between shale and chert in the reactor experiments using Dry Valley Mine samples than were observed with Smoky Canyon Mine samples. This may reflect differences in the Mn-oxidizing microbial communities between the two mine sites or local differences in mineralogy. The occurrence of soluble Mn with low concentrations of Fe under micro-aerophilic, denitrifying conditions (such as those observed in early time steps in the reactors) are consistent with groundwater

chemistry monitored at GW7D and GW11 (Table S5-2). The redox chemistry of Mn in sedimentary pore water is complex, with potential for the intermediate $Mn^{3+}$ to serve as both oxidant and reductant [55-57]. Rapid cycling between $Mn^{2+}$ and $Mn^{4+}$ likely occurs in suboxic sediments at redox boundaries similar to those studied in the backfill environment[58], with consequences for the redox cycling of Fe, and potentially Se [59, 60]. Such changes potentially affect the oxidation state and solubility of Fe and may explain the low concentrations of dissolved Fe late in the experiments (e.g., as $Mn^{4+}$ is reduced, $Fe^{2+}$ is reoxidized, promoting the precipitation of iron oxide minerals). Recognizing that the rapid cycling of Fe drives denitrification and hydrocarbon degradation in suboxic subsurface systems [61, 62], it is possible that microbially mediated, reactive green-rust minerals develop on a transient basis, promoting the abiotic reduction of Se within the suboxic sedimentary environment.

Nitrogen Concentration During Se Reduction: Nitrate and total nitrogen (N) concentrations are plotted in Figures 19 and 20 for the reactors from the Dry Valley and Smoky Canyon mines, respectively. Although $NO_3^-$ concentrations decreased during the lag phase, it was not completely removed from all reactors, and was observed to increase mid reduction phase in some reactors. Total N was observed to increase mid-reduction phase in all reactors. Interestingly, these data suggest that either a fraction of the $NO_3^-$ persists in some reactors or is produced via re-oxidation of reduced N concentrations (2.5 mg/L) mid-reduction (e.g., t= 90 to 188 hours in Smoky Canyon). Results of the rate experiments indicate that $NO_3^-$ was reduced at the same time that $SeO_4^{2-}$ was reduced to $SeO_3^{2-}$, but because the reactors rely on a mixed community of microbes for biological

reduction, it is not clear whether this was co-metabolism or ongoing $NO_3^-$ reduction that occurred in parallel with $SeO_4^{2-}$ reduction (Table 11). These results suggest that for these systems, $NO_3^-$ does not need to be completely reduced prior to the onset of $SeO_4^{2-}$ reduction. The extent to which the persistence of $NO_3^-$ in the reactors was driven by re-oxidation of reduced N (such as $NO_2^-$ or $NH_4^+$) cannot be addressed with available data, but the identification of abundant ammonia-oxidizing *Archaea*, specifically *Nitrosphaera* and *Nitrospumilus,* in groundwater (data not presented here) suggest that this is possible.

In all of the Dry Valley Mine reactors, total N was observed to peak mid-reduction phase (Figure 21), but not in the killed controls (data not shown). Interestingly, total N shows a somewhat different pattern of increase in the Smoky Canyon Mine reactors, with total N increasing over time. In order to obtain sufficient volume to measure total N, the replicate reactor samples were composited, so it is not possible to report error for these measurements, and it is therefore difficult to be confident in the limited total N results. These data suggest a more complex N-cycle, with the possible decomposition of N-containing compounds (e.g., proteins) formed during the period of greatest Fe and Mn reduction. Preliminary analyses of volatile hydrocarbon compounds that were measured by HS-SPME-GCMS for a select number of samples indicated that some of the compounds detected in the headspace were nitrogenous, but this analysis was only possible for a limited number of samples and provided only qualitative data (Table S5-5; Appendix E). Alternatively, a change in N fixation during the reduction process may be involved. The fact that Se reduction can proceed in spite of

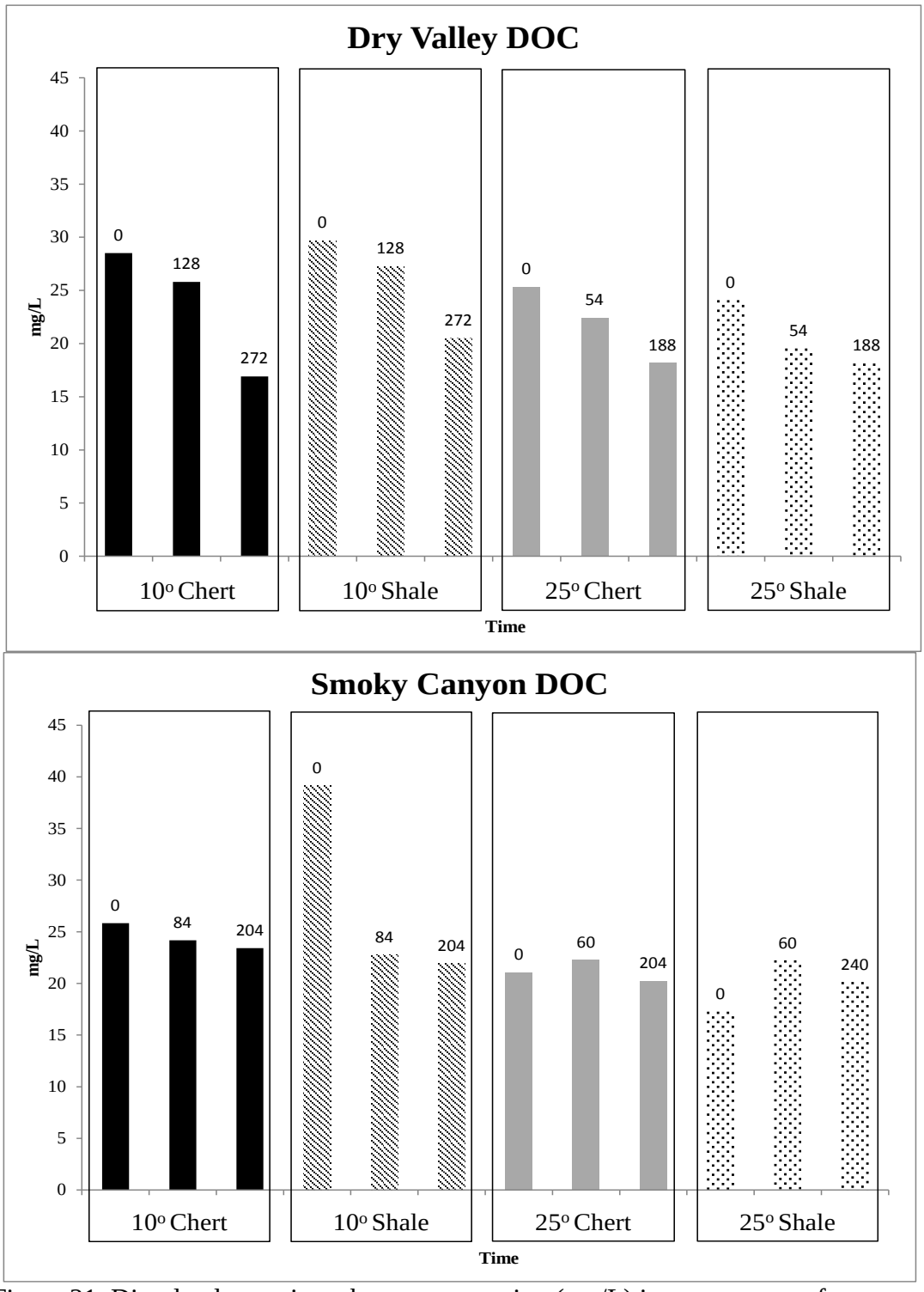



Figure 21. Dissolved organic carbon concentration (mg/L) in rate reactors, for composited sample (n=3) of each lithotype. Numbers above bars indicate time of sampling (in hours).

the presence of soluble N, and $NO_3^-$ in particular, is significant, given its recognized importance as a potential inhibitor of $SeO_4^{2-}$.

Dissolved Organic Carbon during Se Reduction

Figure 22 shows changes in DOC for the Dry Valley and Smoky Canyon mine reactors, respectively. These samples were composites of water from the replicate reactors (e.g., the sample identified as 10 DCL is a composite of samples from Dry Valley Mine Live Chert reactors 1, 2, and 3 at 10°C). In most reactors, a portion of the available DOC was consumed during the reduction process, but less than 40% of native DOC is shown to be consumed in live reactors; no C was consumed in killed controls, indicating biological consumption of the available C compounds. Evidence of lower DOC following reduction in the rate reactor was also observed in the qualitative SPME data (Table S5-5). Further, an overall shift from complex to simpler hydrocarbon compounds is suggested by the SPME results, as the relative proportion of high molecular weight compounds (e.g., C> 15) measured in the head space was lower for samples of water taken from reactors at the end of the reduction process. The molar ratio of Fe to Se in solution during peak reduction increased by as much as two orders of magnitude in the Dry Valley experiments, with somewhat lower Mn to Se ratios, depending upon mine site and temperature (calculations provided in Appendix D1 on CD). This indicates that a much greater mass of Fe and Mn is reduced than Se in the reactors. Given the lack of demonstrable change in overall reactor biomass based on protein analysis during the Se reduction experiments (Appendix D3), and the relatively low abundance of SeRB noted in the microbial community study (Chapter 4), it seems

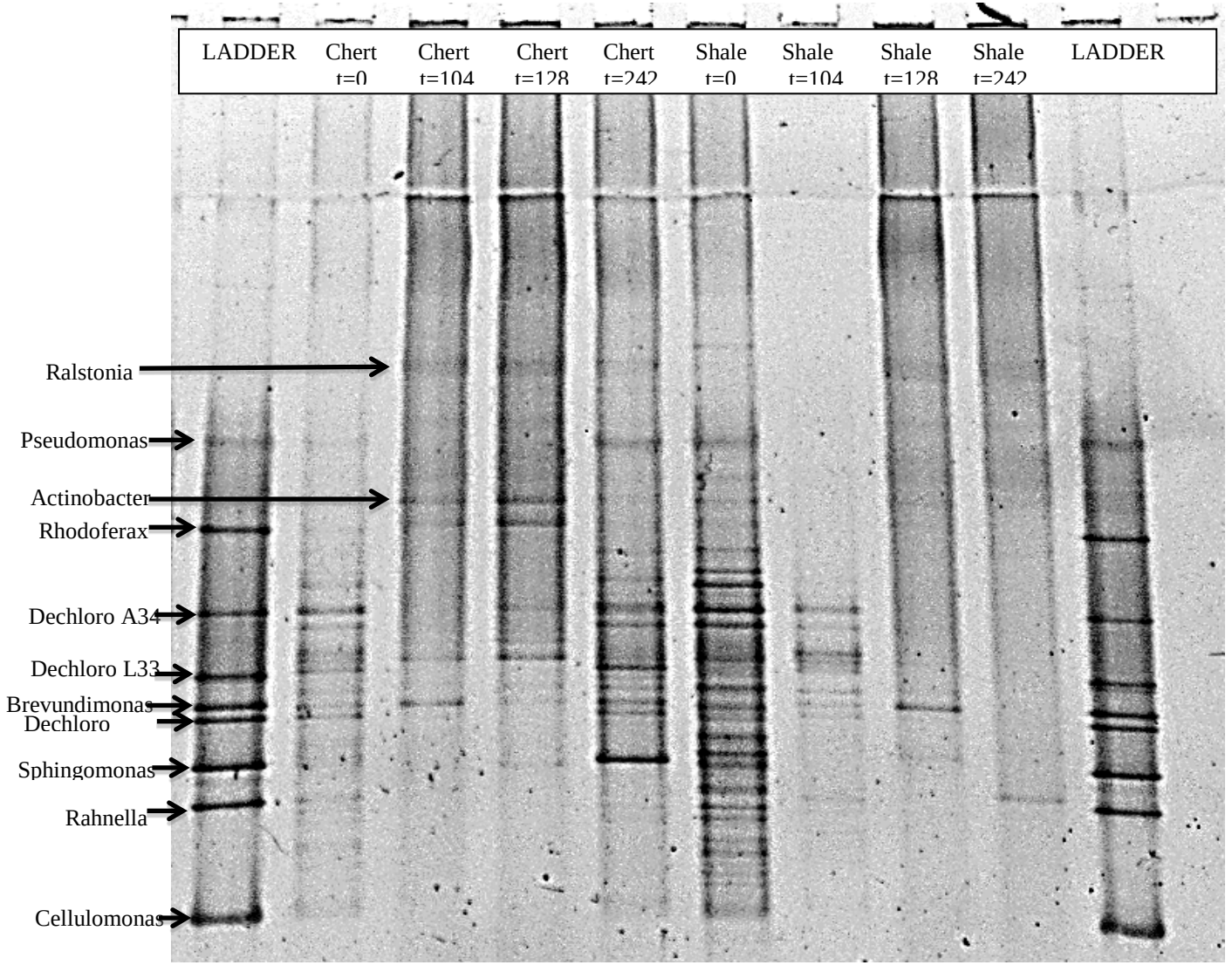



Figure 22. DGGE gel comparing DNA extracted from 10°C reactors, Dry Valley.

multiple members of the native consortium (especially, the Fe and Mn-reducing organisms) and likely not solely coupled to Se reduction. The well-known process of hydrocarbon degradation linked to Fe and Mn reduction under anaerobic conditions [62-64] very likely provides the simpler C compounds that support Se reduction in these reactors, in the absence of added C. Whether the hydrocarbon degradation is directly linked to Se reduction (as opposed to $NO_3^-$, Mn, or Fe reduction) cannot be determined from available data.

Changes in Biomass in Reactors

Efforts to quantify changes in protein in the Smoky Canyon batch reactors during Se reduction were unsuccessful, potentially due to interference of humic compounds in the organic-rich solutions with the colorimetric assay used in the Coomassie method. Results of these analyses are presented in Appendix D3. Use of the Pierce cleanup kit resulted in low protein yield and did not improve the ability to resolve systematic changes in protein within these native sediment reactors. Alternatively, these findings may simply reflect limited relative growth of SeRB organisms within the reactors during the Se reduction process. Samples were spiked with groundwater containing $10^4$ and $10^6$ SeRB per gram of rock (Chapter 4) from Smoky Canyon and Dry Valley mines respectively, and it is likely that there was little subsequent increase in population density to measure as the $SeO_4^{2-}$ reduction process in the reactor proceeded. This is supported by the microbial community analysis, which indicated that the number of SeRB in unsaturated chert and shale sediments is lower than in groundwater, and that the microbial community is dominated by hydrocarbon-oxidizing,and $Fe^{3+}$, $Mn^{4+}$, and $NO_3^-$ reducing

bacteria, rather than SeRB (see Chapter 4). Regardless, the inability to measure changes in protein limits the ability to report a Se reduction rate relative to biomass.

Se Mineralization in Batch Reactors

Characterizing the mineral products that resulted from the Se reduction process is important in understanding the operational potential of *in situ* stabilization of Se in backfilled panels and/or constructed reactive barriers. Minerals produced through microbial activity may have different potential for re-oxidation or desorption of reduced Se, thereby affecting the capacity for Se attenuation. Results of synchrotron XANES and XRD analyses are presented in Appendix F and summarized below.

Results of S-XRD were somewhat limited due to the complexity of the background sample mineralogy. Quartz and microcline feldspar were confirmed with matches to all peaks in the Jade software standard library, while hematite was probably present in samples of both chert and shale, based on matches to multiple peaks in the region of interest within its diffraction spectra. Zaherite ($Al_{12}(SO_4)_5(OH)_{26}\bullet_{20}H_20$) was also identified in a pre-reduction sample of chert. In post-reduction samples of chert, sodium $SeO_3^{2-}$ was confirmed and ferroselite (an $FeSe_2$ mineral) and copper selenide were probably present, with matches to some peaks in known reference spectra. Samples of shale contained probable $Se^0$ (based on matches to 3 or more peaks), with likely organo-Se compounds methyline selenafulvene and toluene selenoic acid, as well as $FeSe_2$. No $SeO_3^{2-}$ bearing minerals were identified in the shale.

Efforts to visualize microbes in association with Se minerals following reduction were unsuccessful using Field Emission Scanning Electron Microscopy (FE-SEM), due

to the abundance of fine grained silts in the samples and the detection limit of approximately 0.1 to 1.0 wt% Se for this method relative to low mass of Se potentially precipitated on the mineral surfaces as a result of these experiments.

For these reasons, Se was analyzed using the more sensitive XANES and S-XRD methods at the Stanford Synchrotron Radiation Light Source. Following the previous analyses of Se using XANES [65-67], several standard Se compounds were included in these analyses as listed in Table S5-6. The x-ray absorption energy edge position was measured for several Dry Valley and Smoky Canyon 10°C reactor samples collected at different time steps. The “shale only” and “chert only” samples represent the natural background mineralogy in rock substrate prior to formation of Se minerals in the batch reactor. One additional “live” end sample was studied, which reflected the composition of the reactor at the time it was decommissioned, several months after the Se reduction process had ended in a sample that was incubated at room temperature. This measurement was made to identify stable mineral products of the reduction process. Reduction products were also compared with the Se solid mineral phases present in abiotic control reactors at the end of the experiment.

Comparison of the energy edge (eV) measured for standards with those measured in the background, live reactor, and control samples are summarized in Table S5-6. The geometry of the uniquely shaped spectra shown in Figures 23a (Dry Valley) and Figure 23b (Smoky Canyon) suggests that different end products result from reduction in chert and shale lithotypes. It is difficult to resolve $Se^0$, SeC, and selenide from one another

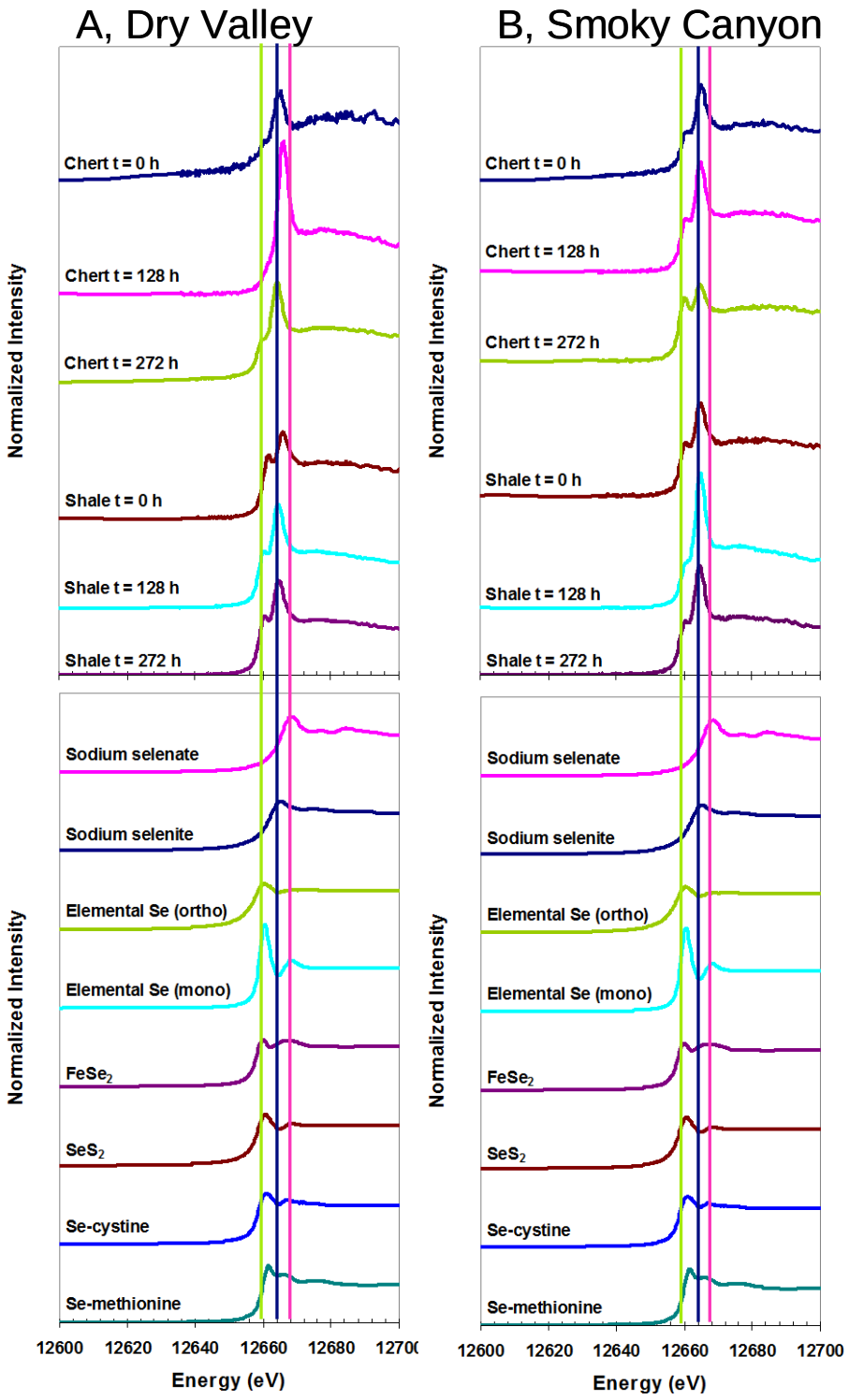



Figure 23. XANES analyses of waste rock from rate reactors for (A) Dry Valley and (B) Smoky Canyon. Colored lines indicate locations of $SeO_4^{2-}$, $SeO_3^{2-}$, and $Se^0$ energy peaks.

based on energy edge alone, so interpretation must also consider the shape of the spectra obtained for each standard.

The energy edge and spectral data in Table S5-6 and Figure 23a show that little if any $SeO_4^{2-}$ was present on the solids in any of the samples, regardless of lithology or mine site studied, and in spite of the elevated concentration known to be present in solution in the reactors at the start of the experiment. This is consistent with the low capacity for $SeO_4^{2-}$ sorption that is reported to exist under neutral to alkaline conditions, which are similar to those that exist in these reactors [18, 20, 28, 68].

Figures 22 a and b show the chert samples from the Dry Valley and Smoky Canyon mines exhibited an overall shift from $SeO_3^{2-}$ to $Se^0$. In Figure 22a, the background and pre-reduction reactor solid phase had a Se edge that matched $SeO_3^{2-}$, but as reduction proceeded, the $SeO_3^{2-}$ peak that is strongly in evidence at 12662 eV at t=128 hours is diminished and a peak aligning with $Se^0$ at 12660 eV appeared on the shoulder of the spectra at t = 272 hours. Selenite (with minor amounts of reduced Se) appears to be the dominant form of Se present in the Smoky Canyon chert in background sediments and pre-reduction samples (t=0). In Figure 22b, the intensity of peaks at 12660 and 12662 eV both increased in mid-reduction phase (t=228), with the $SeO_3^{2-}$ peak at 12662 eV increasing in intensity relative to the reduced phase represented by the “shoulder” peak at 12660 eV. The $SeO_3^{2-}$ peak at 12662 eV declined in intensity relative to the more reduced form in the latest reduction time step (t=272), suggesting a shift toward more reduced Se, although both were present at the end of the reduction process. The rather narrow shape of the more reduced Se peak at 12660 eV suggests the presence of $Se^0$,

rather than the more reduced forms selenide or SeC, which have energy edge values similar to one another, but a broader shaped spectrum. Thus, in the chert reactors of both sites, Se added as $SeO_4^{2-}$ was reduced to $SeO_3^{2-}$, which likely sorbed onto mineral surfaces, and then subsequently transformed to the more reduced, insoluble $Se^0$. From the geometry of the peaks observed in Figure 22, it appears that more Se was transformed to the elemental form in the Smoky Canyon Mine treatments than in the Dry Valley Mine treatments.

The Se characteristics of the shale samples show a somewhat different pattern than those for chert. In background and pre-reduction samples from both mine sites, peaks corresponding to both $SeO_3^{2-}$ at 12662 eV and SeM at 12660 eV are evident. In the Dry Valley Mine reactor (Figure 23a), both peaks grew in intensity in relative proportion to one another, with the "shoulder" peak at 12660 eV shifting towards alignment with the energy edge of the reduced $Se^0$ mineral at mid reduction phase (t=128). At the end of reduction, the peak appears to shift slightly towards a broader shape that is more characteristic of $FeSe_2$, although subtle differences make it difficult to be conclusive. A similar pattern was observed for the shale sample from the Smoky Canyon Mine reactor (Figure 22b). These results suggest that $SeO_4^{2-}$ added to the shale reactors was reduced to $SeO_3^{2-}$ and SeM, followed by transformation of some of each reaction product to a more reduced $FeSe_2$ compound. Two hypotheses to consider as explanations for the observed differences in alternative biogeochemical pathways include (1) shifts in Se methylation enzyme regulation as a toxicity response to the higher salinity of the shale geochemistry, e.g. elevated $SO_4^{2-}$ or metal content and (2) the reaction of reduced $SeO_3^{2-}$ with primary

$FeS_2$ present in the shale, thus forming $Se^0$ and releasing $Fe^{2+}$ into solution [53]. The latter mechanism fits well with the observed increase in dissolved Fe at neutral pH that was observed in association with Se reduction in these experiments.

These results agree with the aqueous speciation data reported in Table 11 for chert and shale, which suggest that $SeO_3^{2-}$ is produced initially and then removed from solution, either through sorption to the solid phase or through further reduction to insoluble $Se^0$ and selenide minerals.

Within the pH range observed for the Dry Valley chert (7.3-7.5) and shale (6.6-6.8) reactors, the majority of Se(IV) should be present as $HSeO_3^-$. In this range, Se(IV) should sorb strongly to Fe oxyhydroxide minerals [18]. It is important to note, however, that average pH observed in the field at Dry Valley was somewhat higher than this value (7.8, Table S5-4, see also Table A1-1 through 3), closer to the $HSeO_3^{2-}/SeO_3^{2-}$ boundary. The extent of pH-dependent sorption predicted based on results from the reactor could therefore vary somewhat in the field setting. Sorption does not explain the relatively faster reduction of Se by the chert sediments, as the higher pH of the chert should be somewhat less likely than shale to promote sorption of negatively charged oxyanions.

Recent work by Martin and others [69] using x-ray fine structure (XAFS) described distinct $SeO_3^{2-}$ and $SeO_4^{2-}$ and organo-Se reduction profiles in lentic sediments. In these environments, low $O_2$ concentrations drive similar Fe-N-Mn cycling and Se-redox transformations, with insoluble $Se^0$ partitioning to the solid phase, and volatile organo-Se and $SeO_3^{2-}$ being released to the aqueous phase. These results are also consistent with those reported by Chen et al. (2009), who described $SeO_3^{2-}$ reduction to

$Se^0$ under concurrent $Fe^{3+}$-reducing conditions, releasing sorbed $SeO_3^{2-}$ into solution where it was available for subsequent microbial reduction to $Se^0$ [70]. It is also possible that desorbed $SeO_3^{2-}$ could be abiotically reduced by biogenically-produced $Fe^{2+}$ [71-73]. The close association of isolated Fe-reducing organisms such as *Rhodoferax* with heterotrophs such as *Cellulomonas* and *Arthobacter* shown to be capable of Se reduction in multiple enrichment cultures from Smoky Canyon and Dry Valley, as well as the elevated $Fe^{2+}$-concentrations associated with $SeO_4^{2-}$ reduction in experiments conducted with native consortia from both mine sites (Figures 19, 20), suggests the existence of a potentially important mechanistic link between Fe and Se reduction. In the absence of green-rust, the only known abiotic catalyst of $SeO_4^{2-}$ reduction [74, 75], the observed association of Fe and Se in these reactors is inferred to be an $Fe^{2+}$-$SeO_3^{2-}$ interaction.

Belzile et al. described a very similar, multi-scale process of biological and chemical Se attenuation [76]. They characterized the remobilization of $SeO_3^{2-}$ initially sorbed onto Fe-Mn oxyhydroxides, which were dissolved through biotic reduction under progressively reducing conditions developed during diagenesis. Iron and Mn-reducing organisms promoted mineralization of organic matter in the sediments and supported the formation of $Se^0$, seleniferous pyrite, and selenides [76].

These results have important implications for the function of a $SeO_4^{2-}$-reducing reactive barrier or backfill environment within waste rock deposits, in that sorbed $SeO_3^{2-}$complexes have potential to be desorbed and/or remobilized from reduced iron oxide substrates. The cycling of Fe (and probably Mn) is likely to influence downstream geochemical pathways, wherein the reduced Fe and Mn produced in these suboxic

environments may also play a direct role in subsequent abiotic $SeO_3^{2-}$ reduction. The long term stability of sorbed $SeO_3^{2-}$ complexes is less certain than that of reduced and insoluble $Se^0$ and $Se^{2-}$ minerals [17, 77]. Although Se reduction may proceed more rapidly in chert due to $SeO_3^{2-}$ sorption, at least initially, the more reduced selenide product observed in the shale potentially offers greater long-term stability.

## Changes in Microbial Community During Se Reduction

DNA extracted from Dry Valley samples collected from chert and shale batch reactors for key reduction time steps was compared using DGGE in Figure 23. Samples of sediment from the most field relevant 10°C chert and shale reactors were compared with a ladder comprised of organisms isolated from groundwater and sediment MPNs. Bands were cut where possible to allow sequencing of the DNA fragments, thus identifying phylotypes with high degree of similarity to the genera *Dechloromonas* (>96%), *Rhodoferax* (>98%), *Ralstonia* (>99%), and *Brevundimonas* (>96%). DGGE results allowed community analysis at the genus level; these results are compared with isolates in the ladder, which were identified at both the genus and in a few cases, the species level.

Changes in band intensity representing changes in operational taxonomic unit (OTU) abundance and, by inference community composition, were evident in the rate reactors for chert and shale during the reduction process. Band alignment in the chert suggests an initial community dominated by multiple organisms with a high degree of similarity to members of the genera *Dechloromonas*, *Brevundimonas*, *Sphingomonas*, and

*Rahnella*, along with other unidentified organisms represented by fainter bands. The community, as suggested by the identified OTUs, appears to transition to a more limited community comprised of genera including *Dechloromonas, Ralstonia, Brevundimonas, Rhodoferax* and members of the *Actinobacteria* class during Se and Fe reduction (t = 104 and 128 hours). Unfortunately, DNA from the earliest time of Se reduction in the chert (e.g., t = 48 hours) was destroyed and was not available for inclusion in this analysis. Community diversity appeared to return to levels comparable to time zero in the chert after the Se was reduced (t = 242 hours). A relatively more diverse community was observed in shale at t=0 in the reactor, which shifted to a much simpler community described by OTUs that aligned with ladder bands from the isolated dechloromanads, which were highly similar to the species *Dechloromonas sp. A34*, *Dechloromonas aromatica*, and *Dechloromonas denitrificans*, along with the *Brevundimonas* spp. isolated at the mid-reduction phase. Faint DNA bands began to appear at t=128 hours that aligned with the band of an isolated *Rhodoferax*. Bands in the shale aligning with a *Brevundimonas* isolate and, to a lesser degree, a *Sphingomona*s isolate, increased in intensity during Se and Fe reduction (t = 104 and 128 hours), while a band aligning with a *Rahnella* isolate intensified at t=242 hours. Community diversity did not appear to return to initial conditions as clearly in the shale at the end of reduction, but the overall faint patterns at t=128 and 242 hours suggest that this may simply reflect lower concentrations of DNA in the gel. Bands that appeared in the upper portion of the gel at t=104 and 128 hours in the chert, and t=128 and 242 hours in the shale, were strongly similar to results obtained in preliminary gels with the genus *Ralstonia.*

The changes in microbial community indicated by the DGGE analysis agree with the aqueous speciation data reported in Table 11 for chert and shale, which show that $SeO_3^{2-}$ is only detected rarely and early in the reduction process. It may be that it is produced initially and then removed from solution, either through sorption to the solid phase or further reduction to insoluble $Se^0$ and $Se^{2-}$ minerals. Overall, Figure 23 provides an indication of microbial community changes as reduction proceeded in the chert and shale reactors for the Dry Valley Mine, under the field relevant temperature of 10°C. These findings suggest the involvement of microbes known to break down hydrocarbons and reduce $SeO_4^{2-}$, $SeO_3^{2-}$, $Fe^{3+}$, $Mn^{4+}$, and $NO_3^-$. Differences in the microbial community appear to exist between the two lithologies, which may in part explain the observed differences in rate, speciation, and reaction pathway. Additional research would be needed to investigate the specifics of these influences. Overall, the phylotypes identified in the DGGE analyses confirm the presence of the otherwise isolated $SeO_4^{2-}$ reducing *Dechloromonas* sp. A34 *(*Childers, unpublished data, this study*)* and a *Rhodoferax* sp. Sequences with a high degree of similarity to the heterotrophic genera *Brevundimonas* and *Sphingomonas*, both of which have members known to degrade aromatic hydrocarbons [78], were identified in chert and shale. Phylotypes strongly similar to the genus *Rahnella*, members of which a reported $SeO_4^{2-}$-reducer [79], and *Actinobacteria* only appeared in the shale. A phylotype highly similar to a *Pseudomonas*, which was present in the initial community in both chert and shale, was evident again following Se reduction in the chert, but not in the shale. The DNA band associated with

the genus *Ralstonia* [80, 81], members of which have been shown to have the ability to reduce Se, was stronger during the mid-reduction phase for both lithotypes.

## Conclusions

Selenate reduction by indigenous microbes using native C in groundwater-saturated phosphate mine overburden was studied under conditions representative of subsurface backfills in S.E. Idaho Phosphate Resource Area, to determine its potential effectiveness for *in situ* source control.

Kinetic experiments were conducted under microaerophilic conditions to evaluate how $SeO_4^{2-}$ reduction proceeds in mineralogically-distinct chert and shale waste lithologies. Analyses of Se, Fe, Mn, N, S, P and C in aqueous and solid phases, together with measurements of pH and $O_2$. Results indicate that biotic $SeO_4^{2}$ reduction began following biogeochemical reduction of $O_2$ and some (but possibly not all) $NO_3^-$, and was significantly associated with concurrent Fe and Mn reduction. Remaining soluble $NO_3^-$ was reduced as $SeO_4^{2-}$ reduction began, but there was an apparent increase in total dissolved N as reduction proceeded. Observed reduction in DOC suggests that carbon obtained through degradation of hydrocarbons present in the rock supported the observed reduction of Se as well as Fe and Mn, with a fraction of the DOC consumed during the reduction process. It is possible that other electron donors also supported the Se reduction. Sulfate was not reduced in either rock type, nor was phosphate measured in solution. The pH was relatively constant within each reactor during the reduction process; pH was lower in shale treatments than in chert at Dry Valley, however.

Selenium was reduced in a step-wise process, from $SeO_4^{2-}$ to $SeO_3^{2-}$ in both chert and shale, at rates influenced by temperature and possibly different $SeO_3^{2-}$ reduction pathways and microbial communities that yielded different mineral products. Reduction occurred more quickly in chert (4.3 to 10.2 µg Se/kg rock/hr), which had lower $SO_4^{2-}$ concentrations (approximately 300 mg/L) and pH values between 7.2 and 7.8, using natural hydrocarbon with relatively fewer aromatic compounds, and produced $SeO_3^{2-}$ and $Se^0$. Reduction occurred more slowly in shale (3.0 to 6.3 µg Se/kg rock/hour) at concentrations of $SO_4^{2-}$ between 900 and 1300 mg/L and pH values between 6.7 and 6.9, using a mixture of natural aromatic and alkane hydrocarbon, and producing $SeO_3^{2-}$, methylated Se, and $Se^{-2}$ minerals as reduction products. In spite of differences in rate and end-products, reduction in both lithologies within 100 hours reduced the majority of $SeO_4^{2-}$ to insoluble forms when saturated, low $O_2$ (less than 0.3 mg/L) conditions were developed.

Changes in the bacterial community were evident between treatments at different temperatures, and to a lesser degree, rock type. Changes in DNA banding patterns indicate that higher numbers of a *Rhodoferax*-like bacterium were present early in the $SeO_4^{2-}$ reduction process. The banding pattern showed increases in phylotypes representative of the genera *Dechloromonas* (known $SeO_4^{2-}$ reducers) in shale and *Ralstonia* (also known $SeO_3^{2-}$ reducers) in both lithologies as reduction proceeded. This analysis of community response to $SeO_4^{2-}$ exposure under microaerophilic conditions suggests hydrocarbon degradation may be coupled with denitrification and Fe-reduction, in association with Se reduction. The biogeochemical factors driving mineralization to

different endpoints should be confirmed with further analysis, perhaps using pyrosequencing and fine scale XAFS tools to better resolve the microbial community and the biomineralization pathways.

Results of these experiments show that facultative members of this microbial community are likely to couple oxidation of native carbon to $O_2$ reduction, quickly driving the saturated microcosms to microaerophilic and ultimately, conditions that support $NO_3^-$, Fe, Mn, and Se reduction. Capacity for *in situ* Se reduction by indigenous organisms using electron donors present in the rock was documented using samples from two mine sites, located 15 miles apart, which produce phosphate from the same geological formation. Results of this study explain observed differences in Se concentrations between wells completed in backfill at the two mine sites, which could not previously be explained based solely on solid phase equilibria (TetraTech, 2007). Although lithology affected the reduction rate and mineral end product, Se reduction within either rock type was relatively rapid under low $O_2$ conditions (on the order of weeks) when compared with the probable residence time of water within a backfilled mine facility, which typically ranges from months to years. Although Se reduction rates and mechanisms differ subtly between the two mine sites in these experiments, likely reflecting different microbial communities and/or abundance resulting from the different *in situ* $O_2$ conditions, the native microbes from both locations at Dry Valley (GW7D) and Smoky Canyon (GW11) were able to provide effective Se-reducing capacity when grown on either chert or shale under saturated, microaerophilic conditions. It is likely, however, that shale will offer better moisture retention and capacity to consume $O_2$ within a

backfilled panel or a constructed reactive barrier zone, due to finer grain size and higher organic C content, as well as promote the formation of more reduced mineral products which have lower potential for re-oxidation and remobilization. These results suggest that successful designs relying on microbial reduction as source control within the S.E. Idaho Phosphate Resource Area overburden deposits are possible. Successful designs will depend most significantly on the management of rock and water to develop and maintain suitably microaerophilic and moist conditions.

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# Chapter 5 Supplementary Information

## Overburden and Groundwater Sampling Methods

Representative samples of overburden and groundwater were collected from backfilled panels at the Dry Valley and Smoky Canyon mines. Multiple samples of shale and chert were collected from sonic drill core and near surface excavations to represent the range of mineralogy, texture, and geochemistry of backfilled overburden deposits. Samples were air-dried, flailed, and graded through sieve and hydrometer analysis. Sub 1/4 inch rock was composited, split, and digested with aqua regia followed by analysis of multiple elements by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS). Methylene chloride extractable organic C species were also identified for two composited samples of chert and shale from the Smoky Canyon Mine using EPA method 3350, followed by Gas Chromotography Mass Spectroscopy (GC-MS) following EPA method 8270C.

Groundwater samples were collected from a well completed in saturated backfill at the base of backfilled panel B at the Dry Valley Mine (GW7D2a) and from a well completed in partially (and intermittently) saturated backfill in panel A at the Smoky Canyon Mine (GW11). Water was bailed manually using a disposable plastic bailer weighted to facilitate sediment recovery during sampling, as a means of collecting both planktonic and attached microbes. Samples were transferred immediately into sterile glass or polypropylene bottles and stored at 10°C in the dark without headspace. Groundwater pH, dissolved $O_2$ content (Hach model AQ4, Loveland, Colo) and temperature were measured at the time of sampling. Groundwater samples were collected

as close as possible to the start of each series of reactor experiments to limit the effects of storage on chemistry and microbial communities.

Less frequently, samples from each well were submitted for a comprehensive analysis of major and trace element chemistry; chemistries reported in 2007 are provided in Table S5-1. These samples were collected using low flow pumping techniques and preserved without headspace for analysis of dissolved metals using 0.45 µm filtration and nitric acid; total metals using nitric acid; nutrients using hydrochloric acid; and major ions/alkalinity (no filtration or reagent addition). Relative constant chemistry, with minor seasonal variation, has been observed in each of the wells over time. Dissolved organic carbon (DOC, by standard method 5310) and volatile aqueous C species (by Head Space-Solid Phase Micro Extraction-Gas Chromatography-Mass Spectrometry, HS-SPME-GC-MS) were also measured in groundwater collected from GW7D2A.

Total Element Analysis (ICP-MS)
Following Aqua Regia Digestion

Samples of chert, shale, and a run-of-mine mixture containing 55% shale, 35% chert, and 10% mudstone were analyzed for 51 elements by ICP-MS following aqua regia extraction at ALS Chemex laboratories (MEMS41).

Organic Carbon Speciation in Rock

Solvent extractable organic carbon was extracted using methylene chloride by EPA method 3550B and analyzed by EPA Method 8270C at Energy Laboratories in Billings, MT. Chromatographic spectra from unknowns were identified tentatively by comparison with the NIST standard reference database by Energy Laboratories.

Table S5-1. XRD mineralogy of chert and shale used in rate reactors.

| | |
|---|---|
| CHERT | Quartz<br>Hematite<br>Clay<br>Aluminum sulfate<br>Carbonate |
| SHALE | Quartz<br>Microcline<br>Hematite<br>Fluoroapatite<br>Zinc Sulfide |

Water-extractable Se, Fe, Mn, $NO_3^-$ and DOC

Leachable Se, Fe, Mn, and $NO_3^-$ were measured from bottle-roll extracted solutions by ICP and Ion chromatography. Rock was leached in a 3:1 ratio of distilled water to solid in an aerated 24 hour bottle roll at room temperature. Samples were allowed to stand for 1 hour, followed by centrifugation to remove solids and filtered to 0.20 μM prior to analysis. Total Se, Fe, and Mn were measured by ICP. DOC and total N were measured as described above in the methods section.

## Rock and Groundwater Geochemistry Characterization

Representative samples of rock and groundwater used to construct batch reactors were characterized for total and leachable Se, Fe, Mn, $SO_4^{2-}$, $NO_3^-$, and organic C content and speciation. A homogenized, representative composite of chert and shale was developed for each of the two mine sites using 24 mono-lithologic samples of overburden collected from backfill excavations at the Smoky Canyon Mine. An additional 34 samples were collected from an archived core of unconsolidated backfill from the Dry

Valley Mine drilled hole GW7D2. The bulk composites were prepared by compositing samples in equal proportions. *In situ* and analytical geochemical data reported previously for select samples from this hole [38] (see also Appendix A1) were used to guide sampling and composite development, in addition to data collected during this study. This provided a homogenized and characterized substrate for experiments that represents the range of mineralogical, textural, and geochemical variation in pit backfill.

XRD Analysis of Rock

Samples of rock used to construct reactors, and samples of rock following reduction of Se in batch reactors, were analyzed using the Scintag Inc. (USA) Advanced diffraction system XL. Randomly packed powder was analyzed from 5 to 75 degrees in 0.02 degree steps, at a power of 1.8 kW using an energy of 45 kV and current set at 40 mA. Spectra were interpreted via comparison using a database of known XRD spectra provided by Scintag.

The bench top XRD analysis provided a baseline analysis of major rock forming minerals, as shown in Table S5-1. Trace quantities of Se phases were identified, but no clear trends with reduction in samples from the batch reactors could be established.

Total Element Analysis (ICP-MS) Following Aqua Regia Digestion: The total (Se, Mn, Fe, in mg/kg and S, in wt%) and extracted soluble Se, Mn, Fe, $NO_3^-$ and organic

Table S5-2. Geochemistry of overburden from Dry Valley and Smoky Canyon mines used in batch reactor rate experiments.

| | | | | Dry Valley Mine Well GW7D2A | | |
|---|---|---|---|---|---|---|
| | | | | **chert** | **shale** | **ROM** |
| **No. of Samples** | | | | **4** | **21** | composite |
| | **Method** | **Lab** | **Source** | | | composite |
| **Se, mg/kg** | Aqua Regia/ICPMS MEMS 41 | ALS Chemex | a | 15 | 86 | 56.8 |
| **Mn, mg/kg** | Aqua Regia/ICPMS MEMS 41 | ALS Chemex | a | 345 | 271 | 314 |
| **Fe, wt%** | Aqua Regia/ICPMS MEMS 41 | ALS Chemex | a | 1.61 | 1.68 | 1.65 |
| **S, wt%** | Aqua Regia/ICPMS MEMS 41 | ALS Chemex | a | 0.17 | 0.86 | 0.6 |
| **Soluble Se, mg/L** | EPA 1312/6020 | Energy Labs | b | 0.006 | 0.089 | 0.054 |
| **Soluble Mn, mg/L** | EPA 1312/6020 | Energy Labs | b | 0.228 | 0.082 | 0.137 |
| **Soluble Fe, mg/L** | EPA 1312/6020 | Energy Labs | b | 2.1 | 1 | 1.5 |
| **Soluble Nitrate, mg/L** | 3:1 DI bottle roll extract Method 4500 | MSU | a | 44.3 | 9.2 | nd |
| **Soluble Sulfate, mg/L** | 3:1 DI bottle roll extract method 4500 B | MSU | a | 15 | 413 | nd |
| **Leachable Organic Carbon mg/L** | EPA method 5310 GCMS | Energy Labs | a | 16.8 | 72.1 | nd |
| **TOT Organic Carbon, wt%** | Walkley Black | Energy Labs | b | 0.37 | 3.43 | nd |
| | | | | **Smoky Canyon Mine D and E panel excavation Well GW11** | | |
| | | | | **chert** | **shale** | **ROM [1]** |
| **No. of Samples** | | | | **15** | **12** | composite |
| | **Method** | **Lab** | **Source** | | | |
| **Se, mg/kg** | Aqua Regia/ICPMS MEMS 41 | ALS Chemex | a | 8 | 44 | 28.9 |
| **Mn, mg/kg** | Aqua Regia/ICPMS MEMS 41 | ALS Chemex | a | 438 | 289 | 393 |
| **Fe, wt%** | Aqua Regia/ICPMS MEMS 41 | ALS Chemex | a | 1.15 | 1.32 | 1.31 |
| **S, wt%** | Aqua Regia/ICPMS MEMS 41 | ALS Chemex | a | 0.08 | 0.40 | 0.26 |
| **Soluble Se, mg/L** | saturated paste extract | Energy Labs | c | <0.01 | 0.22 | 0.13 |
| **Soluble Mn, mg/L** | saturated paste extract | Energy Labs | c | 0.150 | 0.100 | 0.12 |
| **Soluble Fe, mg/L** | saturated paste extract | Energy Labs | c | nd | nd | nd |
| **Soluble Nitrate, mg/L** | saturated paste extract | Energy Labs | c | nd | nd | nd |
| **Soluble Sulfate, mg/L** | saturated paste extract | Energy Labs | c | 8 | 232 | 135 |
| **Leachable Organic Carbon mg/L** | 3:1 DI bottle roll extract Method 4500 infrared | MSU | a | 43.7 | 84.5 | nd |
| **TOT Organic Carbon, wt%** | saturated paste extract | Energy Labs | c | 0.36 | 4.63 | nd |

ROM: Run-of-Mine Mixed rock. nd: not determined

SOURCE: supporting data in Appendix A2

a this study, b Tt/Maxim and Geomatrix, 2008, c Smoky Canyon B & C EIS

C content (mg/L) of the shale and chert composites are described in Table S5-2. These results indicate higher total Se, S, and TOC content in shale, compared to chert, with concentrations at the Dry Valley Mine twice those measured at the Smoky Canyon Mine. Comparable concentrations of Fe were observed between rock types (e.g., both chert and shale) at the Smoky Canyon Mine, with values again higher at the Dry Valley Mine. Total Mn concentration was higher in the chert sample than in shale at both mine sites, with slightly higher overall concentrations at the Smoky Canyon Mine. These results are relatively consistent with the variation known to exist within the Phosphoria Formation [82]. At the Smoky Canyon Mine, soluble $NO_3^-$ was relatively consistent in paste extracts between rock units, and significantly lower than the values reported for bottle roll extracts from the Dry Valley Mine. Both are reported. Dissolved organic C was lower in chert than in shale extracts, and comparable between mine sites.

Total organic C (Table S5-3) was an order of magnitude higher in shale than in chert at both mine sites. Leachable organic C was also higher in shale at both mines. At the Smoky Canyon Mine, shale had a higher concentration of aromatic hydrocarbons and an overall higher ratio of aromatic to alkane and alkene compounds (Table S5-4) relative to chert. As the total organic C content in chert and shale was consistent between the two mine sites, and due to the cost of speciation analyses, extractable organic C was speciated only for samples from the Smoky Canyon Mine.

Table S5-4 statistically describes the long term chemistry of groundwater collected at the Dry Valley and Smoky Canyon mines, along with water quality data for samples of groundwater analyzed close to the time that samples were collected for use as

Table S5-3. Hydrocarbon extracted from composited overburden, using methylene chloride extraction followed by GC-MS.

| | **Shale** | | **Chert** | |
|---|---|---|---|---|
| | n = 1 *mg/kg* | *common compounds* | n = 1 *mg/kg* | *common compounds* |
| **Solvent Extractable Organic Carbon** | 72.1 | | 16.8 | |
| **Non-Aromatic** | 41.4 | | 15.1 | |
| **Aromatic** | 30.7 | | 1.9 | |
| **Ratio Aromatic/Total** | 43% | | 12% | |
| **Alcohol** | *nd* | | 1.2 | hexadecanol |
| **Alkane** | 32.2 | decane, hexane | 9.7 | decane, eicosane |
| **Alkene** | 0.6 | octadecene | *nd* | octadecene |
| **Amide** | 7.7 | decanamide | 3.6 | decanamide |
| **Aldehyde** | 0.5 | octadecenal | 0.4 | dimethyl octenal |
| **Heterocyclic** | 0.3 | azetidine | 0.2 | tetrahydropyran |
| **Monoaromatic** | 14.8 | phthalate, benzene, toluene | 1.9 | phthalate, benzene |
| **Diaromatic** | 9.9 | naphthalene | *nd* | |
| **Polyaromatic** | 6.0 | dibenzothiophene, phenanthrene | *nd* | |

innoculum in July 2007 (see methods described in Appendix A1). Sediments from the Dry Valley Mine GW7D groundwater samples incubated under anaerobic conditions averaged $4.6 \times 10^6$ SeRB per gram of rock, values that were considerably higher than the $1.7 \times 10^4$ SeRB per gram in the Smoky Canyon Mine monitoring well GW11 (Chapter 4).

Dissolved organic C was speciated using HS-SPME-GC-MS for a sample of groundwater from the Dry Valley Mine monitoring well GW7D2a. A sample could not be obtained from GW11 for this analysis, as the well had been abandoned due to construction of additional backfill in this location. Naturally-occurring, volatile organic C compounds detected in the head space are summarized in Appendix E and include benzene, butanone, pentane, furan, and hexadecane. These compounds are reported as a % of C detected in vapor.

Table S5-4. Groundwater chemistry at the Dry Valley and Smoky Canyon mines.

| 2007 Groundwater samples used in batch reactors | | Dry Valley Mine GW7D2A | | | Smoky Canyon Mine GW11 | | |
|---|---|---|---|---|---|---|---|
| | Depth, feet | 180 | | | 85 | | |
| | Lithology | Run-of-mine mix | | | Run-of-mine mix | | |
| | no. of samples | Average (n=8) | | | Average (n=3, diss Se only) | | |
| *In situ* conditions | T°C | 9.8 | | | 7.7 | | |
| | Moisture Content | saturated | | | saturated | | |
| | *In situ* $O_2$, mg/L | 0.20 | | | 5.5 | | |
| | pH | 7.8 | | | 7.3 | | |
| | | *MIN* | *2007* | *MAX* | *MIN* | *2007* | *MAX* |
| Groundwater Chemistry ICPMS | $NO_3^-$, mg/L | 0.11 | 0.3 | 11 | nd | 0.16 | nd |
| | Diss Se, ug/L | 0.01 | 0.021 | 0.065 | 0.299 | 1.01 | 1.06 |
| | $SO_4^{2-}$, mg/L | 680 | 710 | 830 | nd | 1666 | nd |
| | Fe, mg/L | 0.11 | 0.20 | 0.31 | nd | 3.3 | nd |
| | Mn, mg/L | 0.394 | 0.47 | 0.55 | nd | 0.45 | nd |
| DOC, mg/L | | 10.0 | | | 5.57 | | |
| Organic C Speciation HS-SPME-GCMS | Example compound | % | | | | | |
| | benzene | 16 | | | | | |
| | butanone | 25 | | | | | |
| | pentane | 19 | | | | | |
| | furan | 16 | | | | | |
| | hexadecane | 24 | | | | | |

nd not determined

Dissolved Organic Carbon Speciation by HS-SPME-GC-MS: Volatile organic C compounds were extracted from acidified aqueous samples by head space-solid phase microextraction (Vas and Veke, 2004) using a Supelco carbox/polydimethylsiloxane fiber, with adsorption time fixed at 45 min with stirring. Analyses were run by the RJ Lee Group, Center for Laboratory Sciences. The fiber was desorbed in the injector at 240°C with helium carrier gas at 1.0 mL/min., followed by GC-MS analysis using EPA Method 8260 BFB tuning criteria. The oven temperature was ramped from 40°C to 230°C at 5°C/min. Although it is possible to quantify volatile organic C compounds based on

equilibrium partitioning coefficients, this was not attempted, as the intended use of the data was qualitative identification of C compounds. HS-SPME-GCMS analyses were run to qualitatively identify volatile C species for eight samples including groundwater, lithology, specific groundwater extracts, and rate reactor water at the start and end of one set of replicate experiments. Compounds were tentatively identified using the NIST05 library[83] and results were edited to remove any compounds introduced with the standards. The tentative identifications provided by the laboratory were reviewed to ensure data quality by limiting identified compounds to well defined peaks with greater than 80% similarity to known compounds, within 0.2 minutes variance of known retention time. The relative amount of identified species was quantified by comparison with a 4-bromofluorobenzene internal standard calibration curve delivered in methanol over the concentration range of 1.2 to 78 ng. Results are summarized in Appendix E.

Volatile C compounds were measured by HS-SPME-GC-MS for samples of groundwater, as well as water from live and killed control reactors for both chert and shale at the end of reduction (Table S5-5). This method allowed qualitative assessment of C speciation in the aqueous headspace only for volatile compounds, based on comparison of peak area with the mass of a known internal standard. These data can only indicate shifts in relative frequency of C species. Also, limited available sample restricted the number of these analyses, unfortunately, so that it was not possible to determine sample reproducibility. As preliminary results, however, the data presented in Table S5-5 suggest that the majority of C in the reactors came from the solid phase, rather than groundwater, which had relatively low initial concentrations of alkane and aromatic compounds. These

data also suggest that relatively larger numbers of shorter chain (fewer C) alkane compounds were initially present in the chert reactor than in the shale reactor and that concentrations of volatile hydrocarbons in the aqueous phase decreased as Se reduction proceeded.

Table S5-5. Dissolved organic carbon speciation by HS-SPME-GC-MS for select samples.

| | | | | | GW DV7D2A | ROM mix media | SCD chert 5 | SCD shale 75 | 10C control | 10C live end | 10 S control | 10 S live end |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Example Compound** | **# Carbons** | **# Nitrogens** | **Chemical Formula** | **ng in vapor** | | | | | | | |
| Alkane | Ethanol | 2 | 0 | $C_2H_6O$ | | | | 14.20 | | | | |
| Alkane | ethane, 1,1 -oxybis- | 4 | 0 | $C_2H_6$ | | | | 40.02 | | | | |
| Alkane | Pentanal | 5 | 0 | $C_5H_{10}O$ | | | | | 36.36 | 13.31 | | |
| Alkane | cyclopentane, methyl- | 6 | 0 | $C_6H_{12}$ | 1.27 | 7.51 | 14.68 | 39.62 | 49.73 | 12.63 | 36.53 | 28.48 |
| Alkane | Pentane, 3-methyl- | 6 | 0 | $C_6H_{14}$ | | | | 7.71 | | 11.45 | | 10.60 |
| Alkane | 2-pentanone, 4-methyl- | 6 | 0 | $C_6H_{12}O$ | | | 2.77 | 1.75 | 9.16 | | | |
| Alkane | Hexane | 6 | 0 | $C_6H_{14}$ | | | 3.60 | 23.44 | 17.46 | | 32.00 | 13.10 |
| Alkane | 2-butanone, 3-hydroxy-3-methyl- | 6 | 0 | $C_5H_{10}O_2$ | 1.27 | 1.70 | | | 18.17 | | | |
| Alkane | Acetic acid, butyl ester | 6 | 0 | $C_6H_{12}O_2$ | | | | 2.09 | | | | |
| Alkane | Acetic acid, 1,1-dimethylethyl ester | 6 | 0 | $C_6H_{12}O_2$ | | | 3.58 | | | | | |
| Alkane | 2-Pentanone, 3-methyl- | 6 | 0 | $C_6H_{14}O$ | | | | 2.17 | | | | |
| Alkane | 3- hexanone | 6 | 0 | $C_6H_{12}O$ | | | | | 4.94 | | 4.53 | 4.78 |
| Alkane | Hexane, 3-iodo | 6 | 0 | $C_6H_{13}$ | | 5.81 | 2.39 | 2.46 | | 1.18 | | |
| Alkane | Propane, 2-ethoxy-2-methyl- | 6 | 0 | $C_9H_{14}O$ | | | 2.34 | | | | | |
| Alkane | 3-pentanone, 2,4-dimethyl- | 7 | 0 | $C_5H_{10}O$ | | 5.40 | | | 31.80 | | | |
| Alkane | 1-pentanol, 2,2-dimethyl- | 7 | 0 | $C_7H_{16}O$ | | | | | | | | |
| Alkane | 2-heptanol, 2-methyl- | 8 | 0 | $C_8H_{18}O$ | | | | | 26.11 | | | |
| Alkane | 2-heptanone, 4-methyl- | 8 | 0 | $C_8H_{16}O$ | | | | | 17.98 | | | |
| Alkane | Nonanal | 9 | 0 | $C_9H_{18}O$ | | 3.60 | 7.87 | | 21.63 | 12.28 | 7.09 | |
| Alkane | heptane, 4,4-dimethyl- | 9 | | $C_9H_{20}$ | | 1.45 | | | 9.74 | 1.32 | | |
| Alkane | Undecane, 2,4, -dimethyl- | 9 | 0 | $C_{11}H_{24}$ | | | | | | | | |
| Alkane | 3-heptanone, 2,4-dimethyl- | 9 | 0 | $C_9H_{18}O$ | | | | | | | | |
| Alkane | octanal, 7-hydroxy-3,7-dimethyl- | 10 | 0 | $C_{10}H_{20}O_2$ | | | | | 17.27 | | | |
| Alkane | decanal | 10 | 0 | $C_{10}H_{20}O$ | | 4.61 | 7.97 | | 27.09 | 1.38 | 7.40 | 7.49 |

Table S5-5. Dissolved Organic Carbon Speciation by HS-SPME-GCMS for Select Samples, continued.

| | | | | | GW DV7D2A | ROM mix media | SCD chert 5 | SCD shale 75 | 10C control | 10C live end | 10 S control | 10 S live end |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Example Compound** | **# Carbons** | **# Nitrogens** | **Chemical Formula** | **ng in vapor** | | | | | | | |
| Alkane | Dodecane | 12 | 0 | $C_{12}H_{26}$ | | | | 2.32 | 5.71 | 0.90 | 24.21 | |
| Alkane | Dodecane, 1-iodo- | 12 | 0 | $C_{12}H_{25}I$ | | | | | | | 12.29 | |
| Alkane | 3-Dodecanol | 12 | 0 | $C_{12}H_{26}O$ | | | | | 5.71 | | | |
| Alkane | 3,6- dimethyldecane | 12 | 0 | $C_{12}H_{22}$ | | | | | | | 11.92 | |
| Alkane | Propanoic acid, 2-methyl-, 2-methyl | 12 | 1 | $C_{12}H_{15}NO_4$ | | | | 2.32 | | | | |
| Alkane | 9-Undecen-2-one, 6,10-dimethyl- | 13 | 0 | $C_{13}H_{24}O$ | | 3.63 | | | | 1.05 | 4.85 | |
| Alkane | Undecane, 4,6-dimethyl- | 13 | 0 | $C_{13}H_{28}$ | | | | | | | 4.85 | |
| Alkane | hexane, 1,1-[ehtylidenebis(oxy) | 14 | 0 | $C_6H_{14}$ | | 6.43 | | 2.14 | 5.27 | | 20.67 | |
| Alkane | Decane, 2,3,5,8-tetramethyl- | 14 | 0 | $C_{14}H_{30}$ | | | | | | | 7.32 | |
| Alkane | Tetradecane | 14 | 0 | $CH_3(CH_2)_{12}CH_3$ | | | | | | | 4.66 | |
| Alkane | Tridecane, 5-methyl- | 14 | 0 | $C_{14}H_{30}$ | | 6.43 | | | | | | |
| Alkane | 2-nonanone,9-[(tetrahydro-2h-py | 14 | 0 | $C_{14}H_{22}O_2$ | | | | | 5.27 | | | |
| Alkane | Dodecane, 4,6-dimethyl- | 14 | 0 | $C_{14}H_{30}$ | | | | | | | 8.69 | |
| Alkane | Pentadecane | 15 | 0 | $C_{15}H_{32}$ | | | | | | | 10.79 | |
| Alkane | hexadecane | 16 | 0 | $C_{16}H_{34}$ | 1.21 | | | 13.39 | | | | |
| Alkane | Tridecane, 6-propyl- | 16 | 0 | $C_{16}H_{34}$ | | | | 1.88 | | | | |
| Alkane | propanoic acid, 2-methyl-, 1-(1 | 16 | 0 | $C_{16}H_{32}NO_4$ | | | | 11.51 | | | | |
| Alkane | octadecane | 18 | 0 | $C_{18}H_{38}$ | | | | | | | 4.89 | |
| Alkane | Tricosane | 23 | 0 | $C_{23}H_{48}$ | | | | | | | 5.09 | |
| Alkane | Octosane | 28 | 0 | $C_{28}H_{48}$ | | | | | | | 7.97 | |
| Alkane | triacontane | 30 | 0 | $C_{30}H_{62}$ | | | | | | | 27.31 | |
| Alkane | hexatriacontane | 36 | 0 | $C_{36}H_{74}$ | | | | | | | 4.64 | |
| **Total Alkane** | | | | | **2.48** | **31.18** | **30.52** | **111.69** | **203.70** | **41.55** | **161.44** | **35.97** |

Table S5-5. Dissolved Organic Carbon Speciation by HS-SPME-GCMS for Select Samples, continued.

| | | | | | GW DV7D2A | ROM mix media | SCD chert 5 | SCD shale 75 | 10C control | 10C live end | 10 S control | 10 S live end |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Example Compound** | **# Carbons** | **# Nitrogens** | **Chemical Formula** | **ng in vapor** | | | | | | | |
| Alkene | 1-Pentene, 2,4,4 trimethyl- | 8 | 0 | $C_5H_{10}$ | | | | | | 0.77 | | |
| Alkene | 2-Pentene, 3-ethyl-4,4-dimethyl- | 9 | 0 | $C_5H_{10}$ | | | | 1.68 | | | | |
| Alkene | 1,2,6 Heptatriene, 2,5,5-trimethyl | 10 | 0 | $C_9H_{12}$ | | | | 1.89 | | | | |
| Alkene | 4,4,7,7 -Tetramethyldeca-1,9-dien | 14 | 0 | $C_{14}H_{24}O_2$ | | | | | 7.47 | | | |
| **Total Alkene** | | | | | | | | **3.57** | **7.47** | **0.77** | | |
| Aromatic | Pyrimidine, 5-bromo- | 4 | 2 | $C_4H3BrN_2$; | | 1.25 | | | | | | |
| Aromatic | 1-H-Pyrazole, 4,5-dihydro-3,5,5-t | 6 | 3 | $C_{29}H_{22}F_3N_3O_2$ | | | | | 20.66 | | | |
| Aromatic | benzene, methyl- | 7 | 0 | $C_6H_6$ | 0.81 | | | 1.77 | | | | 2.94 |
| Aromatic | Furan, tetrahydro-2,2,5,5-tetram | 8 | 0 | $C_4H_4O$ | 0.79 | | | | 17.00 | | 4.36 | |
| Aromatic | Benzenemethanol, 4-hydroxy-.al | 9 | 0 | $C_8H_{10}O$ | | 1.20 | 7.27 | | | | | |
| Aromatic | Benzenedicarboxylic acid, di | 12 | 0 | $C_8H_6O_4$ | | | | 2.54 | | | | |
| Aromatic | Benzenedicarboxylic acid, butyl | 20 | 0 | $C_7H_6O_2$ | | | | 2.48 | | | | |
| **Total Aromatic** | | | | | **1.60** | **2.45** | **7.27** | **6.79** | **37.66** | **0.00** | **4.36** | **2.94** |
| Cyclic | cyclopentane, methyl- | 6 | 0 | $C_5H_{10}$ | | 1.33 | 6.32 | 7.26 | | 1.83 | | 9.59 |
| Cyclic | 1,2 cyclopentadiol, 1-(1-methyl | 8 | 0 | $C_6H_{12}O_2$ | | | | | | | | 7.51 |
| Cyclic | 1,8- cineole | 10 | 0 | $C_{10}H_{18}O$ | | | 3.68 | | 22.53 | | | |
| Cyclic | fenchone | 10 | 0 | $C_{10}H_{16}O$ | | | | | 8.83 | | | |
| Cyclic | (+)- isomethol | 10 | 0 | $C_{10}H_{20}O$ | | | | | 4.87 | | | |
| Cyclic | Cyclohexane, 1,2-diethyl-3-methy | 11 | 0 | $C_6H_{12}$ | | | | | | | 16.27 | |
| Cyclic | Cyclohexane, 1-ethyl-2-propyl | 11 | 0 | $C_{11}H_{22}$ | | | | | | | 7.29 | |

Table S5-5. Dissolved Organic Carbon Speciation by HS-SPME-GCMS for Select Samples, continued.

| | | | | | GW DV7D2A | ROM mix media | SCD chert 5 | SCD shale 75 | 10C control | 10C live end | 10 S control | 10 S live end |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Example Compound** | **# Carbons** | **# Nitrogens** | **Chemical Formula** | **ng in vapor** | | | | | | | |
| Cyclic | Cyclooctane,butyl- | 12 | 0 | $C_6H_{12}$ | | | | | | | 6.98 | |
| Cyclic | cyclohexane, octyl- | 14 | 0 | $C_{14}H_{28}$ | | | | | 4.90 | | | |
| **Total Cyclic** | | | | | | **1.33** | **10.00** | **7.26** | **41.13** | **1.83** | **30.54** | **17.10** |
| Amine | 2-Propanamine | 3 | 1 | $C_3H_9N$ | | | 9.38 | | | | | |
| ketone | 2-Propanone | 3 | 0 | $C_3H_6O$ | | | 2.09 | | | | | |
| | Plumbane, tetramethyl- | 4 | 0 | $C_4H_{12}Pb$ | | | | | 6.45 | | | 2.04 |
| | 4-Nitro-1-methylimidazole | 4 | 2 | $C_4H_6N_2$ | | | | | 6.45 | | | |
| Hetero-cyclic | 2,2,3,3-Tetramethyl-1-d1-aziridine | 4 | | | | | | | 13.40 | | | 13.82 |
| | 1,2,3- Oxazaborolane, 2-butyl- | 6 | 1 | $C_6H_{14}BNO$ | | | | | | | | 13.82 |
| ketone | Camphor | 10 | 0 | $C_{10}H_{16}O$ | | | | 2.04 | 14.54 | | | |
| | Hydroxylamine, o-decyl- | 10 | 1 | $C_{10}H_{23}NO$ | | | | 2.04 | | | | |
| terpene | Farnesol | 15 | 0 | $C_{15}H_{26}O$ | | | | | | | 4.58 | |
| | | | | | | | | | | | | |

In blank cells, compound was not detected.

These analyses represent GCMS speciation of the aqueous hydrocarbons using SPME methods - volatiles stirred out of aqueous phase, collected on siloxane fiber that is destructively sampled in GCMS. See Appendix E.

This table summarizes #C compounds within each major class of hydrocarbon (alkane, alkene, aromatic, cyclic, misc)

Samples are groundwater GWDV7D2a and aqueous GWTOC extract (bottle roll);two of the bottle roll extracts used for the MPN work, DMSo and DC5; starting (killed) and ending (live) solutions from the 10 Chert and 10 shale (Dry Valley).

Table S5-6. XANES Analysis of Se in Rate Reactor Mineral Samples

| **Standard** | **Edge position (eV)** |
|---|---|
| Sodium selenite | 12 659.5 |
| Sodium selenate | 12 662.6 |
| Elemental selenium – red | 12 657.5 |
| Elemental selenium – grey | 12 655.5 |
| $SeS_2$ | 12 656.4 |
| $FeSe_2$ | 12 657.0 |
| Selenium cysteine | 12 657.3 |
| Selenium methionine | 12 658.4 |
| | |
| **Sample** | **Edge position (eV)** |
| ***Dry Valley Shale*** | |
| Shale only | 12 658.7 |
| 0 h – 10°C | 12 658.4 |
| 128 h – 10°C | 12 658.0 |
| 272h – 10°C | 12 657.8 |
| Live end – 25°C | 12 657.5 |
| Killed end – 25°C | 12 657.7 |
| | |
| ***Dry Valley Chert*** | |
| Chert only | 12 659.1 |
| 0 h – 10°C | 12 657.6 |
| 128 h – 10°C | 12 660.6 |
| 272 h – 10°C | 12 657.4 |
| Live end – 25°C | 12 658.5 |
| Killed end – 25°C | 12 659.5 |
| | |
| ***Smoky Canyon Shale*** | |
| 0 h – 10°C | 12 658.2 |
| 108 h – 10°C | 12 658.5 |
| 246 h – 10°C | 12 657.9 |
| | |
| ***Smoky Canyon Chert*** | |
| 0 h – 10°C | 12 657.8 |
| 108 h – 10°C | 12 658.1 |
| 246 h – 10°C | 12 657.8 |

*Data from Appendix F*

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# CHAPTER SIX

## SUMMARY AND CONCLUSIONS FOR SELENIUM SOURCE CONTROL IN MINED OVERBURDEN

Improved capacity for *in situ* source control of selenium (Se) in mine-affected water is of increasing importance to mining operators working in a variety of commodities, including phosphate, coal, and metals [1, 2]. Microbial source control of Se within deposits of phosphate mine waste in the S.E. Idaho Phosphate Resource Area is an important potential strategy for mine waste management and protection of water resources. Observations of apparent *in situ* Se reduction in backfill at multiple locations, and the results of controlled microbiological and kinetic geochemical experiments presented here, support the conclusion that it is possible to intentionally develop conditions within saturated and organic-rich backfilled mine waste that will support Se reduction. Microbes living in backfilled mine overburden deposits can, under conditions that have been documented within full-scale mine backfill and dump facilities, reduce soluble and toxic $SeO_4^{2-}$ and $SeO_3^{2-}$ to insoluble and less toxic $Se^0$ and $Se^{2-}$ minerals. More than ten years of *in situ* monitoring data from Dry Valley GW7D describing suboxic conditions in partially saturated to saturated backfill demonstrate, at the broadest scale, that this is true. In this study, the biogeochemical conditions observed at this site have been linked with microbial communities and geochemical processes active at the pore scale, and contrasted with those in other S.E. Idaho Phosphate Resource Area waste deposits at Smoky Canyon and Enoch Valley, as a foundation for the design of facilities that integrate biogeochemistry across these scales. The following discussion summarizes

the findings of this study, and outlines the questions it raises regarding the implementation of *in situ* microbial source control of Se within phosphate overburden deposits.

This study applies a variety of existing knowledge regarding the biogeochemistry of Se to questions of mine waste management. For example, the existence of Se-reducing bacteria (SeRB), and the impact they have on the kinetics of Se cycling in the environment, is well documented [3]. A number of microbes capable of reducing Se in the environment are known and many commercial water treatment processes (active or passive) rely on microbial Se reduction with addition of carbon (C) and nutrients [4]. Similarly, the potential for reactive barriers to intercept contaminants in groundwater is also well known [5] and Se remediation using this approach has been the focus of other, ongoing investigations [6]. However, the intentional design of mine facilities (backfills and overburden dumps among others) to act as operational sinks by facilitating biological reduction of Se remains relatively unexplored. This study begins at the hydrogeochemical field scale (Chapter 2 and Figure 3) and works back to a microscale understanding of microbial geochemistry (Figure 4) based on the identity, number, and reduction capabilities of native microbes (including SeRB) under field relevant lithotype, chemistry, and moisture conditions.

This study has identified a variety of native SeRB which function within a community of hydrocarbon-oxidizing and $NO_3^-$, $Fe^{3+,}$ and $Mn^{4+-}$reducing, aerobic and facultative anaerobic microbes. The SeRB include several $SeO_4^{2-}$respiring *Dechloromonads*, as well as other SeRB including *Anaeromyxobacter* spp. and *Stenotrophomonas* spp., also known to reduce $SeO_4^{2-}$ for detoxification or other non-

respiratory reasons. Only *Dechloromonas* was shown to grow on $SeO_4^{2-}$ and reduce it to $Se^0$ under anaerobic conditions (Childers, unpublished data this study). Also in this study, other SeRB isolates were shown to reduce $SeO_3^{2-}$, but not $SeO_4^{2-}$, including members of of the *Arthrobacter*, *Pseudomonas*, *Cellulomonas*, and *Sphingomonas* genera. Other bacteria genera that were not isolated, but which were identified in molecular work, including *Anaeromyxobacter* and *Ralstonia*, are also known $SeO_3^{2-}$-reducers.

Results presented here suggest that *Dechloromonas* spp. can reduces $SeO_4^{2-}$ in a stepwise fashion to $SeO_3^{2-}$ and then $Se^0$, using complex native hydrocarbon and dominates the SeRB community in groundwater samples from both Dry Valley and Smoky Canyon. Based on the absence of dechloromonads in clone libraries and most dilute positive MPN cultures, however, this genus appears to be present in relatively low numbers in unsaturated sediments, where the SeRB were shown to include *Anaeromyxobacter*, *Stenotrophomonas*, and other heterotrophs. Interestingly, phylotypes highly similar to the Fe-reducing genus *Rhodoferax* occur frequently in the clone libraries and DGGE analyses of unsaturated sediment enrichments, in association with the heterotrophic SeRB (Chapter 4), but rarely in the groundwater samples. This raises interesting questions which should be further investigated regarding the role that *Rhodoferax* spp. may play in providing $Fe^{2+}$- to serve as an electron shuttle in support of biotic Se reduction (or perhaps abiotic $SeO_3^{2-}$ reduction) in unsaturated environments.

A number of opportunities remain to learn more about this complex microbial ecology and its influence on the mineralization endpoints of reduction in this system. A more comprehensive analysis of the diversity and abundance of microorganisms using state-of –the-art methods is essential to obtain a more statistically robust analysis. This

work was conducted at a time when high throughput genomic methods (such as 454 pyrosequencing) were far more expensive than they are today, and these methods were therefore unavailable to this project. The limitations of the DGGE method, relatively small clone libraries, and inconsistencies in identified SeRB between various data subsets (e.g., MPN cultures, isolate populations, and clone libraries), suggest that a more comprehensive approach using methods such as pyrosequencing of rock and groundwater samples would be highly informative.

It would also be interesting to conduct more focused rate experiments using a defined mixed culture consisting of key members of the identified SeRB and a *Rhodoferax* isolate, with one select member of the hydrocarbon-degrading microbial community, under controlled conditions of $NO_3^-$ addition and more carefully tracked changes in $NO_3^-$, $NO_2^-$, $Mn^{4+}$, $Mn^{3+}$, $Mn^{2+}$, $Fe^{3+}$, and $Fe^{2+}$ concentrations. Isolates to be included could be chosen from the community results defined with DGGE. Experiments should be conducted in the presence of known hydrocarbon electron donors and select Fe and Mn mineral substrates, to reduce the complexity of the experimental system. Outstanding questions that could be addressed in these experiments include:

1. Is the observed increase in dissolved $Fe^{2+}$ reproducible, and is it associated with an increase in the *Rhodoferax* (or another Fe/Mn-reducing microbial) population? Does it reflect an abiotic reaction of selenite with primary $FeS_2$ mineralization?
2. Can the isolated members of the *Rhodoferax* genus, which did not reduce $SeO_4^{2-}$ to $Se^0$ in culture, be shown to specifically facilitate the ability of other organisms to reduce $SeO_4^{2-}$ to $SeO_3^{2-}$, and thus contribute to Se

reduction through community level interactions? Does this occur in the presence of specific Fe-oxide substrates?

3. Why does the aqueous total N concentration increase during the middle of the Se reduction process, as suggested by the results shown in Figures 19 and 20? How does this relate to the observed increase in dissolved Fe concentration? Is this associated with increased numbers of $N_2$-fixing organisms in the microbial community?
4. Are the microbial community changes indicated in Figure 23 reproducible? Or, is there a change in the community within a consistent set of functional microbial niches that correspond with other biogeochemical variables, such as moisture or lithologically-controlled geochemistry? How might the observed biogeochemistry inform the management of such a microbial community in a pilot or field scale facility?

The most favorable conditions for Se reduction appear to be in saturated or moist conditions (close to field capacity) where sufficient soluble Se and organic C is available to support higher numbers of SeRB. The SeRB were present in greatest numbers ($5 \times 10^6$ SeRB per gram of rock) in moist, fine-grained crushed shale that were Se and C rich. The number of SeRB was negligible in chert and mudstone lithotypes ($3 \times 10^2$ SeRB per gram of rock), and no Se reduction was observed in aerobic MPN experiments for any lithotypes.

The greatest numbers of SeRB in sediment collected from turbid groundwater were from the Dry Valley backfill well GW7D2a (4.6 x$10^6$ SeRB per gram of rock), which had less than 0.2 mg/L initial dissolved $O_2$. There were approximately 300 times more SeRB at in saturated sediments collected from groundwater Dry Valley than from Smoky Canyon (1.6 x $10^4$ SeRB per gram of rock), which had elevated concentrations of dissolved $O_2$ (5 mg/L). Many of the identified SeRB bacteria are facultative and therefore can use $O_2$ and/or $NO_3^-$ until they are depleted, and $SeO_4^{2-}$ reduction can occur.

Results of this study indicate that members of the native microbial consortia make use of variable $O_2$ and moisture conditions within the mined overburden for both aerobic and anaerobic degradation of complex shale hydrocarbons. This is reflected by the diversity of identified organisms, which reduce $SeO_4^{2-}$ using the available, naturally-occurring hydrocarbon compounds under $Fe^{3+}$, $Mn^{4+}$, and $NO_3^-$ reducing conditions. Bacteria capable of aerobic hydrocarbon degradation (e.g., *Polaromonas)* and anaerobic hydrocarbon degradation coupled to $NO_3^-$, $Fe^{3+}$, and $Mn^{4+}$ reduction (e.g., *Rhodoferax, Pelosinus, Geothrix,* and *Dechloromonas*) have been identified. It is likely that degradation of complex shale hydrocarbons by aerobic members of the community decreases available $O_2$, thus creating conditions favorable for $SeO_4^{2-}$ reduction by facultative anaerobes like *Dechloromonas* and *Stenotrophomonas,* as well as other heterotrophic SeRB.

Experiments to determine rates of $O_2$, $NO_3^-$, $Fe^{3+}$, $Mn^{4+}$ and $SO_4^{2-}$ reduction were conducted under saturated, microaerophilic conditions. The results presented here showed that following depletion of $O_2$ (via biotic and abiotic mechanisms), biological $NO_3^-$, $Fe^{3+}$, and $Mn^{4+}$ reduction occurred together with Se reduction, but $SO_4^{2-}$ reduction

was not observed. Natural C of mixed complexity (including alkanes, cyclic, and aromatic compounds) from the solid phase was solubilized and a fraction (less than 40% of soluble C) was consumed. Reduction of Se occurred more slowly in shale than chert, at Dry Valley, at both temperatures, and was slower at field-relevant 10°C than at room temperature; no difference in rate (Table 10) was observed between lithology at Smoky Canyon experiments. In the experiments conducted for both sites, reduction occurs within 100 hours once low $O_2$ conditions were developed in both lithotypes. As the residence time of groundwater movement in field scale facilities is likely to be on the order of months or years, rather than days, these data suggest that it is likely possible to design facilities to retain water long enough to deplete residual $O_2$ and reduce available $SeO_4^{2-}$ and $SeO_3^{2-}$. The extent to which this is true will depend upon balancing the flux of oxidants into the system against its capacity for reduction, which is in turn dependent on the rate of air ($O_2$) flow, water flow, and available C, and will need to be considered on a site specific basis. The $O_2$ demand and replenishment rate can potentially be altered, where necessary, through amendment (e.g., addition of clays) or placement of rock in short, compacted lifts to increase water retention and reduce water flux rates. Other strategies targeting the creation of suboxic zones include the use of textural discontinuities and capillarity [7], construction of efficient store and release covers [8], and addition of C to consume oxygen and thereby promote the development of desired $O_2$ gradients. Potential health and safety risks associated with $CO_2$ accumulation and seasonal displacement from constructed facilities could require monitoring and management with institutional controls [9, 10]. This study has not evaluated the potential for re-oxidation of reduced Se minerals, but this risk should be addressed in tandem with

*in situ* demonstration studies, to consider a realistic flux of oxidants (e.g., $O_2$ and $NO_3^-$) as well as potential for re-oxidation by soluble $Fe^{3+}$ and $Mn^{4+}$.

The aqueous speciation of Se and reduced secondary Se mineralization described in the reactor experiments support chert- and shale-specific biogeochemical pathways at Dry Valley. In the chert reactor, $SeO_4^{2-}$ was reduced to aqueous $HSeO_3^-$ /$SeO_3^{2-}$ that was detected early in the reduction process. The fact that it was not detected subsequently suggests that it was rapidly removed from solution, either through adsorption to a solid phase mineral, probably clay or iron oxide, or through further reduction. The $HSeO_3^-$ /$SeO_3^{2-}$ was subsequently partially reduced to $Se^0$, although the amount of $HSeO_3^-$ /$SeO_3^{2-}$ reduction to $Se^0$ varied between the two mine sites; the extent to which this was related to remobilization of $HSeO_3^-$ /$SeO_3^{2-}$ during the concurrent reduction of $Fe^{3+}$ is not clear. It may be that higher concentrations of Fe-oxide and clay minerals present in the chert (Table S5-1) provided greater substrate for initial $HSeO_3^-$ /$SeO_3^{2-}$ sorption, or that differences in pH (Appendix D1) between the two lithotypes subtly influence the extent of $HSeO_3^-$ /$SeO_3^{2-}$ sorption, with consequence for subsequent $HSeO_3^-$ /$SeO_3^{2-}$ availability for reduction. However, it is difficult to determine if sorption is the primary control defining the different pathways between the chert and shale, as in spite of the pH differences between the two lithotypes in reactors at Dry Valley, both have pH below 8 and should support $HSeO_3^-$ /$SeO_3^{2-}$ sorption[11]. The sorption of the $HSeO_3^-$ /$SeO_3^{2-}$ thus has the potential to alter the trajectory of the subsequent biogeochemical reduction pathway. It is also possible that the different concentrations of dissolved $SO_4^{2-}$ between the chert and shale influenced the $SeO_3^{2-}$ reduction pathway, as described by Hockin and Gadd [12]. Another possibility is that there is a difference in the availability of free $Ca^{2+}$

(as opposed to $CaSO_4^0{}_{aq}$) lithotypes, as a result of the different amount of available $SO_4^{2-}$ between the two lithotypes in the reactors. This could influence the interim solubility of $CaSeO_3xH_2O$ (Figure 5) and have a similar effect on the availability of $SeO_3^{2-}$ for subsequent biological reduction. This would be an interesting question to address from a modeling perspective.

One way to address the significance of these factors on the reduction endpoint could be further examined in batch sorption experiments, with addition of $NaSeO_3$ under a controlled range of pH and $SO_4^{2-}$ concentrations, using defined sorption substrates of known surface area including iron oxide mineral and/or clay. Initial abiotic experiments conducted with speciation of Se, Fe, and Mn, followed by mineralogical analysis to identify surface complexation, could then be inoculated with select members of the Se-, Fe-, and Mn-reducing microbial community to evaluate potential differences in subsequence Se biomineralization.

In the Dry Valley shale, $SeO_4^{2-}$ was more slowly reduced, with some evidence of interim $SeO_3^{2-}$ and selenomethionine (SeM) formation, followed by reduction to Se(-II) and precipitation of $FeSe_2$ as shown by S-XRD and XAFS analyses. It is likely that the $Se^0$ and Se(-II) reduction products will be less reversible and more stable than adsorbed $SeO_3^{2-}$. The potential for reoxidation of reduced Se minerals should not be discounted, however, and should be considered in design.

Further study of the biogeochemical pathways that influence the differential production of $Se^0$ and Se(-II) in chert and shale, respectively, may identify engineering strategies that can optimize the stabilization of Se for most effective long-term remediation. Preliminary aqueous data support the formation of SeM as an interim

reduction product, but these data require replication. The observed decline in dissolved Fe concentration agrees with field scale observations, and is explained by the identification of $FeSe_2$ in shale, but the comparable drop in Fe concentration in chert reactors that do not show evidence for Se(-II) formation at the end of reduction suggests that this should be examined further.

Questions remain about the extent of reduction under unsaturated, but oxygen-depleted conditions, in layered or mixed mine waste. At Enoch Valley, no groundwater saturated conditions were identified, yet suboxic conditions were identified. Much of the Intermountain West hosts mine waste deposits in high evaporation environments where the dominant waste condition is unsaturated. If suboxic conditions can be reliably and cost-effectively created under unsaturated conditions, through the use of capillary break, compaction, organic amendment, or other design parameters, there is significant potential benefit to be gained through $NO_3^-$, $SO_4^{2-}$ and metal reduction.

Selenium reduction rate experiments were conducted using samples from two mine sites which produce phosphate from the same formation under different hydrologic conditions, located approximately 15 miles apart from one another. In spite of the relatively drier conditions in backfill at Smoky Canyon, zones were identified in the sediments of the panel A dump that hosted relatively higher numbers of SeRB, comparable to those observed in Dry Valley groundwater where Se reduction has been observed *in situ* for over 10 years. Within unsaturated sediments, the number of SeRB varies within the shale, and differences in microbial community are observed both within the shales, and between the chert, shale, and mudstone lithotypes. In spite of these variations, the $O_2$, moisture, and geochemical conditions identified in this study that were

needed to support biological Se reduction lie within the range of *in situ* conditions identified in phosphate overburden at all three of the studied S.E. Idaho phosphate mine facilities. These results do not indicate a need to add Fe, $NO_3^-$, or C to promote the *in situ* reduction of Se by native organisms, within a suboxic, steady state low flow environment in backfilled sediments comparable to that developed at Dry Valley.

Collectively, the results of the microbial community, enumeration, and saturated rate reactor studies agree with *in situ* monitoring results, wherein low concentrations of soluble Se exists under dry conditions in unsaturated backfill, with low numbers of associated SeRB, as described at Enoch Valley [13]. Low dissolved concentrations of Se also exist under moist (but, not necessarily saturated) and low $O_2$, but C-rich conditions, where the number of SeRB is greater, as has been observed at the Dry Valley B pit over time[14]. Higher concentrations of dissolved Se are evident where Se rich shales are exposed to $O_2$ and water, promoting release of Se that is not locally re-reduced *in situ,* such as Smoky Canyon [15]. In facilities where saturation occurs in deep backfill, $O_2$ is limited and local Se release following sulfide oxidation is low. In saturated environments, these results demonstrate that although sulfide is oxidized, native microbes can actively reduce soluble Se, Fe, and Mn. When saturation would not be possible, construction of facilities to limit $O_2$ recharge using placement of rock by individual dump trucks on lifts (as done at Enoch Valley, Dry Valley, and Luxor) or compaction/amendment of Se and C rich shales to promote higher moisture retention and Se reduction (as indicated by the high number of SeRB in the external panel A dump samples AS113 and AS71) may be of value. The extent to which the creation of suboxic conditions can be accomplished operationally, in a cost effective manner, is unclear, but these results suggest that it is

possible and potentially, highly effective. Reduction of Fe and Mn raises potential for changing concentrations of these elements downgradient, but potential for reoxidation of both (as well as precipitation of oxide minerals with capacity for further sorption of other metals) is high and also of potential environmental management value.

Further, our results suggest that the native microbial community is capable of adaptive metabolism, consuming $O_2$ while degrading hydrocarbons that further support SeRB and other reducing bacteria. When exposed to elevated $O_2$ concentrations, the SeRB did not efficiently reduce Se in groundwater monitored at GW11. However, fewer facultative bacteria capable of this process were detected at that location and were successfully stimulated in a closed, microaerophilic reactor, demonstrating that operational management of water and rock to create suboxia can make SeRB more efficient. The fact that reduction proceeds within chert when it is saturated with groundwater under anaerobic conditions, in spite of the inherently low numbers of SeRB that exist in that lithotype, suggests that reduction would proceed within mixed lithology backfills.

The ability of the system to tolerate repeated application of high Se water has not been studied in these experiments, but the availability of excess C and the long term monitoring record at Dry Valley suggest that *in situ* reduction of Se within overburden can be stable over a prolonged period of time as long as $O_2$ levels remain low and water flux rates (hence, $O_2$ transport) are minimized. Regardless, the goal of the *in situ* stabilization process described here is not to treat multiple volumes of water in rapid succession, but rather to stabilize Se within the mined overburden, thereby preventing the

need for such treatment. Treatment of multiple volumes of water would require consideration of the rate of C transformation to ensure sufficient C supply.

Due to the experimental complexity in the construction and sampling of reactors that maintain partially saturated, anaerobic conditions at field relevant temperatures, and the scale-dependent character of the $O_2$ consumption and replenishment processes, it seems more appropriate to further demonstrate the potential for *in situ* reduction of Se using paired laboratory and field experiments. This would allow laboratory experiments to reflect field conditions most realistically. Data characterizing scale-dependent parameters, such as those influencing the $O_2$ consumption and replenishment processes, can be measured in instrumented meso- or full-scale field facilities and used to address questions requiring greater experimental control in column or flow reactor microcosms. Some aspects of the biogeochemical kinetics and microbial community analysis will require further laboratory study, however, but should be conducted using $O_2$, C, and other oxidant and reductant flux measurements determined from field investigations.

## Microbial Ecology in Mine Waste Facility Design

The understanding of the environmental and financial risk posed by mine waste facilities, and their biogeochemistry, continues to evolve. Thirty years ago, mined lands had only begun to be recognized for their potential to affect water resources. Overburden dumps located merely to limit the cost of removing rock from extraction areas began to undergo active design, first to limit risk of hazard due to geotechnical failure, and then to manage water to prevent erosion and downstream sedimentation. More recently, the geochemistry and dynamics of water and gas flux within these facilities has been

characterized through geochemical testing and modeling, to predict the nature of potential impacts and evaluate alternative management options. Increasingly sophisticated yet primarily abiotic conceptual models of elemental fate and transport drive the understanding of future aqueous chemical conditions and subsequent evaluation of environmental and financial risk. Uncertainty about the site-specific efficacy of available, affordable management options continually challenges mining operations because of the inherent difficulty in predicting chemical and hydrodynamic changes across geospatial and temporal dimensions of such magnitude. Ever more comprehensive, complex, and expensive sampling and modeling efforts reflect the industrial and regulatory commitment to overcome these challenges, but do not necessarily provide better answers. Meanwhile, failure to identify risks associated with facility design (however unintentional) has put the social license of the industry to operate and grow in peril. Cast against growing demand for essential raw materials, and escalating environmental expectations, this situation puts the mining industry between a proverbial “rock and a hard place”. Pressure to produce mineral resources, while maintaining the universal availability of clean water, presents mine operators, regulators, and stakeholders with increasingly difficult choices.

A growing commitment to design and manage mining operations “sustainably” appears to offer a meaningful path forward, based on the principle that “ongoing creation of financial and social wealth should protect the future aesthetic and productive capacity of natural capital for future generations” [16, 17]. This concept recognizes the importance of a triple “bottom-line” decision framework that integrates social, environmental, and economic criteria. Decisions made in this context place a premium on options that can

effectively improve facility design for source control, thereby limiting down-gradient impact. Innovative designs that utilize the capacity of native microbial communities of mined materials, relying on management of available rock and water, to influence the biogeochemical processes that control rates of metal release and sequestration, may well become an important component of future mine design.

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# APPENDICES

# APPENDIX A

## OVERBURDEN AND GROUNDWATER CHARACTERIZATION DATA

## IDAHO PHOSPHATE MINE

# APPENDIX A

## OVERBURDEN AND GROUNDWATER CHARACTERIZATION DATA IDAHO PHOSPHATE MINE

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On CD: Excerpts from Agrium 2007 Groundwater Validation study
Groundwater sampling protocol
To request DVD copies contact your local public or university library to place an interlibrary loan request to Montana State University. Questions call 406-994-3161.

A-2 : 2005 Overburden Characterization Data
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Table A2-2. 2005 Overburden sample sieve results

On CD: Field sampling notes, photos, protocols from 2005
Energy Labs report – GCMS analysis of extracted hydrocarbon
ALS Chemex reports – Aqua Regia digestion, ICPMS analyses
To request DVD copies contact your local public or university library to place an interlibrary loan request to Montana State University. Questions call 406-994-3161.

A-3: 2006 Field Sampling protocol
Table A3-1. Sample Summary

On CD: Photo log (powerpoint) and Field Notes
TetraTech, 2008 – Geochemical Characterization of Phosphate Mining Report, with well installation details for SCA, SCD, and MEV.
To request DVD copies contact your local public or university library to place an interlibrary loan request to Montana State University. Questions call 406-994-3161.

Table A1-1. Dry Valley Monitoring Well GW-7D

| | | | | | | |
|---|---|---|---|---|---|---|
| | **Station ID** | GW-7D | GW-7D | GW-7D | GW-7D | GW-7D |
| | **Sample Date** | 11/1/1998 | 9/23/1999 | 11/7/2000 | 10/30/2001 | 10/28/2002 |
| | **Field Sample ID** | GW-7D | GW-7D | GW-7D | GW-7D | GW-7D |
| | **Lab Sample ID** | | | | | |
| **Parameter** | **Result Units** | | | | | |
| Alkalinity (as CaCO3) | mg/l | 210 | 210 | 231 | 241 | 260 |
| Bicarbonate (as CaCO3) | mg/l | 210 | 210 | 282 | 241 | 260 |
| Hardness (as CaCO3) | mg/l | nr | nr | nr | nr | nr |
| Calcium, dissolved | mg/l | nr | nr | nr | nr | nr |
| Calcium, total | mg/l | 248 | 286 | 266 | 252 | 244 |
| Magnesium, dissolved | mg/l | nr | nr | nr | nr | nr |
| Magnesium, total | mg/l | 49.5 | 60.1 | 54.7 | 51.3 | 57.8 |
| Sodium, dissolved | mg/l | nr | nr | nr | nr | nr |
| Sodium, total | mg/l | 13.2 | 16.2 | 16.2 | 13.7 | 14.4 |
| Potassium, dissolved | mg/l | nr | nr | nr | nr | nr |
| Potassium, total | mg/l | 1.4 | 1.4 | 1.4 | 1.7 | 2.2 |
| Chloride | mg/l | 8 | 9 | 7.7 | 8.6 | 7.5 |
| Fluoride | mg/l | 2.6 | 0.2 | 0.1 | 0.2 | 0.3 |
| Sulfate | mg/l | 830 | 790 | 650 | 583 | 574 |
| Phosphorus, total as P | mg/l | 0.1 | 0.3 | 0.1 | 0.09 | 0.1 |
| Nitrate as N, dissolved | mg/l | 3.9 | 3.3 | 0.29 | 0.18 | <0.1 |
| Nitrite as N, dissolved | mg/l | nr | nr | nr | nr | nr |
| Nitrate/Nitrite as N, dissolved | mg/l | nr | nr | nr | nr | nr |
| Nitrogen, ammonia | mg/l | <0.6 | 1.4 | <0.6 | <0.6 | <0.6 |
| Total Dissolved Solids | mg/l | 1300 | 1400 | 1280 | 1230 | 1210 |
| Total Suspended Solids | mg/l | nr | nr | nr | nr | nr |
| Iron, total | mg/l | 0.073 | <0.065 | <0.05 | <0.05 | <0.05 |
| Manganese, total | mg/l | 0.56 | 0.41 | 0.352 | 0.382 | 0.399 |
| Selenium, dissolved | mg/l | 0.0081 | 0.046 | 0.044 | 0.025 | 0.018 |
| Selenium, total | mg/l | nr | 0.043 | nr | nr | nr |
| Zinc, dissolved | mg/l | nr | nr | nr | nr | nr |
| Zinc, total | mg/l | 1.3 | 1.5 | 1.66 | 1.46 | 1.36 |
| Temperature, Field | deg C | 3.8 | 8.7 | 7 | 7.3 | 7.4 |
| E.C., Field, 25C | umhos/cm | nr | 1580 | 1492 | 1386 | 1281 |
| pH, Field | s.u. | nr | 6.6 | 7.01 | 6.57 | 6.85 |
| Dissolved oxygen | mg/l | nr | nr | nr | nr | nr |
| ORP, Field | millivolts | nr | nr | nr | nr | nr |
| Depth to Water | ft | nr | nr | nr | nr | nr |
| Sum of Anions | meq/l | nr | nr | nr | nr | nr |
| Sum of Cations | meq/l | nr | nr | nr | nr | nr |
| Cation-Anion Balance | % | nr | nr | nr | nr | nr |
| TDS (calculated) | mg/l | nr | nr | nr | nr | nr |

nr = not reported

Table A1-1. Dry Valley Monitoring Well GW-7D (continued)

| | | | | | | |
|---|---|---|---|---|---|---|
| | **Station ID** | GW-7D | GW-7D | GW-7D | GW-7D | GW-7D |
| | **Sample Date** | 10/22/2003 | 10/7/2004 | 10/14/2005 | 10/23/2006 | 10/4/2007 |
| | **Field Sample ID** | GW-7D | GW-7D | GW-7D | GW-7D | GW-7D |
| | **Lab Sample ID** | | | L53888-05 | L60021-07 | L65749-03 |
| **Parameter** | **Result Units** | | | | | |
| Alkalinity (as CaCO3) | mg/l | 251 | 252 | 263 | 262 | 267 |
| Bicarbonate (as CaCO3) | mg/l | 251 | 252 | 263 | 262 | 267 |
| Hardness (as CaCO3) | mg/l | nr | nr | 922 | 877 | 942 |
| Calcium, dissolved | mg/l | nr | nr | 272 | 257 | 277 |
| Calcium, total | mg/l | 257 | 210 | nr | nr | nr |
| Magnesium, dissolved | mg/l | nr | nr | 59 | 57.1 | 60.6 |
| Magnesium, total | mg/l | 56.8 | 58 | nr | nr | nr |
| Sodium, dissolved | mg/l | nr | nr | 15.4 | 14.6 | 15 |
| Sodium, total | mg/l | 14.7 | 16 | nr | nr | nr |
| Potassium, dissolved | mg/l | nr | nr | 1.1 | 1 | 1.2 |
| Potassium, total | mg/l | <1 | 1.2 | nr | nr | nr |
| Chloride | mg/l | 7.3 | 8.76 | 8 | 8 | 9 |
| Fluoride | mg/l | 0.2 | 0.21 | <0.1 | 0.3 | 0.3 |
| Sulfate | mg/l | 581 | 615 | 650 | 670 | 650 |
| Phosphorus, total as P | mg/l | 3.3 | <0.05 | 0.15 | 0.13 | 0.14 |
| Nitrate as N, dissolved | mg/l | <0.1 | <0.03 | <0.02 | 0.02 | <0.02 |
| Nitrite as N, dissolved | mg/l | nr | nr | <0.01 | <0.01 | <0.01 |
| Nitrate/Nitrite as N, dissolved | mg/l | nr | nr | <0.02 | 0.02 | <0.02 |
| Nitrogen, ammonia | mg/l | <0.6 | 0.31 | <0.05 | 0.16 | <0.05 |
| Total Dissolved Solids | mg/l | 1220 | 1200 | 1250 | 1220 | 1210 |
| Total Suspended Solids | mg/l | nr | nr | <5 | <5 | <5 |
| Iron, total | mg/l | 2.88 | 0.012 | <0.02 | <0.02 | <0.02 |
| Manganese, total | mg/l | 0.417 | 0.3716 | 0.424 | 0.471 | 0.428 |
| Selenium, dissolved | mg/l | 0.026 | 0.0172 | 0.026 | 0.027 | 0.0214 |
| Selenium, total | mg/l | nr | nr | 0.019 | 0.0247 | 0.0131 |
| Zinc, dissolved | mg/l | nr | nr | nr | nr | nr |
| Zinc, total | mg/l | 1.62 | 1.062 | 1.38 | 1.41 | 1.1 |
| Temperature, Field | deg C | 8 | 7.6 | 8.01 | 8.15 | 8.24 |
| E.C., Field, 25C | umhos/cm | 1164 | 1211 | 1035 | 1610 | 1529 |
| pH, Field | s.u. | 7.13 | 7.13 | 6.64 | 6.83 | 6.71 |
| Dissolved oxygen | mg/l | nr | nr | 0.43 | 0.18 | 0.25 |
| ORP, Field | millivolts | nr | nr | nr | nr | nr |
| Depth to Water | ft | nr | nr | nr | nr | nr |
| Sum of Anions | meq/l | nr | nr | 19.1 | 19.6 | 19.3 |
| Sum of Cations | meq/l | nr | nr | 19.1 | 18.2 | 19.5 |
| Cation-Anion Balance | % | nr | nr | nr | -3.7 | 0.5 |
| TDS (calculated) | mg/l | nr | nr | 1160 | 1170 | 1170 |

nr = not reported

Table A1-1. Dry Valley Monitoring Well GW-7D (continued)

| | | | |
|---|---|---|---|
| | **Station ID** | GW-7D | GW-7D |
| | **Sample Date** | 9/23/2008 | 10/12/2009 |
| | **Field Sample ID** | GW-7D | GW-7D |
| | **Lab Sample ID** | L70172-05 | L78778-02 |
| **Parameter** | **Result Units** | | |
| Alkalinity (as CaCO3) | mg/l | 262 | 271 |
| Bicarbonate (as CaCO3) | mg/l | 262 | 271 |
| Hardness (as CaCO3) | mg/l | 899 | 959 |
| Calcium, dissolved | mg/l | 261 | 282 |
| Calcium, total | mg/l | nr | nr |
| Magnesium, dissolved | mg/l | 59.9 | 61.8 |
| Magnesium, total | mg/l | nr | nr |
| Sodium, dissolved | mg/l | 15 | 15.3 |
| Sodium, total | mg/l | nr | nr |
| Potassium, dissolved | mg/l | 1.1 | 1.1 |
| Potassium, total | mg/l | nr | nr |
| Chloride | mg/l | 9 | 8 |
| Fluoride | mg/l | 0.3 | 0.3 |
| Sulfate | mg/l | 570 | 680 |
| Phosphorus, total as P | mg/l | 0.2 | 0.23 |
| Nitrate as N, dissolved | mg/l | 0.04 | 0.04 |
| Nitrite as N, dissolved | mg/l | <0.01 | <0.01 |
| Nitrate/Nitrite as N, dissolved | mg/l | 0.04 | 0.04 |
| Nitrogen, ammonia | mg/l | <0.05 | <0.05 |
| Total Dissolved Solids | mg/l | 1250 | 1350 |
| Total Suspended Solids | mg/l | <0.05 | <5 |
| Iron, total | mg/l | 0.07 | <0.02 |
| Manganese, total | mg/l | 0.416 | 0.48 |
| Selenium, dissolved | mg/l | 0.0292 | 0.0417 |
| Selenium, total | mg/l | 0.0255 | 0.0382 |
| Zinc, dissolved | mg/l | nr | nr |
| Zinc, total | mg/l | 1.02 | 1.32 |
| Temperature, Field | deg C | 8.24 | 7.98 |
| E.C., Field, 25C | umhos/cm | 1454 | nr |
| pH, Field | s.u. | 6.77 | 6.62 |
| Dissolved oxygen | mg/l | 0.35 | 0.29 |
| ORP, Field | millivolts | nr | 122.8 |
| Depth to Water | ft | nr | 108.15 |
| Sum of Anions | meq/l | 17.5 | 19.9 |
| Sum of Cations | meq/l | 18.7 | 19.9 |
| Cation-Anion Balance | % | 3.3 | nr |
| TDS (calculated) | mg/l | 1070 | 1210 |

nr = not reported

Table A1-2. Dry Valley Monitoring Well GW7D-2A/2B

| | | | | | | |
|---|---|---|---|---|---|---|
| | **Station ID** | GW-7D-2A | GW-7D-2A | GW-7D-2A | GW-7D-2A | GW-7D-2A |
| | **Sample Date** | 6/15/2005 | 10/14/2005 | 3/3/2006 | 5/25/2006 | 10/25/2006 |
| | **Field Sample ID** | GW-7D-2A | GW-7D-2A | GW-7D-2A | GW-7D-2A | GW-7D-2A |
| | **Lab Sample ID** | L51895-03 | L53888-06 | L55672-02 | L56910-01 | L60021-09 |
| **Parameter** | **Result Units** | | | | | |
| pH, Lab | s.u. | nr | nr | 7.7 | 7.8 | nr |
| E.C., Lab | umhos/cm | nr | nr | 1580 | 1480 | nr |
| Alkalinity (as CaCO3) | mg/l | 225 | 231 | 249 | 230 | 232 |
| Bicarbonate (as CaCO3) | mg/l | 225 | 231 | 249 | 230 | 232 |
| Carbonate as CaCO3 | mg/l | <2 | <2 | <2 | <2 | <2 |
| Hydroxide (as CaCO3) | mg/l | <2 | <2 | <2 | <2 | <2 |
| Hardness (as CaCO3) | mg/l | 1040 | 920 | 969 | 947 | 936 |
| Calcium, dissolved | mg/l | 302 | 266 | 279 | 272 | 265 |
| Calcium, total | mg/l | nr | nr | nr | nr | nr |
| Magnesium, dissolved | mg/l | 68.8 | 62 | 66 | 65 | 66.5 |
| Magnesium, total | mg/l | nr | nr | nr | nr | nr |
| Sodium, dissolved | mg/l | 14.5 | 13.4 | 14.5 | 13.7 | 13.6 |
| Sodium, total | mg/l | nr | nr | nr | nr | nr |
| Potassium, dissolved | mg/l | 1.3 | 1.5 | 1.5 | 1.4 | 1.7 |
| Potassium, total | mg/l | nr | nr | nr | nr | nr |
| Chloride | mg/l | 5 | 7 | 9 | 7 | 7 |
| Fluoride | mg/l | 0.4 | 0.2 | 0.3 | 0.2 | 0.4 |
| Sulfate | mg/l | 740 | 690 | 680 | 720 | 690 |
| Sulfide as S, dissolved | mg/l | nr | nr | <0.02 | <0.02 | nr |
| Phosphorus, total as P | mg/l | 0.47 | 0.76 | 0.47 | 0.55 | 0.55 |
| Nitrate as N, dissolved | mg/l | 0.11 | 0.2 | nr | nr | 0.21 |
| Nitrite as N, dissolved | mg/l | <0.01 | <0.01 | nr | nr | <0.01 |
| Nitrate/Nitrite as N, dissolved | mg/l | 0.11 | 0.2 | 0.39 | 0.14 | 0.21 |
| Nitrogen, ammonia | mg/l | <0.05 | <0.05 | <0.05 | <0.05 | 0.12 |
| Total Dissolved Solids | mg/l | 1360 | 1240 | 1250 | 1330 | 1240 |
| Total Suspended Solids | mg/l | <5 | 8 | <5 | <5 | <5 |
| Iron, dissolved | mg/l | nr | nr | 0.07 | 0.1 | nr |
| Iron, total | mg/l | 0.12 | 0.29 | 0.17 | 0.11 | 0.16 |
| Manganese, dissolved | mg/l | nr | nr | 0.421 | 0.438 | nr |
| Manganese, total | mg/l | 0.445 | 0.394 | 0.41 | 0.448 | 0.468 |
| Selenium, dissolved | mg/l | 0.026 | 0.013 | 0.013 | 0.0167 | 0.0102 |
| Selenium, total | mg/l | 0.023 | 0.009 | 0.0114 | 0.018 | 0.0087 |
| Zinc, dissolved | mg/l | nr | nr | nr | nr | nr |
| Zinc, total | mg/l | 0.34 | 0.28 | 0.29 | 0.28 | 0.27 |
| Sum of Anions | meq/l | 20.2 | 19.3 | 19.5 | 19.9 | 19.4 |
| Sum of Cations | meq/l | 21.4 | 19 | 20.1 | 19.6 | 19.4 |
| Cation-Anion Balance | % | 2.9 | -0.8 | 1.5 | -0.8 | nr |
| TDS (calculated) | mg/l | 1270 | 1180 | nr | nr | 1180 |

nr = not reported

Table A1-2. Dry Valley Monitoring Well GW7D-2A/2B, continued

| | | | | | | |
|---|---|---|---|---|---|---|
| **Station ID** | | GW-7D-2A | GW-7D-2A | GW-7D-2B | GW-7D-2B | GW-7D-2B |
| **Sample Date** | | 6/14/2007 | 10/4/2007 | 6/15/2005 | 10/14/2005 | 3/3/2006 |
| **Field Sample ID** | | GW-7D-2A | GW-7D-2A | GW-7D-2B | GW-7D-2B | GW-7D-2B |
| **Lab Sample ID** | | L63345-02 | L65750-01 | L51895-05 | L53888-07 | L55672-03 |
| **Parameter** | **Result Units** | | | | | |
| pH, Lab | s.u. | nr | nr | nr | nr | 7.8 |
| E.C., Lab | umhos/cm | nr | nr | nr | nr | 1570 |
| Alkalinity (as CaCO3) | mg/l | 236 | 241 | 88 | 126 | 143 |
| Bicarbonate (as CaCO3) | mg/l | 236 | 241 | 82 | 126 | 143 |
| Carbonate as CaCO3 | mg/l | <2 | <2 | 6 | <2 | <2 |
| Hydroxide (as CaCO3) | mg/l | <2 | <2 | <2 | <2 | <2 |
| Hardness (as CaCO3) | mg/l | 915 | 975 | 1160 | 963 | 905 |
| Calcium, dissolved | mg/l | 264 | 283 | 344 | 283 | 264 |
| Calcium, total | mg/l | nr | nr | nr | nr | nr |
| Magnesium, dissolved | mg/l | 62 | 65.1 | 73.6 | 62.1 | 59.5 |
| Magnesium, total | mg/l | nr | nr | nr | nr | nr |
| Sodium, dissolved | mg/l | 13.3 | 13.4 | 15.5 | 14.2 | 13.9 |
| Sodium, total | mg/l | nr | nr | nr | nr | nr |
| Potassium, dissolved | mg/l | 1.6 | 1.3 | 1.3 | 1.5 | 1.3 |
| Potassium, total | mg/l | nr | nr | nr | nr | nr |
| Chloride | mg/l | 6 | 7 | 5 | 7 | 8 |
| Fluoride | mg/l | 0.4 | 0.4 | 1.8 | 1.6 | 1.3 |
| Sulfate | mg/l | 710 | 710 | 940 | 830 | 770 |
| Sulfide as S, dissolved | mg/l | nr | nr | nr | nr | 0.02 |
| Phosphorus, total as P | mg/l | 0.51 | 0.54 | 1.36 | 1.25 | 1.51 |
| Nitrate as N, dissolved | mg/l | 0.3 | 0.32 | <0.02 | <0.02 | nr |
| Nitrite as N, dissolved | mg/l | <0.01 | <0.01 | <0.01 | <0.01 | nr |
| Nitrate/Nitrite as N, dissolved | mg/l | 0.3 | 0.32 | <0.02 | <0.02 | 0.04 |
| Nitrogen, ammonia | mg/l | <0.05 | <0.05 | <0.05 | <0.05 | 0.07 |
| Total Dissolved Solids | mg/l | 1280 | 1300 | 1550 | 1360 | 1310 |
| Total Suspended Solids | mg/l | <5 | <5 | 16 | 14 | 12 |
| Iron, dissolved | mg/l | nr | nr | nr | nr | 7.23 |
| Iron, total | mg/l | 0.22 | 0.21 | 10.1 | 8.72 | 37.9 |
| Manganese, dissolved | mg/l | nr | nr | nr | nr | 1.04 |
| Manganese, total | mg/l | 0.47 | 0.462 | 1.44 | 1.26 | 5.27 |
| Selenium, dissolved | mg/l | 0.0213 | 0.0144 | 0.001 | 0.001 | <0.001 |
| Selenium, total | mg/l | 0.0218 | 0.0163 | <0.001 | <0.001 | 0.0003 |
| Zinc, dissolved | mg/l | nr | nr | nr | nr | nr |
| Zinc, total | mg/l | 0.28 | 0.3 | 4.29 | 3.2 | 10.9 |
| Sum of Anions | meq/l | 19.8 | 20 | 21.7 | 20.2 | 19.3 |
| Sum of Cations | meq/l | 18.9 | 20.1 | 24 | 19.9 | 19.2 |
| Cation-Anion Balance | % | -2.3 | 0.2 | 5 | -0.7 | -0.3 |
| TDS (calculated) | mg/l | 1200 | 1230 | 1440 | 1270 | nr |

nr = not reported

| | | | | |
|---|---|---|---|---|
| | **Station ID** | GW-7D-2B | GW-7D-2B | GW-7D-2B |
| | **Sample Date** | 5/25/2006 | 6/12/2007 | 10/4/2007 |
| | **Field Sample ID** | GW-7D-2B | GW-7D-2B | GW-7D-2B |
| | **Lab Sample ID** | L56906-08 | L63345-04 | L65750-02 |
| **Parameter** | **Result Units** | | | |
| pH, Lab | s.u. | 7.5 | nr | nr |
| E.C., Lab | umhos/cm | 1570 | nr | nr |
| Alkalinity (as CaCO3) | mg/l | 159 | 160 | 158 |
| Bicarbonate (as CaCO3) | mg/l | 159 | 160 | 158 |
| Carbonate as CaCO3 | mg/l | <2 | <2 | <2 |
| Hydroxide (as CaCO3) | mg/l | <2 | <2 | <2 |
| Hardness (as CaCO3) | mg/l | 993 | 889 | 954 |
| Calcium, dissolved | mg/l | 287 | 259 | 280 |
| Calcium, total | mg/l | nr | nr | nr |
| Magnesium, dissolved | mg/l | 67.1 | 58.8 | 61.8 |
| Magnesium, total | mg/l | nr | nr | nr |
| Sodium, dissolved | mg/l | 14.9 | 13.3 | 13.6 |
| Sodium, total | mg/l | nr | nr | nr |
| Potassium, dissolved | mg/l | 1.6 | 1.2 | 1.4 |
| Potassium, total | mg/l | nr | nr | nr |
| Chloride | mg/l | 8 | 8 | 8 |
| Fluoride | mg/l | 1.2 | 1.1 | 1.1 |
| Sulfate | mg/l | 850 | 790 | 790 |
| Sulfide as S, dissolved | mg/l | <0.02 | nr | nr |
| Phosphorus, total as P | mg/l | 1.38 | 1.32 | 1.6 |
| Nitrate as N, dissolved | mg/l | nr | 0.03 | <0.02 |
| Nitrite as N, dissolved | mg/l | nr | <0.01 | <0.01 |
| Nitrate/Nitrite as N, dissolved | mg/l | 0.02 | 0.03 | <0.02 |
| Nitrogen, ammonia | mg/l | <0.05 | <0.05 | <0.05 |
| Total Dissolved Solids | mg/l | 1460 | 1330 | 1340 |
| Total Suspended Solids | mg/l | 16 | <5 | <5 |
| Iron, dissolved | mg/l | 7.26 | nr | nr |
| Iron, total | mg/l | 7.33 | 6.29 | 7.27 |
| Manganese, dissolved | mg/l | 1.06 | nr | nr |
| Manganese, total | mg/l | 1.09 | 0.967 | 1.06 |
| Selenium, dissolved | mg/l | 0.0002 | 0.0001 | <0.0001 |
| Selenium, total | mg/l | 0.0007 | 0.0002 | 0.0001 |
| Zinc, dissolved | mg/l | nr | nr | nr |
| Zinc, total | mg/l | 1.98 | 1.51 | 1.58 |
| Sum of Anions | meq/l | 21.3 | 20.1 | 20 |
| Sum of Cations | meq/l | 21 | 18.4 | 19.7 |
| Cation-Anion Balance | % | -0.7 | -4.4 | -0.8 |
| TDS (calculated) | mg/l | nr | 1230 | 1250 |

nr = not reported

Table A1-3. Smoky Canyon Groundwater Monitoring Well GW11

| | Panel A pit backfill well | 10/30/2003 | IDL | FLAG | 3/24/2005 |
|---|---|---|---|---|---|
| Alkalinity | mg/L | 198 | 1 | | |
| Bicarbonate as CaCO3 | mg/L | 198 | 1 | | |
| Calcium, Dissolved | mg/L | 618 | 0.0083 | | |
| Carbonate as CaCO3 | mg/L | 1 | 1 | | |
| Cation-Anion Balance | % | -0.31 | 0.01 | B | |
| Chloride | mg/L | 109 | 5 | | |
| Iron, Dissolved | mg/L | 0.0045 | 0.0045 | | |
| Magnesium, Dissolved | mg/L | 118 | 0.005 | | |
| Manganese, Dissolved | mg/L | 0.0108 | 0.0007 | | |
| Nitrate + Nitrite (as N) | mg/L | 0.16 | 0.02 | J | |
| Phosphorus, Total | mg/L | 38.1 | 0.01 | | |
| Potassium, Dissolved | mg/L | 2.2 | 0.025 | J | |
| Selenium, Dissolved | mg/L | 1.01 | 0.02 | | |
| Selenium, Total | mg/L | 0.421 | 0.02 | | |
| Sodium, Dissolved | mg/L | 16.6 | 0.0054 | | |
| Sulfate (as SO4) | mg/L | 1666 | 30 | | |
| Sum of Anions | meq/L | 41.73 | 0.01 | | |
| Sum of Cations | meq/L | 41.47 | 0.01 | | |
| TDS | mg/L | 2470 | 10 | UJ | |
| Zinc, Dissolved | mg/L | 3.85 | 0.0018 | UJ | |
| Conductivity at 25° C | µmhos/cm | 1691 | | J | 3160 |
| DTW | Feet | 100.25 | | UJ | |
| DTW | Feet | 105.6 | | | 103 |
| Iron, Ferrous | mg/L | 3.3 | | J | |
| Iron, Total | mg/L | 3.3 | | | |
| ORP | mV | 157.1 | | J | |
| Oxygen, Dissolved | mg/L | 5.57 | | J | 5 |
| pH | SU | 6.5 | | | 5.95 |
| Temperature | C | 6.9 | | | 4 |
| Turbidity | NTU | 1000 | | | |

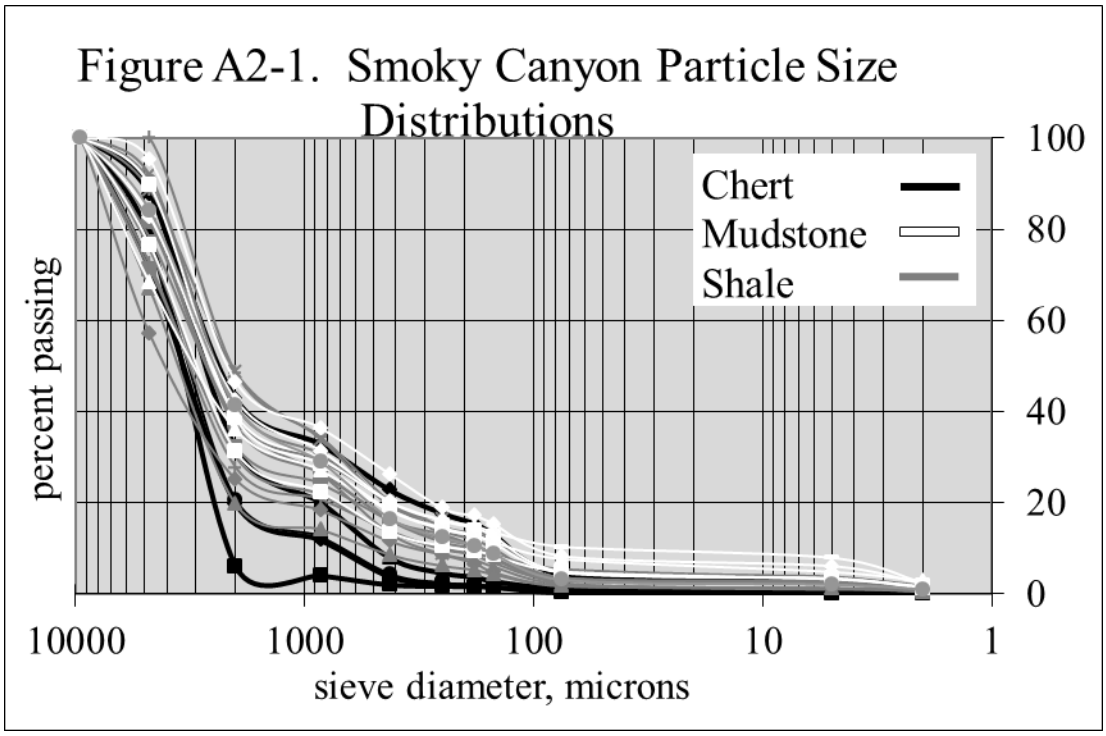



Figure A2-1. Smoky Canyon Particle Size Distributions

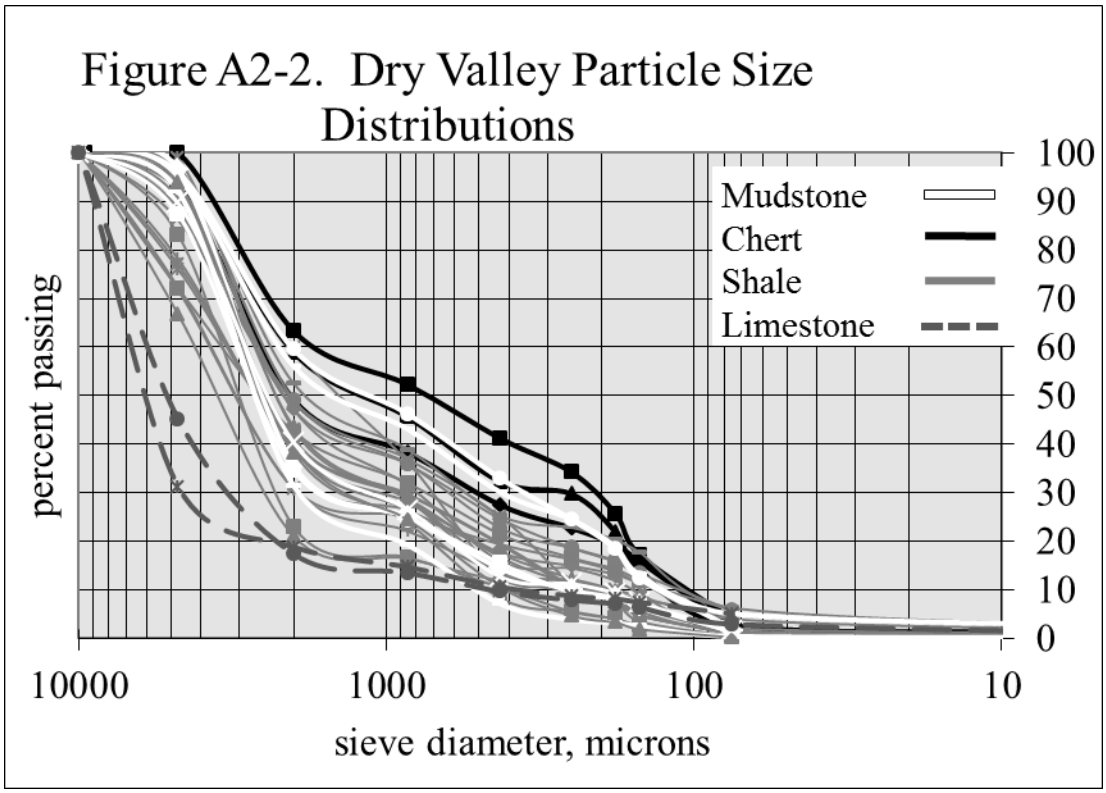



Figure A2-2. Dry Valley Particle Size Distributions

Table A2-1. Dry Valley GW7D2 Drillcore Geochemical Data

| Identification | Lith-ology[1] | Clay | Sand | Tex-ture[2] | Organic Carbon | Total Sulfur | Acid/Base Potential | pH | Cadmium | Manganese | Selenium | Cadmium | Manganese | Selenium |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Saturated Paste Extraction | | | | Whole Rock Digestion | | |
| | | Wt % | Wt % | | Wt % | Wt % | t/kt | s.u. | mk/kg[3] | mk/kg[3] | mk/kg[3] | mk/kg | mk/kg | mk/kg |
| GW-7D-2-1 | Chert | 16 | 50 | L | 0.37 | 0.20 | 417 | 7.7 | < 0.1 | < 0.01 | 0.03 | 71.8 | 170 | 12.8 |
| GW-7D-2-2 | HWM | 23 | 44 | LS | 0.51 | 0.17 | 75 | 7.2 | < 0.1 | 0.1 | 0.02 | 59.9 | 465 | 11.4 |
| GW-7D-2-3 | CWS | 6 | 78 | SCL | 2.01 | 0.52 | 206 | 7.4 | < 0.1 | 0.3 | 0.19 | 8.82 | 110 | 34.8 |
| GW-7D-2-4 | HWM | 11 | 76 | L | 0.86 | 0.39 | 37 | 7.2 | < 0.1 | 1.5 | 0.13 | 6.22 | 180 | 26.8 |
| GW-7D-2-5 | Chert | 7 | 80 | SL | 0.58 | 0.16 | 18 | 6.9 | < 0.1 | 2.0 | 0.06 | 2.86 | 130 | 9.6 |
| GW-7D-2-6 | CWS | 11 | 72 | L | 0.96 | 0.34 | 77 | 7.3 | < 0.1 | 0.8 | 0.06 | 25.2 | 400 | 23.4 |
| GW-7D-2-7 | CWSr | 13 | 64 | SCL | 8.25 | 0.90 | 81 | 6.8 | < 0.1 | < 0.01 | 1.71 | 90.2 | 45 | 98.4 |
| GW-7D-2-8B | CWSr | 11 | 62 | SL | 7.26 | 1.49 | 50 | 6.4 | < 0.1 | 0.8 | 2.70 | 27.6 | 95 | 125.5 |
| GW-7D-2-8G | CWS | 15 | 48 | SL | 2.76 | 0.81 | 56 | 6.3 | < 0.1 | 0.2 | 0.43 | 25.2 | 145 | 40.6 |
| GW-7D-2-8R | CWSox | 15 | 58 | SL | 0.61 | 0.16 | 55 | 7.0 | < 0.1 | 0.2 | 0.01 | 21.9 | 150 | 14.0 |
| GW-7D-2-9 | CWS | 9 | 70 | SL | 2.14 | 0.57 | 407 | 7.7 | < 0.1 | 0.2 | 0.14 | 51.4 | 275 | 44.4 |
| GW-7D-2-10 | FWM | 25 | 48 | L | 0.30 | 0.10 | 275 | 7.6 | < 0.1 | 0.1 | < 0.01 | 25.7 | 180 | 14.0 |
| GW-7D-2-11 | LST | 19 | 51 | SL | 0.36 | 0.11 | 388 | 7.8 | < 0.1 | < 0.01 | < 0.01 | 44.0 | 190 | 9.8 |
| GW-7D-2-12 | Chert | 23 | 52 | L | 0.16 | 0.05 | 91 | 7.6 | < 0.1 | < 0.01 | < 0.01 | 31.0 | 485 | 16.6 |

[1] Key to lithologic descriptions: Chert = Rex chert, HWM = hanging wall mud, CWS = center waste shale, CWSr = reduced center waste shale, CWSox = oxidized center waste shale, FWM = footwall mud, LST = Wells/Grandeur limestone.

[2] C = Clay, L = Loam(y), S = Sand(y)

[3] Concentrations expressed as mass of analyte per mass of solid extracted in saturated paste. Mg/kg = milligrams per kilogram.

[4] ALS Chemex method MEMS41, aqua-regia like digestion.

Table A2-2. 2005 Overburden Sample Sieve Analyses.

| | | | | | gravel | | sand | | | | | | fines | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SUB 1/2 inch | | | µM | 12700 | 10000 | 4750 | 2000 | 850 | 425 | 250 | 180 | 150 | 75 | 5 | 2 | | | |
| | | | | 1/2 inch | % pass 0.375in | %pass 4 | %pass 10 | %pass 20 | %pass 40 | %pass 60 | %pass 80 | %pass 100 | %pass 200 | silt | clay | gravel | sand | silt/ clay |
| | SCHT1 | 5 | 14 | 100 | 100 | 87.9 | 42.8 | 32.8 | 22.8 | 17.6 | 15.3 | 13.1 | 4.3 | 3.0 | 1.3 | 57 | 38 | 4 |
| | SCHT2 | 5 | 14 | 100 | 100 | 78.1 | 6.0 | 4.0 | 2.0 | 1.7 | 1.6 | 1.5 | 0.2 | 0.1 | 0.1 | 94 | 6 | 0 |
| | SCHT3 | 5 | 1 | 100 | 100 | 88.2 | 32.0 | 20.0 | 7.9 | 4.6 | 3.6 | 2.7 | 1.2 | 0.6 | 0.6 | 68 | 31 | 1 |
| | SCHT4 | 5 | 2 | 100 | 100 | 71.4 | 19.8 | 11.8 | 3.8 | 2.2 | 1.8 | 1.4 | 0.8 | 0.4 | 0.4 | 80 | 19 | 1 |
| | SCHT5 | 5 | 3 | 100 | 100 | 76.5 | 20.7 | 12.6 | 4.4 | 2.5 | 2.0 | 1.5 | 0.8 | 0.4 | 0.4 | 79 | 20 | 1 |
| **AVG** | **SCHT** | | | **100** | **100** | **80.4** | **24.3** | **16.2** | **8.2** | **5.7** | **4.9** | **4.0** | **3.2** | **0.9** | **0.6** | **76** | **21** | **3** |
| | SCWS1 | 5 | 16 | 100 | 100 | 90.5 | 41.2 | 30.6 | 20.0 | 13.9 | 11.9 | 10.0 | 3.6 | 2.5 | 1.1 | 59 | 38 | 4 |
| | SCWS2 | 5 | 1 | 100 | 100 | 100.0 | 48.5 | 34.4 | 20.3 | 13.0 | 10.6 | 8.1 | 2.3 | 1.0 | 1.4 | 51 | 46 | 2 |
| | SCWS3 | 5 | 23 | 100 | 100 | 75.6 | 30.1 | 21.8 | 13.5 | 10.1 | 8.5 | 6.8 | 2.9 | 2.1 | 0.8 | 70 | 27 | 3 |
| | SCWS4 | 5 | 15 | 100 | 100 | 82.0 | 37.0 | 26.6 | 16.3 | 13.1 | 10.3 | 7.6 | 3.2 | 2.2 | 1.0 | 63 | 34 | 3 |
| | SCWS5 | 5 | 21 | 100 | 100 | 57.0 | 25.1 | 18.3 | 11.5 | 8.6 | 6.6 | 4.7 | 1.8 | 1.3 | 0.5 | 75 | 23 | 2 |
| | SCWS6 | 5 | 3 | 100 | 100 | 71.2 | 32.2 | 24.2 | 16.1 | 12.1 | 10.5 | 9.0 | 3.5 | 2.8 | 0.7 | 68 | 29 | 4 |
| | SCWS7 | 5 | 2 | 100 | 100 | 66.9 | 19.7 | 14.1 | 8.5 | 6.1 | 5.2 | 4.3 | 1.7 | 1.2 | 0.5 | 80 | 18 | 2 |
| | SCWS8 | 5 | 17 | 100 | 100 | 91.9 | 39.5 | 26.4 | 13.2 | 8.8 | 7.4 | 6.0 | 2.1 | 1.2 | 0.9 | 61 | 37 | 2 |
| | SCWS9 | 5 | 21 | 100 | 100 | 88.7 | 48.6 | 33.9 | 19.3 | 16.3 | 14.2 | 12.0 | 4.9 | 3.8 | 1.1 | 51 | 44 | 5 |
| | SCWS10 | 5 | 4 | 100 | 100 | 81.5 | 36.7 | 26.4 | 16.2 | 11.3 | 10.0 | 8.6 | 5.4 | 3.5 | 1.9 | 63 | 31 | 5 |
| | SCWS11 | 5 | 5 | 100 | 100 | 72.5 | 27.8 | 20.8 | 13.8 | 9.8 | 8.8 | 7.8 | 3.2 | 2.3 | 0.9 | 72 | 25 | 3 |
| | SCWS12 | 5 | 22 | 100 | 100 | 78.2 | 38.2 | 29.2 | 20.3 | 14.8 | 13.2 | 11.7 | 4.9 | 3.4 | 1.5 | 62 | 33 | 5 |
| **AVG** | **SCWS** | | | **100** | **100** | **79.7** | **35.4** | **25.6** | **15.7** | **11.5** | **9.8** | **8.1** | **3.3** | **2.3** | **1.0** | **65** | **32** | **3** |
| | SHWM1 | 5 | 19 | 100 | 100 | 83.5 | 39.2 | 29.7 | 20.2 | 16.2 | 14.9 | 13.6 | 10.3 | 7.9 | 2.4 | 61 | 29 | 10 |
| | SHWM2 | 5 | 9 | 100 | 100 | 95.3 | 46.5 | 36.4 | 26.3 | 18.8 | 17.1 | 15.4 | 8.3 | 6.1 | 2.2 | 53 | 38 | 8 |
| | SHWM3 | 5 | 3 | 100 | 100 | 89.7 | 38.5 | 28.5 | 18.5 | 14.5 | 12.9 | 11.3 | 4.6 | 3.4 | 1.3 | 62 | 34 | 5 |
| | SHWM4 | 5 | 18 | 100 | 100 | 68.3 | 36.0 | 27.1 | 18.2 | 14.2 | 12.8 | 11.4 | 7.6 | 4.8 | 2.8 | 64 | 28 | 8 |
| | SHWM5 | 5 | 13 | 100 | 100 | 76.6 | 31.2 | 22.4 | 13.5 | 10.5 | 9.5 | 8.4 | 4.9 | 3.2 | 1.6 | 69 | 26 | 5 |
| | SHWM6 | 5 | 12 | 100 | 100 | 82.7 | 42.6 | 31.4 | 20.2 | 16.0 | 14.4 | 12.8 | 4.8 | 3.4 | 1.5 | 57 | 38 | 5 |
| | SHWM7 | 5 | 11 | 100 | 100 | 84.1 | 41.3 | 28.9 | 16.5 | 12.3 | 10.6 | 8.8 | 3.1 | 2.0 | 1.0 | 59 | 38 | 3 |
| **AVG** | **SHWM** | | | **100** | **100** | **82.9** | **39.3** | **29.2** | **19.1** | **14.6** | **13.2** | **11.7** | **6.2** | **4.4** | **1.8** | **61** | **33** | **6** |
| | DCHT1 | 2.5 | 5 | 100 | 100 | 93.2 | 49.1 | 38.2 | 27.4 | 22.7 | 19.3 | 15.8 | 2.1 | | | 51 | 47 | 2 |
| | DCHT2 | 150 | 153 | 100 | 100 | 100.0 | 63.3 | 52.2 | 41.1 | 34.2 | 25.6 | 17.1 | 3.9 | | | 37 | 59 | 4 |

Table A2-2. 2005 Overburden Sample Sieve Analyses, continued

| | | | | | gravel | | sand | | | | | | fines | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SUB 1/2 inch | | | µM | 12700 | 10000 | 4750 | 2000 | 850 | 425 | 250 | 180 | 150 | 75 | 5 | 2 | | | |
| | | | | 1/2 inch | % pass 0.375in | %pass 4 | %pass 10 | %pass 20 | %pass 40 | %pass 60 | %pass 80 | %pass 100 | %pass 200 | silt | clay | gravel | sand | silt/ clay |
| | DCHT3 | 157 | 160 | 100 | 100 | 95.4 | 58.9 | 45.5 | 32.2 | 29.7 | 21.8 | 14.0 | 2.7 | 1.4 | 1.2 | 41 | 56 | 3 |
| **AVG** | **DCHT** | | | **100** | **100** | **96.2** | **57.1** | **45.3** | **33.6** | **28.9** | **22.2** | **15.6** | **2.9** | | | **43** | **54** | **3** |
| | DCWS0 | 36 | 38 | 100 | 100 | 93.8 | 38.3 | 24.4 | 10.5 | 4.7 | 3.3 | 1.9 | 0.2 | | | 62 | 38 | 0 |
| | DCWS1 | 11 | 13 | 100 | 100 | 92.6 | 59.7 | 36.2 | 12.7 | 11.4 | 10.0 | 8.6 | 4.7 | 1.8 | 2.9 | 40 | 55 | 5 |
| | DCWS2 | 13 | 16 | 100 | 100 | 98.9 | 43.0 | 28.7 | 14.3 | 10.8 | 8.9 | 7.0 | 1.8 | | | 57 | 41 | 2 |
| | DCWS3 | 41 | 43 | 100 | 100 | 92.1 | 39.2 | 23.7 | 8.2 | 5.6 | 3.9 | 2.3 | 0.4 | 0.1 | 0.3 | 61 | 39 | 0 |
| | DCWS4 | 43 | 46 | 100 | 100 | 71.7 | 30.7 | 22.1 | 13.5 | 11.8 | 9.2 | 6.6 | 2.1 | 0.9 | 1.2 | 69 | 29 | 2 |
| | DCWS5 | 46 | 48 | 100 | 100 | 88.5 | 40.9 | 28.8 | 16.7 | 11.2 | 8.7 | 6.2 | 1.4 | | | 59 | 40 | 1 |
| | DCWS6 | 48 | 51 | 100 | 100 | 90.5 | 39.2 | 26.3 | 13.3 | 10.5 | 8.0 | 5.5 | 1.5 | 0.6 | 0.9 | 61 | 38 | 2 |
| | DCWS7 | 56 | 58 | 100 | 100 | 93.6 | 35.1 | 26.3 | 17.6 | 14.9 | 13.2 | 11.4 | 5.8 | | | 65 | 29 | 6 |
| | DCWS8 | 60 | 68 | 100 | 100 | 83.1 | 22.9 | 16.7 | 10.4 | 5.5 | 4.1 | 2.7 | 0.6 | | | 77 | 22 | 1 |
| | DCWS9 | 67 | 69 | 100 | 100 | 95.6 | 43.5 | 28.4 | 13.3 | 12.1 | 9.8 | 7.4 | 1.1 | | | 56 | 42 | 1 |
| | DCWS10 | 76 | 79 | 100 | 100 | 77.0 | 39.9 | 29.7 | 19.5 | 10.8 | 7.8 | 4.7 | 0.9 | | | 60 | 39 | 1 |
| | DCWS11 | 76 | 79 | 100 | 100 | 76.1 | 39.8 | 29.5 | 19.3 | 10.3 | 7.4 | 4.6 | 1.2 | | | 60 | 39 | 1 |
| | DCWS12 | 81 | 83.5 | 100 | 100 | 96.4 | 49.1 | 35.9 | 22.7 | 18.6 | 16.0 | 13.3 | 5.6 | | | 51 | 43 | 6 |
| | DCWS13 | 96 | 98.5 | 100 | 100 | 78.1 | 38.5 | 28.0 | 17.4 | 12.2 | 8.5 | 4.8 | 0.9 | | | 61 | 38 | 1 |
| | DCWS14 | 104 | 106 | 100 | 100 | 93.4 | 47.1 | 31.4 | 15.8 | 14.4 | 11.9 | 9.3 | 1.3 | 0.6 | 0.7 | 53 | 46 | 1 |
| | DCWS15 | 111 | 113 | 100 | 100 | 89.7 | 47.1 | 35.8 | 24.6 | 18.7 | 15.8 | 12.9 | 1.9 | | | 53 | 45 | 2 |
| | DCWS16 | 126 | 128 | 100 | 100 | 97.1 | 52.5 | 38.8 | 25.2 | 22.8 | 20.3 | 17.7 | 6.3 | 2.2 | 4.1 | 48 | 46 | 6 |
| | DCWS17 | 131 | 133 | 100 | 100 | 72.1 | 42.5 | 32.0 | 21.4 | 16.6 | 14.3 | 11.9 | 4.0 | 1.6 | 2.4 | 57 | 39 | 4 |
| | DCWS18 | 141 | 143 | 100 | 100 | 66.7 | 21.0 | 16.4 | 11.8 | 9.8 | 8.9 | 8.1 | 4.7 | | | 79 | 16 | 5 |
| | DCWS19 | 143 | 146 | 100 | 100 | 86.2 | 48.8 | 36.9 | 25.0 | 11.3 | 7.4 | 3.5 | 0.3 | | | 51 | 48 | 0 |
| | DCWS20 | 172 | 174 | 100 | 100 | 72.3 | 30.9 | 25.1 | 19.3 | 16.2 | 13.8 | 11.3 | 3.9 | | | 69 | 27 | 4 |
| **AVG** | **DCWS** | | | **100** | **100** | **85.6** | **40.6** | **28.8** | **17.1** | **12.8** | **10.4** | **8.0** | **2.5** | | | **59** | **38** | **3** |
| | DHWM10 | 162 | 167 | 100 | 100 | 96.0 | 59.5 | 46.2 | 32.9 | 24.5 | 18.4 | 12.3 | 2.1 | | | 40 | 57 | 2 |
| | DFWM2 | 167 | 170 | 100 | 100 | 95.5 | 63.3 | 46.9 | 30.5 | 27.4 | 20.1 | 12.8 | 1.6 | 0.7 | 0.9 | 37 | 62 | 2 |
| | DHWM30 | 176 | 178.5 | 100 | 100 | 95.0 | 56.0 | 43.0 | 30.1 | 24.5 | 18.8 | 13.0 | 5.0 | 2.3 | 2.7 | 44 | 51 | 5 |
| | DHWM | 7.5 | 10 | 100 | 100 | 92.2 | 31.4 | 19.2 | 7.0 | 3.9 | 3.2 | 2.5 | 0.4 | 0.2 | 0.2 | 69 | 31 | 0 |
| | DHWM | 21 | 23 | 100 | 100 | 96.7 | 35.6 | 25.0 | 14.4 | 9.9 | 8.3 | 6.8 | 1.4 | | | 64 | 34 | 1 |
| | DHWM | 83.5 | 86 | 100 | 100 | 87.3 | 34.6 | 25.0 | 15.4 | 9.5 | 8.1 | 6.8 | 1.3 | | | 65 | 33 | 1 |

Table A2-2. 2005 Overburden Sample Sieve Analyses, continued

| | | | | | gravel | | sand | | | | | | fines | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SUB 1/2 inch | | | µM | 12700 | 10000 | 4750 | 2000 | 850 | 425 | 250 | 180 | 150 | 75 | 5 | 2 | | | |
| | | | | 1/2 inch | % pass 0.375in | %pass 4 | %pass 10 | %pass 20 | %pass 40 | %pass 60 | %pass 80 | %pass 100 | %pass 200 | silt | clay | gravel | sand | silt/ clay |
| | DHWM/CHT | 26 | 28 | 100 | 100 | 89.6 | 40.3 | 26.5 | 12.6 | 11.2 | 9.3 | 7.3 | 1.5 | | | 60 | 39 | 1 |
| | **DMUD** | | | **100** | **100** | **93.2** | **45.8** | **33.1** | **20.4** | **15.8** | **12.3** | **8.8** | **1.9** | | | **54** | **44** | **2** |
| | DLST | 169 | 172 | 100 | 100 | 31.2 | 18.8 | 14.7 | 10.5 | 8.8 | 8.1 | 7.4 | 5.0 | | | 81 | 14 | 5 |
| | DLST | 174 | 176 | 100 | 100 | 45.1 | 17.3 | 13.5 | 9.6 | 7.9 | 7.1 | 6.4 | 2.9 | 1.0 | 1.9 | 83 | 14 | 3 |
| AVG | **DLST** | | | **100** | **100** | **38.2** | **18.1** | **14.1** | **10.1** | **8.3** | **7.6** | **6.9** | **4.0** | | | **82** | **14** | **4** |

Table A3-1. 2006 Microbial Geochemistry Sample Summary.

| | Smoky Canyon panel D backfill | | | | | Smoky Canyon panel A dump | | | | | Monsanto Enoch Valley backfill | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **T in hole is** | | **11.7°C** | **(1 week)** | | **T in hole is** | | **8.6°C** | **(compl)** | | | | | | |
| hole depth | logged lith | DNA samples | T°C sample | interval sampled | | logged lith | DNA samples | T°C sample | interval sampled | | logged lith | DNA samples | T°C sample | interval sampled | |
| 0 | C | cup | nd | 3-5 | C | soil, C | 3,4 | 7.1 | | | S | | nd | | |
| 5 | C | cht1-3 ? | nd | | | S | 7.5 | nd | 5-7 | S | S | | 10 | 5-7 | S |
| 10 | C | | nd | | | S | 10 | 9.1 | | | M | | nd | | |
| 15 | C | | nd | | | S | | nd | | | M | 13,14 | nd | | |
| 20 | C | cht4 ? | nd | | | S | | 11.7 | | | M | 16 | nd | | |
| 25 | C | | nd | | | S | 24 | | | | M | | nd | | |
| 30 | C | | nd | | | S | | 14.5 | | | M | | 25 | 32-35 | M |
| 35 | C | 33 | nd | | | S | | | | | M | 36 | nd | | |
| 40 | C | | nd | | | S | | 14.9 | | | M | 40 | nd | | |
| 45 | C | 49.5 | nd | | | S | 45 | | | | M | | nd | | |
| 50 | M | 52.5 | 14 | 50-54 | M | S | | 17.3 | | | M | 47,50 | nd | | |
| 55 | S | | 12 | | | S | | | | | M | 52,58 | nd | | |
| 60 | S | 61,62,64 | 19 | | | S | | 16.7 | | | S | 61 | nd | | |
| 65 | S | 69 | 16 | | | S | | | | | S | 64 | nd | | |
| 70 | S | 70 | 22 | | | S | | 17 | 71-73 | S | S | | 32 | 73-77 | S |
| 75 | S | 76,79 | 17 | 75-77 | S | S | 74 | | | | S | 75 | nd | | |
| 80 | S | | 24.5 | | | S | | 23.2 | | | S | | nd | | |
| 85 | S | 86 | 24 | | | S | 84 | | | | S | 81 | nd | | |
| 90 | S | 94 | 23.5 | | | S | | 53.6 | | | S | | nd | | |
| 95 | S | | 23.4 | | | S | 93 | | | | S | 96,98 | nd | | |
| 100 | S | | 28.5 | | | S | 97 | 29 | | | S | 102,104, | nd | | |
| 105 | S | 109 | 28.5 | | | S | 101, 104 | | | | S | 108 | nd | | |
| 110 | S | | nd | | | S | 108 | 33.4 | 113-115 | S | S | 110, 113 | nd | | |
| 115 | S | | nd | | | S | 113, 116 | | | | S | 117 | nd | | |
| 120 | C | 122 | 27.5 | 123-125 | C | S | | 28 | | | S | 124, 125 | nd | | |
| 125 | S | | 26.5 | | | C | | | | | S | | nd | | |
| 130 | S | 130 | 28 | | | C | 130, 132 | 40 | 125-127 | C | L | | nd | | |
| 135 | S | 136 | 26.2 | | | C | 136 | 68 | | | S | | nd | | |
| 140 | S | | 82 | 142-143 | S* | C | | 32 | | | S | 143 | nd | | |
| 145 | bedrock | TD | 80 | 143 | | M | 146 | 36 | 145-146 | M | S | 146 | 32 | | |
| 150 | 5,81 | | | | | bedrock | TD | | 147 | | S | | 54 | | |
| 155 | | | | | | | | | | | S | 154 | 35 | | |

Table A3-1. 2006 Microbial Geochemistry Sample Summary, continued

| | Smoky Canyon panel D backfill | | | | Smoky Canyon panel A dump | | | | Monsanto Enoch Valley backfill | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **T in hole is** | | **11.7°C** | **(1 week)** | **T in hole is** | | **8.6°C** | **(compl)** | | | | | |
| hole depth | logged lith | DNA samples | T°C sample | interval sampled | logged lith | DNA samples | T°C sample | interval sampled | logged lith | DNA samples | T°C sample | interval sampled | |
| 160 | | | | | | | | | S | 160 | 38 | | |
| 165 | | | | | | | | | S | | 50 | | |
| 170 | | | | | | | | | S | 173 | 65 | | |
| 175 | | | | | | | | | L | 176 | nd | | |
| 180 | | | | | | | | | S | | 35 | 178-180 | M |
| 185 | | | | | | | | | L | | nd | | |
| 190 | | | | | | | | | S | | 45 | | |
| 195 | | | | | | | | | L | | nd | | |
| 200 | | | | | | | | | S | | 34 | | |
| 200 | | | | | | | | | L | | 54 | | |
| 205 | | | | | | | | | L | | nd | | |
| 210 | | | | | | | | | S | | nd | | |
| 215 | | | | | | | | | L | | nd | | |
| 220 | | | | | | | | | S | | nd | | |
| 225 | | | | | | | | | S | 226 | nd | | |
| 230 | | | | | | | | | S | 228, 230 | nd | | |
| 235 | | | | | | | | | S | 236 | nd | | |
| 240 | | | | | | | | | L | | nd | | |
| 245 | | | | | | | | | L | | nd | | |
| 250 | | | | | | | | | L | | nd | | |
| 255 | | | | | | | | | L | 258 | nd | | |
| 260 | | | | | | | | | L | | 35 | 261-263 | L |
| 265 | | | | | | | | | S | | nd | | |
| 270 | | | | | | | | | L | | nd | | |
| 275 | | | | | | | | | S | | nd | | |
| 280 | | | | | | | | | S | | nd | | |
| 285 | | | | | | | | | S | 288 | 27 | 285-287 | S |
| 290 | | | | | | | | | S | 290 | nd | | |
| 295 | | | | | | | | | L | | nd | | |
| 300 | | | | | | | | | L | | nd | | |
| 305 | | | | | | | | | S | 302 | nd | | |
| 310 | | | | | | | | | S | 306 | nd | | |
| 315 | | | | | | | | | L | | nd | | |

# APPENDIX B

## MOST PROBABLE NUMBER DATA

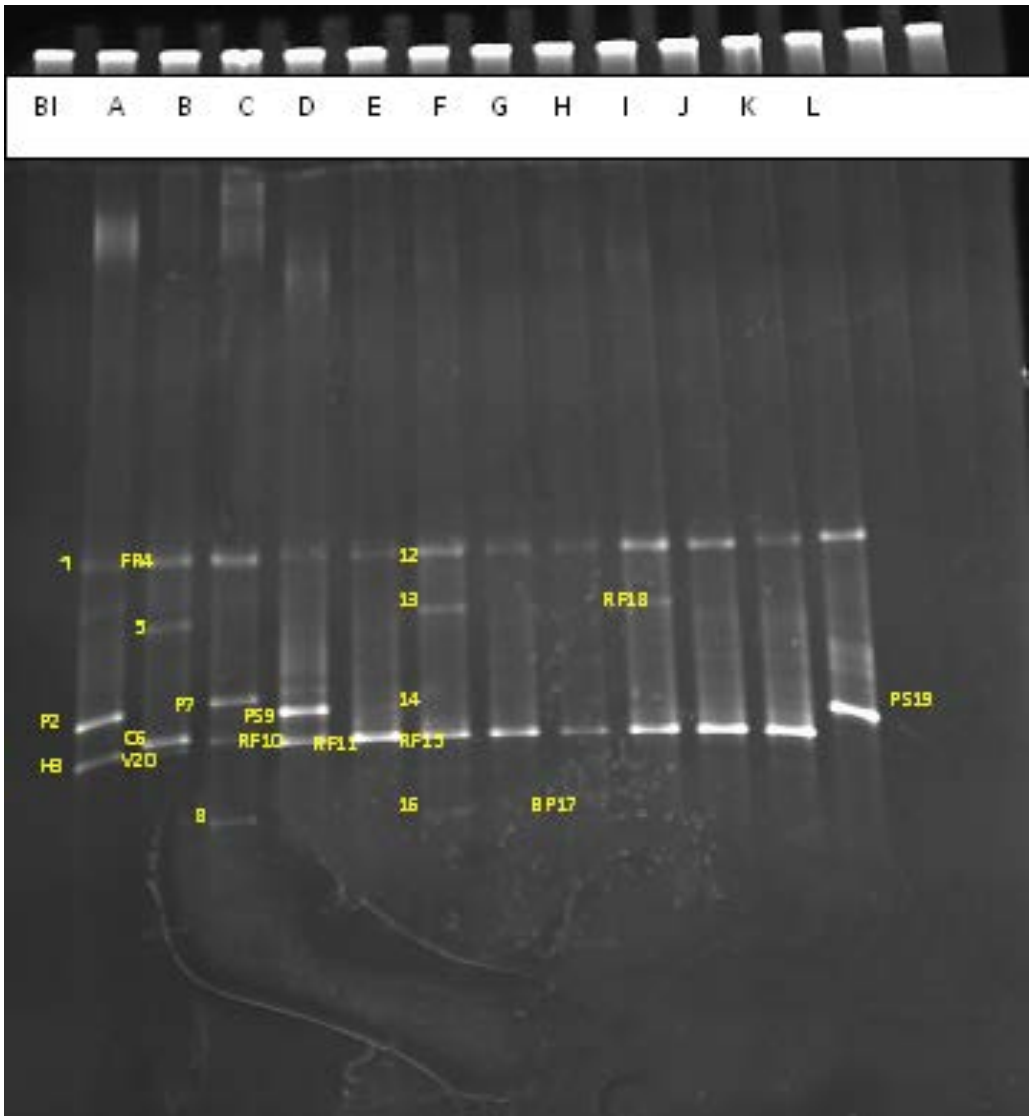
# APPENDIX B

## MOST PROBABLE NUMBER DATA

B-1: Groundwater Chemistry for Dry Valley and Smoky Canyon
Figure B1-1. DGGE analysis of DNA from MPN Enrichments

Table B1-1. MPN Bottle Roll Extract Selenium Analyses
Table B1-2. MPN Bottle Roll Carbon and Nitrogen Analyses
Table B1-3. Summary of MPN Rankings
Table B1-4. MPN DGGE band DNA sequences
Table B1-5. List of MPN ICP-MS data files on CD

On DVD: ICP-MS data supporting MPN analyses
To request DVD copies contact your local public or university library to place an interlibrary loan request to Montana State University. Questions call 406-994-3161.




Hole ID = Hole_Mine_incubation_dilution_replicate

For example, AS5-A1-4C is from drill hole SCA, Shale at 5 feet of depth, anaerobic incubation replicate 1 – 10-4 diluted replicate C

| | |
|---|---|
| Bl | blank |
| A | AS5-A1-4C |
| B | AS5-A1-5A |
| C | AS71-A2-3B |
| D | AS113-A1-3E |
| E | AS113-A1-7C |
| F | AS113-A1-8E |
| G | MS285-A2-7D |
| H | MS73-A1-6A |
| I | MS5-A1-5B |
| J | MS285-N1-8E |
| K | AM145-N1-3B |
| L | Mm32 |

| | |
|---|---|
| P2, P7 | Polaromonas |
| RF10, RF11, RF15 | R. Ferrireducens T118 |
| RF18 | Rhodoferax Sp. AsD |
| FR4 | Rhodoferax Fermentans |
| C6 | Comamonadacea |
| PS9, PS19 | Pelosinus |
| V20 | Varivorax |
| H3 | Herminiimonas |
| BP17 | Uncult Betaproteo |

Figure B1-1. DGGE analysis of DNA from MPN enrichments.

Table B1-1. Selenium concentration of Rock Extracts Used for MPNs in Experiments.

| 2006 | | ppb blank | C125 SCA | SCA S113 | SCA M145 | SCD M50 | SCD S75 | SCD C3 | SCD C123 | MEV S55 | MEV M32 | MEV M178 | MEV L261 | MEV S285 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 4/19 | Se74 | 2.82 | na | na | na | na | na | na | na | na | na | na | na | na |
| 4/19 | Se76 | 4.389 | na | na | na | na | na | na | na | na | na | na | na | na |
| 4/19 | Se77 | 4.462 | na | na | na | na | na | na | na | na | na | na | na | na |
| 4/19 | Se78 | 4.452 | na | na | na | na | na | na | na | na | na | na | na | na |
| 4/19 | Se80 | 4.562 | na | na | na | na | na | na | na | na | na | na | na | na |
| 4/19 | Se82 | 4.997 | na | na | na | na | na | na | na | na | na | na | na | na |
| 4/20 | Total Se | | 2041 | 2079 | 2167 | 1832 | 1743 | 2414 | 2106 | 2098 | 2224 | 2098 | 1900 | 2059 |
| 4/20 | Se IV as Se | ppb | 419 | 371 | 402 | 370 | 404 | 470 | 419 | 414 | 508 | 419 | 380 | 355 |
| 4/20 | Se VI as Se | ppb | 1622 | 1708 | 1765 | 1462 | 1339 | 1944 | 1687 | 1684 | 1716 | 1679 | 1520 | 1704 |
| 4/20 | SeO4 as Se | mM | 0.021 | 0.022 | 0.022 | 0.019 | 0.017 | 0.025 | 0.021 | 0.021 | 0.022 | 0.021 | 0.019 | 0.022 |
| 4/20 | SeO3 as Se | mM | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 | 0.006 | 0.005 | 0.005 | 0.006 | 0.005 | 0.005 | 0.004 |

Extracted at 2.75 : 1 ratio distilled water to rock, filter sterilized 0.22 uM
Contrast with reported maximum SE Idaho field concentration 12 mg/L
na = not analyzed

Table B1-2. MPN Bottle Roll C N Chemistry.

| Sample ID | NPOC mg $L^{-1}$ | TN mg $L^{-1}$ | TN peak area | TN corrected mg $L^{-1}$ | TOT C mg $L^{-1}$ | dilution factor | DIC dil factor corrected | NPOC dil factor corrected | TN dil factor corrected |
|---|---|---|---|---|---|---|---|---|---|
| DS 75 | 23.88 | 2.946 | 69.34 | 3.688 | 27.6 | 3.7 | 13.9 | 88.36 | 13.65 |
| DC 3 | 19.7 | 2.355 | 55.44 | 2.949 | 20.1 | 4 | 1.7 | 78.80 | 11.80 |
| DM 50 | 20.69 | 2.269 | 53.42 | 2.841 | 21.5 | 3.4 | 2.7 | 70.35 | 9.66 |
| DC 123 | 33.88 | 4.783 | 112.6 | 5.989 | 32.9 | 2.9 | < 0.3 | 98.25 | 17.37 |
| AM 145 | 25.43 | 3.114 | 73.3 | 3.899 | 31.1 | 3.9 | 22.1 | 99.18 | 15.21 |
| AC 125 | 18.22 | 14.23 | 334.9 | 17.814 | 19.2 | 4.2 | 4.1 | 76.52 | 74.82 |
| MS 5 | 24.27 | 3.529 | 83.07 | 4.419 | 25.2 | 3.9 | 3.7 | 94.65 | 17.23 |
| MM 32 | 23.87 | 2.568 | 60.45 | 3.215 | 25.8 | 2.5 | 4.7 | 59.68 | 8.04 |
| MM 178 | 18.53 | 2.725 | 64.14 | 3.412 | 24.4 | 4.4 | 26.0 | 81.53 | 15.01 |
| MS 285 | 23.4 | 2.511 | 59.11 | 3.144 | 26.3 | 4.6 | 13.3 | 107.64 | 14.46 |
| ML 261 | 30.25 | 3.332 | 78.43 | 4.172 | 29.2 | 3.2 | < 0.3 | 96.80 | 13.35 |

Table B1-3. Summary of MPN Rankings.

| Sample ID | Treat-ment | Dilution $10^{-1}$ abcde | Dilution $10^{-2}$ abcde | Dilution $10^{-3}$ abcde | Dilution $10^{-4}$ abcde | Dilution $10^{-5}$ abcde | Dilution $10^{-6}$ abcde | Dilution $10^{-7}$ abcde | Dilution $10^{-8}$ abcde | MPN Score | 95% c.l. | 95% c.l. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ANAEROBIC Most Probable Number Experiments | | | | | | | | | | | | |
| GW11 | A1 | +++++ | +++++ | +++++ | +-++- | ----- | ----- | +---- | +---- | 16,803 | 6,451 | 43,925 |
| GW11 | A2 | +++++ | +++++ | +++++ | +--+- | +-+-- | --+-- | -+--- | +---- | 16,730 | 7,809 | 35,967 |
| SC-GW7D | A1 | +++++ | +++++ | +++++ | +++++ | +++++ | +++-+ | ++++- | ++++- | 837,196 | 314,433 | 2,250,783 |
| SC-GW7D | A2 | +++++ | +++++ | +++++ | +++++ | +++++ | +++++ | +++++ | +---- | 5,384,114 | 23,500,511 | 12,404,417 |
| | | | | | | | | | | | | |
| AS5 | A1 | +++++ | +++++ | +++++ | -+++- | ++++- | -+--- | ----- | ----- | 24293 | 11513 | 51447 |
| AS5 | A2 | +++++ | +++++ | +++++ | --+++ | --+-- | ----- | ----- | ----- | 10,676 | 3883 | 29,453 |
| AS71 | A1 | +++++ | +++++ | +++++ | +++++ | +++++ | +++++ | +++++ | +++++ | 10,359,566 | 5,323,065 | 20,279,141 |
| AS71 | A2 | +++++ | +++++ | ----- | ----- | ----- | ----- | ----- | ----- | 230 | 78 | 681 |
| AS113 | A1 | +++++ | +++++ | +++++ | +++++ | +++++ | --+-+ | ----+ | ----- | 594,643 | 206,113 | 1,723,803 |
| AS113 | A2 | +++++ | +++++ | +++++ | +++++ | -+++- | ++++- | ----- | +---- | 260,016 | 111,469 | 609,254 |
| AC123 | A1 | +++++ | --+-+ | ----+ | ----- | ----- | ----- | ----- | ----- | 67 | 22 | 208 |
| AC123 | A2 | +++++ | ---+- | ----- | --+-- | ----- | ----- | ----- | ----- | 45 | 15 | 136 |
| AM145 | A1 | +++++ | +++++ | +++++ | ----- | ----- | ----- | ----- | ----- | 2,302 | 780 | 6,812 |
| AM145 | A2 | +++++ | +++++ | -++++ | ----- | ----- | ----- | ----- | ----- | 1,273 | 497 | 3,632 |
| DC3 | A1 | +++++ | +++++ | --++- | ----- | ----- | ----- | +---- | ----+ | 488 | 154 | 1553 |
| DC3 | A2 | ++++- | +++++ | ----- | ----- | ----- | ----- | ----- | ----- | 230 | 78 | 681 |
| DM50 | A1 | +++++ | +---+ | ----- | ----- | ----- | ----- | ----- | ----- | 49 | 15 | 155 |
| DM50 | A2 | +++++ | +++++ | ---+- | ----- | ----- | ----- | ----- | ----- | 327 | 109 | 982 |
| DS75 | A1 | +++++ | +++++ | -++++ | -+--- | -+--+ | ----- | ----- | ----- | 2,576 | 1,106 | 6,015 |
| DS75 | A2 | +++++ | +++-+ | ++-++ | +---- | ----- | ----- | ----- | ----- | 334 | 146 | 761 |
| DC123 | A1 | +++++ | +++++ | ----- | ----- | ----- | ----+ | ----- | ----- | 311 | 107 | 706 |
| DC123 | A2 | +++++ | +++++ | ----- | ----- | ----- | ----- | ----- | ----- | 230 | 78 | 681 |
| MS5 | A1 | +++++ | +++++ | +++-+ | ++++- | -+-+- | -+--- | ----- | ----- | 3,165 | 1,407 | 7,138,234 |
| MS5 | A2 | +++++ | +++++ | +++-+ | -++++ | +---- | ----- | ----- | ----- | 3,967 | 1,807 | 8,738 |
| MM32 | A1 | +++++ | +++-- | +---- | ----- | ----- | ----- | ----- | ----- | 107 | 39 | 295 |
| MM32 | A2 | +++++ | +++++ | -++++ | ----- | ----- | +++-- | +---- | ----- | 1,651 | 635 | 4,301 |
| MS73 | A2 | +++++ | +++++ | +++++ | +-+++ | ----- | ----- | ----- | ----- | 756 | 407 | 1410 |
| MS73 | A1 | +++++ | +++++ | ++-++ | +-+-- | ----- | ----- | ----- | ----- | 12720 | 4472 | 36305 |
| MS285 | A1 | +++++ | +++++ | +++++ | +++++ | +++++ | ----- | ----- | ----+ | 302,742 | 105,654 | 871,435 |
| MS285 | A2 | +++++ | +++++ | +++++ | +++++ | +---- | ----- | +++-+ | ++--- | 16461 | 6342 | 42876 |

abcde: 5 replicates
based on calculator using Standard MPN tables
See BAMMPN.xls file in Appendix B.

Table B1-3. Summary of MPN Rankings, continued.

| Sample ID | Treat-ment | Dilution $10^{-1}$ abcde | Dilution $10^{-2}$ abcde | Dilution $10^{-3}$ abcde | Dilution $10^{-4}$ abcde | Dilution $10^{-5}$ abcde | Dilution $10^{-6}$ abcde | Dilution $10^{-7}$ abcde | Dilution $10^{-8}$ abcde | MPN Score | 95% c.l. | 95% c.l. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AEROBIC Most Probable Number Experiments | | | | | | | | | | | | |
| SC-GW11 | 01 | +++++ | ++--- | +--+- | +----- | ----+ | ----- | ----- | ----- | 4 | 1 | 18 |
| SC-GW11 | O2 | +++++ | +++++ | ++++- | +---- | ----- | ----- | ----- | ----- | 122 | 79 | 205 |
| DV-GW7D | 02 | +++++ | +++++ | ++++- | +---- | ----- | ----- | ----- | ----- | 1,684 | 649 | 4,402 |
| DV-GW7D | 01 | ---+- | ---++ | ----- | ----- | ----- | ----- | -+--- | ----- | 8 | 3 | 22 |
| | | | | | | | | | | | | |
| AS5 | 01 | +---- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | 2 | 0.3 | 14 |
| AS5 | 02 | -+-++ | ----- | ----- | ----- | ----- | ----- | ----- | ----- | 2 | 0.3 | 14 |
| AS71 | 01 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | 0 | 0 | 0 |
| AS71 | 02 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | 0 | 0 | 0 |
| AS113 | 01 | +----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | 2 | 0.3 | 14 |
| AS113 | 02 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | 0 | 0 | 0 |
| AC123 | 01 | +-+-- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | 4 | 1 | 18 |
| AC123 | 02 | +--++ | +--+- | ----- | ----- | ----- | ----- | ----- | ----- | 14 | 6 | 35 |
| AM145 | 01 | +++++ | ---+- | ----- | ----- | ----- | ----- | ----- | ----- | 49 | 15 | 155 |
| AM145 | 02 | +-+++ | +-+-+ | ----+ | ----- | ----- | --+-- | ----+ | ----- | 105 | 38 | 290 |
| DC3 | 01 | +-+++ | ----- | ----- | ----- | ----- | ----- | ----- | ----- | 13 | 4 | 36 |
| DC3 | 02 | +++++ | ----- | ----- | ----- | ----- | ----- | ----- | ----- | 23 | 8 | 68 |
| DM50 | 01 | -+--+ | ----- | ----- | ----- | ----- | ----- | ----- | ----- | 4 | 1 | 18 |
| DM50 | 02 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | 0 | 0 | 0 |
| DS75 | 01 | +++++ | -+-++ | ----- | ----- | ----- | ----- | ----- | ----- | 78 | 25 | 243 |
| DS75 | 02 | +++++ | ---+- | ----- | ----- | ----- | ----- | ----- | ----- | 37 | 10 | 98 |
| DC123 | 01 | ----- | ----- | ----- | ----- | ----- | ----- | ----- | ----- | 0 | 0 | 0 |
| DC123 | 02 | ----+ | ----- | ----- | ----- | ----- | ----- | ----- | ----- | 2 | 0.3 | 14 |
| MS5 | 01 | +++-+ | ---+- | ----- | ----- | ----- | ----- | ----- | ----- | 17 | 6 | 44 |
| MS5 | 02 | +++++ | -+--- | ---+- | ----- | ----- | ----- | ----- | ----- | 45 | 15 | 137 |
| MM32 | 01 | ----- | ----- | ----- | -+--- | -+--- | ----- | ----- | ----- | 4 | 1 | 14 |
| MM32 | 02 | +---- | ----- | ----- | ----- | ----- | ++--- | ----- | ----- | 1 | 0 | 14 |
| MS73 | 01 | +++++ | -+++- | ----- | ----- | ----- | ----- | ----- | ----- | 230 | 78 | 681 |
| MS73 | 02 | +++++ | -++++ | --+-- | ----- | +---- | ----- | ----- | +---- | 210 | 86 | 516 |
| MM178 | 01 | +++++ | ----- | +---- | ----- | ----- | -++-- | -++++ | +++++ | 31 | 10 | 91 |
| MM178 | 02 | +-+++ | ----- | --+-- | ----- | ----- | ----- | ----- | ++--+ | 12 | 4 | 36 |
| MS285 | 01 | -++++ | -++++ | -+++- | ----+ | ----- | ----- | ----- | ----- | 61 | 31 | 121 |
| MS285 | O2 | +++++ | -++++ | ++-++ | ----+ | --+-+ | ----+ | -+--- | ---+- | 756 | 407 | 1410 |

abcde: 5 replicates
based on calculator using Standard MPN tables
See BAMMPN.xls file in Appendix B.

Table B1-4. MPN DGGE Band DNA Sequences – July 2009

| MPN DGGE bands | | ID | Coverage | Identity | in clone library | in sed DGGE |
|---|---|---|---|---|---|---|
| 1 | Polaromonas sp. UF008 | AB426569.1 | 83% | 90% | x | x |
| 2 | Polaromonas sp. UF008 | AB426569.1 | 98% | 100% | x | x |
| 3 | Herminiimonas fonticola | AB512142.1 | 93% | 100% | | |
| 4 | Rhodoferax fermentans strain FR2 | D16212.1 | 98% | 97% | x | |
| 5 | Uncultured Betaproteobacteria | CU926297.1 | 98% | 100% | | |
| 6 | Comamonadaceae bacterium ED16 | FJ755906.1 | 100% | 98% | | |
| 7 | Polaromonas sp. UF008 gene | AB426569.1 | 100% | 100% | x | x |
| 8 | Pseudomonas sp. RF-58 16S | GQ205100.1 | 95% | 95% | x | |
| 9 | Pelosinus sp. UFO1 | EU215386.1 | 96% | 92% | x | x |
| 10 | Rhodoferax ferrireducens T118 | CP000267.1 | 99% | 99% | x | x |
| 11 | Rhodoferax ferrireducens T118 | CP000267.1 | 98% | 99% | x | x |
| 12 | Uncultured bacterium gene | EU499692.1 | 99% | 96% | | |
| 13 | Uncultured bacterium gene | AB504940.1 | 99% | 97% | | |
| 14 | Uncultured bacterium clone 3BH-10FF | EU937983.1 | 100% | 97% | | |
| 15 | Rhodoferax ferrireducens T118 | CP000267.1 | 100% | 97% | x | x |
| 16 | Bacterium TG141 gene | AB308367.1 | 100% | 88% | | |
| 17 | Uncultured beta proteobacterium clone MBMV10 | FJ538157.1 | 99% | 97% | | |
| 18 | Rhodoferax sp. Asd M2A1 | FM955857.1 | 98% | 97% | x | x |
| 19 | Pelosinus sp. UFO1 | EU215386.1 | 99% | 99% | x | x |
| 20 | Variovorax paradoxus S110 | CP001635.1 | 100% | 99% | x | |

Table B1-5. List of MPN ICPMS data files on CD.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| 101607 Aquant | App B MPN/4_MPN/GW11_7D MPN | 10/6/2007 | Se 74, 76, 77, 78, 80, and 82 |
| 101607 Aquant | App B MPN/4_MPN/GW11_7D MPN | 10/6/2007 | Se 74, 76, 77, 78, 80, and 82 |
| GW7dC | App B MPN/4_MPN/GW11_7D MPN | 7/5, 7/9/2007 | Turbid, Sediment, Reduction, None |
| GW11 | App B MPN/4_MPN/GW11_7D MPN | 7/5/07 and 7/9/07 | Turbid, Sediment, Reduction, None |
| gw inventory July 2007 | App B MPN/4_MPN/GW11_7D MPN | NA | Description of samples collected |
| MPN Gw7d | App B MPN/4_MPN/GW11_7D MPN | 7/13/07, 7/18/2007. 7/20/07, 8/1/07, 8/3/07, 8/6/07, 8/10/07, 8/14/07, 8/20/07, and 8/24/07 | Turbid, Sediment, Reduction, None |
| MPN Gw7D N | App B MPN/4_MPN/GW11_7D MPN | 7/13/07, 7/20/07, 8/6/07, and 8/24/07 | Turbid, Sediment, Reduction, None |
| MPN Gw11 N | App B MPN/4_MPN/GW11_7D MPN | 7/13/07, 7/20/07, 8/6/07, and 8/24/07 | Turbid, Sediment, Reduction, None |
| MPN gw11 | App B MPN/4_MPN/GW11_7D MPN | 7/13/07, 7/18/07, 7/20/07, 7/24/07, 8/1/07, 8/3/07, 8/6/07, 8/10/07, 8/14/07, 8/20/07, and 8/24/07 | Turbid, Sediment, Reduction, None |
| 070806quant | App B MPN/4_MPN/ICPMS | 7/8/2006 | Se 74, 76, 77, 78, 80, and 82 |
| 092906quant | App B MPN/4_MPN/ICPMS | 9/29/2006 | Se 74, 76, 77, 78, 80, and 82 |
| 092906quant | App B MPN/4_MPN/ICPMS | 9/29/2006 | Se 74, 76, 77, 78, 80, and 82 |
| 093006quant | App B MPN/4_MPN/ICPMS | 9/29 and 9/30/06 | Se 74, 76, 77, 78, 80, and 82 |
| 100406quant | App B MPN/4_MPN/ICPMS | 10/4/2006 | Se 74, 76, 77, 78, 80, and 82 |
| calib0406 | App B MPN/4_MPN/ICPMS | NA | Various dates- Se curves |
| DCUP | App B MPN/4_MPN/ICPMS | 6/26/06, 6/27/06, 7/5/06, 7/10/06, 7/28/06, 7/24/06, and 8/9/06 | Turbid, Sediment, Reduction, None |
| DMup AC | App B MPN/4_MPN/ICPMS | 6/27/2006 | Turbid, Sediment, Reduction, None |
| DSUP | App B MPN/4_MPN/ICPMS | 6/26/2006 | Turbid, Sediment, Reduction, None |
| extract041606 | App B MPN/4_MPN/ICPMS | 4/19/2006 | Se 78 |
| LA071106quant | App B MPN/4_MPN/ICPMS | 7/11/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk010507quant | App B MPN/4_MPN/ICPMS | 1/4/2007 | Se 74, 76, 77, 78, 80, and 82 |
| lk012307quant | App B MPN/4_MPN/ICPMS | 1/26 and 1/27/07 | Se 74, 76, 77, 78, 80, and 82 |
| lk012607quant | App B MPN/4_MPN/ICPMS | 1/26 and 1/27/07 | Se 74, 76, 77, 78, 80, and 82 |

Table B1-5. List of MPN ICPMS data files on CD, continued.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| lk012707quant | App B MPN/4_MPN/ICPMS | 1/27/2007 | Se 74, 76, 77, 78, 80, and 82 |
| lk012807quant | App B MPN/4_MPN/ICPMS | 1/27 and 1/28/07 | Se 74, 76, 77, 78, 80, and 82 |
| LK070806quant | App B MPN/4_MPN/ICPMS | 7/8/2008 | Se 74, 76, 77, 78, 80, and 82 |
| LK071106quant | App B MPN/4_MPN/ICPMS | 7/11/2008 | Se 74, 76, 77, 78, 80, and 82 |
| LK72006quant | App B MPN/4_MPN/ICPMS | 7/20/2006 | Se 78 and 82 |
| lk081406quant | App B MPN/4_MPN/ICPMS | 8/14 and 8/15/06 | Se 74, 76, 77, 78, 80, and 82 |
| lk91206Aquant | App B MPN/4_MPN/ICPMS | 9/12 and 9/13/06 | Se 74, 76, 77, 78, 80, and 82 |
| LK091206quant | App B MPN/4_MPN/ICPMS | 9/12/2006 | Se 74, 76, 77, 78, 80, and 82 |
| LK91606Aquant | App B MPN/4_MPN/ICPMS | 9/12 and 9/13/06 | Se 74, 76, 77, 78, 80, and 82 |
| LK092206quant | App B MPN/4_MPN/ICPMS | 9/22/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk092906quant | App B MPN/4_MPN/ICPMS | 9/29 and 9/30/06 | Se 74, 76, 77, 78, 80, and 82 |
| lk093006quant | App B MPN/4_MPN/ICPMS | 9/30/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk100406quant | App B MPN/4_MPN/ICPMS | 10/4/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk102306quant | App B MPN/4_MPN/ICPMS | 10/22/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk102406quant | App B MPN/4_MPN/ICPMS | 10/22 and 10/23/06 | Se 74, 76, 77, 78, 80, and 82 |
| lk102606quant | App B MPN/4_MPN/ICPMS | 10/26 and 10/27/06 | Se 74, 76, 77, 78, 80, and 82 |
| LK102707 | App B MPN/4_MPN/ICPMS | NA | List of Samples (no analysis) |
| lk102806quant | App B MPN/4_MPN/ICPMS | 10/28/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk110306quant | App B MPN/4_MPN/ICPMS | 11/4/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk110406quant | App B MPN/4_MPN/ICPMS | 11/4 and 11/5/06 | Se 74, 76, 77, 78, 80, and 82 |
| lk110506quant | App B MPN/4_MPN/ICPMS | 11/5/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk111006quant | App B MPN/4_MPN/ICPMS | 11/10/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk180906quant | App B MPN/4_MPN/ICPMS | 8/9/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk180906quant (2) | App B MPN/4_MPN/ICPMS | NA | Same as lk180906, but with some data copied into new worksheets |
| lk12110806quant | App B MPN/4_MPN/ICPMS | 11/8/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lkl1114d6quant | App B MPN/4_MPN/ICPMS | 11/14/2006 | Se 74, 76, 77, 78, 80, and 82 |
| May282006 | App B MPN/4_MPN/ICPMS | NA | Data and figures from may 28 and 29 2006 |
| MPN071506 | App B MPN/4_MPN/ICPMS | 7/8 and 7/11/06 | Se 74, 76, 77, 78, 80, and 82 |

Table B1-5. List of MPN ICPMS data files on CD, continued.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| MPN review | App B MPN/4_MPN/ICPMS | NA | uncertain eactly what this is- appears to be some review of classes of samples. |
| MPNs saved | App B MPN/4_MPN/ICPMS | NA | Master list of sample IDs? |
| MPNSUMM | App B MPN/4_MPN/ICPMS | NA | Results sorted with each sample on separate worksheet. |
| MPNSUMM_A | App B MPN/4_MPN/ICPMS | NA | Results sorted with each sample on separate worksheet. |
| MPNSUMM_B | App B MPN/4_MPN/ICPMS | NA | Results sorted with each sample on separate worksheet. |
| MPNSUMM_D | App B MPN/4_MPN/ICPMS | NA | Results sorted with each sample on separate worksheet. |
| MPNSUMM_M | App B MPN/4_MPN/ICPMS | NA | Results sorted with each sample on separate worksheet. |
| SCA M145 | App B MPN/4_MPN/ICPMS | 5/10/2006, 5/15/2006, 5/22/2006, 5/28/2006, 6/26/2006, 7/5/2007, 7/10/2006, 7/18/2006, 7/24/2006 | Turbid, Sediment, Reduction, None |
| SCA M145 (version 1) | App B MPN/4_MPN/ICPMS | 5/10/2006, 5/15/2006, 5/22/2006, 5/25/2006, 5/28/2006, 6/26/2006, 7/5/2007, 7/10/2006, 7/18/2006, 7/24/2006 | Turbid, Sediment, Reduction, None |
| Sca S113 | App B MPN/4_MPN/ICPMS | 5/10/2006, 5/14/2006, 5/15/2006, 5/22/2006, 5/25/2006, 5/26/2006, 5/27/2006, 5/28/2006, 5/31/2006, 6/26/2006, 6/27/2006, 7/5/2007, 7/6/2006, 7/10/2006, 7/18/2006, 7/24/2006 | Turbid, Sediment, Reduction, None |
| Sca S113 (version 1) | App B MPN/4_MPN/ICPMS | 5/10/2006, 5/14/2006, 5/15/2006, 5/22/2006, 5/25/2006, 5/26/2006, 5/27/2006, 5/28/2006, 5/31/2006, 6/26/2006, 6/27/2006, 7/5/2007, 7/6/2006, 7/10/2006, 7/18/2006, 7/24/2006 | Turbid, Sediment, Reduction, None |
| SCA Smid | App B MPN/4_MPN/ICPMS | 6/26/2006, 7/5/2006, 7/10/2006, 7/18/2006, 7/24/2006, 8/2/2006, 8/7/2006 | Turbid, Sediment, Reduction, None |
| SCA Smid (version 1) | App B MPN/4_MPN/ICPMS | 6/26/2006, 7/5/2006, 7/10/2006, 7/18/2006 | Turbid, Sediment, |

Table B1-5. List of MPN ICPMS data files on CD, continued.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| | | | Reduction, None |
| SCD Cup | App B MPN/4_MPN/ICPMS | 4/26/2006, 4/29/2006, 5/2/2006, 5/5/2006, 5/7/2006, 5/15/2006, 5/22/2006, 5/31/2006, 6/27/2006, 7/5/2006, 7/10/2006, 8/7/2006 | Turbid, Sediment, Reduction, None |
| SCD week 1 | App B MPN/4_MPN/ICPMS | NA | Summary of Week 1 data (tables and figures) |
| SCDCup CO | App B MPN/4_MPN/ICPMS | 6/26/2006, 7/5/2006, 7/10/2006, 7/18/2006, 7/24/2006, 7/31/2006 | Turbid, Sediment, Reduction, None |
| 070806quant | App B MPN/4_MPN | 7/8/2006 | Se 74, 76, 77, 78, 80, and 82 |
| BAM-MPN | App B MPN/4_MPN | NA | ? |
| LA071106quant | App B MPN/4_MPN | 7/11 and 7/12/2006 | Se 74, 76, 77, 78, 80, and 82 |
| LK070806quant | App B MPN/4_MPN | 7/8/2006 | Se 74, 76, 77, 78, 80, and 82 |
| LK071106quant | App B MPN/4_MPN | 7/11/2006 | Se 74, 76, 77, 78, 80, and 82 |
| MPN071506 | App B MPN/4_MPN | 7/8 and 7/11/06 | Se 74, 76, 77, 78, 80, and 82 |
| MPNserumchemistry | App B MPN/4_MPN | ? | ? |
| rock extracts | App B MPN/4_MPN | ? | ? |
| setupinventory | App B MPN/4_MPN | NA | ? |
| setupinventoryv2 | App B MPN/4_MPN | NA | ? |
| MPN DGGE library sequences_July2009 | App B MPN/4_MPN | NA | ? |
| MPN DNA 5809 | App B MPN/4_MPN | 2/3/2009, 12/29/2008, 12/5/2009, 1/9/2009, 12/2/2008 | ? |
| LK071106quant | App B MPN/4_MPN | 7/11 and 7/12/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk1111A6quant | App B MPN/4_MPN/ICPMS/12-20 ICP Files | 11/11/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk1111B6quant | App B MPN/4_MPN/ICPMS/12-20 ICP Files | 11/11/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk1111C6quant | App B MPN/4_MPN/ICPMS/12-20 ICP Files | 11/11/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk1112A6quant | App B MPN/4_MPN/ICPMS/12-20 ICP Files | 11/12/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk1112B6quant | App B MPN/4_MPN/ICPMS/12-20 ICP Files | 11/12/2006 | Se 74, 76, 77, 78, 80, and 82 |

Table B1-5. List of MPN ICPMS data files on CD, continued.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| lk1114A6quant | App B MPN/4_MPN/ICPMS/12-20 ICP Files | 11/14/2006 | Se 74, 76, 77, 78, 80, and 82 |
| LK070806quant | App B MPN/4_MPN/ICPMS/12-20 ICP Files | 7/8/2006 | Se 74, 76, 77, 78, 80, and 82 |
| LK72006quant | App B MPN/4_MPN/ICPMS/12-20 ICP Files | 7/20/2006 | Se 78 and 82 |
| LK91606Aquant | App B MPN/4_MPN/ICPMS/12-20 ICP Files | 9/12 and 9/13/06 | Se 74, 76, 77, 78, 80, and 82 |
| lk093006quant | App B MPN/4_MPN/ICPMS/12-20 ICP Files | 9/30/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lkl1114d6quant | App B MPN/4_MPN/ICPMS/12-20 ICP Files | 11/14/2006 | Se 74, 76, 77, 78, 80, and 82 |
| 070809quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 7/8/2006 | Se 74, 76, 77, 78, 80, and 82 |
| 092906quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 9/29 and 9/30/06 | Se 74, 76, 77, 78, 80, and 82 |
| LA071106quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 7/11 and 7/12/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk010507quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 1/4/2007 | Se 74, 76, 77, 78, 80, and 82 |
| lk012807quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 1/28 and 1/29/2007 | Se 74, 76, 77, 78, 80, and 82 |
| LK070806quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 7/8/2006 | Se 74, 76, 77, 78, 80, and 82 |
| LK071106quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 7/11/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk081406quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 8/14 and 8/15/06 | Se 74, 76, 77, 78, 80, and 82 |
| lk91206Aquant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 9/12 and 9/13/06 | Se 74, 76, 77, 78, 80, and 82 |
| LK092206quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 9/22 and 9/23/2006 | Se 74, 76, 77, 78, 80, and 82 |
| LK091206quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 9/12/2006 | Se 74, 76, 77, 78, 80, and 82 |

Table B1-5. List of MPN ICPMS data files on CD, continued.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| lk092906quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 9/29 and 9/30/06 | Se 74, 76, 77, 78, 80, and 82 |
| lk093006quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 9/30/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk100406quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 10/4/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk102306quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 10/22/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk102406quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 10/22 and 10/23/06 | Se 74, 76, 77, 78, 80, and 82 |
| lk102606quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 10/26 and 10/27/06 | Se 74, 76, 77, 78, 80, and 82 |
| lk102806quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 10/28/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk110406quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 11/4 and 11/5/06 | Se 74, 76, 77, 78, 80, and 82 |
| lk111006quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 11/10/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk180906quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 8/9/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk12110806quant | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 11/8/2006 | Se 74, 76, 77, 78, 80, and 82 |
| May282006 | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 5/28 and 5/29/2006 | ? |
| MPN071506 | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | 7/8 and 7/11/06 | Se 74, 76, 77, 78, 80, and 82 |
| SCD week 1 | App B MPN/4_MPN/ICPMS/ICPdata78_711_06 | ? | ? |
| lk081406quant | App B MPN/4_MPN/MPN2006/Reports52806/LK081406.B | 8/14 and 8/15/06 | Se 74, 76, 77, 78, 80, and 82 |
| 070806quant | App B MPN/4_MPN/MPN2006/NOT FINAL MPNS | 7/8/2006 | ? |

Table B1-5. List of MPN ICPMS data files on CD, continued.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| 070806quant | App B MPN/4_MPN/MPN2006/NOT FINAL MPNS | 7/8/2006 | ? |
| LA071106quant | App B MPN/4_MPN/MPN2006/NOT FINAL MPNS | 7/11 and 7/12/2006 | Se 74, 76, 77, 78, 80, and 82 |
| LA071106quant | App B MPN/4_MPN/MPN2006/NOT FINAL MPNS | 7/11 and 7/12/2006 | Se 74, 76, 77, 78, 80, and 82 |
| LK070806quant | App B MPN/4_MPN/MPN2006/NOT FINAL MPNS | 7/8/2006 | Se 74, 76, 77, 78, 80, and 82 |
| LK071106quant | App B MPN/4_MPN/MPN2006/NOT FINAL MPNS | 7/11/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk100406quant | App B MPN/4_MPN/MPN2006/NOT FINAL MPNS | 10/4/2006 | Se 74, 76, 77, 78, 80, and 82 |
| LK102707 | App B MPN/4_MPN/MPN2006/NOT FINAL MPNS | ? | ? |
| lk12110806quant | App B MPN/4_MPN/MPN2006/NOT FINAL MPNS | 11/8/2006 | Se 74, 76, 77, 78, 80, and 82 |
| AML261 | App B MPN/4_MPN/MPN2006/Mpn | 7/28/2006 | Turbid, Sediment, Reduction, None |
| AS5 A | App B MPN/4_MPN/MPN2006/Mpn | 7/20/2006 | Turbid, Sediment, Reduction, None |
| AS5 O | App B MPN/4_MPN/MPN2006/Mpn | 7/28 and 8/8/2006 | Turbid, Sediment, Reduction, None |
| AS113 | App B MPN/4_MPN/MPN2006/Mpn | 7/28/2006 | Turbid, Sediment, Reduction, None |
| DCUP | App B MPN/4_MPN/MPN2006/Mpn | 6/26, 7/5, 7/10, 7/28, 7/24, 8/9/2006 | Turbid, Sediment, Reduction, None |
| Dcup AC | App B MPN/4_MPN/MPN2006/Mpn | 7/20 and 7/28/2006 | Turbid, Sediment, Reduction, None |
| DMup A | App B MPN/4_MPN/MPN2006/Mpn | 7/20 and 7/28/2006 | Turbid, Sediment, Reduction, None |
| DMup AC | App B MPN/4_MPN/MPN2006/Mpn | 6/27/2006 | Turbid, Sediment, Reduction, None |
| DSUP | App B MPN/4_MPN/MPN2006/Mpn | 6/26/2006 | Turbid, Sediment, Reduction, None |
| Dsup AC | App B MPN/4_MPN/MPN2006/Mpn | 7/20 and 7/28/2006 | Turbid, Sediment, Reduction, None |

Table B1-5. List of MPN ICPMS data files on CD, continued.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| M145 A | App B MPN/4_MPN/MPN2006/Mpn | 7/20 and 7/28/2006 | Turbid, Sediment, Reduction, None |
| m145 O1, O2 | App B MPN/4_MPN/MPN2006/Mpn | 6/28, 7/5, 7/13, 7/19, 7/31, 8/7, 8/15/2006 | Turbid, Sediment, Reduction, None |
| Mev Sup O | App B MPN/4_MPN/MPN2006/Mpn | 8/8/2006 | Turbid, Sediment, Reduction, None |
| ML31 Ac | App B MPN/4_MPN/MPN2006/Mpn | 7/28/2006 | Turbid, Sediment, Reduction, None |
| ML261 A | App B MPN/4_MPN/MPN2006/Mpn | 7/28/2006 | Turbid, Sediment, Reduction, None |
| ML261 | App B MPN/4_MPN/MPN2006/Mpn | 7/28, 8/8, 8/14/2006 | Turbid, Sediment, Reduction, None |
| MM32 O | App B MPN/4_MPN/MPN2006/Mpn | 8/1/2006 | Turbid, Sediment, Reduction, None |
| Mm32 | App B MPN/4_MPN/MPN2006/Mpn | 7/28/2006 | Turbid, Sediment, Reduction, None |
| mm178 o | App B MPN/4_MPN/MPN2006/Mpn | 8/9, 9/14/2006 | Turbid, Sediment, Reduction, None |
| MS5 O | App B MPN/4_MPN/MPN2006/Mpn | 7/14/2006 | Turbid, Sediment, Reduction, None |
| MS5 | App B MPN/4_MPN/MPN2006/Mpn | Blank | Blank |
| Ms285 O | App B MPN/4_MPN/MPN2006/Mpn | 8/9, 8/24/2006 | Turbid, Sediment, Reduction, None |
| MS 73 O | App B MPN/4_MPN/MPN2006/Mpn | 8/8, 8/14/2006 | Turbid, Sediment, Reduction, None |
| SCA C10 (version 1) | App B MPN/4_MPN/MPN2006/Mpn | 7/5, 7/10, 7/18, 7/24/2006 | Turbid, Sediment, Reduction, None |
| SCA C10 | App B MPN/4_MPN/MPN2006/Mpn | 7/5, 7/10, 7/24, 7/30, 8/7, 8/14/2006 | Turbid, Sediment, Reduction, None |
| SCA M145 | App B MPN/4_MPN/MPN2006/Mpn | 5/10, 5/15, 5/22, 5/25, 5/26, 5/28, 6/26, 7/5, 7/10, 8/1, 8/8, 8/14/2006 | Turbid, Sediment, Reduction, None |
| SCA S113 | App B MPN/4_MPN/MPN2006/Mpn | 7/5, 7/10, 7/24, 8/2, 8/14/2006 | Turbid, Sediment, Reduction, None |
| SCA Smid | App B MPN/4_MPN/MPN2006/Mpn | 6/26, 7/5, 7/10, 7/18, 7/24, 8/2, 8/7, 8/14/2006 | Turbid, Sediment, Reduction, None |
| SCA SUP O1, O2, OC | App B MPN/4_MPN/MPN2006/Mpn | 8/8/2006 | Turbid, Sediment, Reduction, None |

Table B1-5. List of MPN ICPMS data files on CD, continued.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| SCD C10, 01, 02 | App B MPN/4_MPN/MPN2006/Mpn | 4/26, 5/7, 5/15, 6/28, 7/5, 7/13, 7/19, 7/24, 7/31, 8/7, 8/14/2006 | Turbid, Sediment, Reduction, None |
| SCD CLO | App B MPN/4_MPN/MPN2006/Mpn | 7/20, 7/24/2006 | Turbid, Sediment, Reduction, None |
| SCD Clo A1, A2 | App B MPN/4_MPN/MPN2006/Mpn | 7/19/2006 | Turbid, Sediment, Reduction, None |
| SCD MUP 01, 02 | App B MPN/4_MPN/MPN2006/Mpn | 6/28, 7/5, 7/13, 7/19, 7/25, 7/31, 8/7/2006 | Turbid, Sediment, Reduction, None |
| SCD MUP A | App B MPN/4_MPN/MPN2006/Mpn | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |
| SCD sup 01, 02 | App B MPN/4_MPN/MPN2006/Mpn | 4/26, 5/7, 5/23, 6/28, 7/5, 7/13, 7/19, 7/24, 7/31, 8/7/2006 | Turbid, Sediment, Reduction, None |
| lk180906quant | App B MPN/4_MPN/MPN2006/LK180906.B | 8/9/2006 | Turbid, Sediment, Reduction, None |
| lk180906quant | App B MPN/4_MPN/MPN2006/ICPdata78_711_06 | 8/9/2006 | Turbid, Sediment, Reduction, None |
| May282006 | App B MPN/4_MPN/MPN2006/ICPdata78_711_06 | 5/28, 5/29/2006 | ? |
| MPN071506 | App B MPN/4_MPN/MPN2006/ICPdata78_711_06 | 7/8, 7/11/2006 | Se 74, 76, 77, 78, 80, and 82 |
| SCD week 1 | App B MPN/4_MPN/MPN2006/ICPdata78_711_06 | ? | ? |
| lk180906quant | App B MPN/4_MPN/MPN2006/ICPdata78_711_06/lk180906.B | 8/9/2006 | Se 74, 76, 77, 78, 80, and 82 |
| 092906quant | App B MPN/4_MPN/MPN2006/MPN FINAL DATA | 9/30/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk010507quant | App B MPN/4_MPN/MPN2006/MPN FINAL DATA | 1/4/2007 | Se 74, 76, 77, 78, 80, and 82 |
| lk012307quant | App B MPN/4_MPN/MPN2006/MPN FINAL DATA | 1/23, 1/24/2007 | Se 74, 76, 77, 78, 80, and 82 |

Table B1-5. List of MPN ICPMS data files on CD, continued.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| lk012707quant | App B MPN/4_MPN/MPN2006/MPN FINAL DATA | 1/27/2007 | Se 74, 76, 77, 78, 80, and 82 |
| lk012807quant | App B MPN/4_MPN/MPN2006/MPN FINAL DATA | 1/28, 1/29/2007 | Se 74, 76, 77, 78, 80, and 82 |
| LK091206quant | App B MPN/4_MPN/MPN2006/MPN FINAL DATA | 9/12/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk110306quant | App B MPN/4_MPN/MPN2006/MPN FINAL DATA | 11/4/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk110506quant | App B MPN/4_MPN/MPN2006/MPN FINAL DATA | 11/5/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk111006quant | App B MPN/4_MPN/MPN2006/MPN FINAL DATA | 11/10/2006 | Se 74, 76, 77, 78, 80, and 82 |
| AML261 | App B MPN/4_MPN/MPN by name | 7/28/2006 | Turbid, Sediment, Reduction, None |
| AS5 A | App B MPN/4_MPN/MPN by name | 7/20/2006 | Turbid, Sediment, Reduction, None |
| AS5 O | App B MPN/4_MPN/MPN by name | 7/28, 8/8/2006 | Turbid, Sediment, Reduction, None |
| AS5 | App B MPN/4_MPN/MPN by name | 10/22, 10/23/2006 | Se 74, 76, 77, 78, 80, and 82 |
| AS71 | App B MPN/4_MPN/MPN by name | 10/28, 11/5/2006 | Se 74, 76, 77, 78, 80, and 82 |
| AS113 | App B MPN/4_MPN/MPN by name | 11/4/2006 | Se 74, 76, 77, 78, 80, and 82 |
| DC3 | App B MPN/4_MPN/MPN by name | 8/9, 8/14, 8/15/2006 | Se 74, 76, 77, 78, 80, and 82 |
| DC123 | App B MPN/4_MPN/MPN by name | 9/29, 9/30, 10/4/2006 | Se 74, 76, 77, 78, 80, and 82 |
| DCUP | App B MPN/4_MPN/MPN by name | 6/26, 7/5, 7/10, 7/28, 7/24, 8/9/2006 | Turbid, Sediment, Reduction, None |
| Dcup AC | App B MPN/4_MPN/MPN by name | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |
| DM50 | App B MPN/4_MPN/MPN by name | 9/12, 9/13, 9/22, 9/23/2006 | Turbid, Sediment, Reduction, None |
| Dmup A | App B MPN/4_MPN/MPN by name | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |
| Dmup AC | App B MPN/4_MPN/MPN by name | 6/27/2006 | Turbid, Sediment, Reduction, None |
| DS75 | App B MPN/4_MPN/MPN by name | 9/23, 9/29, 9/30/2006 | Se 74, 76, 77, 78, 80, and 82 |
| DSUP | App B MPN/4_MPN/MPN by name | 6/26/2009 | Turbid, Sediment, Reduction, None |

Table B1-5. List of MPN ICPMS data files on CD, continued.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| Dsup AC | App B MPN/4_MPN/MPN by name | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |
| M145 | App B MPN/4_MPN/MPN by name | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |
| m145 O1, O2 | App B MPN/4_MPN/MPN by name | 6/28, 7/5, 7/13, 7/18, 7/25, 7/31, 8/7, 8/15/2006 | Turbid, Sediment, Reduction, None |
| Mev Sup O | App B MPN/4_MPN/MPN by name | 8/8/2006 | Turbid, Sediment, Reduction, None |
| ML31 Ac | App B MPN/4_MPN/MPN by name | 7/28/2006 | Turbid, Sediment, Reduction, None |
| ML261 A | App B MPN/4_MPN/MPN by name | 7/28/2006 | Turbid, Sediment, Reduction, None |
| ML261 | App B MPN/4_MPN/MPN by name | 1/24, 1/26, 1/27, 1/28, 1/29/2007 | Se 74, 76, 77, 78, 80, and 82 |
| MM32 O | App B MPN/4_MPN/MPN by name | 7/31, 8/1/2006 | Turbid, Sediment, Reduction, None |
| MM32 | App B MPN/4_MPN/MPN by name | 1/4, 1/23, 1/24, 1/27/2007 | Se 74, 76, 77, 78, 80, and 82 |
| mm178 o | App B MPN/4_MPN/MPN by name | 8/9, 8/14/2006 | Turbid, Sediment, Reduction, None |
| MS5 O | App B MPN/4_MPN/MPN by name | 7/14, 8/9, 8/14/2006 | Turbid, Sediment, Reduction, None |
| MS5 | App B MPN/4_MPN/MPN by name | 11/4, 11/5/2006 | Se 74, 76, 77, 78, 80, and 82 |
| MS73 | App B MPN/4_MPN/MPN by name | 1/27/2007 | Se 74, 76, 77, 78, 80, and 82 |
| Ms285 O | App B MPN/4_MPN/MPN by name | 8/9, 8/14, 8/24/2006 | Turbid, Sediment, Reduction, None |
| MS285 | App B MPN/4_MPN/MPN by name | 11/10/2006 | Se 74, 76, 77, 78, 80, and 82 |
| MS 73 O | App B MPN/4_MPN/MPN by name | 8/8, 8/14/2006 | Turbid, Sediment, Reduction, None |
| SCA C10 (version 1) | App B MPN/4_MPN/MPN by name | 7/5, 7/10, 7/18, 7/24/2006 | Turbid, Sediment, Reduction, None |
| SCA C10 | App B MPN/4_MPN/MPN by name | 7/5, 7/10, 7/18, 7/24, 7/30, 8/1, 8/7, 8/14, 8/24/2006 | Turbid, Sediment, Reduction, None |
| SCA M145 | App B MPN/4_MPN/MPN by name | 5/15, 5/25, 5/26, 5/28, 6/26, 7/5, 7/10, 8/1, 8/10, 8/14/2006 | Turbid, Sediment, Reduction, None |
| Sca S113 | App B MPN/4_MPN/MPN by name | 5/26, 5/31, 6/27, 7/5, 7/6, 7/10, 7/18, 7/24, 8/2, 8/8, 8/14/2006 | Turbid, Sediment, Reduction, None |

Table B1-5. List of MPN ICPMS data files on CD, continued.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| SCA Smid | App B MPN/4_MPN/MPN by name | 6/26, 7/5, 7/10, 7/18, 7/24, 8/2, 8/7, 8/14, 8/15/2006 | Turbid, Sediment, Reduction, None |
| SCA SUP O1, O2, OC | App B MPN/4_MPN/MPN by name | 8/1, 8/8/2006 | Turbid, Sediment, Reduction, None |
| SCD C10, 01, 02 | App B MPN/4_MPN/MPN by name | 4/26, 5/7, 5/15, 6/28, 7/5, 7/13, 7/19, 7/24, 7/31, 8/7, 8/14/2006 | Turbid, Sediment, Reduction, None |
| SCD CLO | App B MPN/4_MPN/MPN by name | 7/20, 7/24/2006 | Turbid, Sediment, Reduction, None |
| SCD Clo A1, A2 | App B MPN/4_MPN/MPN by name | 7/19, 7/20/2006 | Turbid, Sediment, Reduction, None |
| SCD MUP 01, 02 | App B MPN/4_MPN/MPN by name | 5/7, 5/10, 5/17, 5/20, 5/23, 5/31, 6/28, 7/5, 7/13, 7/19, 7/25, 7/31, 8/7/2006 | Turbid, Sediment, Reduction, None |
| SCD MUP A | App B MPN/4_MPN/MPN by name | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |
| SCD sup O1, O2 | App B MPN/4_MPN/MPN by name | 4/26, 5/7, 5/23, 6/28, 7/5, 7/13, 7/19, 7/24, 7/31, 8/7 | Turbid, Sediment, Reduction, None |
| AML261 | App B MPN/4_MPN/MPN by name/Mpn | 7/26/2006 | Turbid, Sediment, Reduction, None |
| AS5 A | App B MPN/4_MPN/MPN by name/Mpn | 7/20/2006 | Turbid, Sediment, Reduction, None |
| AS5 O | App B MPN/4_MPN/MPN by name/Mpn | 7/28, 8/8/2006 | Turbid, Sediment, Reduction, None |
| AS113 | App B MPN/4_MPN/MPN by name/Mpn | 7/28/2006 | Turbid, Sediment, Reduction, None |
| DCUP | App B MPN/4_MPN/MPN by name/Mpn | 6/26, 6/27, 7/5, 7/10, 7/28, 7/24, 8/9/2006 | Turbid, Sediment, Reduction, None |
| Dcup AC | App B MPN/4_MPN/MPN by name/Mpn | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |
| Dmup A | App B MPN/4_MPN/MPN by name/Mpn | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |
| Dmup AC | App B MPN/4_MPN/MPN by name/Mpn | 6/27/2006 | Turbid, Sediment, Reduction, None |
| DSUP | App B MPN/4_MPN/MPN by name/Mpn | 6/26/2006 | Turbid, Sediment, Reduction, None |

Table B1-5. List of MPN ICPMS data files on CD, continued.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| Dsup AC | App B MPN/4_MPN/MPN by name/Mpn | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |
| M145 A | App B MPN/4_MPN/MPN by name/Mpn | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |
| m145 O1, O2 | App B MPN/4_MPN/MPN by name/Mpn | 6/28, 7/5, 7/13, 7/18, 7/25, 7/31, 8/7, 8/15/2006 | Turbid, Sediment, Reduction, None |
| Mev Sup O | App B MPN/4_MPN/MPN by name/Mpn | 8/8/2006 | Turbid, Sediment, Reduction, None |
| ML31 Ac | App B MPN/4_MPN/MPN by name/Mpn | 7/28/2006 | Turbid, Sediment, Reduction, None |
| ML261 A | App B MPN/4_MPN/MPN by name/Mpn | 7/28/2006 | Turbid, Sediment, Reduction, None |
| ML261 | App B MPN/4_MPN/MPN by name/Mpn | 7/28, 8/1, 8/8, 8/14, 2006 | Turbid, Sediment, Reduction, None |
| MM32 O | App B MPN/4_MPN/MPN by name/Mpn | 7/31, 8/1/2006 | Turbid, Sediment, Reduction, None |
| MM32 | App B MPN/4_MPN/MPN by name/Mpn | 7/28/2006 | Turbid, Sediment, Reduction, None |
| mm178 o | App B MPN/4_MPN/MPN by name/Mpn | 8/9, 9/14/2006 | Turbid, Sediment, Reduction, None |
| MS5 O | App B MPN/4_MPN/MPN by name/Mpn | 7/14, 8/9, 8/14/2006 | Turbid, Sediment, Reduction, None |
| MS5 | App B MPN/4_MPN/MPN by name/Mpn | Blank | Blank |
| Ms285 O | App B MPN/4_MPN/MPN by name/Mpn | 8/9, 8/14, 8/24/2006 | Turbid, Sediment, Reduction, None |
| MS 73 O | App B MPN/4_MPN/MPN by name/Mpn | 8/8, 8/14/2006 | Turbid, Sediment, Reduction, None |
| SCA C10 (version 1) | App B MPN/4_MPN/MPN by name/Mpn | 7/5, 7/10, 7/18, 7/24/2006 | Turbid, Sediment, Reduction, None |
| SCA C10 | App B MPN/4_MPN/MPN by name/Mpn | 7/5, 7/10, 7/18, 7/24/2006 | Turbid, Sediment, Reduction, None |
| SCA M145 | App B MPN/4_MPN/MPN by name/Mpn | 5/15, 5/25, 5/26, 5/28, 6/26, 7/5, 7/10, 8/1, 8/8, 8/14/2006 | Turbid, Sediment, Reduction, None |

Table B1-5. List of MPN ICPMS data files on CD, continued.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| Sca S113 | App B MPN/4_MPN/MPN by name/Mpn | 5/26, 5/31, 6/27, 7/5, 7/10, 7/18, 7/24, 8/2, 8/8, 8/14/2006 | Turbid, Sediment, Reduction, None |
| SCA Smid | App B MPN/4_MPN/MPN by name/Mpn | 6/26, 7/5, 7/10, 7/18, 7/24, 8/2, 8/7, 8/14/2006 | Turbid, Sediment, Reduction, None |
| SCA SUP O1, O2, OC | App B MPN/4_MPN/MPN by name/Mpn | 8/1, 8/8/2006 | Turbid, Sediment, Reduction, None |
| SCD C10, 01, 02 | App B MPN/4_MPN/MPN by name/Mpn | 4/26, 5/7, 5/15, 6/28, 7/5, 7/13, 7/19, 7/24, 7/31, 8/7, 8/14/2006 | Turbid, Sediment, Reduction, None |
| SCD CLO | App B MPN/4_MPN/MPN by name/Mpn | 7/20, 7/24/2006 | Turbid, Sediment, Reduction, None |
| SCD Clo A1, A2 | App B MPN/4_MPN/MPN by name/Mpn | 7/19, 7/20/2006 | Turbid, Sediment, Reduction, None |
| SCD MUP 01, 02 | App B MPN/4_MPN/MPN by name/Mpn | 5/7, 5/10, 5/17, 5/20, 5/23, 5/31, 6/28, 7/5, 7/13, 7/19, 7/25, 7/31, 8/7/2006 | Turbid, Sediment, Reduction, None |
| SCD MUP A | App B MPN/4_MPN/MPN by name/Mpn | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |
| SCD sup O1, O2 | App B MPN/4_MPN/MPN by name/Mpn | 4/26, 5/7, 5/23, 6/28, 7/5, 7/13, 7/19, 7/24, 7/31, 8/7/2006 | Turbid, Sediment, Reduction, None |
| 070806quant | App B MPN/4_MPN/MPNs | 7/8/2006 | Se 74, 76, 77, 78, 80, and 82 |
| 100406quant | App B MPN/4_MPN/MPNs | 10/4/2006 | Se 74, 76, 77, 78, 80, and 82 |
| AML261 | App B MPN/4_MPN/MPNs | 7/28/2006 | Turbid, Sediment, Reduction, None |
| AS5A | App B MPN/4_MPN/MPNs | 7/20, 8/22/2006 | Turbid, Sediment, Reduction, None |
| AS5O | App B MPN/4_MPN/MPNs | 7/28, 8/8, 9/7, 9/8/2006 | Turbid, Sediment, Reduction, None |
| AS113 | App B MPN/4_MPN/MPNs | 7/28, 8/22/2006 | Turbid, Sediment, Reduction, None |
| A-S5O | App B MPN/4_MPN/MPNs | 7/28, 8/8/2006 | Turbid, Sediment, Reduction, None |
| D-CUP | App B MPN/4_MPN/MPNs | 6/26, 6/27, 6/28, 7/5, 7/10, 7/28, 7/31, 8/14, 8/21, 9/1, 9/7, 9/14, 9/22/2006 | Turbid, Sediment, Reduction, None |
| DcupAC | App B MPN/4_MPN/MPNs | 7/20, 7/28, 8/22/2006 | Turbid, Sediment, Reduction, None |
| DmupA | App B MPN/4_MPN/MPNs | 7/20, 7/28, 8/22/2006 | Turbid, Sediment, Reduction, None |

Table B1-5. List of MPN ICPMS data files on CD, continued.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| Ds-Pac | App B MPN/4_MPN/MPNs | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |
| DsupAC | App B MPN/4_MPN/MPNs | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |
| LA071106quant | App B MPN/4_MPN/MPNs | 7/11, 7/12/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk010507quant | App B MPN/4_MPN/MPNs | 1/4/2007 | Se 74, 76, 77, 78, 80, and 82 |
| lk012807quant | App B MPN/4_MPN/MPNs | 1/28, 1/29/2007 | Se 74, 76, 77, 78, 80, and 82 |
| LK070806quant | App B MPN/4_MPN/MPNs | 7/8/2006 | Se 74, 76, 77, 78, 80, and 82 |
| LK071106quant | App B MPN/4_MPN/MPNs | 7/11/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk081406quant | App B MPN/4_MPN/MPNs | 8/14, 8/15/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk91206Aquant | App B MPN/4_MPN/MPNs | 9/12, 9/13/2006 | Se 74, 76, 77, 78, 80, and 82 |
| LK091206quant | App B MPN/4_MPN/MPNs | 9/12/2006 | Se 74, 76, 77, 78, 80, and 82 |
| LK092206quant | App B MPN/4_MPN/MPNs | 9/22, 9/23/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk092906quant | App B MPN/4_MPN/MPNs | 9/29, 9/30/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk093006quant | App B MPN/4_MPN/MPNs | 9/30/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk100406quant | App B MPN/4_MPN/MPNs | 10/4/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk102306quant | App B MPN/4_MPN/MPNs | 10/22/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk102406quant | App B MPN/4_MPN/MPNs | 10/22, 10/23/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk102606quant | App B MPN/4_MPN/MPNs | 10/26, 10/27/2006 | Se 74, 76, 77, 78, 80, and 82 |
| LK102707 | App B MPN/4_MPN/MPNs | ? | ? |
| lk102806quant | App B MPN/4_MPN/MPNs | 10/28/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk110306quant | App B MPN/4_MPN/MPNs | 11/4/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk110406quant | App B MPN/4_MPN/MPNs | 11/4, 11/5/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk110506quant | App B MPN/4_MPN/MPNs | ? | Se 80, 82 |
| lk111006quant | App B MPN/4_MPN/MPNs | 11/10/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk180906quant | App B MPN/4_MPN/MPNs | 8/9/2006 | Se 74, 76, 77, 78, 80, and 82 |
| lk12110806quant | App B MPN/4_MPN/MPNs | 11/8/2006 | Se 74, 76, 77, 78, 80, and 82 |
| M145A | App B MPN/4_MPN/MPNs | 7/20, 7/28, 8/22/2006 | Turbid, Sediment, Reduction, None |
| m145O1, O2 | App B MPN/4_MPN/MPNs | 6/28, 7/5, 7/13, 7/19, 7/31, 8/7, 8/15, 8/21, 9/1, 9/7, 9/14, 9/22/2006 | Turbid, Sediment, Reduction, None |
| May282006 | App B MPN/4_MPN/MPNs | 5/28, 5/29/2006 | ? |
| MevSupO | App B MPN/4_MPN/MPNs | 8/8, 8/21, 9/1, 9/22/2006 | Turbid, Sediment, Reduction, None |

Table B1-5. List of MPN ICPMS data files on CD, continued.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| ML31 Ac | App B MPN/4_MPN/MPNs | 7/28/2006 | Turbid, Sediment, Reduction, None |
| ML261 | App B MPN/4_MPN/MPNs | 7/28, 8/8, 8/14, 8/21, 9/1, 9/8, 9/14, 9/22/2006 | Turbid, Sediment, Reduction, None |
| Ml261A | App B MPN/4_MPN/MPNs | 7/28, 8/22/2006 | Turbid, Sediment, Reduction, None |
| Mm32 | App B MPN/4_MPN/MPNs | 7/28, 8/22/2006 | Turbid, Sediment, Reduction, None |
| MM320 | App B MPN/4_MPN/MPNs | 8/1, 8/14, 9/1, 9/8, 9/14, 9/22/2006 | Turbid, Sediment, Reduction, None |
| mm178 o | App B MPN/4_MPN/MPNs | 8/9, 8/14, 9/1, 9/7, 9/14, 9/22/2006 | Turbid, Sediment, Reduction, None |
| mm-178o | App B MPN/4_MPN/MPNs | 8/9, 8/14, 8/21/2006 | Turbid, Sediment, Reduction, None |
| MS5 | App B MPN/4_MPN/MPNs | 8/22/2006 | Turbid, Sediment, Reduction, None |
| MS5O | App B MPN/4_MPN/MPNs | 8/9, 8/14, 8/21, 9/1, 9/7/2006 | Turbid, Sediment, Reduction, None |
| MS73O | App B MPN/4_MPN/MPNs | 8/8, 8/14, 8/21, 9/1, 9/8, 9/14, 9/22/2006 | Turbid, Sediment, Reduction, None |
| MS285A | App B MPN/4_MPN/MPNs | 8/22/2006 | Turbid, Sediment, Reduction, None |
| Ms285O | App B MPN/4_MPN/MPNs | 8/9, 8/14, 8/21, 9/7, 9/8, 9/14, 9/22/2006 | Turbid, Sediment, Reduction, None |
| Ms-285O | App B MPN/4_MPN/MPNs | 8/9, 8/14, 8/24, 9/1, 9/8/2006 | Turbid, Sediment, Reduction, None |
| SCAC10(version1) | App B MPN/4_MPN/MPNs | 7/5, 7/10, 7/18, 7/24/2006 | Turbid, Sediment, Reduction, None |
| SCAC10 | App B MPN/4_MPN/MPNs | 7/5, 7/10, 7/24, 7/30, 8/7, 8/14, 9/8, 9/14/2006 | Turbid, Sediment, Reduction, None |
| SCA-C10 | App B MPN/4_MPN/MPNs | 7/5, 7/10, 7/18, 7/24, 8/1, 8/21, 8/24, 9/14/2006 | Turbid, Sediment, Reduction, None |
| SCAM145 | App B MPN/4_MPN/MPNs | 5/15, 5/25, 5/26, 5/28, 6/26, 7/5, 7/10, 8/1, 8/8, 8/14, 8/21, 9/1, 9/7, 9/14, 9/22/2006 | Turbid, Sediment, Reduction, None |
| ScaS113 | App B MPN/4_MPN/MPNs | 5/26, 5/31, 6/27, 7/5, 7/10, 7/18, 7/24, 8/2, 8/8, 8/14, 8/21, 9/1, 9/8, 9/22/2006 | Turbid, Sediment, Reduction, None |
| SCASmid | App B MPN/4_MPN/MPNs | 6/26, 7/5, 7/10, 7/18, 7/24, 8/2, 8/7, 8/14, 9/8, 9/14, 9/22/2006 | Turbid, Sediment, Reduction, None |

Table B1-5. List of MPN ICPMS data files on CD, continued.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| SCASUPO1, O2, OC | App B MPN/4_MPN/MPNs | 8/1, 8/14, 8/21, 9/1, 9/7, 9/14, 9/22/2006 | Turbid, Sediment, Reduction, None |
| SCDC1002,02 | App B MPN/4_MPN/MPNs | 6/28, 7/5, 7/13, 7/19, 7/25, 7/31, 8/7, 8/14, 8/21, 9/1, 9/7, 9/14, 9/22/2006 | Turbid, Sediment, Reduction, None |
| SCDCLO | App B MPN/4_MPN/MPNs | 7/20, 7/24/2006 | Turbid, Sediment, Reduction, None |
| SCDCloA1, A2 | App B MPN/4_MPN/MPNs | 7/19, 7/22/2006 | Turbid, Sediment, Reduction, None |
| SCDMUP01.02 | App B MPN/4_MPN/MPNs | 5/7, 5/10, 5/17, 5/20, 5/23, 5/31, 6/28, 7/5, 7/13, 7/19, 7/25, 7/31, 8/7, 8/14, 8/21, 9/1, 9/7, 9/14, 9/22/2006 | Turbid, Sediment, Reduction, None |
| SCDMUPA | App B MPN/4_MPN/MPNs | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |
| SCDsupO1, O2 | App B MPN/4_MPN/MPNs | 6/28, 7/5, 7/13, 7/19, 7/25, 7/31, 8/7, 8/14, 8/21, 9/1, 9/7, 9/14, 9/22/2006 | Turbid, Sediment, Reduction, None |
| Setupinventory | App B MPN/4_MPN/MPNs | ? | ? |
| AML261 | App B MPN/Mpn | 7/28/2006 | Turbid, Sediment, Reduction, None |
| AS5 A | App B MPN/Mpn | 7/20/2006 | Turbid, Sediment, Reduction, None |
| AS5 O | App B MPN/Mpn | 7/28, 8/8/2006 | Turbid, Sediment, Reduction, None |
| AS113 | App B MPN/Mpn | 7/28/2006 | Turbid, Sediment, Reduction, None |
| DCUP | App B MPN/Mpn | 6/26, 7/5, 7/10, 7/28, 7/24, 8/9/2006 | Turbid, Sediment, Reduction, None |
| Dcup AC | App B MPN/Mpn | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |
| Dmup A | App B MPN/Mpn | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |
| Dmup AC | App B MPN/Mpn | 6/27/2006 | Turbid, Sediment, Reduction, None |
| DSUP | App B MPN/Mpn | 6/26/2006 | Turbid, Sediment, Reduction, None |
| Dsup AC | App B MPN/Mpn | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |
| M145 A | App B MPN/Mpn | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |

Table B1-5. List of MPN ICPMS data files on CD, continued.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| m145 O1, O2 | App B MPN/Mpn | 6/28, 7/5, 7/13, 7/19, 7/31, 8/7, 8/15/2006 | Turbid, Sediment, Reduction, None |
| Mev Sup O | App B MPN/Mpn | 8/8/2006 | Turbid, Sediment, Reduction, None |
| ML31 Ac | App B MPN/Mpn | 7/28/2006 | Turbid, Sediment, Reduction, None |
| Ml261 | App B MPN/Mpn | 7/28/2006 | Turbid, Sediment, Reduction, None |
| MM32 O | App B MPN/Mpn | 8/21/2006 | Turbid, Sediment, Reduction, None |
| Mm 32 | App B MPN/Mpn | 7/28/2006 | Turbid, Sediment, Reduction, None |
| mm178 o | App B MPN/Mpn | 8/9, 9/14/2006 | Turbid, Sediment, Reduction, None |
| MS5 O | App B MPN/Mpn | 7/14, 8/9, 8/14/2006 | Turbid, Sediment, Reduction, None |
| MS5 | App B MPN/Mpn | Blank | Blank |
| Ms285 O | App B MPN/Mpn | 8/9, 8/14, 8/24/2006 | Turbid, Sediment, Reduction, None |
| MS 73 O | App B MPN/Mpn | 8/8, 8/14/2006 | Turbid, Sediment, Reduction, None |
| SCA C10 (version 1) | App B MPN/Mpn | 7/5, 7/10, 7/18, 7/24/2006 | Turbid, Sediment, Reduction, None |
| SCA C10 | App B MPN/Mpn | 7/5, 7/10, 7/18, 7/24, 8/1, 8/24/2006 | Turbid, Sediment, Reduction, None |
| SCA M145 | App B MPN/Mpn | 5/15, 5/25, 5/26, 5/28, 6/26, 7/5, 7/10, 8/1, 8/8, 8/14/2006 | Turbid, Sediment, Reduction, None |
| Sca S113 | App B MPN/Mpn | 5/26, 5/31, 6/27, 7/5, 7/10, 7/18, 7/24, 8/2, 8/8, 8/14/2006 | Turbid, Sediment, Reduction, None |
| SCA Smid | App B MPN/Mpn | 6/26, 7/5, 7/10, 7/18, 7/24, 8/2, 8/7, 8/14/2006 | Turbid, Sediment, Reduction, None |
| SCA SUP O1, O2, OC | App B MPN/Mpn | 8/1, 8/8/2006 | Turbid, Sediment, Reduction, None |
| SCD C10, 01, 02 | App B MPN/Mpn | 4/26, 5/7, 5/15, 6/28, 7/5, 7/13, 7/19, 7/24, 7/31, 8/7, 8/14/2006 | Turbid, Sediment, Reduction, None |
| SCD CLO | App B MPN/Mpn | 7/20, 7/24/2006 | Turbid, Sediment, Reduction, None |
| SCD Clo A1, A2 | App B MPN/Mpn | 7/19, 7/20/2006 | Turbid, Sediment, |

Table B1-5. List of MPN ICPMS data files on CD, continued.

| File Name (Excel) | Current Location on Kirk CD | Date(s) of analysis | Analytes |
|---|---|---|---|
| | | | Reduction, None |
| SCD MUP 01, 02 | App B MPN/Mpn | 5/7, 5/10, 5/17, 5/20, 5/23, 5/31, 6/28, 7/5, 7/13, 7/19, 7/25, 7/31, 8/7/2006 | Turbid, Sediment, Reduction, None |
| SCD MUP A | App B MPN/Mpn | 7/20, 7/28/2006 | Turbid, Sediment, Reduction, None |
| SCD sup O1, O2 | App B MPN/Mpn | 4/26, 5/7, 5/23, 6/28, 7/5, 7/13, 7/19, 7/24, 7/31, 8/7/2006 | |
| MPN extract041606 | App B MPN/ | no file | |
| rock extracts | App B MPN/ | no file | |

# APPENDIX C

## MICROBIAL COMMUNITY CHARACTERIZATION DATA

# APPENDIX C

## MICROBIAL COMMUNITY CHARACTERIZATION DATA

C-1: Isolates

Table C1-1. Dry Valley GW7D Enrichment Series 1, March 2007
Table C1-2. Dry Valley GW7D Enrichment Series 2, June 2007
Table C1-3. Enrichment Results for Drilling Shale Samples
Table C1-4. Summary of Unique Isolates from this SE Idaho Se Study

On DVD:
C1.1 Isolation
Methods (multiple files with notes, photos)
Results (spreadsheets)
C1.2 Enrichments
Results (photographs)
To request DVD copies contact your local public or university library to place an interlibrary loan request to Montana State University. Questions call 406-994-3161.

C-2: On DVD: DNA Sequence Data
To request DVD copies contact your local public or university library to place an interlibrary loan request to Montana State University. Questions call 406-994-3161.

C-3: Clone Libraries

Figure C3.1 As113 Archaeal Rarefaction Curve for Clone Library
Figure C3.2 As71 Bacterial Rarefaction Curve for Clone Library
Figure C3.3 As113 Bacterial Rarefaction Curve for Clone Library
Table C3-1. Smoky Canyon Sample AS71 Bacterial Clone Library
Table C3-2. Smoky Canyon Sample AS113 Bacterial Clone Library

On DVD:
C3.1 Clone Library Methods and Data
C3.2 Bioinformatics
To request DVD copies contact your local public or university library to place an interlibrary loan request to Montana State University. Questions call 406-994-3161.

C-4: On DVD: DGGE Images
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Table C1-1. Dry Valley GW7D Enrichment Series 1

**Dry Valley GW7D Enrichments March 2007**
GW diluted serially with filter sterilized GW
Specific electron donor, 3 concentrations

| Carbon | Slide | SeO4, mM | -1 | -2 | -3 | -4 |
|---|---|---|---|---|---|---|
| NATC | 1 | 0.2 | +oj/b | +oj/b | - | - |
| NATC | 1 | 2 | +oj | +oj | ++r | +oj |
| NATC | 1 | 10 | +++ | ++r | ++r | +oj |
| LACT | 2 | 0.2 | +b | + | ++ | + |
| LACT | 2 | 2 | ++ro | ++r | +lo | ++r |
| LACT | 2 | 10 | +oj | +oj | +lo | ++ro |
| ACET | 3 | 0.2 | +b | +b | +r | +oj |
| ACET | 3 | 2 | +++r | +++r | ++oj | +oj |
| ACET | 3 | 10 | ++oj | ++oj | +oj | ++ro |
| PYRUV | 4 | 0.2 | +pb | +ob | +b | +rb |
| PYRUV | 4 | 2 | broken | +++r | +r | r |
| PYRUV | 4 | 10 | ++r | +r | +r | +r |
| H2/CO2 | 5 | 0.2 | +oj | +oj | ++oj | +oj |
| H2/CO2 | 5 | 2 | ++oj | +oj | +oj | ++++red |
| H2/CO2 | 5 | 10 | ++oj | ++++red | ++++red | ++oj |

*- indicates negative for selenate reduction*
*+ indicates positive for selenate reduction.*

| | | | |
|---|---|---|---|
| | | *r* | *red* |
| | | *ro* | *red orange* |
| + | *slight color* | *lo* | *light orange* |
| ++ | *mod color* | *b* | *black* |
| +++ | *strong color* | *oj* | *orange* |
| ++++ | *str color, turbid* | *rb* | *red black* |

*Note: See attached *.ppt slides 1-5*

Table C1-2. Dry Valley GW7D Enrichment Series 2

**Dry Valley GW7D Enrichments June 2007**

GW diluted serially with filter sterilized GW
Specific electron donor, 3 concentrations

| E donor | Slide | SeO4, mM | -1 | -2 | -3 | -4 |
|---|---|---|---|---|---|---|
| NATC | 6 | 0.2 | +oj/b | - | +oj | - |
| NATC | 6 | 2 | +oj | +ojb | +oj | - |
| NATC | 6 | 10 | ++oj | +++oj | ++oj | +oj |
| LACT | 7 | 0.2 | ++oj | +oj | +roj | ++roj |
| LACT | 7 | 2 | +++r | +oj | +oj | +roj |
| LACT | 7 | 10 | +oj | +++oj | +oj | ++oj |
| ACET | 8 | 0.2 | ++OJ | +r | +p | +p |
| ACET | 8 | 2 | ++r | +r | +oj | ++oj |
| ACET | 8 | 10 | +++roj | ++oj | ++oj | ++oj |
| PYRUV | 9 | 0.2 | +pb | +ob | +b | +r |
| PYRUV | 9 | 2 | +oj | +oj | +r | +oj |
| PYRUV | 9 | 10 | ++r | +r | +r | +r |
| H2/CO2 | 10 | 0.2 | +ojb | +oj | +oj | +oj |
| H2/CO2 | 10 | 2 | ++ojr | +oj | +oj | +oj |
| H2/CO2 | 10 | 10 | ++oj | +++oj | ++++oj | ++++oj |

*- indicates negative for selenate reduction*
*+ indicates positive for selenate reduction.*

| | | | |
|---|---|---|---|
| | | *r* | *red* |
| + | *slight color* | *ro* | *red orange* |
| ++ | *mod color* | *lo* | *light oj* |
| +++ | *strong color* | *b* | *black* |
| ++++ | *str color, turbid* | *oj* | *orange* |
| | | *rb* | *red black* |

*Note: See attached *.ppt slides 5-10*

Table C1-3. Enrichment Results for Drilling Shale Samples.

live shale enrichments started 2/27/07
filter sterilized groundwater with selenate added, 5 mM;
C cocktail (native C, acetate, pyruvate, lactate) 1.25 mM each Σ5 Mm
nitrogen purged headspace with half of volume replaced with 1:1 H2 and CO2.
incubated at 11oC

| | | | | 2/27/07 enr | | | | | 4-12-07 dil |
|---|---|---|---|---|---|---|---|---|---|
| ID | Slide* | Mine | Depth | -1 | -2 | -3 | -4 | -5 | redilute |
| MS5 | 11 | Monsanto Enoch Valley | 5 | ? | + | + | + | - | +3 1:10, 1:100 |
| MS73 | 12 | Monsanto Enoch Valley | 73 | ? | + | + | + | + | +4 1:100 |
| MS285 | 13 | Monsanto Enoch Valley | 285 | ? | + | + | -/+ | - | +3 1:10, 1:100 |
| AS5 | 14 | Smoky Canyon A Dump | 5 | ? | ? | + | - | - | +3 1:100 |
| AS71 | 15 | Smoky Canyon A Dump | 71 | ? | + | - | - | - | +3 1:10 |
| AS113 | 16 | Smoky Canyon A Dump | 113 | ? | ? | + | + | - | +3 1:10, 1:100 |
| DS75 | 17 | Smoky Canyon D Backfill | 75 | ? | ? | - | - | - | |

control
control no C added

? Indicates cannot be determined visually
+ indicates positive for selenate reduction, - indicates negative for selenate

Table C1-4. List of Unique Isolates Obtained for this Project in SE Idaho.

| Tag | Genus | Se4 Grw | Se4 Rdn | Se6 Grw | Se6 Rdn | Source |
|---|---|---|---|---|---|---|
| A34 | Dechloromonas | | | | | from H. Knotek-Smith Smoky Canyon sample |
| L33 | Dechloromonas | | | | | from H. Knotek-Smith Smoky Canyon sample |
| L35 | Dechloromonas | | | | | from H. Knotek-Smith Smoky Canyon sample |
| LK1 | Dechloromonas | +++ | red | +++ | | from Lisa 120728 |
| E51Y | Dechloromonas | +++ | slight | +++ | | from variably weathered shales collected by Lisa in 2005 |
| CMS | Dechloromonas | | | | | from variably weathered shales collected by Lisa in 2005 |
| R. ferrireducens | | + | weak | ++ | | from Lovley lab |
| | | | | | | |
| AV1a | Sphingomonas | +++ | dk red | +++ | none | from Lisa pure culture plate sent to me ("Acidovorax") |
| AV3 | Oleomonas | +++ | red | +++ | none | from Lisa pure culture plate sent to me ("Acidovorax") |
| RF3 | Sphingobium | +++ | med red | +++ | none | from Lisa pure culture plate sent to me ("Rhodoferax ferrireducens") |
| CNT5 | Pseudomonas | +++ | lt red | +++ | none | from Lisa 100768 |
| E5-4a | Cellulomonas | +++ | dk red | +++ | none | from variably weathered shales collected by Lisa in 2005 |
| DV1a | Cellulomonas | +++ | some | +++ | none | soil from Dry Valley reclaimed site; collected Aug 2008 |
| DV1b | Nocardiodes | + | weak | ++ | none | same as above |
| DV4 | Sporosarcina | + | weak | +++ | none | same as above |
| DV5a | Cellulomonas | +++ | lt red | +++ | none | same as above |
| DV6 | Arthrobacter | +++ | dk red | +++ | none | same as above |
| DV9 | Arthro. chlorophenolicus | +++ | dk red | +++ | none | same as above |
| | | *growth determined on R2A plates; aerobic incubation | | | | |
| | | | | | | |
| P93(pl) C6A | Stenotrophomonas maltophilia | | | | | from Lisa's liquid cultures labeled "P93(plate)"--mailed to me |
| P93(pl) C6B | Stenotrophomonas maltophilia | | | | | same as above |
| P93(pl) C7 | Stenotrophomonas maltophilia | | | | | same as above |
| P93(pl) C8 | Rahnella | | | | | same as above |
| P93-0 A1 | Pseudomonas | | | | | from Lisa's liquid culture labeled P93sub0--mailed to me |
| P93-0 A2 | Pseudomonas | | | | | same as above |
| | | | | | | |
| RF1 B3 | Stenotrophomonas maltophilia | | | | | from Lisa plate of "Rhodoferax ferrireducens" pure culture-sent to UI |
| RF1 B3a | Stenotrophomonas maltophilia | | | | | same as above |
| RF1 B4a | Rahnella | | | | | same as above |
| RF1 B4b | Rahnella | | | | | same as above |

Table C1-4. List of Unique Isolates Obtained for this Project in SE Idaho, continued.

| Tag | Genus | Se4 Grw | Se4 Rdn | Se6 Grw | Se6 Rdn | Source |
|---|---|---|---|---|---|---|
| RF1 B4c | Rahnella | | | | | same as above |
| RF1 B5 | Stenotrophomonas maltophilia | | | | | same as above |
| | | | | | | |
| AS113N1 7E1 | Rhodoferax ferrireducens | | | | | from MPN culture Lisa sent-AS113N17E |
| AS113N1 7E2 | (Actinobacterium) | | | | | from MPN culture Lisa sent-AS113N17E |
| AS113N1 7E3 | Rhodoferax ferrireducens | | | | | from MPN culture Lisa sent-AS113N17E |
| AS113N1 7E4b | Cellulomonas | | | | | from MPN culture Lisa sent-AS113N17E |
| AS113N1 7E4c | Cellulomonas | | | | | from MPN culture Lisa sent-AS113N18A |
| AS113N2 8A1A | Rhodoferax | | | | | from MPN culture Lisa sent-AS113N18A |
| AS113N2 8A1B | Rhodoferax | | | | | from MPN culture Lisa sent-AS113N18A |
| AS113N2 8A2 | Actinobacteria | | | | | from MPN culture Lisa sent-AS113N18A |
| AS113N2 8A4a | Cellulomonas | | | | | from MPN culture Lisa sent-AS113N18A |
| AS113N2 8A4c | Cellulomonas | | | | | from MPN culture Lisa sent-AS113N18A |
| | | | | | | |
| L2A1 X | Stenotrophomonas | | | | | from Lisa 120929 |
| L2A1 Y | Microbacterium | | | | | from Lisa 120929 |
| L2E | Stenotrophomonas | | | | | from Lisa 120929 |
| L2P | Stenotrophomonas | | | | | from Lisa 120929 |
| L5C1-A | Stenotrophomonas | | | | | from Lisa 100768 |
| L5C1-B | Stenotrophomonas | | | | | from Lisa 100768 |
| L5C2 | Stenotrophomonas | | | | | from Lisa 100768 |
| L5E1 | Stenotrophomonas | | | | | from Lisa 100768 |
| L5R | Microbacterium | | | | | from Lisa 100768 |
| L12H | Stenotrophomonas | | | | | from Lisa 100715 |
| L12N | Microbacterium | | | | | from Lisa 100715 |
| L12F | Sphingobium yanoikuyae | | | | | from Lisa 100715 |
| L12W | Rhodopseudomonas palustris | | | | | from Lisa 100715 |
| L12J2-A | Brevundimonas | | | | | from Lisa 100715 |
| L12J2-B | Brevundimonas | | | | | from Lisa 100715 |

Figure C3-1. Rarefaction curve for archaeal library of AS113.

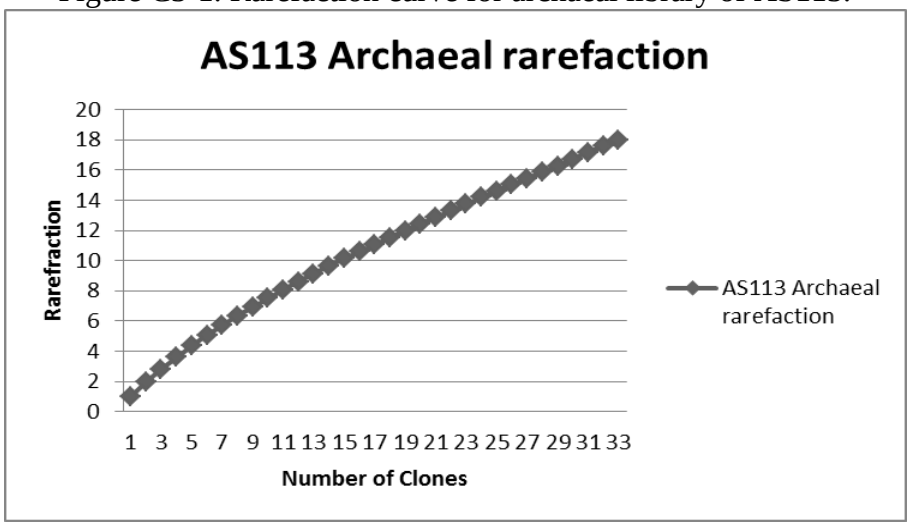



Figure C3-2. Rarefaction Curve for bacterial library of AS71.

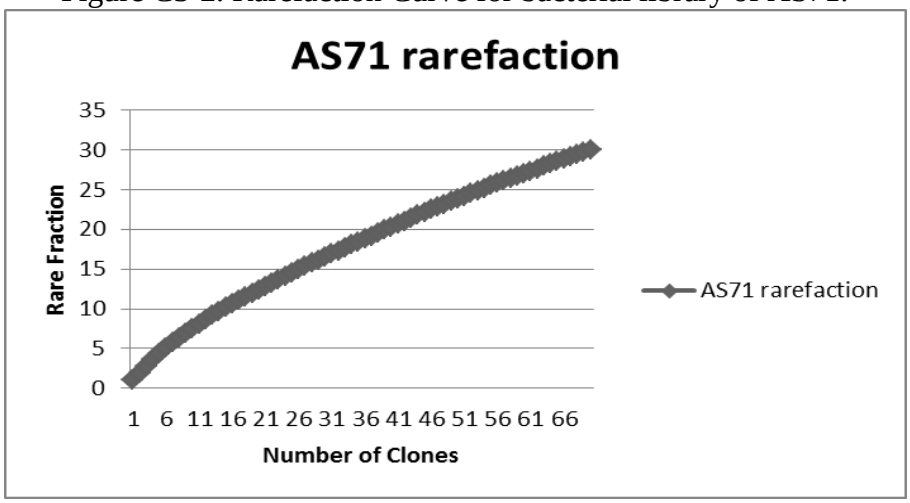



Figure C3-3. Rarefaction Curve for bacterial library of AS113.

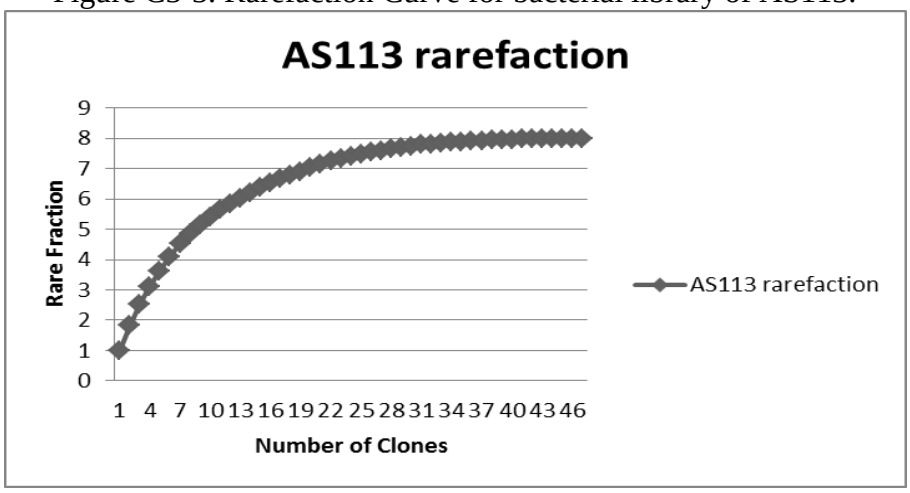


Table C3-1. Smoky Canyon Sample AS71 Clone Library.

| Sample ID | Accession | Description | Max Score | Total Score | Query Coverage | E Value | Max Ident |
|---|---|---|---|---|---|---|---|
| AS71-7 | AB166733.1 | Thiothrix NKBI-C gene for 16S rRNA, partial sequence | 564 | 564 | 96% | 1.00E-157 | 95% |
| AS71-46 | AB426569.1 | Polaromonas UF008 gene for 16S ribosomal RNA, partial sequence | 647 | 647 | 96% | 0 | 100% |
| AS71-56 | AB426569.1 | Polaromonas UF008 gene for 16S ribosomal RNA, partial sequence | 647 | 647 | 96% | 0.00E+00 | 100% |
| AS71-59 | AB426569.1 | Polaromonas UF008 gene for 16S ribosomal RNA, partial sequence | 647 | 647 | 96% | 0.00E+00 | 100% |
| AS71-61 | AB426569.1 | Polaromonas UF008 gene for 16S ribosomal RNA, partial sequence | 647 | 647 | 100% | 0.00E+00 | 100% |
| AS71-63 | AB426569.1 | Polaromonas UF008 gene for 16S ribosomal RNA, partial sequence | 641 | 641 | 96% | 0.00E+00 | 99% |
| AS71-69 | AB426569.1 | Polaromonas UF008 gene for 16S ribosomal RNA, partial sequence | 647 | 647 | 97% | 0.00E+00 | 100% |
| AS71-73 | AB426569.1 | Polaromonas UF008 gene for 16S ribosomal RNA, partial sequence | 647 | 647 | 96% | 0.00E+00 | 100% |
| AS71-74 | AB426569.1 | Polaromonas UF008 gene for 16S ribosomal RNA, partial sequence | 647 | 647 | 96% | 0.00E+00 | 100% |
| AS71-2 | AF293013.1 | Uncultured Green Bay ferromanganous micronodule bacterium MNA3 16S ribosomal RNA gene, partial sequence | 616 | 616 | 96% | 3.00E-173 | 98% |
| AS71-28 | AF293013.1 | Uncultured Green Bay ferromanganous micronodule bacterium MNA3 16S ribosomal RNA gene, partial sequence | 610 | 610 | 96% | 1.00E-171 | 98% |
| AS71-39 | AF482687.1 | Myxobacterium KC 16S ribosomal RNA gene, partial sequence | 625 | 625 | 100% | 5.00E-176 | 98% |
| AS71-43 | AF482687.1 | Myxobacterium KC 16S ribosomal RNA gene, partial sequence | 619 | 619 | 100% | 2.00E-174 | 98% |
| AS71-47 | AF482687.1 | Myxobacterium KC 16S ribosomal RNA gene, partial sequence | 619 | 619 | 100% | 2.00E-174 | 100% |
| AS71-49 | AF482687.1 | Myxobacterium KC 16S ribosomal RNA gene, partial sequence | 630 | 630 | 100% | 1.00E-177 | 99% |
| AS71-50 | AF482687.1 | Myxobacterium KC 16S ribosomal RNA gene, partial sequence | 614 | 614 | 100% | 1.00E-172 | 98% |
| AS71-51 | AF482687.1 | Myxobacterium KC 16S ribosomal RNA gene, partial sequence | 625 | 625 | 100% | 5.00E-176 | 98% |
| AS71-53 | AF482687.1 | Myxobacterium KC 16S ribosomal RNA gene, partial sequence | 630 | 630 | 100% | 1.00E-177 | 99% |
| AS71-54 | AF482687.1 | Myxobacterium KC 16S ribosomal RNA gene, partial sequence | 625 | 625 | 100% | 5.00E-176 | 98% |
| AS71-57 | AF482687.1 | Myxobacterium KC 16S ribosomal RNA gene, partial sequence | 630 | 630 | 100% | 1.00E-177 | 99% |
| AS71-60 | AF482687.1 | Myxobacterium KC 16S ribosomal RNA gene, partial sequence | 627 | 627 | 100% | 1.00E-176 | 98% |
| AS71-68 | AF482687.1 | Myxobacterium KC 16S ribosomal RNA gene, partial sequence | 630 | 630 | 100% | 1.00E-177 | 99% |
| AS71-71 | AF482687.1 | Myxobacterium KC 16S ribosomal RNA gene, partial sequence | 630 | 630 | 100% | 1.00E-177 | 99% |
| AS71-25 | AJ414655.1 | Methylobacter tundripaludum 16S ribosomal RNA, type strain SV96T | 532 | 532 | 96% | 3.00E-148 | 92% |
| AS71-58 | AM934876.1 | Uncultured Nitrospirales bacterium partial 16S rRNA gene, clone AMJC5 | 636 | 636 | 96% | 2.00E-179 | 99% |
| AS71-9 | AM935618.1 | Uncultured Desulfuromonadaceae bacterium partial 16S rRNA gene, clone AMDF7 | 617 | 617 | 92% | 9.00E-174 | 100% |
| AS71-67 | AM936431.1 | Uncultured Rhodobacterales bacterium partial 16S rRNA gene, clone CM38A2 | 608 | 608 | 100% | 5.00E-171 | 98% |
| AS71-62 | AM990839.1 | Arenimonas MOLA 64 partial 16S rRNA gene, culture collection MOLA:64 | 619 | 619 | 100% | 0.00E+00 | 98% |
| AS71-10 | AY491577.1 | Uncultured bacterium clone oc34 16S ribosomal RNA gene, partial sequence | 593 | 593 | 96% | 1.00E-166 | 96% |
| AS71-19 | CP000698.1 | Geobacter uraniireducens Rf4, complete genome | 621 | 1243 | 96% | 7.00E-175 | 98% |
| AS71-17 | D84568.2 | Pseudomonas S21027 gene for 16S ribosomal RNA, partial sequence | 448 | 448 | 90% | 1.00E-122 | 87% |
| AS71-38 | D84645.2 | Variovorax S24561 gene for 16S ribosomal RNA, partial sequence | 641 | 641 | 96% | 0 | 99% |
| AS71-44 | D84645.2 | Variovorax S24561 gene for 16S ribosomal RNA, partial sequence | 641 | 641 | 96% | 0 | 100% |

Table C3-1. Smoky Canyon Sample AS71 Clone Library, continued.

| Sample ID | Accession | Description | Max Score | Total Score | Query Coverage | E Value | Max Ident |
|---|---|---|---|---|---|---|---|
| AS71-45 | D84645.2 | Variovorax S24561 gene for 16S ribosomal RNA, partial sequence | 641 | 641 | 96% | 0.00E+00 | 100% |
| AS71-3 | DQ378240.1 | Uncultured soil bacterium clone M20_Pitesti 16S ribosomal RNA gene, complete sequence | 599 | 599 | 96% | 3.00E-168 | 97% |
| AS71-37 | DQ489306.1 | Aquabacterium hongkongensis strain CA5 16S ribosomal RNA gene, partial sequence | 532 | 532 | 85% | 4.00E-148 | 91% |
| AS71-34 | DQ837236.1 | Uncultured candidate division OP10 bacterium clone 49S1_2B_10 16S ribosomal RNA gene, partial sequence | 619 | 619 | 96% | 2.00E-174 | 99% |
| AS71-13 | DQ837241.1 | Uncultured Firmicutes bacterium clone 49S1_2B_19 16S ribosomal RNA gene, partial sequence | 608 | 608 | 96% | 5.00E-171 | 97% |
| AS71-72 | EF220453.1 | Uncultured actinobacterium clone FI-2F_H01 16S gene, partial sequence | 623 | 623 | 96% | 2.00E-175 | 98% |
| AS71-24 | EF467590.1 | Uncultured bacterium clone lka50b 16S ribosomal RNA gene, partial sequence | 643 | 643 | 96% | 0 | 99% |
| AS71-16 | EU194898.1 | Methylobacillus M8 16S ribosomal RNA gene, partial sequence | 167 | 281 | 50% | 4.00E-38 | 98% |
| AS71-65 | EU215386.1 | Pelosinus UFO1 16S ribosomal RNA pseudogene, complete sequence | 652 | 652 | 100% | 0.00E+00 | 100% |
| AS71-55 | EU266783.1 | Uncultured Thiotrichaceae bacterium clone D10_10 small subunit riboso | 647 | 647 | 100% | 0.00E+00 | 100% |
| AS71-66 | FJ711197.1 | Afipia KC-IT-F4 16S ribosomal RNA gene, partial sequence | 647 | 647 | 100% | 0.00E+00 | 100% |
| AS71-22 | FJ713034.1 | Uncultured Acidobacteria bacterium clone 49 16S ribosomal RNA gene, partial sequence | 652 | 652 | 96% | 0 | 100% |
| AS71-21 | FJ823826.1 | Uncultured Rhodocyclaceae bacterium clone MFC68E10 16S ribosomal RNA gene, partial sequence | 534 | 534 | 73% | 1.00E-148 | 91% |
| AS71-40 | FJ939131.1 | Anaeromyxobacter IN2 16S ribosomal RNA gene, partial sequence | 285 | 285 | 90% | 5.00E-74 | 97% |
| AS71-42 | FM955859.1 | Polaromonas Asd M3-1 16S rRNA gene, strain Asd M3-1 | 647 | 647 | 96% | 0 | 100% |
| AS71-52 | FM955859.1 | Polaromonas Asd M3-1 16S rRNA gene, strain Asd M3-1 | 647 | 647 | 96% | 0.00E+00 | 100% |
| AS71-64 | FM955859.1 | Polaromonas Asd M3-1 16S rRNA gene, strain Asd M3-1 | 625 | 625 | 96% | 5.00E-176 | 98% |
| AS71-70 | FM955859.1 | Polaromonas Asd M3-1 16S rRNA gene, strain Asd M3-1 | 647 | 647 | 96% | 0.00E+00 | 100% |
| AS71-33 | EF019585.1 | Uncultured Mycobacteriaceae bacterium clone Elev_16S_1104 16S ribosomal RNA gene, partial sequence | 617 | 617 | 95% | 9.00E-174 | 99% |
| AS71-5 | DQ837241.1 | Uncultured Firmicutes bacterium clone 49S1_2B_19 16S ribosomal RNA gene, partial sequence | 608 | 608 | 96% | 5.00E-171 | 97% |
| AS71-23 | DQ837241.1 | Uncultured Firmicutes bacterium clone 49S1_2B_19 16S ribosomal RNA gene, partial sequence | 597 | 597 | 96% | 1.00E-167 | 96% |
| AS71-27 | DQ837241.1 | Uncultured Firmicutes bacterium clone 49S1_2B_19 16S ribosomal RNA gene, partial sequence | 614 | 614 | 96% | 1.00E-172 | 98% |
| AS71-30 | DQ837241.1 | Uncultured Firmicutes bacterium clone 49S1_2B_19 16S ribosomal RNA gene, partial sequence | 608 | 608 | 96% | 5.00E-171 | 97% |
| AS71-48 | AF387301.2 | Iron-oxidizing acidophile m-1 16S ribosomal RNA gene, partial sequence | 575 | 575 | 100% | 5.00E-161 | 96% |
| AS71-12 | AF387301.2 | Iron-oxidizing acidophile m-1 16S ribosomal RNA gene, partial sequence | 577 | 577 | 96% | 1.00E-161 | 96% |
| AS71-11 | AB252945.1 | Uncultured Nitrospirae bacterium gene for 16S rRNA, partial sequence, clone: 356 | 601 | 601 | 96% | 9.00E-169 | 97% |
| AS71-15 | AB252945.1 | Uncultured Nitrospirae bacterium gene for 16S rRNA, partial sequence, clone: 356 | 601 | 601 | 96% | 9.00E-169 | 97% |
| AS71-29 | EU266874.1 | Uncultured Syntrophaceae bacterium clone D15_37 small subunit ribosomal RNA gene, partial sequence | 584 | 584 | 96% | 9.00E-164 | 95% |

Table C3-1. Smoky Canyon Sample AS71 Clone Library, continued.

| Sample ID | Accession | Description | Max Score | Total Score | Query Coverage | E Value | Max Ident |
|---|---|---|---|---|---|---|---|
| AS71-36 | EU266874.1 | Uncultured Syntrophaceae bacterium clone D15_37 small subunit ribosomal RNA gene, partial sequence | 521 | 622 | 96% | 7.00E-145 | 100% |
| AS71-41 | EU202763.1 | Uncultured Acidobacteriales bacterium clone Plot29-2C11 16S ribosomal RNA gene, partial sequence | 599 | 599 | 100% | 3.00E-168 | 97% |
| AS71-31 | AM690820.1 | Uncultured actinobacterium partial 16S rRNA gene, clone TH1-16 | 608 | 608 | 96% | 5.00E-171 | 97% |
| AS71-32 | AM690820.1 | Uncultured actinobacterium partial 16S rRNA gene, clone TH1-16 | 608 | 608 | 96% | 5.00E-171 | 97% |
| AS71-8 | EU266808.1 | Uncultured Thiotrichaceae bacterium clone D10_44 small subunit ribosomal RNA gene, partial sequence | | | | | |
| AS71-18 | FJ802331.1 | Iron-reducing bacterium enrichment culture clone FEA_2_A7 16S ribosomal RNA gene, partial sequence | 643 | 643 | 96% | 0 | 99% |
| AS71-4 | EU266808.1 | Uncultured Thiotrichaceae bacterium clone D10_44 small subunit ribosomal RNA gene, partial sequence | 599 | 599 | 96% | 3.00E-168 | 97% |
| AS71-6 | EU266808.1 | Uncultured Thiotrichaceae bacterium clone D10_44 small subunit ribosomal RNA gene, partial sequence | 604 | 604 | 96% | 7.00E-170 | 97% |
| AS71-14 | EU266808.1 | Uncultured Thiotrichaceae bacterium clone D10_44 small subunit ribosomal RNA gene, partial sequence | 604 | 604 | 96% | 7.00E-170 | 97% |
| AS71-26 | EU266808.1 | Uncultured Thiotrichaceae bacterium clone D10_44 small subunit ribosomal RNA gene, partial sequence | | | | | |
| AS71-20 | AB473787.1 | Uncultured Rhodoferax gene for 16S rRNA, partial sequence, clone: AA_05_UNI | 544 | 544 | 96% | 1.00E-151 | 92% |

Table C3-2. Smoky Canyon Sample AS113 Clone Library.

| Sample ID | Accession | Description | Max Score | Total Score | Query Coverage | E Value | Max Ident |
|---|---|---|---|---|---|---|---|
| AS113-2 | AB426569.1 | Polaromonas sp. UF008 gene for 16S ribosomal RNA, partial sequence | 647 | 647 | 96% | 0 | 100% |
| AS113-15 | AB426569.1 | Polaromonas sp. UF008 gene for 16S ribosomal RNA, partial sequence | 647 | 647 | 96% | 0.00E+00 | 100% |
| AS113-18 | AB426569.1 | Polaromonas sp. UF008 gene for 16S ribosomal RNA, partial sequence | 647 | 647 | 96% | 0.00E+00 | 100% |
| AS113-24 | AB426569.1 | Polaromonas sp. UF008 gene for 16S ribosomal RNA, partial sequence | 647 | 647 | 96% | 0.00E+00 | 100% |
| AS113-26 | AB426569.1 | Polaromonas sp. UF008 gene for 16S ribosomal RNA, partial sequence | 647 | 647 | 96% | 0.00E+00 | 100% |
| AS113-27 | AB426569.1 | Polaromonas sp. UF008 gene for 16S ribosomal RNA, partial sequence | 641 | 641 | 96% | 0.00E+00 | 99% |
| AS113-37 | AB426569.1 | Polaromonas sp. UF008 gene for 16S ribosomal RNA, partial sequence | 647 | 647 | 96% | 0.00E+00 | 100% |
| AS113-6 | AF529125.1 | Uncultured Acidobacterium group bacterium clone FTLM5 16S ribosomal RNA gene, partial sequence | 652 | 652 | 96% | 0 | 100% |
| AS113-7 | AF529125.1 | Uncultured Acidobacterium group bacterium clone FTLM5 16S ribosomal RNA gene, partial sequence | 652 | 652 | 96% | 0 | 100% |
| AS113-14 | AB425279.1 | Sporotalea colonica gene for 16S rRNA, partial sequence | 652 | 652 | 100% | 0.00E+00 | 100% |
| AS113-10 | AM258974.1 | Sporotalea propionica partial 16S rRNA gene, strain TmPM3 | 649 | 649 | 96 | 0 | 100% |
| AS113-5 | CP000267.1 | Rhodoferax ferrireducens T118, complete genome | 641 | 1283 | 96% | 0 | 99% |
| AS113-8 | CP000267.1 | Rhodoferax ferrireducens T118, complete genome | 636 | 1272 | 96% | 2.00E-179 | 99% |
| AS113-12 | CP000267.1 | Rhodoferax ferrireducens T118, complete genome | 647 | 1294 | 96% | 0.00E+00 | 100% |
| AS113-23 | CP000267.1 | Rhodoferax ferrireducens T118, complete genome | 647 | 1294 | 96% | 0.00E+00 | 100% |
| AS113-25 | CP000267.1 | Rhodoferax ferrireducens T118, complete genome | 647 | 1294 | 96% | 0.00E+00 | 100% |
| AS113-30 | CP000267.1 | Rhodoferax ferrireducens T118, complete genome | 623 | 1296 | 98% | 2.00E-175 | 99% |
| AS113-31 | CP000267.1 | Rhodoferax ferrireducens T118, complete genome | 647 | 1294 | 96% | 0.00E+00 | 100% |
| AS113-41 | CP000267.1 | Rhodoferax ferrireducens T118, complete genome | 647 | 1294 | 96% | 0.00E+00 | 100% |
| AS113-43 | CP000267.1 | Rhodoferax ferrireducens T118, complete genome | 647 | 1294 | 96% | 0.00E+00 | 100% |
| AS113-44 | CP000267.1 | Rhodoferax ferrireducens T118, complete genome | 647 | 1294 | 96% | 0.00E+00 | 100% |
| AS113-45 | CP000267.1 | Rhodoferax ferrireducens T118, complete genome | 647 | 1294 | 96% | 0.00E+00 | 100% |
| AS113-17 | DQ145536.1 | Pelosinus fermentans strain R7 16S ribosomal RNA gene, partial sequence | 641 | 641 | 100% | 0.00E+00 | 99% |
| AS113-32 | DQ145536.1 | Pelosinus fermentans strain R7 16S ribosomal RNA gene, partial sequence | 652 | 652 | 100% | 0.00E+00 | 100% |
| AS113-36 | DQ145536.1 | Pelosinus fermentans strain R7 16S ribosomal RNA gene, partial sequence | 641 | 641 | 100% | 0.00E+00 | 99% |
| AS113-39 | DQ145536.1 | Pelosinus fermentans strain R7 16S ribosomal RNA gene, partial sequence | 652 | 652 | 100% | 0.00E+00 | 100% |

Table C3-2. Smoky Canyon Sample AS113 Clone Library, continued.

| Sample ID | Accession | Description | Max Score | Total Score | Query Coverage | E Value | Max Ident |
|---|---|---|---|---|---|---|---|
| AS113-1 | EU215386.1 | Pelosinus sp. UFO1 16S ribosomal RNA pseudogene, complete sequence | 654 | 654 | 73% | 0 | 100% |
| AS113-3 | EU215386.1 | Pelosinus sp. UFO1 16S ribosomal RNA pseudogene, complete sequence | 580 | 580 | 98% | 1.00E-162 | 100% |
| AS113-9 | EU215386.1 | Pelosinus sp. UFO1 16S ribosomal RNA pseudogene, complete sequence | 654 | 654 | 96% | 0 | 100% |
| AS113-11 | EU215386.1 | Pelosinus sp. UFO1 16S ribosomal RNA pseudogene, complete sequence | 654 | 654 | 96% | 0 | 100% |
| AS113-19 | EU215386.1 | Pelosinus sp. UFO1 16S ribosomal RNA pseudogene, complete sequence | 652 | 652 | 100% | 0.00E+00 | 100% |
| AS113-20 | EU215386.1 | Pelosinus sp. UFO1 16S ribosomal RNA pseudogene, complete sequence | 652 | 652 | 100% | 0.00E+00 | 100% |
| AS113-21 | EU215386.1 | Pelosinus sp. UFO1 16S ribosomal RNA pseudogene, complete sequence | 630 | 630 | 100% | 1.00E-177 | 98% |
| AS113-22 | EU215386.1 | Pelosinus sp. UFO1 16S ribosomal RNA pseudogene, complete sequence | 647 | 647 | 100% | 0.00E+00 | 99% |
| AS113-28 | EU215386.1 | Pelosinus sp. UFO1 16S ribosomal RNA pseudogene, complete sequence | 652 | 652 | 100% | 0.00E+00 | 100% |
| AS113-35 | EU215386.1 | Pelosinus sp. UFO1 16S ribosomal RNA pseudogene, complete sequence | 647 | 647 | 99% | 0.00E+00 | 99% |
| AS113-38 | EU215386.1 | Pelosinus sp. UFO1 16S ribosomal RNA pseudogene, complete sequence | 652 | 652 | 100% | 0.00E+00 | 100% |
| AS113-40 | EU215386.1 | Pelosinus sp. UFO1 16S ribosomal RNA pseudogene, complete sequence | 652 | 652 | 100% | 0.00E+00 | 100% |
| AS113-47 | EU215386.1 | Pelosinus sp. UFO1 16S ribosomal RNA pseudogene, complete sequence | 652 | 652 | 100% | 0.00E+00 | 100% |
| AS113-34 | FJ939131.1 | Anaeromyxobacter sp. IN2 16S ribosomal RNA gene, partial sequence | 641 | 641 | 100% | 0.00E+00 | 99% |
| AS113-13 | U41563.1 | Geothrix fermentans 16S rRNA gene, partial sequence | 647 | 647 | 96% | 0.00E+00 | 99% |
| AS113-16 | U41563.1 | Geothrix fermentans 16S rRNA gene, partial sequence | 545 | 545 | 100% | 4.00E-152 | 94% |
| AS113-29 | U41563.1 | Geothrix fermentans 16S rRNA gene, partial sequence | 647 | 647 | 96% | 0.00E+00 | 99% |
| AS113-33 | U41563.1 | Geothrix fermentans 16S rRNA gene, partial sequence | 647 | 647 | 96% | 0.00E+00 | 99% |
| AS113-42 | U41563.1 | Geothrix fermentans 16S rRNA gene, partial sequence | 647 | 647 | 96% | 0.00E+00 | 99% |
| AS113-46 | U41563.1 | Geothrix fermentans 16S rRNA gene, partial sequence | 647 | 647 | 96% | 0 | 99% |

# APPENDIX D

## SATURATED RATE EXPERIMENTAL DATA

# APPENDIX D

## SATURATED RATE EXPERIMENTAL DATA

D1: ICP-MS ANALYSES OF TOTAL Fe, Mn, and Se Concentrations

Tables contain replicate ICP-MS data for the Dry Valley Mine and Smoky Canyon Mine, 10°C and 25°C treatments, and killed controls. Replicates were averaged to create Chapter 4, Figure 13 and Chapter 5, Figures 18-20.


Table D1-1. Selenium ICP-MS data for Dry Valley Mine
- D1-1.1. DV 10 LIVE SELENIUM.
- D1-1.2. DV 25 LIVE SELENIUM.
- D1-1.3. DV 10 KILLED SELENIUM.
- D1-1.4. DV 25 KILLED SELENIUM.

Table D1-2. Iron and Manganese ICP-MS data for Dry Valley Mine
- D1-2.1. DV 10 LIVE IRON/MANGANESE.
- D1-2.2. DV 25 LIVE IRON/MANGANESE.
- D1-2.3. DV 10 KILLED IRON/MANGANESE.
- D1-2.4. DV 25 KILLED IRON/MANGANESE.

Table D1-3. Selenium ICP-MS data for Smoky Canyon Mine
- D1-3.1. SC 10 LIVE SELENIUM.
- D1-3.2. SC 25 LIVE SELENIUM.
- D1-3.3. SC 10 KILLED SELENIUM.
- D1-3.4. SC 25 KILLED SELENIUM.

Table D1-4. Iron and Manganese ICP-MS data for Smoky Canyon Mine
- D1-4.1. SC 10 LIVE IRON/MANGANESE.
- D1-4.2. SC 25 LIVE IRON/MANGANESE.
- D1-4.3. SC 10 KILLED IRON/MANGANESE.
- D1-4.4. SC 25 KILLED IRON/MANGANESE.

D2: ION CHROMATOGRAPHY DATA
- Table D2-1. Dry Valley Ion Chromotography Data
- Table D2-2. Smoky Canyon Ion Chromotography Data

D3: PROTEIN ASSAY DATA
- Table D3-1. Dry Valley Protein Assay – Coomassie/Qbit Method.
- Table D3-2. Smoky Canyon Protein Assay – Coomassie Method.

Table D1-1. Selenium ICP-MS data for Dry Valley Mine Saturated Rate Experiments.
D1-1.1. DV 10 LIVE SELENIUM.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 10DCL1 | 0 | 10DCL1-0 | 9.811 | 490.5 | 20.7 | 0.09 | 10150 | 10150 | 021309cps |
| 10DCL1 | 8 | 10DCL1-08 | 9.812 | 490.6 | 20.2 | 0.09 | 9923 | 9923 | 021309cps |
| 10DCL1 | 20 | 10DCL1-20 | 9.818 | 490.9 | 21.1 | 0.09 | 10367 | 10367 | 021309cps |
| 10DCL1 | 32 | 10DCL1-32 | 9.796 | 489.8 | 18.2 | 0.11 | 8905 | 8905 | 032009SeLtd |
| 10DCL1 | 53 | 10DCL1-53 | 9.841 | 492.1 | 19.1 | 0.21 | 9404 | 9404 | 0214B |
| 10DCL1 | 70 | 10DCL1-70 | 9.606 | 480.3 | 23.3 | 0.84 | 11171 | 11171 | 21509 |
| 10DCL1 | 80 | 10DCL1-80 | 9.811 | 490.6 | 13.2 | 0.16 | 6487 | 6487 | 021609Bcps |
| 10DCL1 | 104 | 10DCL1-104 | 9.796 | 489.8 | 3.6 | 0.08 | 1744 | 1744 | 021709cps |
| 10DCL1 | 128 | 10DCL1-128 | 9.795 | 489.8 | 1.5 | 0.16 | 757 | 757 | 021809copy |
| 10DCL1 | 140 | 10DCL1-140 | 9.793 | 489.6 | 1.6 | 0.16 | 793 | 793 | 021809copy |
| 10DCL1 | 164 | 10DCL1-164 | 9.750 | 487.5 | 0.4 | 0.12 | 210 | 210 | 022309cps |
| 10DCL1 | 188 | 10DCL1-188 | 9.788 | 489.4 | 0.4 | 0.12 | 182 | 182 | 022309cps |
| 10DCL1 | 212 | 10DCL1-212 | 9.781 | 489.1 | 0.6 | 0.12 | 297 | 297 | 022309cps |
| 10DCL1 | 272 | 10DCL1-272 | 9.729 | 486.4 | 0.4 | 0.14 | 178 | 178 | 022509Bcps |
| 10DCL2 | 0 | 10DCL2-0 | 9.836 | 491.8 | 21.1 | 0.09 | 10364 | 10364 | 021309cps |
| 10DCL2 | 8 | 10DCL2-08 | 9.827 | 491.4 | 20.4 | 0.09 | 10046 | 10046 | 021309cps |
| 10DCL2 | 20 | 10DCL2-20 | 9.836 | 491.8 | 19.5 | 0.09 | 9603 | 9603 | 021309cps |
| 10DCL2 | 32 | 10DCL2-32 | 9.783 | 489.1 | 21.6 | 0.11 | 10581 | 10581 | 032009SeLtd |
| 10DCL2 | 53 | 10DCL2-53 | 9.840 | 492.0 | 20.7 | 0.21 | 10179 | 10179 | 0214B |
| 10DCL2 | 70 | 10DCL2-70 | 9.773 | 488.6 | 24.4 | 0.84 | 11908 | 11908 | 21509 |
| 10DCL2 | 80 | 10DCL2-80 | 9.790 | 489.5 | 16.3 | 0.16 | 7966 | 7966 | 021609Bcps |
| 10DCL2 | 104 | 10DCL2-104 | 9.796 | 489.8 | 11.1 | 0.08 | 5426 | 5426 | 021709cps |
| 10DCL2 | 128 | 10DCL2-128 | 9.797 | 489.9 | 3.5 | 0.16 | 1731 | 1731 | 021809copy |
| 10DCL2 | 140 | 10DCL2-140 | 9.791 | 489.5 | 3.9 | 0.16 | 1902 | 1902 | 021809copy |
| 10DCL2 | 164 | 10DCL2-164 | 9.814 | 490.7 | 0.6 | 0.12 | 298 | 298 | 022309cps |
| 10DCL2 | 188 | 10DCL2-188 | 9.837 | 491.9 | 0.3 | 0.12 | 172 | 172 | 022309cps |
| 10DCL2 | 212 | 10DCL2-212 | 9.797 | 489.9 | 0.5 | 0.12 | 252 | 252 | 022309cps |
| 10DCL2 | 272 | 10DCL2-272 | 9.783 | 489.2 | 0.3 | 0.14 | 165 | 165 | 022509Bcps |

D1-1.1. DV 10 LIVE SELENIUM, continued.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 10DCL3 | 0 | 10DCL3-0 | 9.820 | 491.0 | 17.6 | 0.09 | 8664 | 8664 | 021309cps |
| 10DCL3 | 8 | 10DCL3-08 | 9.808 | 490.4 | 19.9 | 0.09 | 9745 | 9745 | 021309cps |
| 10DCL3 | 20 | 10DCL3-20 | 9.808 | 490.4 | 20.1 | 0.09 | 9876 | 9876 | 021309cps |
| 10DCL3 | 32 | 10DCL3-32 | 9.800 | 490.0 | 20.0 | 0.11 | 9821 | 9821 | 032009SeLtd |
| 10DCL3 | 53 | 10DCL3-53 | 9.821 | 491.0 | 18.3 | 0.21 | 9006 | 9006 | 0214B |
| 10DCL3 | 70 | 10DCL3-70 | 9.769 | 488.5 | 22.1 | 0.84 | 10796 | 10796 | 21509 |
| 10DCL3 | 80 | 10DCL3-80 | 9.792 | 489.6 | 14.5 | 0.16 | 7089 | 7089 | 021609Bcps |
| 10DCL3 | 104 | 10DCL3-104 | 9.780 | 489.0 | 13.9 | 0.08 | 6792 | 6792 | 021709cps |
| 10DCL3 | 128 | 10DCL3-128 | 9.794 | 489.7 | 13.7 | 0.16 | 6722 | 6722 | 021809copy |
| 10DCL3 | 140 | 10DCL3-140 | 9.790 | 489.5 | 13.3 | 0.16 | 6491 | 6491 | 021809copy |
| 10DCL3 | 164 | 10DCL3-164 | 9.810 | 490.5 | 7.8 | 0.12 | 3805 | 3805 | 022309cps |
| 10DCL3 | 188 | 10DCL3-188 | 9.803 | 490.1 | 4.9 | 0.12 | 2411 | 2411 | 022309cps |
| 10DCL3 | 212 | 10DCL3-212 | 9.783 | 489.2 | 4.4 | 0.12 | 2130 | 2130 | 022309cps |
| 10DCL3 | 272 | 10DCL3-272 | 9.794 | 489.7 | 2.0 | 0.14 | 978 | 978 | 022509Bcps |
| 10DRL1 | 0 | 10DRL1-0 | 9.836 | 491.8 | 20.2 | 0.09 | 9931 | 9931 | 021309cps |
| 10DRL1 | 8 | 10DRL1-08 | 9.828 | 491.4 | 18.7 | 0.09 | 9202 | 9202 | 021309cps |
| 10DRL1 | 20 | 10DRL1-20 | 9.832 | 491.6 | 20.5 | 0.09 | 10058 | 10058 | 021309cps |
| 10DRL1 | 32 | 10DRL1-32 | 9.799 | 489.9 | 22.1 | 0.11 | 10807 | 10807 | 032009SeLtd |
| 10DRL1 | 53 | 10DRL1-53 | 9.828 | 491.4 | 18.6 | 0.21 | 9142 | 9142 | 0214B |
| 10DRL1 | 70 | 10DRL1-70 | 9.755 | 487.8 | 22.4 | 0.84 | 10928 | 10928 | 21509 |
| 10DRL1 | 80 | 10DRL1-80 | 9.793 | 489.6 | 18.2 | 0.16 | 8910 | 8910 | 021609Bcps |
| 10DRL1 | 104 | 10DRL1-104 | 9.801 | 490.0 | 14.3 | 0.08 | 6985 | 6985 | 021709cps |
| 10DRL1 | 128 | 10DRL1-128 | 9.771 | 488.5 | 7.9 | 0.16 | 3861 | 3861 | 021809copy |
| 10DRL1 | 140 | 10DRL1-140 | 9.789 | 489.5 | 5.6 | 0.16 | 2752 | 2752 | 021809copy |
| 10DRL1 | 164 | 10DRL1-164 | 9.833 | 491.7 | 1.9 | 0.12 | 930 | 930 | 022309cps |
| 10DRL1 | 188 | 10DRL1-188 | 9.797 | 489.9 | 0.7 | 0.12 | 338 | 338 | 022309cps |
| 10DRL1 | 212 | 10DRL1-212 | 9.798 | 489.9 | 0.6 | 0.12 | 310 | 310 | 022309cps |
| 10DRL1 | 272 | 10DRL1-272 | 9.785 | 489.2 | 0.3 | 0.14 | 154 | 154 | 022509Bcps |
| 10DRL2 | 0 | 10DRL2-0 | 9.840 | 492.0 | 18.9 | 0.09 | 9318 | 9318 | 021309cps |
| 10DRL2 | 8 | 10DRL2-08 | 9.847 | 492.4 | 17.9 | 0.09 | 8819 | 8819 | 021309cps |

D1-1.1. DV 10 LIVE SELENIUM, continued.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 10DRL2 | 20 | 10DRL2-20 | 9.830 | 491.5 | 19.5 | 0.09 | 9590 | 9590 | 021309cps |
| 10DRL2 | 32 | 10DRL2-32 | 9.799 | 489.9 | 18.5 | 0.11 | 9062 | 9062 | 032009SeLtd |
| 10DRL2 | 53 | 10DRL2-53 | 9.828 | 491.4 | 19.0 | 0.21 | 9345 | 9345 | 0214B |
| 10DRL2 | 70 | 10DRL2-70 | 9.767 | 488.4 | 20.0 | 0.84 | 9763 | 9763 | 21509 |
| 10DRL2 | 80 | 10DRL2-80 | 9.798 | 489.9 | 20.4 | 0.16 | 10013 | 10013 | 021609Bcps |
| 10DRL2 | 104 | 10DRL2-104 | 9.800 | 490.0 | 16.3 | 0.08 | 8003 | 8003 | 021709cps |
| 10DRL2 | 128 | 10DRL2-128 | 9.784 | 489.2 | 8.1 | 0.16 | 3970 | 3970 | 021809copy |
| 10DRL2 | 140 | 10DRL2-140 | 9.799 | 490.0 | 6.8 | 0.16 | 3354 | 3354 | 021809copy |
| 10DRL2 | 164 | 10DRL2-164 | 9.811 | 490.6 | 1.8 | 0.12 | 865 | 865 | 022309cps |
| 10DRL2 | 188 | 10DRL2-188 | 9.796 | 489.8 | 0.6 | 0.12 | 275 | 275 | 022309cps |
| 10DRL2 | 212 | 10DRL2-212 | 9.804 | 490.2 | 0.6 | 0.12 | 310 | 310 | 022309cps |
| 10DRL2 | 272 | 10DRL2-272 | 9.781 | 489.0 | 0.3 | 0.14 | 156 | 156 | 022509Bcps |
| 10DRL3 | 0 | 10DRL3-0 | 9.831 | 491.6 | 17.8 | 0.09 | 8756 | 8756 | 021309cps |
| 10DRL3 | 8 | 10DRL3-08 | 9.823 | 491.2 | 19.3 | 0.09 | 9482 | 9482 | 021309cps |
| 10DRL3 | 20 | 10DRL3-20 | 9.809 | 490.4 | 21.2 | 0.09 | 10397 | 10397 | 021309cps |
| 10DRL3 | 32 | 10DRL3-32 | 9.786 | 489.3 | 19.1 | 0.11 | 9359 | 9359 | 032009SeLtd |
| 10DRL3 | 53 | 10DRL3-53 | 9.822 | 491.1 | 19.8 | 0.21 | 9718 | 9718 | 0214B |
| 10DRL3 | 70 | 10DRL3-70 | 9.805 | 490.2 | 20.7 | 0.84 | 10153 | 10153 | 21509 |
| 10DRL3 | 80 | 10DRL3-80 | 9.791 | 489.6 | 17.1 | 0.16 | 8363 | 8363 | 021609Bcps |
| 10DRL3 | 104 | 10DRL3-104 | 9.796 | 489.8 | 14.2 | 0.08 | 6962 | 6962 | 021709cps |
| 10DRL3 | 128 | 10DRL3-128 | 9.799 | 489.9 | 9.6 | 0.16 | 4694 | 4694 | 021809copy |
| 10DRL3 | 140 | 10DRL3-140 | 9.781 | 489.1 | 7.3 | 0.16 | 3577 | 3577 | 021809copy |
| 10DRL3 | 164 | 10DRL3-164 | 9.803 | 490.2 | 1.9 | 0.12 | 933 | 933 | 022309cps |
| 10DRL3 | 188 | 10DRL3-188 | 0.821 | 41.0 | 0.6 | 0.12 | 24 | 24 | 022309cps |
| 10DRL3 | 212 | 10DRL3-212 | 9.800 | 490.0 | 0.5 | 0.12 | 240 | 240 | 022309cps |
| 10DRL3 | 272 | 10DRL3-272 | 9.793 | 489.7 | 0.3 | 0.14 | 167 | 167 | 022509Bcps |
| 10DSL1 | 0 | 10DSL1-0 | 9.728 | 486.4 | 20.1 | 0.09 | 9773 | 9773 | 021309cps |
| 10DSL1 | 8 | 10DSL1-08 | 9.798 | 489.9 | 19.4 | 0.09 | 9507 | 9507 | 021309cps |
| 10DSL1 | 20 | 10DSL1-20 | 9.804 | 490.2 | 19.1 | 0.09 | 9375 | 9375 | 021309cps |
| 10DSL1 | 32 | 10DSL1-32 | 9.789 | 489.4 | 16.3 | 0.11 | 7997 | 7997 | 032009SeLtd |

D1-1.1. DV 10 LIVE SELENIUM, continued.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 10DSL1 | 53 | 10DSL1-53 | 9.810 | 490.5 | 24.3 | 0.21 | 11935 | 11935 | 0214B |
| 10DSL1 | 70 | 10DSL1-70 | 9.794 | 489.7 | 20.6 | 0.84 | 10108 | 10108 | 21509 |
| 10DSL1 | 80 | 10DSL1-80 | 9.809 | 490.5 | 18.6 | 0.16 | 9137 | 9137 | 021609Bcps |
| 10DSL1 | 104 | 10DSL1-104 | 9.808 | 490.4 | 10.8 | 0.08 | 5285 | 5285 | 021809copy |
| 10DSL1 | 128 | 10DSL1-128 | 9.805 | 490.3 | 5.7 | 0.16 | 2818 | 2818 | 021809copy |
| 10DSL1 | 140 | 10DSL1-140 | 9.721 | 486.1 | 4.0 | 0.16 | 1954 | 1954 | 021809copy |
| 10DSL1 | 164 | 10DSL1-164 | 9.824 | 491.2 | 0.8 | 0.12 | 415 | 415 | 022309cps |
| 10DSL1 | 188 | 10DSL1-188 | 9.805 | 490.2 | 0.7 | 0.12 | 327 | 327 | 022309cps |
| 10DSL1 | 212 | 10DSL1-212 | 9.803 | 490.2 | 0.7 | 0.12 | 344 | 344 | 022309cps |
| 10DSL1 | 272 | 10DSL1-272 | 9.784 | 489.2 | 0.4 | 0.14 | 220 | 220 | 022509Bcps |
| 10DSL2 | 0 | 10DSL2-0 | 9.817 | 490.8 | 20.6 | 0.09 | 10114 | 10114 | 021309cps |
| 10DSL2 | 8 | 10DSL2-08 | 9.822 | 491.1 | 19.5 | 0.09 | 9589 | 9589 | 021309cps |
| 10DSL2 | 20 | 10DSL2-20 | 9.801 | 490.1 | 17.0 | 0.09 | 8337 | 8337 | 021309cps |
| 10DSL2 | 32 | 10DSL2-32 | 9.777 | 488.9 | 18.2 | 0.11 | 8882 | 8882 | 032009SeLtd |
| 10DSL2 | 53 | 10DSL2-53 | 9.807 | 490.3 | 24.4 | 0.21 | 11950 | 11950 | 0214B |
| 10DSL2 | 70 | 10DSL2-70 | 9.763 | 488.1 | 21.5 | 0.84 | 10509 | 10509 | 21509 |
| 10DSL2 | 80 | 10DSL2-80 | 9.813 | 490.6 | 17.9 | 0.16 | 8791 | 8791 | 021609Bcps |
| 10DSL2 | 104 | 10DSL2-104 | 9.794 | 489.7 | 14.0 | 0.08 | 6860 | 6860 | 021809copy |
| 10DSL2 | 128 | 10DSL2-128 | 9.791 | 489.6 | 8.3 | 0.16 | 4054 | 4054 | 021809copy |
| 10DSL2 | 140 | 10DSL2-140 | 9.800 | 490.0 | 8.6 | 0.16 | 4208 | 4208 | 021809copy |
| 10DSL2 | 164 | 10DSL2-164 | 9.811 | 490.6 | 2.1 | 0.12 | 1050 | 1050 | 022309cps |
| 10DSL2 | 188 | 10DSL2-188 | 9.811 | 490.5 | 1.5 | 0.12 | 726 | 726 | 022309cps |
| 10DSL2 | 212 | 10DSL2-212 | 9.788 | 489.4 | 1.3 | 0.12 | 615 | 615 | 022309cps |
| 10DSL2 | 272 | 10DSL2-272 | 9.814 | 490.1 | 0.6 | 0.14 | 277 | 277 | 022509Bcps |
| 10DSL3 | 0 | 10DSL3-0 | 9.821 | 491.1 | 20.6 | 0.09 | 10105 | 10105 | 021309cps |
| 10DSL3 | 8 | 10DSL3-08 | 9.828 | 491.4 | 19.9 | 0.09 | 9798 | 9798 | 021309cps |
| 10DSL3 | 20 | 10DSL3-20 | 9.824 | 491.2 | 19.3 | 0.09 | 9496 | 9496 | 021309cps |
| 10DSL3 | 32 | 10DSL3-32 | 9.792 | 489.6 | 21.8 | 0.11 | 10660 | 10660 | 032009SeLtd |
| 10DSL3 | 53 | 10DSL3-53 | 9.793 | 489.6 | 21.6 | 0.21 | 10562 | 10562 | 0214B |
| 10DSL3 | 70 | 10DSL3-70 | 9.793 | 489.6 | 21.1 | 0.84 | 10327 | 10327 | 21509 |

D1-1.1. DV 10 LIVE SELENIUM, continued.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 10DSL3 | 80 | 10DSL3-80 | 9.810 | 490.5 | 16.0 | 0.16 | 7827 | 7827 | 021609Bcps |
| 10DSL3 | 104 | 10DSL3-104 | 9.806 | 490.3 | 10.3 | 0.08 | 5033 | 5033 | 021809copy |
| 10DSL3 | 128 | 10DSL3-128 | 9.789 | 489.5 | 7.0 | 0.16 | 3442 | 3442 | 021809copy |
| 10DSL3 | 140 | 10DSL3-140 | 9.807 | 490.4 | 3.8 | 0.16 | 1880 | 1880 | 021809copy |
| 10DSL3 | 164 | 10DSL3-164 | 9.804 | 490.2 | 0.8 | 0.12 | 414 | 414 | 022309cps |
| 10DSL3 | 188 | 10DSL3-188 | 9.812 | 490.6 | 0.6 | 0.12 | 310 | 310 | 022309cps |
| 10DSL3 | 212 | 10DSL3-212 | 9.805 | 490.3 | 0.7 | 0.12 | 327 | 327 | 022309cps |
| 10DSL3 | 272 | 10DSL3-272 | 9.803 | 490.1 | 0.7 | 0.14 | 366 | 366 | 022509Bcps |

D1-1.2. DV 25 LIVE SELENIUM.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 25DCL1 | 0 | 25DCL1-0 | 9.8 | 490.5 | 21.2 | 0.14 | 10420 | 10420 | 022509Bcps |
| 25DCL1 | 8 | 25DCL1-8 | 9.7 | 487.4 | 21.3 | 0.14 | 10400 | 10400 | 022509Bcps |
| 25DCL1 | 20 | 25DCL1-20 | 9.8 | 491.8 | 20.9 | 0.14 | 10277 | 10277 | 022509Bcps |
| 25DCL1 | 32 | 25DCL1-32 | 9.8 | 490.2 | 17.5 | 0.11 | 8565 | 8565 | 032009SeLtd |
| 25DCL1 | 54 | 25DCL1-54 | 9.8 | 490.2 | 2.4 | 0.11 | 1173 | 1173 | 032009SeFeMn |
| 25DCL1 | 66 | 25DCL1-66 | 9.8 | 489.6 | 1.2 | 0.11 | 599 | 599 | 032009SeLtd |
| 25DCL1 | 90 | 25DCL1-90 | 9.8 | 490.3 | 0.3 | 0.11 | 168 | 168 | 032009SeLtd |
| 25DCL1 | 120 | 25DCL1-120 | 9.8 | 489.8 | 0.2 | 0.11 | 120 | 120 | 032009SeLtd |
| 25DCL1 | 140 | 25DCL1-140 | 9.8 | 489.9 | 0.3 | 0.11 | 141 | 141 | 032009SeLtd |
| 25DCL1 | 188 | 25DCL1-188 | 10.0 | 499.5 | 0.10 | 0.11 | 49 | 55 | 032009SeFeMn |
| 25DCL2 | 0 | 25DCL2-0 | 9.8 | 491.0 | 22.7 | 0.14 | 11147 | 11147 | 022509Bcps |
| 25DCL2 | 8 | 25DCL2-8 | 9.8 | 490.9 | 21.8 | 0.14 | 10710 | 10710 | 022509Bcps |
| 25DCL2 | 20 | 25DCL2-20 | 9.8 | 491.7 | 21.7 | 0.14 | 10691 | 10691 | 022509Bcps |
| 25DCL2 | 32 | 25DCL2-32 | 9.8 | 488.6 | 19.3 | 0.11 | 9410 | 9410 | 032009SeLtd |
| 25DCL2 | 54 | 25DCL2-54 | 9.8 | 490.2 | 4.0 | 0.11 | 1982 | 1982 | 032009SeFeMn |
| 25DCL2 | 66 | 25DCL2-66 | 9.8 | 489.9 | 0.6 | 0.11 | 285 | 285 | 032009SeLtd |
| 25DCL2 | 90 | 25DCL2-90 | 9.8 | 490.2 | 0.3 | 0.11 | 130 | 130 | 032009SeLtd |
| 25DCL2 | 120 | 25DCL2-120 | 9.8 | 489.9 | 0.2 | 0.11 | 88 | 88 | 032009SeLtd |
| 25DCL2 | 140 | 25DCL2-140 | 9.8 | 490.3 | 0.3 | 0.11 | 168 | 168 | 032009SeLtd |
| 25DCL2 | 188 | 25DCL2-188 | 9.8 | 489.3 | 0.0 | 0.11 | 20 | 54 | 032009SeFeMn |
| 25DCL3 | 0 | 25DCL3-0 | 9.8 | 491.1 | 22.8 | 0.14 | 11216 | 11216 | 022509Bcps |
| 25DCL3 | 8 | 25DCL3-8 | 9.8 | 491.6 | 21.3 | 0.14 | 10453 | 10453 | 022509Bcps |
| 25DCL3 | 20 | 25DCL3-20 | 9.8 | 491.8 | 21.8 | 0.14 | 10717 | 10717 | 022509Bcps |
| 25DCL3 | 32 | 25DCL3-32 | 9.8 | 489.8 | 19.3 | 0.11 | 9443 | 9443 | 032009SeLtd |
| 25DCL3 | 54 | 25DCL3-54 | 9.8 | 489.9 | 3.4 | 0.11 | 1646 | 1646 | 032009SeFeMn |
| 25DCL3 | 66 | 25DCL3-66 | 9.8 | 490.5 | 0.9 | 0.11 | 451 | 451 | 032009SeLtd |
| 25DCL3 | 90 | 25DCL3-90 | 9.8 | 490.9 | 0.3 | 0.11 | 136 | 136 | 032009SeLtd |
| 25DCL3 | 120 | 25DCL3-120 | 9.8 | 490.5 | 0.2 | 0.11 | 77 | 77 | 032009SeLtd |
| 25DCL3 | 140 | 25DCL3-140 | 9.7 | 487.2 | 0.07 | 0.11 | 34 | 54 | 032009SeLtd |

D1-1.2. DV 25 LIVE SELENIUM, continued.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 25DCL3 | 188 | 25DCL3-188 | 9.8 | 489.2 | 0.02 | 0.11 | 12 | 54 | 032009SeFeMn |
| 25DRL1 | 0 | 25DRL1-0 | 9.8 | 491.2 | 21.0 | 0.14 | 10298 | 10298 | 022509Bcps |
| 25DRL1 | 8 | 25DRL1-8 | 9.8 | 491.3 | 20.8 | 0.14 | 10231 | 10231 | 022509Bcps |
| 25DRL1 | 20 | 25DRL1-20 | 9.8 | 491.2 | 20.3 | 0.14 | 9975 | 9975 | 022509Bcps |
| 25DRL1 | 32 | 25DRL1-32 | 9.8 | 489.7 | 18.8 | 0.11 | 9223 | 9223 | 032009SeLtd |
| 25DRL1 | 54 | 25DRL1-54 | 9.8 | 489.4 | 4.8 | 0.11 | 2336 | 2336 | 032009SeFeMn |
| 25DRL1 | 66 | 25DRL1-66 | 9.8 | 490.4 | 0.7 | 0.11 | 349 | 349 | 032009SeLtd |
| 25DRL1 | 90 | 25DRL1-90 | 9.8 | 490.5 | 0.3 | 0.11 | 130 | 130 | 032009SeLtd |
| 25DRL1 | 120 | 25DRL1-120 | 9.8 | 490.8 | 0.15 | 0.11 | 72 | 72 | 032009SeLtd |
| 25DRL1 | 140 | 25DRL1-140 | 9.8 | 489.3 | 0.2 | 0.11 | 109 | 109 | 032009SeLtd |
| 25DRL1 | 188 | 25DRL1-188 | 9.8 | 489.9 | 0.10 | 0.11 | 50 | 54 | 032009SeFeMn |
| 25DRL2 | 0 | 25DRL2-0 | 9.8 | 491.6 | 21.5 | 0.14 | 10593 | 10593 | 022509Bcps |
| 25DRL2 | 8 | 25DRL2-8 | 9.8 | 490.1 | 21.8 | 0.14 | 10662 | 10662 | 022509Bcps |
| 25DRL2 | 20 | 25DRL2-20 | 9.8 | 490.9 | 21.5 | 0.14 | 10550 | 10550 | 022509Bcps |
| 25DRL2 | 32 | 25DRL2-32 | 9.8 | 489.1 | 16.6 | 0.11 | 8103 | 8103 | 032009SeLtd |
| 25DRL2 | 54 | 25DRL2-54 | 9.8 | 489.5 | 5.5 | 0.11 | 2704 | 2704 | 032009SeFeMn |
| 25DRL2 | 66 | 25DRL2-66 | 9.8 | 489.2 | 0.5 | 0.11 | 269 | 269 | 032009SeLtd |
| 25DRL2 | 90 | 25DRL2-90 | 9.8 | 490.9 | 0.2 | 0.11 | 120 | 120 | 032009SeLtd |
| 25DRL2 | 120 | 25DRL2-120 | 9.8 | 490.4 | 0.15 | 0.11 | 72 | 72 | 032009SeLtd |
| 25DRL2 | 140 | 25DRL2-140 | 9.8 | 489.9 | 0.2 | 0.11 | 114 | 114 | 032009SeLtd |
| 25DRL2 | 188 | 25DRL2-188 | 9.8 | 490.2 | 0.066 | 0.11 | 33 | 54 | 032009SeFeMn |
| 25DRL3 | 0 | 25DRL3-0 | 9.8 | 490.1 | 20.8 | 0.14 | 10208 | 10208 | 022509Bcps |
| 25DRL3 | 8 | 25DRL3-8 | 9.8 | 492.0 | 20.9 | 0.14 | 10295 | 10295 | 022509Bcps |
| 25DRL3 | 20 | 25DRL3-20 | 9.8 | 491.5 | 21.2 | 0.14 | 10442 | 10442 | 022509Bcps |
| 25DRL3 | 32 | 25DRL3-32 | 9.8 | 489.9 | 17.5 | 0.11 | 8570 | 8570 | 032009SeLtd |
| 25DRL3 | 54 | 25DRL3-54 | 9.8 | 489.4 | 5.9 | 0.11 | 2904 | 2904 | 032009SeFeMn |
| 25DRL3 | 66 | 25DRL3-66 | 9.8 | 490.8 | 0.9 | 0.11 | 457 | 457 | 032009SeLtd |
| 25DRL3 | 90 | 25DRL3-90 | 9.8 | 490.6 | 0.4 | 0.11 | 205 | 205 | 032009SeLtd |
| 25DRL3 | 120 | 25DRL3-120 | 9.8 | 490.4 | 0.14 | 0.11 | 66 | 66 | 032009SeLtd |
| 25DRL3 | 140 | 25DRL3-140 | 9.8 | 489.3 | 3.0 | 0.11 | 1457 | 1457 | 032009SeLtd |

D1-1.2. DV 25 LIVE SELENIUM, continued.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 25DRL3 | 188 | 25DRL3-188 | 9.8 | 487.8 | 0.06 | 0.11 | 28 | 54 | 032009SeFeMn |
| 25DSL1 | 0 | 25DSL1-0 | 9.8 | 490.8 | 23.6 | 0.14 | 11592 | 11592 | 022509Bcps |
| 25DSL1 | 8 | 25DSL1-8 | 9.8 | 490.5 | 23.3 | 0.14 | 11426 | 11426 | 022509Bcps |
| 25DSL1 | 20 | 25DSL1-20 | 9.8 | 491.6 | 24.2 | 0.14 | 11883 | 11883 | 022509Bcps |
| 25DSL1 | 32 | 25DSL1-32 | 9.8 | 490.3 | 22.9 | 0.11 | 11246 | 11246 | 032009SeLtd |
| 25DSL1 | 54 | 25DSL1-54 | 9.8 | 489.9 | 11.8 | 0.11 | 5761 | 5761 | 032009SeFeMn |
| 25DSL1 | 66 | 25DSL1-66 | 9.8 | 490.0 | 5.4 | 0.11 | 2622 | 2622 | 032009SeLtd |
| 25DSL1 | 90 | 25DSL1-90 | 9.8 | 490.2 | 1.3 | 0.11 | 632 | 632 | 032009SeLtd |
| 25DSL1 | 120 | 25DSL1-120 | 9.8 | 489.8 | 0.3 | 0.11 | 141 | 141 | 032009SeLtd |
| 25DSL1 | 140 | 25DSL1-140 | 9.8 | 489.7 | 0.3 | 0.11 | 168 | 168 | 032009SeLtd |
| 25DSL1 | 188 | 25DSL1-188 | 9.8 | 490.4 | 0.04 | 0.11 | 18 | 54 | 032009SeFeMn |
| 25DSL2 | 0 | 25DSL2-0 | 9.8 | 491.3 | 25.0 | 0.14 | 12297 | 12297 | 022509Bcps |
| 25DSL2 | 8 | 25DSL2-8 | 9.8 | 491.2 | 23.5 | 0.14 | 11523 | 11523 | 022509Bcps |
| 25DSL2 | 20 | 25DSL2-20 | 9.8 | 491.6 | 23.5 | 0.14 | 11567 | 11567 | 022509Bcps |
| 25DSL2 | 32 | 25DSL2-32 | 9.8 | 490.3 | 20.7 | 0.11 | 10125 | 10125 | 032009SeLtd |
| 25DSL2 | 54 | 25DSL2-54 | 9.8 | 490.3 | 12.4 | 0.11 | 6089 | 6089 | 032009SeFeMn |
| 25DSL2 | 66 | 25DSL2-66 | 9.8 | 489.9 | 6.0 | 0.11 | 2957 | 2957 | 032009SeLtd |
| 25DSL2 | 90 | 25DSL2-90 | 9.8 | 489.8 | 2.0 | 0.11 | 989 | 989 | 032009SeLtd |
| 25DSL2 | 120 | 25DSL2-120 | 9.8 | 489.3 | 0.4 | 0.11 | 189 | 189 | 032009SeLtd |
| 25DSL2 | 140 | 25DSL2-140 | 9.8 | 490.2 | 0.2 | 0.11 | 82 | 82 | 032009SeLtd |
| 25DSL2 | 188 | 25DSL2-188 | 9.8 | 489.9 | 0.06 | 0.11 | 31 | 54 | 032009SeFeMn |
| 25DSL3 | 0 | 25DSL3-0 | 9.8 | 490.6 | 23.3 | 0.14 | 11430 | 11430 | 022509Bcps |
| 25DSL3 | 8 | 25DSL3-8 | 9.8 | 491.7 | 23.3 | 0.14 | 11466 | 11466 | 022509Bcps |
| 25DSL3 | 20 | 25DSL3-20 | 9.8 | 491.0 | 22.8 | 0.14 | 11205 | 11205 | 022509Bcps |
| 25DSL3 | 32 | 25DSL3-32 | 9.8 | 490.3 | 18.8 | 0.11 | 9202 | 9202 | 032009SeLtd |
| 25DSL3 | 54 | 25DSL3-54 | 9.8 | 489.0 | 10.2 | 0.11 | 5009 | 5009 | 032009SeFeMn |
| 25DSL3 | 66 | 25DSL3-66 | 9.8 | 490.1 | 4.5 | 0.11 | 2206 | 2206 | 032009SeLtd |
| 25DSL3 | 90 | 25DSL3-90 | 9.8 | 489.7 | 0.9 | 0.11 | 450 | 450 | 032009SeLtd |
| 25DSL3 | 120 | 25DSL3-120 | 9.8 | 490.1 | 0.3 | 0.11 | 162 | 162 | 032009SeLtd |
| 25DSL3 | 140 | 25DSL3-140 | 9.8 | 489.4 | 0.3 | 0.11 | 130 | 130 | 032009SeLtd |

D1-1.2. DV 25 LIVE SELENIUM, continued.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 25DSL3 | 188 | 25DSL3-188 | 9.8 | 489.6 | 0.10 | 0.11 | 48 | 54 | 032009SeFeMn |

D1-1.3. DV 10 KILLED SELENIUM.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 10DCK1 | 0 | 10DCK1-0 | 9.79 | 489.6 | 17.09 | 0.18 | 8367 | 8367 | 032109cps_SeFeMn |
| 10DCK1 | 24 | 10DCK1-24 | 9.87 | 493.7 | 13.33 | 0.53 | 6581 | 6581 | 040409cps_SeLtdEnd |
| 10DCK1 | 66 | 10DCK1-66 | 9.81 | 490.5 | 29.12 | 0.54 | 14283 | 14283 | 032109cps_SeLtd |
| 10DCK1 | 139 | 10DCK1-139 | 9.80 | 489.9 | 25.73 | 0.54 | 12602 | 12602 | 032109cps_SeLtd |
| 10DCK1 | 162 | 10DCK1-162 | 9.88 | 493.9 | 21.11 | 0.53 | 10426 | 10426 | 040409cps_SeLtdEnd |
| 10DCK1 | 190 | 10DCK1-190 | 9.80 | 490.1 | 7.42 | 0.18 | 3636 | 3636 | 032109cps_SeFeMn |
| 10DCK1 | 270 | 10DCK1-270 | 9.86 | 493.2 | 22.04 | 0.53 | 10869 | 10869 | 040409cps_SeLtdEnd |
| 10DCK2 | 0 | 10DCK2-0 | 9.78 | 488.9 | 20.02 | 0.18 | 9786 | 9786 | 032109cps_SeFeMn |
| 10DCK2 | 24 | 10DCK2-24 | 9.85 | 492.5 | 21.11 | 0.53 | 10396 | 10396 | 040409cps_SeLtdEnd |
| 10DCK2 | 66 | 10DCK2-66 | 9.81 | 490.3 | 28.55 | 0.54 | 13999 | 13999 | 032109cps_SeLtd |
| 10DCK2 | 139 | 10DCK2-139 | 9.82 | 491.0 | 25.12 | 0.54 | 12335 | 12335 | 032109cps_SeLtd |
| 10DCK2 | 162 | 10DCK2-162 | 9.85 | 492.4 | 14.44 | 0.53 | 7110 | 7110 | 040409cps_SeLtdEnd |
| 10DCK2 | 190 | 10DCK2-190 | 9.82 | 491.1 | 4.36 | 0.18 | 2141 | 2141 | 032109cps_SeFeMn |
| 10DCK2 | 270 | 10DCK2-270 | 9.82 | 490.91 | 17.2 | 0.53 | 8443.7 | 8444 | 040409cps_SeLtdEnd |
| 10DCK3 | 0 | 10DCK3-0 | 9.78 | 488.9 | 20.22 | 0.18 | 9883 | 9883 | 032109cps_SeFeMn |
| 10DCK3 | 24 | 10DCK3-24 | 9.86 | 493.2 | 30.00 | 0.53 | 14797 | 14797 | 040409cps_SeLtdEnd |
| 10DCK3 | 66 | 10DCK3-66 | 9.82 | 490.9 | 26.87 | 0.54 | 13189 | 13189 | 032109cps_SeLtd |
| 10DCK3 | 139 | 10DCK3-139 | 9.81 | 490.4 | 25.64 | 0.54 | 12575 | 12575 | 032109cps_SeLtd |
| 10DCK3 | 162 | 10DCK3-162 | 9.85 | 492.4 | 38.89 | 0.53 | 19151 | 19151 | 040409cps_SeLtdEnd |
| 10DCK3 | 190 | 10DCK3-190 | 9.82 | 491.2 | 6.15 | 0.18 | 3021 | 3021 | 032109cps_SeFeMn |
| 10DCK3 | 270 | 10DCK3-270 | 9.88 | 493.76 | 35.29 | 0.53 | 17425 | 17425 | 040409cps_SeLtdEnd |
| 10DRK1 | 0 | 10DRK1-0 | 9.81 | 490.4 | 18.55 | 0.18 | 9094 | 9094 | 032109cps_SeFeMn |
| 10DRK1 | 24 | 10DRK1-24 | 9.86 | 493.1 | 6.67 | 0.53 | 3289 | 3289 | 040409cps_SeLtdEnd |
| 10DRK1 | 66 | 10DRK1-66 | 9.82 | 491.1 | 26.91 | 0.54 | 13217 | 13217 | 032109cps_SeLtd |
| 10DRK1 | 139 | 10DRK1-139 | 9.81 | 490.7 | 25.78 | 0.54 | 12649 | 12649 | 032109cps_SeLtd |
| 10DRK1 | 162 | 10DRK1-162 | 9.85 | 492.6 | 17.78 | 0.53 | 8759 | 8759 | 040409cps_SeLtdEnd |
| 10DRK1 | 190 | 10DRK1-190 | 9.80 | 489.8 | 13.23 | 0.18 | 6480 | 6480 | 032109cps_SeFeMn |
| 10DRK1 | 270 | 10DRK1-270 | 9.82 | 491.1 | 19.64 | 0.53 | 9647 | 9647 | 040409cps_SeLtdEnd |
| 10DRK2 | 0 | 10DRK2-0 | 9.79 | 489.3 | 18.93 | 0.18 | 9261 | 9261 | 032109cps_SeFeMn |

D1-1.3. DV 10 KILLED SELENIUM, continued.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 10DRK2 | 24 | 10DRK2-24 | 9.87 | 493.5 | 36.67 | 0.53 | 18096 | 18096 | 040409cps_SeLtdEnd |
| 10DRK2 | 66 | 10DRK2-66 | 9.80 | 490.0 | 24.86 | 0.54 | 12184 | 12184 | 032109cps_SeLtd |
| 10DRK2 | 139 | 10DRK2-139 | 9.81 | 490.3 | 28.29 | 0.54 | 13870 | 13870 | 032109cps_SeLtd |
| 10DRK2 | 162 | 10DRK2-162 | 9.83 | 491.5 | 44.45 | 0.53 | 21849 | 21849 | 040409cps_SeLtdEnd |
| 10DRK2 | 190 | 10DRK2-190 | 9.79 | 489.5 | 9.15 | 0.18 | 4479 | 4479 | 032109cps_SeFeMn |
| 10DRK2 | 270 | 10DRK2-270 | 9.80 | 490.0 | 18.32 | 0.53 | 8976 | 8976 | 040409cps_SeLtdEnd |
| 10DRK3 | 0 | 10DRK3-0 | 9.79 | 489.7 | 18.11 | 0.18 | 8867 | 8867 | 032109cps_SeFeMn |
| 10DRK3 | 24 | 10DRK3-24 | 9.86 | 492.8 | 12.22 | 0.53 | 6022 | 6022 | 040409cps_SeLtdEnd |
| 10DRK3 | 66 | 10DRK3-66 | 9.82 | 491.0 | 27.83 | 0.54 | 13663 | 13663 | 032109cps_SeLtd |
| 10DRK3 | 139 | 10DRK3-139 | 9.82 | 491.1 | 24.21 | 0.54 | 11887 | 11887 | 032109cps_SeLtd |
| 10DRK3 | 162 | 10DRK3-162 | 9.84 | 492.0 | 44.45 | 0.53 | 21871 | 21871 | 040409cps_SeLtdEnd |
| 10DRK3 | 190 | 10DRK3-190 | 9.82 | 490.9 | 8.80 | 0.18 | 4320 | 4320 | 032109cps_SeFeMn |
| 10DRK3 | 270 | 10DRK3-270 | 9.86 | 493.1 | 20.37 | 0.53 | 10043 | 10043 | 040409cps_SeLtdEnd |
| 10DSK1 | 0 | 10DSK1-0 | 9.80 | 489.8 | 18.44 | 0.18 | 9033 | 9033 | 032109cps_SeFeMn |
| 10DSK1 | 24 | 10DSK1-24 | 9.87 | 493.4 | 47.78 | 0.53 | 23575 | 23575 | 040409cps_SeLtdEnd |
| 10DSK1 | 66 | 10DSK1-66 | 9.79 | 489.6 | 25.57 | 0.54 | 12521 | 12521 | 032109cps_SeLtd |
| 10DSK1 | 139 | 10DSK1-139 | 9.82 | 490.8 | 24.49 | 0.54 | 12016 | 12016 | 032109cps_SeLtd |
| 10DSK1 | 162 | 10DSK1-162 | 9.85 | 492.4 | 10.00 | 0.53 | 4924 | 4924 | 040409cps_SeLtdEnd |
| 10DSK1 | 190 | 10DSK1-190 | 9.82 | 491.0 | 17.80 | 0.18 | 8739 | 8739 | 032109cps_SeFeMn |
| 10DSK1 | 270 | 10DSK1-270 | 9.86 | 493.1 | 23.89 | 0.53 | 11781 | 11781 | 040409cps_SeLtdEnd |
| 10DSK2 | 0 | 10DSK2-0 | 9.78 | 489.2 | 17.10 | 0.18 | 8362 | 8362 | 032109cps_SeFeMn |
| 10DSK2 | 24 | 10DSK2-24 | 9.86 | 493.2 | 15.56 | 0.53 | 7674 | 7674 | 040409cps_SeLtdEnd |
| 10DSK2 | 66 | 10DSK2-66 | 9.82 | 491.2 | 26.08 | 0.54 | 12810 | 12810 | 032109cps_SeLtd |
| 10DSK2 | 139 | 10DSK2-139 | 9.82 | 491.1 | 20.42 | 0.54 | 10029 | 10029 | 032109cps_SeLtd |
| 10DSK2 | 162 | 10DSK2-162 | 9.86 | 493.2 | 7.78 | 0.53 | 3837 | 3837 | 040409cps_SeLtdEnd |
| 10DSK2 | 190 | 10DSK2-190 | 9.81 | 490.3 | 15.14 | 0.18 | 7423 | 7423 | 032109cps_SeFeMn |
| 10DSK2 | 270 | 10DSK2-270 | 9.85 | 492.3 | 43.67 | 0.53 | 21498 | 21498 | 040409cps_SeLtdEnd |
| 10DSK3 | 0 | 10DSK3-0 | 9.80 | 490.1 | 21.67 | 0.18 | 10622 | 10622 | 032109cps_SeFeMn |
| 10DSK3 | 24 | 10DSK3-24 | 9.86 | 493.2 | 27.78 | 0.53 | 13701 | 13701 | 040409cps_SeLtdEnd |
| 10DSK3 | 66 | 10DSK3-66 | 9.81 | 490.7 | 30.36 | 0.54 | 14900 | 14900 | 032109cps_SeLtd |
| 10DSK3 | 139 | 10DSK3-139 | 9.82 | 490.8 | 25.37 | 0.54 | 12451 | 12451 | 032109cps_SeLtd |

D1-1.3. DV 10 KILLED SELENIUM, continued.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 10DSK3 | 162 | 10DSK3-162 | 9.85 | 492.7 | 31.11 | 0.53 | 15329 | 15329 | 040409cps_SeLtdEnd |
| 10DSK3 | 190 | 10DSK3-190 | 9.82 | 491.0 | 21.48 | 0.18 | 10546 | 10546 | 032109cps_SeFeMn |
| 10DSK3 | 270 | 10DSK3-270 | 9.81 | 490.7 | 28.18 | 0.53 | 13828 | 13828 | 040409cps_SeLtdEnd |

D1-1.4. DV 25 KILLED SELENIUM.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 25DCK1 | 0 | 25DCK1-0 | 9.79 | 489.3 | 18.65 | 0.80 | 9124 | 9124 | 020909Aquant |
| 25DCK1 | 72 | 25DCK1-72 | 9.79 | 489.7 | 28.95 | 0.54 | 14176 | 14176 | 032109cps_SeLtd |
| 25DCK1 | 120 | 25DCK1-120 | 9.78 | 488.9 | 19.9 | 0.80 | 9719 | 9719 | 020909Aquant |
| 25DCK1 | 216 | 25DCK1-216 | 9.81 | 490.7 | 23.19 | 0.84 | 11379 | 11379 | 21509 |
| 25DCK1 | 456 | 25DCK1-456 | 9.81 | 490.3 | 20.45 | 0.02 | 10024 | 10024 | 032009SeFeMn |
| 25DCK2 | 0 | 25DCK2-0 | 9.75 | 487.6 | 21.5 | 0.80 | 10476 | 10476 | 020909Aquant |
| 25DCK2 | 72 | 25DCK2-72 | 9.81 | 490.7 | 32.57 | 0.54 | 15982 | 15982 | 032109cps_SeLtd |
| 25DCK2 | 120 | 25DCK2-120 | 9.79 | 489.4 | 20.4 | 0.80 | 9966 | 9966 | 020909Aquant |
| 25DCK2 | 216 | 25DCK2-216 | 9.80 | 490.2 | 24.08 | 0.84 | 11801 | 11801 | 21509 |
| 25DCK2 | 456 | 25DCK2-456 | 9.81 | 490.5 | 22.33 | 0.02 | 10953 | 10953 | 032009SeFeMn |
| 25DCK3 | 0 | 25DCK3-0 | 9.77 | 488.5 | 15.2 | 0.80 | 7412 | 7412 | 020909Aquant |
| 25DCK3 | 72 | 25DCK3-72 | 9.81 | 490.7 | 26.45 | 0.54 | 12977 | 12977 | 032109cps_SeLtd |
| 25DCK3 | 120 | 25DCK3-120 | 9.77 | 488.7 | 19.1 | 0.80 | 9313 | 9313 | 020909Aquant |
| 25DCK3 | 216 | 25DCK3-216 | 9.81 | 490.5 | 21.87 | 0.84 | 10725 | 10725 | 21509 |
| 25DCK3 | 456 | 25DCK3-456 | 9.80 | 490.2 | 17.96 | 0.02 | 8804 | 8804 | 032009SeFeMn |
| 25DRK1 | 0 | 25DRK1-0 | 9.77 | 488.5 | 20.4 | 0.80 | 9947 | 9947 | 020909Aquant |
| 25DRK1 | 72 | 25DRK1-72 | 9.80 | 489.9 | 30.60 | 0.54 | 14990 | 14990 | 032109cps_SeLtd |
| 25DRK1 | 120 | 25DRK1-120 | 9.78 | 488.8 | 21.1 | 0.80 | 10337 | 10337 | 020909Aquant |
| 25DRK1 | 216 | 25DRK1-216 | 9.81 | 490.4 | 27.31 | 0.84 | 13391 | 13391 | 21509 |
| 25DRK1 | 456 | 25DRK1-456 | 9.81 | 490.3 | 23.01 | 0.02 | 11279 | 11279 | 032009SeFeMn |
| 25DRK2 | 0 | 25DRK2-0 | 9.78 | 488.8 | 19.2 | 0.80 | 9406 | 9406 | 020909Aquant |
| 25DRK2 | 72 | 25DRK2-72 | 9.81 | 490.6 | 26.75 | 0.54 | 13124 | 13124 | 032109cps_SeLtd |
| 25DRK2 | 120 | 25DRK2-120 | 9.76 | 488.2 | 21.9 | 0.80 | 10690 | 10690 | 020909Aquant |

D1-1.4. DV 25 KILLED SELENIUM, continued.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 25DRK2 | 216 | 25DRK2-216 | 9.88 | 494.1 | 26.40 | 0.84 | 13043 | 13043 | 21509 |
| 25DRK2 | 456 | 25DRK2-456 | 9.81 | 490.3 | 20.28 | 0.02 | 9944 | 9944 | 032009SeFeMn |
| 25DSK1 | 0 | 25DSK1-0 | 9.78 | 489.1 | 19.8 | 0.80 | 9704 | 9704 | 020909Aquant |
| 25DSK1 | 72 | 25DSK1-72 | 9.80 | 489.8 | 29.46 | 0.54 | 14426 | 14426 | 032109cps_SeLtd |
| 25DSK1 | 120 | 25DSK1-120 | 9.76 | 488.1 | 21.6 | 0.80 | 10560 | 10560 | 020909Aquant |
| 25DSK1 | 216 | 25DSK1-216 | 9.81 | 490.5 | 25.13 | 0.84 | 12328 | 12328 | 21509 |
| 25DSK1 | 456 | 25DSK1-456 | 9.80 | 490.2 | 16.50 | 0.02 | 8089 | 8089 | 032009SeFeMn |
| 25DSK2 | 0 | 25DSK2-0 | 9.80 | 489.9 | 18.4 | 0.80 | 9024 | 9024 | 020909Aquant |
| 25DSK2 | 72 | 25DSK2-72 | 9.81 | 490.3 | 31.75 | 0.54 | 15569 | 15569 | 032109cps_SeLtd |
| 25DSK2 | 120 | 25DSK2-120 | 9.78 | 489.0 | 16.6 | 0.80 | 8096 | 8096 | 020909Aquant |
| 25DSK2 | 216 | 25DSK2-216 | 9.81 | 490.3 | 24.99 | 0.84 | 12250 | 12250 | 21509 |
| 25DSK2 | 456 | 25DSK2-456 | 9.80 | 490.0 | 23.02 | 0.02 | 11281 | 11281 | 032009SeFeMn |
| 25DSK3 | 0 | 25DSK3-0 | 9.76 | 488.0 | 17.1 | 0.80 | 8352 | 8352 | 020909Aquant |
| 25DSK3 | 72 | 25DSK3-72 | 9.80 | 489.9 | 25.87 | 0.54 | 12673 | 12673 | 032109cps_SeLtd |
| 25DSK3 | 120 | 25DSK3-120 | 9.78 | 489.0 | 22.1 | 0.80 | 10815 | 10815 | 020909Aquant |
| 25DSK3 | 216 | 25DSK3-216 | 9.80 | 490.0 | -0.02 | 0.84 | -10 | 412 | 21509 |
| 25DSK3 | 456 | 25DSK3-456 | 9.80 | 490.2 | 20.63 | 0.02 | 10113 | 10113 | 032009SeFeMn |

Table D1-2. Iron and Manganese ICP-MS data for Dry Valley Mine Saturated Rate Experiments.

D1-2.1. DV 10 LIVE IRON/MANGANESE.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 10DCL1 | 0 | 10DCL1-0 | 9.811 | 490.5 | 12.70 | 0.86 | 6228 | 6228 | 1.04 | 0.35 | 512 | 512 | 021309cps |
| 10DCL1 | 8 | 10DCL1-08 | 9.812 | 490.6 | 12.60 | 0.86 | 6183 | 6183 | 1.00 | 0.35 | 490 | 490 | 021309cps |
| 10DCL1 | 20 | 10DCL1-20 | 9.818 | 490.9 | 12.91 | 0.86 | 6338 | 6338 | 0.97 | 0.35 | 474 | 474 | 021309cps |
| 10DCL1 | 32 | 10DCL1-32 | 9.796 | 489.8 | | | | | | | | | |
| 10DCL1 | 53 | 10DCL1-53 | 9.841 | 492.1 | | | | | | | | | |
| 10DCL1 | 70 | 10DCL1-70 | 9.606 | 480.3 | | | | | | | | | |
| 10DCL1 | 80 | 10DCL1-80 | 9.811 | 490.6 | 216.37 | 5.17 | 106144 | 106144 | 2.50 | 0.16 | 1224 | 1224 | 021609Bcps |
| 10DCL1 | 104 | 10DCL1-104 | 9.796 | 489.8 | 240.84 | 1.31 | 117965 | 117965 | 3.37 | 0.22 | 1653 | 1653 | 021709cps |
| 10DCL1 | 128 | 10DCL1-128 | 9.795 | 489.8 | 229.39 | 0.03 | 112346 | 112346 | 1.86 | 0.01 | 909 | 909 | 021809copy |
| 10DCL1 | 140 | 10DCL1-140 | 9.793 | 489.6 | 231.19 | 0.03 | 113198 | 113198 | 2.00 | 0.01 | 979 | 979 | 021809copy |
| 10DCL1 | 164 | 10DCL1-164 | 9.750 | 487.5 | | | | | | | | | |
| 10DCL1 | 188 | 10DCL1-188 | 9.788 | 489.4 | | | | | | | | | |
| 10DCL1 | 212 | 10DCL1-212 | 9.781 | 489.1 | | | | | | | | | |
| 10DCL1 | 272 | 10DCL1-272 | 9.729 | 486.4 | 25.75 | 1.00 | 12528 | 12528 | 1.71 | 0.10 | 832 | 832 | 022509Bcps |
| 10DCL2 | 0 | 10DCL2-0 | 9.836 | 491.8 | 12.56 | 0.86 | 6177 | 6177 | 0.99 | 0.35 | 487 | 487 | 021309cps |
| 10DCL2 | 8 | 10DCL2-08 | 9.827 | 491.4 | 12.52 | 0.86 | 6149 | 6149 | 1.00 | 0.35 | 493 | 493 | 021309cps |
| 10DCL2 | 20 | 10DCL2-20 | 9.836 | 491.8 | 12.47 | 0.86 | 6134 | 6134 | 0.95 | 0.35 | 466 | 466 | 021309cps |
| 10DCL2 | 32 | 10DCL2-32 | 9.783 | 489.1 | | | | | | | | | |
| 10DCL2 | 53 | 10DCL2-53 | 9.840 | 492.0 | | | | | | | | | |
| 10DCL2 | 70 | 10DCL2-70 | 9.773 | 488.6 | | | | | | | | | |
| 10DCL2 | 80 | 10DCL2-80 | 9.790 | 489.5 | 221.42 | 5.17 | 108389 | 108389 | 2.48 | 0.16 | 1213 | 1213 | 021609Bcps |
| 10DCL2 | 104 | 10DCL2-104 | 9.796 | 489.8 | 240.35 | 1.31 | 117721 | 117721 | 3.34 | 0.22 | 1636 | 1636 | 021709cps |
| 10DCL2 | 128 | 10DCL2-128 | 9.797 | 489.9 | 232.80 | 0.03 | 114040 | 114040 | 1.78 | 0.01 | 872 | 872 | 021809copy |
| 10DCL2 | 140 | 10DCL2-140 | 9.791 | 489.5 | 230.68 | 0.03 | 112926 | 112926 | 1.92 | 0.01 | 942 | 942 | 021809copy |
| 10DCL2 | 164 | 10DCL2-164 | 9.814 | 490.7 | | | | | | | | | |
| 10DCL2 | 188 | 10DCL2-188 | 9.837 | 491.9 | | | | | | | | | |
| 10DCL2 | 212 | 10DCL2-212 | 9.797 | 489.9 | | | | | | | | | |

D1-2.1. DV 10 LIVE IRON/MANGANESE, continued.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 10DCL2 | 272 | 10DCL2-272 | 9.783 | 489.2 | 26.04 | 1.00 | 12738 | 12738 | 1.50 | 0.10 | 732 | 732 | 022509Bcps |
| 10DCL3 | 0 | 10DCL3-0 | 9.820 | 491.0 | 12.04 | 0.86 | 5910 | 5910 | 0.99 | 0.35 | 489 | 489 | 021309cps |
| 10DCL3 | 8 | 10DCL3-08 | 9.808 | 490.4 | 11.57 | 0.86 | 5672 | 5672 | 0.99 | 0.35 | 486 | 486 | 021309cps |
| 10DCL3 | 20 | 10DCL3-20 | 9.808 | 490.4 | 11.46 | 0.86 | 5622 | 5622 | 0.97 | 0.35 | 475 | 475 | 021309cps |
| 10DCL3 | 32 | 10DCL3-32 | 9.800 | 490.0 | | | | | | | | | |
| 10DCL3 | 53 | 10DCL3-53 | 9.821 | 491.0 | | | | | | | | | |
| 10DCL3 | 70 | 10DCL3-70 | 9.769 | 488.5 | | | | | | | | | |
| 10DCL3 | 80 | 10DCL3-80 | 9.792 | 489.6 | 220.20 | 5.17 | 107812 | 107812 | 2.42 | 0.16 | 1184 | 1184 | 021609Bcps |
| 10DCL3 | 104 | 10DCL3-104 | 9.780 | 489.0 | 241.83 | 1.31 | 118259 | 118259 | 7.22 | 0.22 | 3529 | 3529 | 021709cps |
| 10DCL3 | 128 | 10DCL3-128 | 9.794 | 489.7 | 231.93 | 0.03 | 113572 | 113572 | 1.83 | 0.01 | 898 | 898 | 021809copy |
| 10DCL3 | 140 | 10DCL3-140 | 9.790 | 489.5 | 233.40 | 0.03 | 114250 | 114250 | 1.84 | 0.01 | 901 | 901 | 021809copy |
| 10DCL3 | 164 | 10DCL3-164 | 9.810 | 490.5 | | | | | | | | | |
| 10DCL3 | 188 | 10DCL3-188 | 9.803 | 490.1 | | | | | | | | | |
| 10DCL3 | 212 | 10DCL3-212 | 9.783 | 489.2 | | | | | | | | | |
| 10DCL3 | 272 | 10DCL3-272 | 9.794 | 489.7 | 28.58 | 1.00 | 13998 | 13998 | 1.93 | 0.10 | 944 | 944 | 022509Bcps |
| 10DRL1 | 0 | 10DRL1-0 | 9.836 | 491.8 | 12.48 | 0.86 | 6138 | 6138 | 3.89 | 0.35 | 1915 | 1915 | 021309cps |
| 10DRL1 | 8 | 10DRL1-08 | 9.828 | 491.4 | 12.47 | 0.86 | 6127 | 6127 | 3.88 | 0.35 | 1908 | 1908 | 021309cps |
| 10DRL1 | 20 | 10DRL1-20 | 9.832 | 491.6 | 12.38 | 0.86 | 6084 | 6084 | 4.32 | 0.35 | 2126 | 2126 | 021309cps |
| 10DRL1 | 32 | 10DRL1-32 | 9.799 | 489.9 | | | | | | | | | |
| 10DRL1 | 53 | 10DRL1-53 | 9.828 | 491.4 | | | | | | | | | |
| 10DRL1 | 70 | 10DRL1-70 | 9.755 | 487.8 | | | | | | | | | |
| 10DRL1 | 80 | 10DRL1-80 | 9.793 | 489.6 | 217.99 | 5.17 | 106736 | 106736 | 5.14 | 0.16 | 2518 | 2518 | 021609Bcps |
| 10DRL1 | 104 | 10DRL1-104 | 9.801 | 490.0 | 238.26 | 1.31 | 116760 | 116760 | 7.11 | 0.22 | 3483 | 3483 | 021709cps |
| 10DRL1 | 128 | 10DRL1-128 | 9.771 | 488.5 | 235.37 | 0.03 | 114983 | 114983 | 4.05 | 0.01 | 1978 | 1978 | 021809copy |
| 10DRL1 | 140 | 10DRL1-140 | 9.789 | 489.5 | 235.23 | 0.03 | 115135 | 115135 | 4.67 | 0.01 | 2285 | 2285 | 021809copy |
| 10DRL1 | 164 | 10DRL1-164 | 9.833 | 491.7 | | | | | | | | | |
| 10DRL1 | 188 | 10DRL1-188 | 9.797 | 489.9 | | | | | | | | | |
| 10DRL1 | 212 | 10DRL1-212 | 9.798 | 489.9 | | | | | | | | | |
| 10DRL1 | 272 | 10DRL1-272 | 9.785 | 489.2 | 26.20 | 1.00 | 12819 | 12819 | 8.08 | 0.10 | 3952 | 3952 | 022509Bcps |

D1-2.1. DV 10 LIVE IRON/MANGANESE, continued.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 10DRL2 | 0 | 10DRL2-0 | 9.840 | 492.0 | 12.54 | 0.86 | 6168 | 6168 | 3.80 | 0.35 | 1871 | 1871 | 021309cps |
| 10DRL2 | 8 | 10DRL2-08 | 9.847 | 492.4 | 12.47 | 0.86 | 6139 | 6139 | 3.62 | 0.35 | 1781 | 1781 | 021309cps |
| 10DRL2 | 20 | 10DRL2-20 | 9.830 | 491.5 | 12.60 | 0.86 | 6195 | 6195 | 3.79 | 0.35 | 1861 | 1861 | 021309cps |
| 10DRL2 | 32 | 10DRL2-32 | 9.799 | 489.9 | | | | | | | | | |
| 10DRL2 | 53 | 10DRL2-53 | 9.828 | 491.4 | | | | | | | | | |
| 10DRL2 | 70 | 10DRL2-70 | 9.767 | 488.4 | | | | | | | | | |
| 10DRL2 | 80 | 10DRL2-80 | 9.798 | 489.9 | 224.71 | 5.17 | 110087 | 110087 | 5.81 | 0.16 | 2847 | 2847 | 021609Bcps |
| 10DRL2 | 104 | 10DRL2-104 | 9.800 | 490.0 | 239.33 | 1.31 | 117270 | 117270 | 7.29 | 0.22 | 3570 | 3570 | 021709cps |
| 10DRL2 | 128 | 10DRL2-128 | 9.784 | 489.2 | 232.36 | 0.03 | 113665 | 113665 | 3.66 | 0.01 | 1792 | 1792 | 021809copy |
| 10DRL2 | 140 | 10DRL2-140 | 9.799 | 490.0 | 251.86 | 0.03 | 123399 | 123399 | 9.86 | 0.01 | 4829 | 4829 | 021809copy |
| 10DRL2 | 164 | 10DRL2-164 | 9.811 | 490.6 | | | | | | | | | |
| 10DRL2 | 188 | 10DRL2-188 | 9.796 | 489.8 | | | | | | | | | |
| 10DRL2 | 212 | 10DRL2-212 | 9.804 | 490.2 | | | | | | | | | |
| 10DRL2 | 272 | 10DRL2-272 | 9.781 | 489.0 | 25.77 | 1.00 | 12604 | 12604 | 7.10 | 0.10 | 3470 | 3470 | 022509Bcps |
| 10DRL3 | 0 | 10DRL3-0 | 9.831 | 491.6 | 11.59 | 0.86 | 5696 | 5696 | 3.73 | 0.35 | 1835 | 1835 | 021309cps |
| 10DRL3 | 8 | 10DRL3-08 | 9.823 | 491.2 | 11.69 | 0.86 | 5741 | 5741 | 5.52 | 0.35 | 2713 | 2713 | 021309cps |
| 10DRL3 | 20 | 10DRL3-20 | 9.809 | 490.4 | 12.96 | 0.86 | 6357 | 6357 | 4.61 | 0.35 | 2261 | 2261 | 021309cps |
| 10DRL3 | 32 | 10DRL3-32 | 9.786 | 489.3 | | | | | | | | | |
| 10DRL3 | 53 | 10DRL3-53 | 9.822 | 491.1 | | | | | | | | | |
| 10DRL3 | 70 | 10DRL3-70 | 9.805 | 490.2 | | | | | | | | | |
| 10DRL3 | 80 | 10DRL3-80 | 9.791 | 489.6 | 222.99 | 5.17 | 109164 | 109164 | 5.55 | 0.16 | 2715 | 2715 | 021609Bcps |
| 10DRL3 | 104 | 10DRL3-104 | 9.796 | 489.8 | 237.98 | 1.31 | 116559 | 116559 | 7.47 | 0.22 | 3659 | 3659 | 021709cps |
| 10DRL3 | 128 | 10DRL3-128 | 9.799 | 489.9 | 237.90 | 0.03 | 116553 | 116553 | 4.28 | 0.01 | 2097 | 2097 | 021809copy |
| 10DRL3 | 140 | 10DRL3-140 | 9.781 | 489.1 | 233.43 | 0.03 | 114160 | 114160 | 4.84 | 0.01 | 2368 | 2368 | 021809copy |
| 10DRL3 | 164 | 10DRL3-164 | 9.803 | 490.2 | | | | | | | | | |
| 10DRL3 | 188 | 10DRL3-188 | 0.821 | 41.0 | | | | | | | | | |
| 10DRL3 | 212 | 10DRL3-212 | 9.800 | 490.0 | | | | | | | | | |
| 10DRL3 | 272 | 10DRL3-272 | 9.793 | 489.7 | 25.24 | 1.00 | 12358 | 12358 | 8.21 | 0.10 | 4019 | 4019 | 022509Bcps |
| 10DSL1 | 0 | 10DSL1-0 | 9.728 | 486.4 | 13.21 | 0.86 | 6428 | 6428 | 5.60 | 0.35 | 2724 | 2724 | 021309cps |

D1-2.1. DV 10 LIVE IRON/MANGANESE, continued.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 10DSL1 | 8 | 10DSL1-08 | 9.798 | 489.9 | 13.67 | 0.86 | 6696 | 6696 | 5.61 | 0.35 | 2746 | 2746 | 021309cps |
| 10DSL1 | 20 | 10DSL1-20 | 9.804 | 490.2 | 13.09 | 0.86 | 6419 | 6419 | 5.62 | 0.35 | 2757 | 2757 | 021309cps |
| 10DSL1 | 32 | 10DSL1-32 | 9.789 | 489.4 | | | | | | | | | |
| 10DSL1 | 53 | 10DSL1-53 | 9.810 | 490.5 | | | | | | | | | |
| 10DSL1 | 70 | 10DSL1-70 | 9.794 | 489.7 | | | | | | | | | |
| 10DSL1 | 80 | 10DSL1-80 | 9.809 | 490.5 | 218.43 | 5.17 | 107134 | 107134 | 6.93 | 0.16 | 3397 | 3397 | 021609Bcps |
| 10DSL1 | 104 | 10DSL1-104 | 9.808 | 490.4 | 236.91 | 1.31 | 116181 | 116181 | 4.14 | 0.22 | 2032 | 2032 | 021809copy |
| 10DSL1 | 128 | 10DSL1-128 | 9.805 | 490.3 | 231.52 | 0.03 | 113506 | 113506 | 4.74 | 0.01 | 2324 | 2324 | 021809copy |
| 10DSL1 | 140 | 10DSL1-140 | 9.721 | 486.1 | 243.58 | 0.03 | 118393 | 118393 | 7.13 | 0.01 | 3464 | 3464 | 021809copy |
| 10DSL1 | 164 | 10DSL1-164 | 9.824 | 491.2 | | | | | | | | | |
| 10DSL1 | 188 | 10DSL1-188 | 9.805 | 490.2 | | | | | | | | | |
| 10DSL1 | 212 | 10DSL1-212 | 9.803 | 490.2 | | | | | | | | | |
| 10DSL1 | 272 | 10DSL1-272 | 9.784 | 489.2 | 25.72 | 1.00 | 12582 | 12582 | 10.35 | 0.10 | 5063 | 5063 | 022509Bcps |
| 10DSL2 | 0 | 10DSL2-0 | 9.817 | 490.8 | 13.71 | 0.86 | 6732 | 6732 | 5.57 | 0.35 | 2734 | 2734 | 021309cps |
| 10DSL2 | 8 | 10DSL2-08 | 9.822 | 491.1 | 13.20 | 0.86 | 6481 | 6481 | 5.47 | 0.35 | 2685 | 2685 | 021309cps |
| 10DSL2 | 20 | 10DSL2-20 | 9.801 | 490.1 | 13.08 | 0.86 | 6412 | 6412 | 5.01 | 0.35 | 2454 | 2454 | 021309cps |
| 10DSL2 | 32 | 10DSL2-32 | 9.777 | 488.9 | | | | | | | | | |
| 10DSL2 | 53 | 10DSL2-53 | 9.807 | 490.3 | | | | | | | | | |
| 10DSL2 | 70 | 10DSL2-70 | 9.763 | 488.1 | | | | | | | | | |
| 10DSL2 | 80 | 10DSL2-80 | 9.813 | 490.6 | 220.43 | 5.17 | 108151 | 108151 | 6.74 | 0.16 | 3308 | 3308 | 021609Bcps |
| 10DSL2 | 104 | 10DSL2-104 | 9.794 | 489.7 | 233.80 | 1.31 | 114490 | 114490 | 4.77 | 0.22 | 2336 | 2336 | 021809copy |
| 10DSL2 | 128 | 10DSL2-128 | 9.791 | 489.6 | 229.19 | 0.03 | 112205 | 112205 | 5.13 | 0.01 | 2511 | 2511 | 021809copy |
| 10DSL2 | 140 | 10DSL2-140 | 9.800 | 490.0 | 234.91 | 0.03 | 115104 | 115104 | 4.75 | 0.01 | 2329 | 2329 | 021809copy |
| 10DSL2 | 164 | 10DSL2-164 | 9.811 | 490.6 | | | | | | | | | |
| 10DSL2 | 188 | 10DSL2-188 | 9.811 | 490.5 | | | | | | | | | |
| 10DSL2 | 212 | 10DSL2-212 | 9.788 | 489.4 | | | | | | | | | |
| 10DSL2 | 272 | 10DSL2-272 | 9.814 | 490.1 | 25.68 | 1.00 | 12586 | 12586 | 8.66 | 0.10 | 4243 | 4243 | 022509Bcps |
| 10DSL3 | 0 | 10DSL3-0 | 9.821 | 491.1 | 11.91 | 0.86 | 5851 | 5851 | 5.45 | 0.35 | 2675 | 2675 | 021309cps |
| 10DSL3 | 8 | 10DSL3-08 | 9.828 | 491.4 | 11.67 | 0.86 | 5736 | 5736 | 4.18 | 0.35 | 2054 | 2054 | 021309cps |

D1-2.1. DV 10 LIVE IRON/MANGANESE, continued.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 10DSL3 | 20 | 10DSL3-20 | 9.824 | 491.2 | 11.99 | 0.86 | 5889 | 5889 | 5.28 | 0.35 | 2594 | 2594 | 021309cps |
| 10DSL3 | 32 | 10DSL3-32 | 9.792 | 489.6 | | | | | | | | | |
| 10DSL3 | 53 | 10DSL3-53 | 9.793 | 489.6 | | | | | | | | | |
| 10DSL3 | 70 | 10DSL3-70 | 9.793 | 489.6 | | | | | | | | | |
| 10DSL3 | 80 | 10DSL3-80 | 9.810 | 490.5 | 220.15 | 5.17 | 107983 | 107983 | 6.20 | 0.16 | 3042 | 3042 | 021609Bcps |
| 10DSL3 | 104 | 10DSL3-104 | 9.806 | 490.3 | 234.59 | 1.31 | 115024 | 115024 | 4.04 | 0.22 | 1981 | 1981 | 021809copy |
| 10DSL3 | 128 | 10DSL3-128 | 9.789 | 489.5 | 235.09 | 0.03 | 115067 | 115067 | 4.43 | 0.01 | 2167 | 2167 | 021809copy |
| 10DSL3 | 140 | 10DSL3-140 | 9.807 | 490.4 | 235.19 | 0.03 | 115331 | 115331 | 8.45 | 0.01 | 4145 | 4145 | 021809copy |
| 10DSL3 | 164 | 10DSL3-164 | 9.804 | 490.2 | | | | | | | | | |
| 10DSL3 | 188 | 10DSL3-188 | 9.812 | 490.6 | | | | | | | | | |
| 10DSL3 | 212 | 10DSL3-212 | 9.805 | 490.3 | | | | | | | | | |
| 10DSL3 | 272 | 10DSL3-272 | 9.803 | 490.1 | 25.90 | 1.00 | 12695 | 12695 | 11.0 | 0.10 | 5410 | 5410 | 022509Bcps |

Shaded fields = data not collected for those samples.

D1-2.2. DV 25 LIVE IRON/MANGANESE.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 25DCL1 | 0 | 25DCL1-0 | 9.8 | 490.5 | 4.1 | 1.0 | 2003 | 2003 | 0.3 | 0.1 | 163 | 163 | 022509Bcps |
| 25DCL1 | 8 | 25DCL1-8 | 9.7 | 487.4 | 0.9 | 1.0 | 442 | 487 | 0.4 | 0.1 | 212 | 212 | 022509Bcps |
| 25DCL1 | 20 | 25DCL1-20 | 9.8 | 491.8 | -0.4 | 1.0 | -214 | 492 | 0.5 | 0.1 | 234 | 234 | 022509Bcps |
| 25DCL1 | 32 | 25DCL1-32 | 9.8 | 490.2 | | | | | | | | | |
| 25DCL1 | 54 | 25DCL1-54 | 9.8 | 490.2 | 17 | 2.7 | 8302 | 8302 | 0.86 | 0.22 | 420 | 420 | 032009SeFeMn |
| 25DCL1 | 66 | 25DCL1-66 | 9.8 | 489.6 | | | | | | | | | |
| 25DCL1 | 90 | 25DCL1-90 | 9.8 | 490.3 | | | | | | | | | |
| 25DCL1 | 120 | 25DCL1-120 | 9.8 | 489.8 | | | | | | | | | |
| 25DCL1 | 140 | 25DCL1-140 | 9.8 | 489.9 | | | | | | | | | |
| 25DCL1 | 188 | 25DCL1-188 | 10.0 | 499.5 | 17 | 2.7 | 8308 | 8308 | 1.39 | 0.22 | 695 | 695 | 032009SeFeMn |
| 25DCL2 | 0 | 25DCL2-0 | 9.8 | 491.0 | 4.3 | 1.0 | 2130 | 2130 | 0.3 | 0.1 | 167 | 167 | 022509Bcps |
| 25DCL2 | 8 | 25DCL2-8 | 9.8 | 490.9 | -0.4 | 1.0 | -218 | 491 | 0.4 | 0.1 | 173 | 173 | 022509Bcps |
| 25DCL2 | 20 | 25DCL2-20 | 9.8 | 491.7 | -0.4 | 1.0 | -196 | 492 | 0.4 | 0.1 | 218 | 218 | 022509Bcps |
| 25DCL2 | 32 | 25DCL2-32 | 9.8 | 488.6 | | | | | | | | | |
| 25DCL2 | 54 | 25DCL2-54 | 9.8 | 490.2 | 16 | 2.7 | 7895 | 7895 | 0.77 | 0.22 | 378 | 378 | 032009SeFeMn |
| 25DCL2 | 66 | 25DCL2-66 | 9.8 | 489.9 | | | | | | | | | |
| 25DCL2 | 90 | 25DCL2-90 | 9.8 | 490.2 | | | | | | | | | |
| 25DCL2 | 120 | 25DCL2-120 | 9.8 | 489.9 | | | | | | | | | |
| 25DCL2 | 140 | 25DCL2-140 | 9.8 | 490.3 | | | | | | | | | |
| 25DCL2 | 188 | 25DCL2-188 | 9.8 | 489.3 | 23 | 2.7 | 11498 | 11498 | 1.59 | 0.22 | 778 | 778 | 032009SeFeMn |
| 25DCL3 | 0 | 25DCL3-0 | 9.8 | 491.1 | 4.5 | 1.0 | 2188 | 2188 | 0.2 | 0.1 | 121 | 121 | 022509Bcps |
| 25DCL3 | 8 | 25DCL3-8 | 9.8 | 491.6 | -0.03 | 1.0 | -14 | 492 | 0.3 | 0.1 | 137 | 137 | 022509Bcps |
| 25DCL3 | 20 | 25DCL3-20 | 9.8 | 491.8 | -0.8 | 1.0 | -418 | 492 | 0.4 | 0.1 | 202 | 202 | 022509Bcps |
| 25DCL3 | 32 | 25DCL3-32 | 9.8 | 489.8 | | | | | | | | | |
| 25DCL3 | 54 | 25DCL3-54 | 9.8 | 489.9 | 17 | 2.7 | 8361 | 8361 | 1.05 | 0.22 | 516 | 516 | 032009SeFeMn |
| 25DCL3 | 66 | 25DCL3-66 | 9.8 | 490.5 | | | | | | | | | |
| 25DCL3 | 90 | 25DCL3-90 | 9.8 | 490.9 | | | | | | | | | |
| 25DCL3 | 120 | 25DCL3-120 | 9.8 | 490.5 | | | | | | | | | |
| 25DCL3 | 140 | 25DCL3-140 | 9.7 | 487.2 | | | | | | | | | |

D1-2.2. DV 25 LIVE IRON/MANGANESE, continued.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 25DCL3 | 188 | 25DCL3-188 | 9.8 | 489.2 | 17 | 2.7 | 8264 | 8264 | 1.37 | 0.22 | 672 | 672 | 032009SeFeMn |
| 25DRL1 | 0 | 25DRL1-0 | 9.8 | 491.2 | 4.1 | 1.0 | 2032 | 2032 | 4.5 | 0.1 | 2200 | 2200 | 022509Bcps |
| 25DRL1 | 8 | 25DRL1-8 | 9.8 | 491.3 | -0.6 | 1.0 | -281 | 491 | 4.7 | 0.1 | 2322 | 2322 | 022509Bcps |
| 25DRL1 | 20 | 25DRL1-20 | 9.8 | 491.2 | 2.8 | 1.0 | 1395 | 1395 | 5.2 | 0.1 | 2532 | 2532 | 022509Bcps |
| 25DRL1 | 32 | 25DRL1-32 | 9.8 | 489.7 | | | | | | | | | |
| 25DRL1 | 54 | 25DRL1-54 | 9.8 | 489.4 | 21 | 2.7 | 10332 | 10332 | 3.69 | 0.22 | 1808 | 1808 | 032009SeFeMn |
| 25DRL1 | 66 | 25DRL1-66 | 9.8 | 490.4 | | | | | | | | | |
| 25DRL1 | 90 | 25DRL1-90 | 9.8 | 490.5 | | | | | | | | | |
| 25DRL1 | 120 | 25DRL1-120 | 9.8 | 490.8 | | | | | | | | | |
| 25DRL1 | 140 | 25DRL1-140 | 9.8 | 489.3 | | | | | | | | | |
| 25DRL1 | 188 | 25DRL1-188 | 9.8 | 489.9 | 17 | 2.7 | 8226 | 8226 | 9.96 | 0.22 | 4877 | 4877 | 032009SeFeMn |
| 25DRL2 | 0 | 25DRL2-0 | 9.8 | 491.6 | 4.1 | 1.0 | 2003 | 2003 | 4.0 | 0.1 | 1969 | 1969 | 022509Bcps |
| 25DRL2 | 8 | 25DRL2-8 | 9.8 | 490.1 | 1.0 | 1.0 | 493 | 493 | 4.3 | 0.1 | 2092 | 2092 | 022509Bcps |
| 25DRL2 | 20 | 25DRL2-20 | 9.8 | 490.9 | -0.5 | 1.0 | -240 | 491 | 4.7 | 0.1 | 2303 | 2303 | 022509Bcps |
| 25DRL2 | 32 | 25DRL2-32 | 9.8 | 489.1 | | | | | | | | | |
| 25DRL2 | 54 | 25DRL2-54 | 9.8 | 489.5 | 16 | 2.7 | 7767 | 7767 | 3.27 | 0.22 | 1601 | 1601 | 032009SeFeMn |
| 25DRL2 | 66 | 25DRL2-66 | 9.8 | 489.2 | | | | | | | | | |
| 25DRL2 | 90 | 25DRL2-90 | 9.8 | 490.9 | | | | | | | | | |
| 25DRL2 | 120 | 25DRL2-120 | 9.8 | 490.4 | | | | | | | | | |
| 25DRL2 | 140 | 25DRL2-140 | 9.8 | 489.9 | | | | | | | | | |
| 25DRL2 | 188 | 25DRL2-188 | 9.8 | 490.2 | 17 | 2.7 | 8438 | 8438 | 6.22 | 0.22 | 3048 | 3048 | 032009SeFeMn |
| 25DRL3 | 0 | 25DRL3-0 | 9.8 | 490.1 | -0.1 | 1.0 | -28 | 490 | 4.5 | 0.1 | 2214 | 2214 | 022509Bcps |
| 25DRL3 | 8 | 25DRL3-8 | 9.8 | 492.0 | -0.6 | 1.0 | -307 | 492 | 4.7 | 0.1 | 2326 | 2326 | 022509Bcps |
| 25DRL3 | 20 | 25DRL3-20 | 9.8 | 491.5 | -0.7 | 1.0 | -362 | 492 | 5.0 | 0.1 | 2474 | 2474 | 022509Bcps |
| 25DRL3 | 32 | 25DRL3-32 | 9.8 | 489.9 | | | | | | | | | |
| 25DRL3 | 54 | 25DRL3-54 | 9.8 | 489.4 | 16 | 2.7 | 7756 | 7756 | 3.96 | 0.22 | 1936 | 1936 | 032009SeFeMn |
| 25DRL3 | 66 | 25DRL3-66 | 9.8 | 490.8 | | | | | | | | | |
| 25DRL3 | 90 | 25DRL3-90 | 9.8 | 490.6 | | | | | | | | | |
| 25DRL3 | 120 | 25DRL3-120 | 9.8 | 490.4 | | | | | | | | | |
| 25DRL3 | 140 | 25DRL3-140 | 9.8 | 489.3 | | | | | | | | | |

D1-2.2. DV 25 LIVE IRON/MANGANESE, continued.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 25DRL3 | 188 | 25DRL3-188 | 9.8 | 487.8 | 17 | 2.7 | 8183 | 8183 | 6.14 | 0.22 | 2995 | 2995 | 032009SeFeMn |
| 25DSL1 | 0 | 25DSL1-0 | 9.8 | 490.8 | -0.4 | 1.0 | -196 | 491 | 6.9 | 0.1 | 3394 | 3394 | 022509Bcps |
| 25DSL1 | 8 | 25DSL1-8 | 9.8 | 490.5 | -0.5 | 1.0 | -260 | 491 | 7.2 | 0.1 | 3547 | 3547 | 022509Bcps |
| 25DSL1 | 20 | 25DSL1-20 | 9.8 | 491.6 | -0.8 | 1.0 | -387 | 492 | 7.8 | 0.1 | 3831 | 3831 | 022509Bcps |
| 25DSL1 | 32 | 25DSL1-32 | 9.8 | 490.3 | | | | | | | | | |
| 25DSL1 | 54 | 25DSL1-54 | 9.8 | 489.9 | 16 | 2.7 | 7901 | 7901 | 5.31 | 0.22 | 2601 | 2601 | 032009SeFeMn |
| 25DSL1 | 66 | 25DSL1-66 | 9.8 | 490.0 | | | | | | | | | |
| 25DSL1 | 90 | 25DSL1-90 | 9.8 | 490.2 | | | | | | | | | |
| 25DSL1 | 120 | 25DSL1-120 | 9.8 | 489.8 | | | | | | | | | |
| 25DSL1 | 140 | 25DSL1-140 | 9.8 | 489.7 | | | | | | | | | |
| 25DSL1 | 188 | 25DSL1-188 | 9.8 | 490.4 | 17 | 2.7 | 8426 | 8426 | 6.65 | 0.22 | 3262 | 3262 | 032009SeFeMn |
| 25DSL2 | 0 | 25DSL2-0 | 9.8 | 491.3 | -0.6 | 1.0 | -288 | 491 | 7.3 | 0.1 | 3566 | 3566 | 022509Bcps |
| 25DSL2 | 8 | 25DSL2-8 | 9.8 | 491.2 | -0.2 | 1.0 | -86 | 491 | 7.5 | 0.1 | 3660 | 3660 | 022509Bcps |
| 25DSL2 | 20 | 25DSL2-20 | 9.8 | 491.6 | 1.5 | 1.0 | 740 | 740 | 7.8 | 0.1 | 3843 | 3843 | 022509Bcps |
| 25DSL2 | 32 | 25DSL2-32 | 9.8 | 490.3 | | | | | | | | | |
| 25DSL2 | 54 | 25DSL2-54 | 9.8 | 490.3 | 18 | 2.7 | 8687 | 8687 | 6.05 | 0.22 | 2965 | 2965 | 032009SeFeMn |
| 25DSL2 | 66 | 25DSL2-66 | 9.8 | 489.9 | | | | | | | | | |
| 25DSL2 | 90 | 25DSL2-90 | 9.8 | 489.8 | | | | | | | | | |
| 25DSL2 | 120 | 25DSL2-120 | 9.8 | 489.3 | | | | | | | | | |
| 25DSL2 | 140 | 25DSL2-140 | 9.8 | 490.2 | | | | | | | | | |
| 25DSL2 | 188 | 25DSL2-188 | 9.8 | 489.9 | 17 | 2.7 | 8391 | 8391 | 7.00 | 0.22 | 3431 | 3431 | 032009SeFeMn |
| 25DSL3 | 0 | 25DSL3-0 | 9.8 | 490.6 | -0.2 | 1.0 | -83 | 491 | 6.8 | 0.1 | 3325 | 3325 | 022509Bcps |
| 25DSL3 | 8 | 25DSL3-8 | 9.8 | 491.7 | -0.3 | 1.0 | -144 | 492 | 7.2 | 0.1 | 3517 | 3517 | 022509Bcps |
| 25DSL3 | 20 | 25DSL3-20 | 9.8 | 491.0 | 0.2 | 1.0 | 106 | 491 | 7.9 | 0.1 | 3863 | 3863 | 022509Bcps |
| 25DSL3 | 32 | 25DSL3-32 | 9.8 | 490.3 | | | | | | | | | |
| 25DSL3 | 54 | 25DSL3-54 | 9.8 | 489.0 | 16 | 2.7 | 7739 | 7739 | 5.01 | 0.22 | 2449 | 2449 | 032009SeFeMn |
| 25DSL3 | 66 | 25DSL3-66 | 9.8 | 490.1 | | | | | | | | | |
| 25DSL3 | 90 | 25DSL3-90 | 9.8 | 489.7 | | | | | | | | | |
| 25DSL3 | 120 | 25DSL3-120 | 9.8 | 490.1 | | | | | | | | | |
| 25DSL3 | 140 | 25DSL3-140 | 9.8 | 489.4 | | | | | | | | | |

D1-2.2. DV 25 LIVE IRON/MANGANESE, continued.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 25DSL3 | 188 | 25DSL3-188 | 9.8 | 489.6 | 17 | 2.7 | 8371 | 8371 | 7.94 | 0.22 | 3885 | 3885 | 032009SeFeMn |

Shaded fields = data not collected for those samples.

D1-2.3. DV 10 KILLED IRON/MANGANESE.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weigh t** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 10DCK1 | 0 | 10DCK1-0 | 9.79 | 489.6 | 15.06 | 1.24 | 7372 | 7372 | 1.12 | 0.14 | 548 | 548 | 032109cps_SeFeMn |
| 10DCK1 | 24 | 10DCK1-24 | 9.87 | 493.7 | | | | | | | | | |
| 10DCK1 | 66 | 10DCK1-66 | 9.81 | 490.5 | | | | | | | | | |
| 10DCK1 | 139 | 10DCK1-139 | 9.80 | 489.9 | | | | | | | | | |
| 10DCK1 | 162 | 10DCK1-162 | 9.88 | 493.9 | | | | | | | | | |
| 10DCK1 | 190 | 10DCK1-190 | 9.80 | 490.1 | 16.73 | 1.24 | 8198 | 8198 | 1.18 | 0.14 | 577 | 577 | 032109cps_SeFeMn |
| 10DCK1 | 270 | 10DCK1-270 | 9.86 | 493.2 | | | | | | | | | |
| 10DCK2 | 0 | 10DCK2-0 | 9.78 | 488.9 | 15.98 | | 7812 | 7812 | 1.13 | 0.14 | 554 | 554 | 032109cps_SeFeMn |
| 10DCK2 | 24 | 10DCK2-24 | 9.85 | 492.5 | | | | | | | | | |
| 10DCK2 | 66 | 10DCK2-66 | 9.81 | 490.3 | | | | | | | | | |
| 10DCK2 | 139 | 10DCK2-139 | 9.82 | 491.0 | | | | | | | | | |
| 10DCK2 | 162 | 10DCK2-162 | 9.85 | 492.4 | | | | | | | | | |
| 10DCK2 | 190 | 10DCK2-190 | 9.82 | 491.1 | 17.63 | | 8657 | 8657 | 1.47 | 0.14 | 724 | 724 | 032109cps_SeFeMn |
| 10DCK2 | 270 | 10DCK2-270 | 9.82 | 490.91 | | | | | | | | | |
| 10DCK3 | 0 | 10DCK3-0 | 9.78 | 488.9 | 15.59 | | 7619 | 7619 | 1.09 | 0.14 | 535 | 535 | 032109cps_SeFeMn |
| 10DCK3 | 24 | 10DCK3-24 | 9.86 | 493.2 | | | | | | | | | |
| 10DCK3 | 66 | 10DCK3-66 | 9.82 | 490.9 | | | | | | | | | |
| 10DCK3 | 139 | 10DCK3-139 | 9.81 | 490.4 | | | | | | | | | |
| 10DCK3 | 162 | 10DCK3-162 | 9.85 | 492.4 | | | | | | | | | |
| 10DCK3 | 190 | 10DCK3-190 | 9.82 | 491.2 | 15.96 | | 7840 | 7840 | 1.19 | 0.14 | 584 | 584 | 032109cps_SeFeMn |
| 10DCK3 | 270 | 10DCK3-270 | 9.88 | 493.76 | | | | | | | | | |
| 10DRK1 | 0 | 10DRK1-0 | 9.81 | 490.4 | 15.79 | | 7745 | 7745 | 2.77 | 0.14 | 1360 | 1360 | 032109cps_SeFeMn |
| 10DRK1 | 24 | 10DRK1-24 | 9.86 | 493.1 | | | | | | | | | |
| 10DRK1 | 66 | 10DRK1-66 | 9.82 | 491.1 | | | | | | | | | |

D1-2.3. DV 10 KILLED IRON/MANGANESE, continued.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Experiment | Time | Name | Weigh t | Dilution | Fe, Measured | d.l. | Fe, µg/L | Fe µg/L, Reported | Mn, Measured | d.l. | Mn, µg/L | Mn, µg/L, Reported | Fe and Mn Source |
| 10DRK1 | 139 | 10DRK1-139 | 9.81 | 490.7 | | | | | | | | | |
| 10DRK1 | 162 | 10DRK1-162 | 9.85 | 492.6 | | | | | | | | | |
| 10DRK1 | 190 | 10DRK1-190 | 9.80 | 489.8 | 16.35 | | 8007 | 8007 | 3.27 | 0.1 4 | 1601 | 1601 | 032109cps_SeFe Mn |
| 10DRK1 | 270 | 10DRK1-270 | 9.82 | 491.1 | | | | | | | | | |
| 10DRK2 | 0 | 10DRK2-0 | 9.79 | 489.3 | 15.18 | | 7428 | 7428 | 2.89 | 0.1 4 | 1415 | 1415 | 032109cps_SeFe Mn |
| 10DRK2 | 24 | 10DRK2-24 | 9.87 | 493.5 | | | | | | | | | |
| 10DRK2 | 66 | 10DRK2-66 | 9.80 | 490.0 | | | | | | | | | |
| 10DRK2 | 139 | 10DRK2-139 | 9.81 | 490.3 | | | | | | | | | |
| 10DRK2 | 162 | 10DRK2-162 | 9.83 | 491.5 | | | | | | | | | |
| 10DRK2 | 190 | 10DRK2-190 | 9.79 | 489.5 | 16.01 | | 7839 | 7839 | 2.75 | 0.1 4 | 1348 | 1348 | 032109cps_SeFe Mn |
| 10DRK2 | 270 | 10DRK2-270 | 9.80 | 490.0 | | | | | | | | | |
| 10DRK3 | 0 | 10DRK3-0 | 9.79 | 489.7 | 14.99 | | 7339 | 7339 | 3.04 | 0.1 4 | 1489 | 1489 | 032109cps_SeFe Mn |
| 10DRK3 | 24 | 10DRK3-24 | 9.86 | 492.8 | | | | | | | | | |
| 10DRK3 | 66 | 10DRK3-66 | 9.82 | 491.0 | | | | | | | | | |
| 10DRK3 | 139 | 10DRK3-139 | 9.82 | 491.1 | | | | | | | | | |
| 10DRK3 | 162 | 10DRK3-162 | 9.84 | 492.0 | | | | | | | | | |
| 10DRK3 | 190 | 10DRK3-190 | 9.82 | 490.9 | 16.24 | | 7972 | 7972 | 2.60 | 0.1 4 | 1278 | 1278 | 032109cps_SeFe Mn |
| 10DRK3 | 270 | 10DRK3-270 | 9.86 | 493.1 | | | | | | | | | |
| 10DSK1 | 0 | 10DSK1-0 | 9.80 | 489.8 | 15.76 | | 7721 | 7721 | 4.17 | 0.1 4 | 2040 | 2040 | 032109cps_SeFe Mn |
| 10DSK1 | 24 | 10DSK1-24 | 9.87 | 493.4 | | | | | | | | | |
| 10DSK1 | 66 | 10DSK1-66 | 9.79 | 489.6 | | | | | | | | | |
| 10DSK1 | 139 | 10DSK1-139 | 9.82 | 490.8 | | | | | | | | | |
| 10DSK1 | 162 | 10DSK1-162 | 9.85 | 492.4 | | | | | | | | | |
| 10DSK1 | 190 | 10DSK1-190 | 9.82 | 491.0 | 16.01 | | 7863 | 7863 | 4.75 | 0.1 4 | 2331 | 2331 | 032109cps_SeFe Mn |
| 10DSK1 | 270 | 10DSK1-270 | 9.86 | 493.1 | | | | | | | | | |

D1-2.3. DV 10 KILLED IRON/MANGANESE, continued.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Experiment | Time | Name | Weigh t | Dilution | Fe, Measured | d.l. | Fe, µg/L | Fe µg/L, Reported | Mn, Measured | d.l. | Mn, µg/L | Mn, µg/L, Reported | Fe and Mn Source |
| 10DSK2 | 0 | 10DSK2-0 | 9.78 | 489.2 | 15.79 | | 7723 | 7723 | 4.12 | 0.14 | 2014 | 2014 | 032109cps_SeFeMn |
| 10DSK2 | 24 | 10DSK2-24 | 9.86 | 493.2 | | | | | | | | | |
| 10DSK2 | 66 | 10DSK2-66 | 9.82 | 491.2 | | | | | | | | | |
| 10DSK2 | 139 | 10DSK2-139 | 9.82 | 491.1 | | | | | | | | | |
| 10DSK2 | 162 | 10DSK2-162 | 9.86 | 493.2 | | | | | | | | | |
| 10DSK2 | 190 | 10DSK2-190 | 9.81 | 490.3 | 16.64 | | 8159 | 8159 | 4.68 | 0.14 | 2293 | 2293 | 032109cps_SeFeMn |
| 10DSK2 | 270 | 10DSK2-270 | 9.85 | 492.3 | | | | | | | | | |
| 10DSK3 | 0 | 10DSK3-0 | 9.80 | 490.1 | 15.30 | | 7500 | 7500 | 4.19 | 0.14 | 2054 | 2054 | 032109cps_SeFeMn |
| 10DSK3 | 24 | 10DSK3-24 | 9.86 | 493.2 | | | | | | | | | |
| 10DSK3 | 66 | 10DSK3-66 | 9.81 | 490.7 | | | | | | | | | |
| 10DSK3 | 139 | 10DSK3-139 | 9.82 | 490.8 | | | | | | | | | |
| 10DSK3 | 162 | 10DSK3-162 | 9.85 | 492.7 | | | | | | | | | |
| 10DSK3 | 190 | 10DSK3-190 | 9.82 | 491.0 | 16.34 | | 8024 | 8024 | 5.06 | 0.14 | 2486 | 2486 | 032109cps_SeFeMn |
| 10DSK3 | 270 | 10DSK3-270 | 9.81 | 490.7 | | | | | | | | | |

D1-2.4. DV 25 KILLED IRON/MANGANESE.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Experiment | Time | Name | Weight | Dilution | Fe, Measured | d.l. | Fe, µg/L | Fe µg/L, Reported | Mn, Measured | d.l. | Mn, µg/L | Mn, µg/L, Reported | Fe and Mn Source |
| 25DCK1 | 0 | 25DCK1-0 | 9.79 | 489.3 | 4.0 | 0.75 | 1950 | 1950 | 1.07 | 0.43 | 525 | 525 | 020909Aquant |
| 25DCK1 | 72 | 25DCK1-72 | 9.79 | 489.7 | | | | | | | | | |
| 25DCK1 | 120 | 25DCK1-120 | 9.78 | 488.9 | 3.6 | 0.75 | 1777 | 1777 | 1.01 | 0.43 | 495 | 495 | 020909Aquant |
| 25DCK1 | 216 | 25DCK1-216 | 9.81 | 490.7 | | | | | | | | | |
| 25DCK1 | 456 | 25DCK1-456 | 9.81 | 490.3 | 5.47 | 3.73 | 2683 | 2683 | 1.94 | 0.15 | 953 | 953 | 032009SeFeMn |
| 25DCK2 | 0 | 25DCK2-0 | 9.75 | 487.6 | 3.8 | 0.75 | 1854 | 1854 | 1.04 | 0.43 | 509 | 509 | 020909Aquant |
| 25DCK2 | 72 | 25DCK2-72 | 9.81 | 490.7 | | | | | | | | | |
| 25DCK2 | 120 | 25DCK2-120 | 9.79 | 489.4 | 3.6 | 0.75 | 1750 | 1750 | 1.00 | 0.43 | 491 | 491 | 020909Aquant |
| 25DCK2 | 216 | 25DCK2-216 | 9.80 | 490.2 | | | | | | | | | |
| 25DCK2 | 456 | 25DCK2-456 | 9.81 | 490.5 | 5.06 | 3.73 | 2481 | 2481 | 1.81 | 0.15 | 889 | 889 | 032009SeFeMn |
| 25DCK3 | 0 | 25DCK3-0 | 9.77 | 488.5 | 3.9 | 0.75 | 1904 | 1904 | 1.07 | 0.43 | 522 | 522 | 020909Aquant |
| 25DCK3 | 72 | 25DCK3-72 | 9.81 | 490.7 | | | | | | | | | |
| 25DCK3 | 120 | 25DCK3-120 | 9.77 | 488.7 | 3.7 | 0.75 | 1799 | 1799 | 1.01 | 0.43 | 495 | 495 | 020909Aquant |
| 25DCK3 | 216 | 25DCK3-216 | 9.81 | 490.5 | | | | | | | | | |
| 25DCK3 | 456 | 25DCK3-456 | 9.80 | 490.2 | 5.34 | 3.73 | 2617 | 2617 | 1.84 | 0.15 | 900 | 900 | 032009SeFeMn |
| 25DRK1 | 0 | 25DRK1-0 | 9.77 | 488.5 | 3.6 | 0.75 | 1742 | 1742 | 3.15 | 0.43 | 1537 | 1537 | 020909Aquant |
| 25DRK1 | 72 | 25DRK1-72 | 9.80 | 489.9 | | | | | | | | | |
| 25DRK1 | 120 | 25DRK1-120 | 9.78 | 488.8 | 3.6 | 0.75 | 1748 | 1748 | 3.45 | 0.43 | 1687 | 1687 | 020909Aquant |
| 25DRK1 | 216 | 25DRK1-216 | 9.81 | 490.4 | | | | | | | | | |
| 25DRK1 | 456 | 25DRK1-456 | 9.81 | 490.3 | 5.46 | 3.73 | 2677 | 2677 | 4.43 | 0.15 | 2173 | 2173 | 032009SeFeMn |
| 25DRK2 | 0 | 25DRK2-0 | 9.78 | 488.8 | 3.7 | 0.75 | 1817 | 1817 | 2.51 | 0.43 | 1229 | 1229 | 020909Aquant |
| 25DRK2 | 72 | 25DRK2-72 | 9.81 | 490.6 | | | | | | | | | |
| 25DRK2 | 120 | 25DRK2-120 | 9.76 | 488.2 | 3.8 | 0.75 | 1862 | 1862 | 3.44 | 0.43 | 1678 | 1678 | 020909Aquant |

D1-2.4. DV 25 KILLED IRON/MANGANESE, continued.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 25DRK2 | 216 | 25DRK2-216 | 9.88 | 494.1 | | | | | | | | | |
| 25DRK2 | 456 | 25DRK2-456 | 9.81 | 490.3 | 5.27 | 3.73 | 2585 | 2585 | 4.02 | 0.15 | 1972 | 1972 | 032009SeFeMn |
| 25DSK1 | 0 | 25DSK1-0 | 9.78 | 489.1 | 3.6 | 0.75 | 1756 | 1756 | 3.09 | 0.43 | 1511 | 1511 | 020909Aquant |
| 25DSK1 | 72 | 25DSK1-72 | 9.80 | 489.8 | | | | | | | | | |
| 25DSK1 | 120 | 25DSK1-120 | 9.76 | 488.1 | 4.0 | 0.75 | 1932 | 1932 | 4.35 | 0.43 | 2121 | 2121 | 020909Aquant |
| 25DSK1 | 216 | 25DSK1-216 | 9.81 | 490.5 | | | | | | | | | |
| 25DSK1 | 456 | 25DSK1-456 | 9.80 | 490.2 | 9.06 | 3.73 | 4443 | 4443 | 5.17 | 0.15 | 2535 | 2535 | 032009SeFeMn |
| 25DSK2 | 0 | 25DSK2-0 | 9.80 | 489.9 | 3.6 | 0.75 | 1769 | 1769 | 3.48 | 0.43 | 1705 | 1705 | 020909Aquant |
| 25DSK2 | 72 | 25DSK2-72 | 9.81 | 490.3 | | | | | | | | | |
| 25DSK2 | 120 | 25DSK2-120 | 9.78 | 489.0 | 3.6 | 0.75 | 1752 | 1752 | 4.35 | 0.43 | 2127 | 2127 | 020909Aquant |
| 25DSK2 | 216 | 25DSK2-216 | 9.81 | 490.3 | | | | | | | | | |
| 25DSK2 | 456 | 25DSK2-456 | 9.80 | 490.0 | 5.63 | 3.73 | 2759 | 2759 | 5.25 | 0.15 | 2573 | 2573 | 032009SeFeMn |
| 25DSK3 | 0 | 25DSK3-0 | 9.76 | 488.0 | 3.8 | 0.75 | 1872 | 1872 | 3.83 | 0.43 | 1869 | 1869 | 020909Aquant |
| 25DSK3 | 72 | 25DSK3-72 | 9.80 | 489.9 | | | | | | | | | |
| 25DSK3 | 120 | 25DSK3-120 | 9.78 | 489.0 | 3.6 | 0.75 | 1764 | 1764 | 4.74 | 0.43 | 2316 | 2316 | 020909Aquant |
| 25DSK3 | 216 | 25DSK3-216 | 9.80 | 490.0 | | | | | | | | | |
| 25DSK3 | 456 | 25DSK3-456 | 9.80 | 490.2 | 5.05 | 3.73 | 2473 | 2473 | 4.96 | 0.15 | 2430 | 2430 | 032009SeFeMn |

Shaded fields = data not collected for those samples.

Table D1-3. Selenium ICP-MS data for Smoky Canyon Mine Saturated Rate Experiments.
D1-3.1. SC 10 LIVE SELENIUM.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 10SCL1 | 0 | 10SCL1-0 | 9.80 | 490.2 | 17.64 | 0.08 | 8647 | 8647 | 032309cpsrecalc |
| 10SCL1 | 12 | 10SCL1-12 | 9.89 | 494.6 | 14.32 | 0.08 | 12647 | 12647 | 032309cpsrecalc |
| 10SCL1 | 24 | 10SCL1-24 | 9.84 | 491.9 | 18.81 | 0.04 | 10655 | 10655 | 032309cps\SeLtdcps |
| 10SCL1 | 36 | 10SCL1-36 | 9.86 | 492.9 | 13.92 | 0.04 | 10677 | 10677 | 032309cps\SeLtdcps |
| 10SCL1 | 48 | 10SCL1-48 | 9.81 | 490.4 | 16.04 | 0.04 | 7914 | 7914 | 062008Aquant |
| 10SCL1 | 60 | 10SCL1-60 | 9.86 | 492.9 | 13.38 | 0.3 | 7600 | 7600 | 2008621_M_quant |
| 10SCL1 | 84 | 10SCL1-84 | 9.85 | 492.4 | 9.127 | 0.3 | 4888 | 4888 | 2008621_M_quant |
| 10SCL1 | 108 | 10SCL1-108 | 9.79 | 489.7 | 3.01 | 0.09 | 1535 | 1535 | 062508recalc |
| 10SCL1 | 132 | 10SCL1-132 | 9.85 | 492.3 | 0.603 | 0.09 | 402 | 402 | 062508recalc |
| 10SCL1 | 156 | 10SCL1-156 | 9.85 | 492.4 | 0.29 | 0.09 | 271 | 271 | 062508recalc |
| 10SCL1 | 246 | 10SCL1-246 | 9.82 | 490.9 | -0.1508 | 0.09 | -74 | 44 | 071408quant |
| 10SCL2 | 0 | 10SCL2-0 | 9.85 | 492.6 | 16.89 | 0.08 | 8320 | 8320 | 032309cpsrecalc |
| 10SCL2 | 12 | 10SCL2-12 | 9.88 | 493.8 | 15.23 | 0.08 | 13703 | 13703 | 032309cpsrecalc |
| 10SCL2 | 24 | 10SCL1-24 | 9.85 | 492.4 | 18.43 | 0.04 | 13488 | 13488 | 032309cps\SeLtdcps |
| 10SCL2 | 36 | 10SCL2-36 | 9.85 | 492.7 | 17.32 | 0.04 | 13114 | 13114 | 032309cps\SeLtdcps |
| 10SCL2 | 48 | 10SCL2-48 | 9.81 | 490.7 | 17.18 | 0.04 | 8479 | 8479 | 032309cps\SeLtdcps |
| 10SCL2 | 60 | 10SCL2-60 | 9.86 | 492.9 | 16.59 | 0.3 | 8374 | 8374 | 2008621_M_quant |
| 10SCL2 | 84 | 10SCL2-84 | 9.86 | 492.8 | 7.355 | 0.3 | 3867 | 3867 | 2008621_M_quant |
| 10SCL2 | 108 | 10SCL2-108 | 9.77 | 488.7 | 1.69 | 0.09 | 1027 | 1027 | 062508recalc |
| 10SCL2 | 132 | 10SCL2-132 | 9.83 | 491.6 | 0.072 | 0.09 | 27 | 44 | 062508recalc |
| 10SCL2 | 156 | 10SCL2-156 | 9.79 | 489.7 | 0.12 | 0.09 | 158 | 158 | 062508recalc |
| 10SCL2 | 246 | 10SCL2-246 | 9.80 | 490.1 | -0.183 | 0.09 | -90 | 44 | 071408quant |
| 10SCL3 | 0 | 10SCL3-0 | 9.85 | 492.5 | 16.46 | 0.08 | 8108 | 8108 | 032309cpsrecalc |
| 10SCL3 | 12 | 10SCL3-12 | 9.87 | 493.7 | 17.72 | 0.08 | 13671 | 13671 | 032309cpsrecalc |
| 10SCL3 | 24 | 10SCL1-24 | 9.84 | 491.8 | 21.93 | 0.04 | 13741 | 13741 | 032309cps\SeLtdcps |
| 10SCL3 | 36 | 10SCL3-36 | 9.86 | 492.8 | 16.16 | 0.04 | 13203 | 13203 | 032309cps\SeLtdcps |
| 10SCL3 | 48 | 10SCL3-48 | 9.79 | 489.6 | 17.00 | 0.04 | 8862 | 8862 | 032309cps\SeLtdcps |
| 10SCL3 | 60 | 10SCL3-60 | 9.86 | 492.8 | 18.8 | 0.3 | 8471 | 8471 | 2008621_M_quant |

D1-3.1. SC 10 LIVE SELENIUM, continued.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 10SCL3 | 84 | 10SCL3-84 | 9.85 | 492.4 | 10.04 | 0. 3 | 5185 | 5185 | 2008621_M_quant |
| 10SCL3 | 108 | 10SCL3-108 | 9.79 | 489.5 | 2.48 | 0.09 | 1475 | 1475 | 062508recalc |
| 10SCL3 | 132 | 10SCL3-132 | 9.84 | 492.2 | 0.202 | 0.09 | 165 | 165 | 062508recalc |
| 10SCL3 | 156 | 10SCL3-156 | 9.81 | 490.6 | 0.24 | 0.09 | 196 | 196 | 062508recalc |
| 10SCL3 | 246 | 10SCL3-246 | 9.84 | 491.8 | 0.02278 | 0.09 | 11 | 44 | 071408quant |
| 10SRL1 | 0 | 10SRL1-0 | 9.85 | 492.4 | 20.51 | 0.08 | 10099 | 10099 | 032309cpsrecalc |
| 10SRL1 | 12 | 10SRL1-12 | 9.95 | 497.3 | 19.43 | 0.08 | 12965 | 12965 | 032309cpsrecalc |
| 10SRL1 | 24 | 10SRL1-24 | 9.80 | 490.0 | 20.37 | 0.04 | 11588 | 11588 | 032309cps\SeLtdcps |
| 10SRL1 | 36 | 10SRL1-36 | 9.86 | 493.0 | 18.25 | 0.04 | 11661 | 11661 | 032309cps\SeLtdcps |
| 10SRL1 | 48 | 10SRL1-48 | 9.80 | 490.0 | 14.59 | 0.04 | 7194 | 7194 | 032309cps\SeLtdcps |
| 10SRL1 | 60 | 10SRL1-60 | 9.85 | 492.6 | 8.294 | 0.3 | 4255 | 4255 | 2008621_M_quant |
| 10SRL1 | 84 | 10SRL1-84 | 9.85 | 492.5 | 0.881 | 0.3 | 1349 | 1349 | 2008621_M_quant |
| 10SRL1 | 108 | 10SRL1-108 | 9.78 | 489.0 | 1.03 | 0.09 | 844 | 844 | 062508recalc |
| 10SRL1 | 132 | 10SRL1-132 | 9.83 | 491.7 | 0.630 | 0.09 | 239 | 239 | 062508recalc |
| 10SRL1 | 156 | 10SRL1-156 | 9.82 | 490.8 | 0.22 | 0.09 | 256 | 256 | 062508recalc |
| 10SRL1 | 246 | 10SRL1-246 | 9.82 | 490.8 | -0.2295 | 0.09 | -113 | 44 | 071408quant |
| 10SRL2 | 0 | 10SRL2-0 | 9.87 | 493.5 | 17.24 | 0.08 | 8508 | 8508 | 032309cpsrecalc |
| 10SRL2 | 12 | 10SRL2-12 | 9.84 | 492.1 | 26.94 | 0.08 | 13169 | 13169 | 032309cpsrecalc |
| 10SRL2 | 24 | 10SRL2-24 | 9.85 | 492.5 | 18.66 | 0.04 | 13992 | 13992 | 032309cps\SeLtdcps |
| 10SRL2 | 36 | 10SRL2-36 | 9.83 | 491.6 | 17.63 | 0.04 | 13619 | 13619 | 032309cps\SeLtdcps |
| 10SRL2 | 48 | 10SRL2-48 | 9.82 | 490.8 | 9.85 | 0.04 | 4875 | 4875 | 032309cps\SeLtdcps |
| 10SRL2 | 60 | 10SRL2-60 | 9.85 | 492.6 | 7.432 | 0.3 | 3747 | 3747 | 2008621_M_quant |
| 10SRL2 | 84 | 10SRL2-84 | 9.86 | 492.9 | 0.9193 | 0.3 | 407 | 407 | 2008621_M_quant |
| 10SRL2 | 108 | 10SRL2-108 | 9.80 | 490.2 | 0.60 | 0.09 | 292 | 292 | 062508recalc |
| 10SRL2 | 132 | 10SRL2-132 | 9.83 | 491.5 | 0.167 | 0.09 | 116 | 116 | 062508recalc |
| 10SRL2 | 156 | 10SRL2-156 | 9.81 | 490.7 | 0.24 | 0.09 | 190 | 190 | 062508recalc |
| 10SRL2 | 246 | 10SRL2-246 | 9.81 | 490.3 | -0.3031 | 0.09 | -149 | 44 | 071408quant |
| 10SRL3 | 0 | 10SRL3-0 | 9.86 | 493.1 | 14.91 | 0.08 | 7354 | 7354 | 032309cpsrecalc |
| 10SRL3 | 12 | 10SRL3-12 | 9.83 | 491.5 | 24.55 | 0.08 | 14440 | 14440 | 032309cpsrecalc |
| 10SRL3 | 24 | 10SRL1-24 | 9.78 | 488.9 | 15.83 | 0.04 | 15024 | 15024 | 032309cps\SeLtdcps |

D1-3.1. SC 10 LIVE SELENIUM, continued.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 10SRL3 | 36 | 10SRL3-36 | 9.85 | 492.6 | 19.75 | 0.04 | 15138 | 15138 | 032309cps\SeLtdcps |
| 10SRL3 | 48 | 10SRL3-48 | 9.82 | 491.2 | 16.09 | 0.04 | 7952 | 7952 | 032309cps\SeLtdcps |
| 10SRL3 | 60 | 10SRL3-60 | 9.85 | 492.7 | 14.14 | 0.3 | 7809 | 7809 | 2008621_M_quant |
| 10SRL3 | 84 | 10SRL3-84 | 9.85 | 492.6 | 4.072 | 0.3 | 2072 | 2072 | 2008621_M_quant |
| 10SRL3 | 108 | 10SRL3-108 | 9.78 | 488.8 | 0.87 | 0.09 | 502 | 502 | 062508recalc |
| 10SRL3 | 132 | 10SRL3-132 | 9.83 | 491.5 | 0.615 | 0.09 | 524 | 524 | 062508recalc |
| 10SRL3 | 156 | 10SRL3-156 | 9.81 | 490.7 | 0.60 | 0.09 | 312 | 312 | 062508recalc |
| 10SRL3 | 246 | 10SRL3-246 | 9.82 | 491.2 | -0.1354 | 0.09 | -67 | 44 | 071408quant |
| 10SSL1 | 0 | 10SSL1-0 | 9.86 | 492.8 | 26.34 | 0.08 | 12983 | 12983 | 032309cpsrecalc |
| 10SSL1 | 12 | 10SSL1-12 | 9.82 | 490.8 | 18.38 | 0.08 | 13600 | 13600 | 032309cpsrecalc |
| 10SSL1 | 24 | 10SSL1-24 | 9.86 | 492.9 | 20.12 | 0.04 | 13753 | 13753 | 032309cps\SeLtdcps |
| 10SSL1 | 36 | 10SSL1-36 | 9.85 | 492.6 | 16.20 | 0.04 | 13743 | 13743 | 032309cps\SeLtdcps |
| 10SSL1 | 48 | 10SSL1-48 | 9.81 | 490.4 | 17.38 | 0.04 | 8572 | 8572 | 032309cps\SeLtdcps |
| 10SSL1 | 60 | 10SSL1-60 | 9.86 | 492.8 | 13.82 | 0.3 | 7382 | 7382 | 2008621_M_quant |
| 10SSL1 | 84 | 10SSL1-84 | 9.87 | 493.3 | 5.209 | 0.3 | 2674 | 2674 | 2008621_M_quant |
| 10SSL1 | 108 | 10SSL1-108 | 9.50 | 475.0 | 1.30 | 0.09 | 627 | 627 | 062508recalc |
| 10SSL1 | 132 | 10SSL1-132 | 9.83 | 491.6 | 0.288 | 0.09 | 407 | 407 | 062508recalc |
| 10SSL1 | 156 | 10SSL1-156 | 9.81 | 490.7 | 0.42 | 0.09 | 287 | 287 | 062508recalc |
| 10SSL1 | 246 | 10SSL1-246 | 9.80 | 489.8 | -0.0557 | 0.09 | -27 | 44 | 071408quant |
| 10SSL2 | 0 | 10SSL2-0 | 9.84 | 491.8 | 17.86 | 0.08 | 8783 | 8783 | 032309cpsrecalc |
| 10SSL2 | 12 | 10SSL2-12 | 9.84 | 492.0 | 14.48 | 0.08 | 12443 | 12443 | 032309cpsrecalc |
| 10SSL2 | 24 | 10SSL1-24 | 9.85 | 492.3 | 14.95 | 0.04 | 11072 | 11072 | 032309cps\SeLtdcps |
| 10SSL2 | 36 | 10SSL2-36 | 9.85 | 492.7 | 14.98 | 0.04 | 11080 | 11080 | 032309cps\SeLtdcps |
| 10SSL2 | 48 | 10SSL2-48 | 9.87 | 493.7 | 10.08 | 0.04 | 5016 | 5016 | 032309cps\SeLtdcps |
| 10SSL2 | 60 | 10SSL2-60 | 9.82 | 490.9 | 8.07 | 0.3 | 5149 | 5149 | 2008621_M_quant |
| 10SSL2 | 84 | 10SSL2-84 | 9.85 | 492.5 | 1.32 | 0.3 | 620 | 620 | 2008621_M_quant |
| 10SSL2 | 108 | 10SSL2-108 | 9.79 | 489.4 | 0.96 | 0.09 | 767 | 767 | 062508recalc |
| 10SSL2 | 132 | 10SSL2-132 | 9.85 | 492.5 | 0.646 | 0.09 | 317 | 317 | 062508recalc |
| 10SSL2 | 156 | 10SSL2-156 | 9.81 | 490.6 | 0.36 | 0.09 | 241 | 241 | 062508recalc |
| 10SSL2 | 246 | 10SSL2-246 | 9.83 | 491.3 | -0.1346 | 0.09 | -66 | 44 | 071408quant |
| 10SSL3 | 0 | 10SSL3-0 | 9.85 | 492.3 | 11.47 | 0.08 | 5649 | 5649 | 032309cpsrecalc |

D1-3.1. SC 10 LIVE SELENIUM, continued.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 10SSL3 | 12 | 10SSL3-12 | 9.84 | 492.1 | 15.39 | 0.08 | 11687 | 11687 | 032309cpsrecalc |
| 10SSL3 | 24 | 10SSL1-24 | 9.84 | 491.8 | 22.32 | 0.04 | 10830 | 10830 | 032309cps\SeLtdcps |
| 10SSL3 | 36 | 10SSL3-36 | 9.85 | 492.6 | 15.72 | 0.04 | 10847 | 10847 | 032309cps\SeLtdcps |
| 10SSL3 | 48 | 10SSL3-48 | 9.81 | 490.5 | 10.23 | 0.04 | 5057 | 5057 | 032309cps\SeLtdcps |
| 10SSL3 | 60 | 10SSL3-60 | 9.84 | 492.2 | 6.766 | 0.3 | 3316 | 3316 | 2008621_M_quant |
| 10SSL3 | 84 | 10SSL3-84 | 9.85 | 492.7 | 1.456 | 0.3 | 706 | 706 | 2008621_M_quant |
| 10SSL3 | 108 | 10SSL3-108 | 9.78 | 489.0 | 0.42 | 0.09 | 516 | 516 | 062508recalc |
| 10SSL3 | 132 | 10SSL3-132 | 9.77 | 488.4 | 0.371 | 0.09 | 278 | 278 | 062508recalc |
| 10SSL3 | 156 | 10SSL3-156 | 9.81 | 490.6 | 0.44 | 0.09 | 268 | 268 | 062508recalc |
| 10SSL3 | 246 | 10SSL3-246 | 9.84 | 491.8 | -0.07792 | 0.09 | -38 | 44 | 071408quant |

D1-3.2. SC 25 LIVE SELENIUM.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 25SCL1 | 0 | 25SCL1-0 | 9.802 | 490.1 | 21.48 | 6 | 10527 | 10527 | 010909Bcps |
| 25SCL1 | 12 | 25SCL1-12 | 9.834 | 491.7 | 16.95 | 6 | 8333 | 8333 | 032309cpsrecalcFeMn |
| 25SCL1 | 24 | 25SCL1-24 | 9.854 | 492.7 | 11.55 | 6 | 5689 | 5689 | 040409Bpart1cpssummary |
| 25SCL1 | 36 | 25SCL1-36 | 9.825 | 491.3 | 7.75 | 6 | 3807 | 3807 | 040409Bpart1cpssummary |
| 25SCL1 | 48 | 25SCL1-48 | 9.562 | 478.1 | 4.62 | 6 | 2208 | 2869 | 040409Bpart1cpssummary |
| 25SCL1 | 60 | 25SCL1-60 | 9.833 | 491.6 | 0.36 | 0.30 | 176 | 176 | 062008Aquant |
| 25SCL1 | 72 | 25SCL1-72 | 9.793 | 489.7 | -0.03 | 0.11 | -13 | 54 | 062008B25SC 60_72 |
| 25SCL1 | 96 | 25SCL1-96 | 9.836 | 491.8 | -0.28 | 0.19 | -135 | 93 | 071408quant |
| 25SCL1 | 120 | 25SCL1-120 | 9.780 | 489.0 | 0.63 | 0.10 | 310 | 310 | 062508recalc |
| 25SCL1 | 144 | 25SCL1-144 | 9.856 | 492.8 | 0.10 | 0.10 | 50 | 50 | 062508recalc |
| 25SCL1 | 168 | 25SCL1-168 | 9.798 | 489.9 | 0.33 | 0.10 | 159 | 159 | 062508recalc |
| 25SCL1 | 228 | 25SCL1-228 | 9.829 | 491.4 | -0.27 | 0.19 | -132 | 93 | 071408quant |
| 25SCL2 | 0 | 25SCL2-0 | 9.830 | 491.5 | 22.81 | 6 | 11211 | 11211 | 010909Bcps |
| 25SCL2 | 12 | 25SCL2-12 | 9.838 | 491.9 | 16.95 | 6 | 8337 | 8337 | 032309cpsrecalcFeMn |
| 25SCL2 | 24 | 25SCL2-24 | 9.842 | 492.1 | 13.36 | 6 | 6575 | 6575 | 040409Bpart1cpssummary |
| 25SCL2 | 36 | 25SCL2-36 | 9.847 | 492.3 | 6.53 | 6 | 3216 | 3216 | 040409Bpart1cpssummary |
| 25SCL2 | 48 | 25SCL2-48 | 9.852 | 492.6 | 5.03 | 6 | 2480 | 2956 | 040409Bpart1cpssummary |
| 25SCL2 | 60 | 25SCL2-60 | 9.841 | 492.0 | 0.73 | 0.30 | 360 | 360 | 062008Aquant |
| 25SCL2 | 72 | 25SCL2-72 | 9.774 | 488.7 | 0.04 | 0.11 | 17 | 54 | 062008B25SC 60_72 |
| 25SCL2 | 96 | 25SCL2-96 | 9.812 | 490.6 | -0.28 | 0.19 | -138 | 93 | 071408quant |
| 25SCL2 | 120 | 25SCL2-120 | 9.771 | 488.5 | 0.48 | 0.10 | 232 | 232 | 062508recalc |
| 25SCL2 | 144 | 25SCL2-144 | 9.852 | 492.6 | 0.10 | 0.10 | 50 | 50 | 062508recalc |
| 25SCL2 | 168 | 25SCL2-168 | 9.812 | 490.6 | 0.49 | 0.10 | 239 | 239 | 062508recalc |
| 25SCL2 | 228 | 25SCL2-228 | 9.793 | 489.7 | -0.31 | 0.19 | -150 | 93 | 071408quant |
| 25SCL3 | 0 | 25SCL3-0 | 9.820 | 491.0 | 24.33 | 6 | 11947 | 11947 | 010909Bcps |
| 25SCL3 | 12 | 25SCL3-12 | 9.841 | 492.1 | 16.95 | 6 | 8339 | 8339 | 032309cpsrecalcFeMn |
| 25SCL3 | 24 | 25SCL3-24 | 9.846 | 492.3 | 21.27 | 6 | 10472 | 10472 | 040409Bpart1cpssummary |
| 25SCL3 | 36 | 25SCL3-36 | 9.840 | 492.0 | 12.17 | 6 | 5986 | 5986 | 040409Bpart1cpssummary |

D1-3.2. SC 25 LIVE SELENIUM, continued.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 25SCL3 | 48 | 25SCL3-48 | 9.805 | 490.3 | 6.65 | 6 | 3258 | 3258 | 040409Bpart1cpssummary |
| 25SCL3 | 60 | 25SCL3-60 | 9.813 | 490.6 | 2.48 | 0.30 | 1218 | 1218 | 062008Aquant |
| 25SCL3 | 72 | 25SCL3-72 | 9.790 | 489.5 | 0.24 | 0.11 | 119 | 119 | 062008B25SC 60_72 |
| 25SCL3 | 96 | 25SCL3-96 | 9.829 | 491.5 | -0.26 | 0.19 | -127 | **93** | 071408quant |
| 25SCL3 | 120 | 25SCL3-120 | 9.771 | 488.6 | 0.48 | 0.10 | 232 | 232 | 062508recalc |
| 25SCL3 | 144 | 25SCL3-144 | 9.817 | 490.9 | 0.01 | 0.10 | 4 | 49 | 062508recalc |
| 25SCL3 | 168 | 25SCL3-168 | 9.813 | 490.6 | 0.27 | 0.10 | 132 | 132 | 062508recalc |
| 25SCL3 | 228 | 25SCL3-228 | 9.815 | 490.7 | -0.23 | 0.19 | -114 | 93 | 071408quant |
| 25SRL1 | 0 | 25SRL1-0 | 9.853 | 492.6 | 24.05 | 6 | 11848 | 11848 | 010909Bcps |
| 25SRL1 | 12 | 25SRL1-12 | 9.837 | 491.9 | 16.95 | 6 | 8336 | 8336 | 032309cpsrecalcFeMn |
| 25SRL1 | 24 | 25SRL1-24 | 9.833 | 491.6 | 17.24 | 6 | 8477 | 8477 | 040409Bpart1cpssummary |
| 25SRL1 | 36 | 25SRL1-36 | 9.838 | 491.9 | 12.80 | 6 | 6298 | 6298 | 040409Bpart1cpssummary |
| 25SRL1 | 48 | 25SRL1-48 | 9.829 | 491.4 | 7.72 | 6 | 3795 | 3795 | 040409Bpart1cpssummary |
| 25SRL1 | 60 | 25SRL1-60 | 9.845 | 492.2 | 0.46 | 0.30 | 224 | 224 | 062008Aquant |
| 25SRL1 | 72 | 25SRL-1-72 | 9.780 | 489.0 | 0.07 | 0.11 | 34 | 54 | 062008B25SC 60_72 |
| 25SRL1 | 96 | 25SRL1-96 | 9.799 | 489.9 | -0.19 | 0.19 | -92 | 93 | 071408quant |
| 25SRL1 | 120 | 25SRL1-120 | 9.796 | 489.8 | 0.40 | 0.10 | 194 | 194 | 062508recalc |
| 25SRL1 | 144 | 25SRL1-144 | 9.848 | 492.4 | 0.10 | 0.10 | 50 | 50 | 062508recalc |
| 25SRL1 | 168 | 25SRL1-168 | 9.816 | 490.8 | 0.15 | 0.10 | 73 | 73 | 062508recalc |
| 25SRL1 | 228 | 25SRL1-228 | 9.822 | 491.1 | -0.21 | 0.19 | -105 | 93 | 071408quant |
| 25SRL2 | 0 | 25SRL2-0 | 9.847 | 492.3 | 25.68 | 6 | 12643 | 12643 | 010909Bcps |
| 25SRL2 | 12 | 25SRL2-12 | 9.861 | 493.1 | 16.95 | 6 | 8356 | 8356 | 032309cpsrecalcFeMn |
| 25SRL2 | 24 | 25SRL2-24 | 9.838 | 491.9 | 15.84 | 6 | 7792 | 7792 | 040409Bpart1cpssummary |
| 25SRL2 | 36 | 25SRL2-36 | 9.838 | 491.9 | 9.10 | 6 | 4476 | 4476 | 040409Bpart1cpssummary |
| 25SRL2 | 48 | 25SRL2-48 | 9.842 | 492.1 | 1.41 | 6 | 696 | 2953 | 040409Bpart1cpssummary |
| 25SRL2 | 60 | 25SRL2-60 | 9.829 | 491.4 | 0.54 | 0.30 | 264 | 264 | 062008Aquant |
| 25SRL2 | 72 | 25SRL-2-72 | 9.804 | 490.2 | 0.11 | 0.11 | 54 | 54 | 062008B25SC 60_72 |
| 25SRL2 | 96 | 25SRL2-96 | 9.814 | 490.7 | -0.20 | 0.19 | -97 | 93 | 071408quant |
| 25SRL2 | 120 | 25SRL2-120 | 9.784 | 489.2 | 0.95 | 0.10 | 465 | 465 | 062508recalc |
| 25SRL2 | 144 | 25SRL2-144 | 9.855 | 492.7 | 0.07 | 0.10 | 35 | 49 | 062508recalc |
| 25SRL2 | 168 | 25SRL2-168 | 9.818 | 490.9 | 0.24 | 0.10 | 117 | 117 | 062508recalc |

D1-3.2. SC 25 LIVE SELENIUM, continued.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 25SRL2 | 228 | 25SRL2-228 | 9.831 | 491.5 | -0.27 | 0.19 | -132 | 93 | 071408quant |
| 25SRL3 | 0 | 25SRL3-0 | 9.859 | 492.9 | 27.79 | 6 | 13699 | 13699 | 010909Bcps |
| 25SRL3 | 12 | 25SRL3-12 | 9.841 | 492.1 | 16.95 | 6 | 8339 | 8339 | 032309cpsrecalcFeMn |
| 25SRL3 | 24 | 25SRL3-24 | 9.855 | 492.8 | 15.30 | 6 | 7539 | 7539 | 040409Bpart1cpssummary |
| 25SRL3 | 36 | 25SRL3-36 | 9.853 | 492.7 | 9.39 | 6 | 4624 | 4624 | 040409Bpart1cpssummary |
| 25SRL3 | 48 | 25SRL3-48 | 9.843 | 492.2 | 3.09 | 6 | 1522 | 2953 | 040409Bpart1cpssummary |
| 25SRL3 | 60 | 25SRL3-60 | 9.835 | 491.8 | 1.37 | 0.30 | 672 | 672 | 062008Aquant |
| 25SRL3 | 72 | 25SRL3-72 | 9.776 | 488.8 | 0.54 | 0.11 | 262 | 262 | 062008B25SC 60_72 |
| 25SRL3 | 96 | 25SRL3-96 | 9.826 | 491.3 | -0.20 | 0.19 | -100 | 93 | 071408quant |
| 25SRL3 | 120 | 25SRL3-120 | 9.669 | 483.5 | 0.32 | 0.10 | 153 | 153 | 062508recalc |
| 25SRL3 | 144 | 25SRL3-144 | 9.807 | 490.3 | 0.10 | 0.10 | 47 | 49 | 062508recalc |
| 25SRL3 | 168 | 25SRL3-168 | 9.818 | 490.9 | 0.40 | 0.10 | 196 | 196 | 062508recalc |
| 25SRL3 | 228 | 25SRL3-228 | 9.826 | 491.3 | -0.15 | 0.19 | -72 | 93 | 071408quant |
| 25SSL1 | 0 | 25SSL1-0 | 9.772 | 488.6 | 26.72 | 6 | 13055 | 13055 | 010909Bcps |
| 25SSL1 | 12 | 25SSL1-12 | 9.830 | 491.5 | 16.95 | 6 | 8330 | 8330 | 032309cpsrecalcFeMn |
| 25SSL1 | 24 | 25SSL1-24 | 9.829 | 491.4 | 19.80 | 6 | 9732 | 9732 | 040409Bpart1cpssummary |
| 25SSL1 | 36 | 25SSL1-36 | 9.809 | 490.5 | 16.14 | 6 | 7916 | 7916 | 040409Bpart1cpssummary |
| 25SSL1 | 48 | 25SSL1-48 | 9.857 | 492.9 | 7.53 | 6 | 3711 | 3711 | 040409Bpart1cpssummary |
| 25SSL1 | 60 | 25SSL1-60 | 9.853 | 492.6 | 6.77 | 0.30 | 3337 | 3337 | 062008Aquant |
| 25SSL1 | 72 | 25SSL-1-72 | 9.499 | 475.0 | 2.83 | 0.11 | 1346 | 1346 | 062008B25SC 60_72 |
| 25SSL1 | 96 | 25SSL1-96 | 9.815 | 490.8 | -0.18 | 0.19 | -86 | 93 | 071408quant |
| 25SSL1 | 120 | 25SSL1-120 | 9.820 | 491.0 | 0.79 | 0.10 | 389 | 389 | 062508recalc |
| 25SSL1 | 144 | 25SSL1-144 | 10.130 | 506.5 | 0.17 | 0.10 | 84 | 84 | 062508recalc |
| 25SSL1 | 168 | 25SSL1-168 | 9.819 | 491.0 | 0.38 | 0.10 | 185 | 185 | 062508recalc |
| 25SSL1 | 228 | 25SSL1-228 | 9.819 | 491.0 | -0.22 | 0.19 | -107 | 93 | 071408quant |
| 25SSL2 | 0 | 25SSL2-0 | 9.888 | 494.4 | 24.66 | 6 | 12193 | 12193 | 010909Bcps |
| 25SSL2 | 12 | 25SSL2-12 | 9.837 | 491.9 | 16.95 | 6 | 8336 | 8336 | 032309cpsrecalcFeMn |
| 25SSL2 | 24 | 25SSL2-24 | 9.839 | 492.0 | 17.73 | 6 | 8722 | 8722 | 040409Bpart1cpssummary |
| 25SSL2 | 36 | 25SSL2-36 | 9.848 | 492.4 | 15.08 | 6 | 7427 | 7427 | 040409Bpart1cpssummary |
| 25SSL2 | 48 | 25SSL2-48 | 9.855 | 492.8 | 6.80 | 6 | 3350 | 3350 | 040409Bpart1cpssummary |

D1-3.2. SC 25 LIVE SELENIUM, continued.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 25SSL2 | 60 | 25SSL2-60 | 9.835 | 491.8 | 3. 57 | 0.30 | 1757 | 1757 | 062008Aquant |
| 25SSL2 | 72 | 25SSL-2-72 | 9.788 | 489.4 | 0.97 | 0.11 | 476 | 476 | 062008B25SC 60_72 |
| 25SSL2 | 96 | 25SSL2-96 | 9.820 | 491.0 | -0.08 | 0.19 | -40 | 93 | 071408quant |
| 25SSL2 | 120 | 25SSL2-120 | 9.763 | 488.1 | 0.71 | 0.10 | 348 | 348 | 062508recalc |
| 25SSL2 | 144 | 25SSL2-144 | 9.636 | 481.8 | 0.18 | 0.10 | 88 | 88 | 062508recalc |
| 25SSL2 | 168 | 25SSL2-168 | 9.763 | 488.1 | 0.44 | 0.10 | 216 | 216 | 062508recalc |
| 25SSL2 | 228 | 25SSL2-228 | 9.838 | 491.9 | -0.25 | 0.19 | -124 | 93 | 071408quant |
| 25SSL3 | 0 | 25SSL3-0 | 9.894 | 494.7 | 28.42 | 6 | 14059 | 14059 | 010909Bcps |
| 25SSL3 | 12 | 25SSL3-12 | 9.845 | 492.3 | 16.95 | 6 | 8343 | 8343 | 032309cpsrecalcFeMn |
| 25SSL3 | 24 | 25SSL3-24 | 9.831 | 491.6 | 21.90 | 6 | 10763 | 10763 | 040409Bpart1cpssummary |
| 25SSL3 | 36 | 25SSL3-36 | 9.857 | 492.8 | 17.49 | 6 | 8618 | 8618 | 040409Bpart1cpssummary |
| 25SSL3 | 48 | 25SSL3-48 | 9.854 | 492.7 | 10.40 | 6 | 5125 | 5125 | 040409Bpart1cpssummary |
| 25SSL3 | 60 | 25SSL3-60 | 9.844 | 492.2 | 6.36 | 0.30 | 3131 | 3131 | 062008Aquant |
| 25SSL3 | 72 | 25SSL-3-72 | 9.780 | 489.0 | 3.00 | 0.11 | 1467 | 1467 | 062008B25SC 60_72 |
| 25SSL3 | 96 | 25SSL3-96 | 9.811 | 490.6 | -0.17 | 0.19 | -84 | 93 | 071408quant |
| 25SSL3 | 120 | 25SSL3-120 | 9.830 | 491.5 | 0.63 | 0.10 | 311 | 311 | 062508recalc |
| 25SSL3 | 144 | 25SSL3-144 | 9.776 | 488.8 | 0.33 | 0.10 | 160 | 160 | 062508recalc |
| 25SSL3 | 168 | 25SSL3-168 | 9.763 | 488.1 | 0.50 | 0.10 | 246 | 246 | 062508recalc |
| 25SSL3 | 228 | 25SSL3-228 | 9.807 | 490.4 | -0.16 | 0.19 | -79 | 93 | 071408quant |

D1-3.3. SC 10 KILLED SELENIUM.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 10SCK1 | 0 | 10SCK1-0 | 9.852 | 492.6 | 5.6 | 0.53 | 2739 | 2739 | 040409B-part1cps |
| 10SCK1 | 48 | 10SCK1-48 | 9.8278 | 491.39 | 34.5 | 0.53 | 16928 | 16928 | 040409B-part1cps |
| 10SCK1 | 96 | 10SCK1-96 | 9.8638 | 493.19 | 24.5 | 0.53 | 12058 | 12058 | 040409B-part1cps |
| 10SCK1 | 144 | 10SCK1-144 | 9.835 | 491.75 | 21.9 | 0.09 | 10764 | 10764 | 062508recalc |
| 10SCK2 | 0 | 10SCK2-0 | 8.721 | 436.05 | 9.2 | 0.19 | 4008 | 4008 | 0718quant |
| 10SCK2 | 48 | 10SCK2-48 | 8.27 | 413.5 | 8.4 | 0.19 | 3480 | 3480 | 0718quant |
| 10SCK2 | 96 | 10SCK2-96 | 8.145 | 407.25 | 9.1 | 0.19 | 3707 | 3707 | 0718quant |
| 10SCK2 | 144 | 10SCK2-144 | 9.8342 | 491.71 | 18.8 | 0.09 | 9222 | 9222 | 062508recalc |
| 10SCK3 | 0 | 10SCK3-0 | 8.58 | 429 | 9.9 | 0.19 | 4238 | 4238 | 0718quant |
| 10SCK3 | 48 | 10SCK3-48 | 8.62 | 431 | 9.0 | 0.19 | 3894 | 3894 | 0718quant |
| 10SCK3 | 96 | 10SCK3-96 | 7.441 | 372.05 | 7.4 | 0.19 | 2741 | 2741 | 0718quant |
| 10SCK3 | 144 | 10SCK3-144 | 9.8343 | 491.715 | 19.7 | 0.09 | 9669 | 9669 | 062508recalc |
| 10SRK1 | 0 | 10SRK1-0 | 9.8471 | 492.355 | 50.0 | 0.53 | 24618 | 24618 | 040409B-part1cps |
| 10SRK1 | 48 | 10SRK1-48 | 9.8667 | 493.335 | 35.6 | 0.53 | 17543 | 17543 | 040409B-part1cps |
| 10SRK1 | 96 | 10SRK1-96 | 9.8649 | 493.245 | 23.3 | 0.53 | 11507 | 11507 | 040409B-part1cps |
| 10SRK1 | 144 | 10SRK1-144 | 9.7213 | 486.065 | 22.7 | 0.09 | 11027 | 11027 | 062508recalc |
| 10SRK2 | 0 | 10SRK2-0 | 8.413 | 420.65 | 8.6 | 0.19 | 3613 | 3613 | 0718quant |
| 10SRK2 | 48 | 10SRK2-48 | 8.046 | 402.3 | 8.7 | 0.19 | 3481 | 3481 | 0718quant |
| 10SRK2 | 96 | 10SRK2-96 | 7.989 | 399.45 | 8.7 | 0.19 | 3458 | 3458 | 0718quant |
| 10SRK2 | 144 | 10SRK2-144 | 9.8498 | 492.49 | 14.1 | 0.09 | 6938 | 6938 | 062508recalc |
| 10SRK3 | 0 | 10SRK3-0 | 8.751 | 437.55 | 9.1 | 0.19 | 3986 | 3986 | 0718quant |
| 10SRK3 | 48 | 10SRK3-48 | 7.513 | 375.65 | 8.6 | 0.19 | 3240 | 3240 | 0718quant |
| 10SRK3 | 96 | 10SRK3-96 | 8.034 | 401.7 | 9.1 | 0.19 | 3658 | 3658 | 0718quant |
| 10SRK3 | 144 | 10SRK3-144 | 9.832 | 491.6 | 17.7 | 0.09 | 8716 | 8716 | 062508recalc |
| 10SSK1 | 0 | 10SSK1-0 | 9.8576 | 492.88 | 28.9 | 0.53 | 14239 | 14239 | 040409B-part1cps |
| 10SSK1 | 48 | 10SSK1-48 | 9.8451 | 492.255 | 45.6 | 0.53 | 22427 | 22427 | 040409B-part1cps |
| 10SSK1 | 96 | 10SSK1-96 | 9.851 | 492.55 | 58.9 | 0.53 | 29006 | 29006 | 040409B-part1cps |
| 10SSK1 | 144 | 10SSK1-144 | 9.8572 | 492.86 | 17.6 | 0.09 | 8653 | 8653 | 062508recalc |
| 10SSK2 | 0 | 10SSK2-0 | 8.334 | 416.7 | 9.4 | 0.19 | 3918 | 3918 | 0718quant |

D1-3.3. SC 10 KILLED SELENIUM, continued.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 10SSK2 | 48 | 10SSK2-48 | 8.664 | 433.2 | 9.3 | 0.19 | 4039 | 4039 | 0718quant |
| 10SSK2 | 96 | 10SSK2-96 | 7.899 | 394.95 | 8.0 | 0.19 | 3166 | 3166 | 0718quant |
| 10SSK2 | 144 | 10SSK2-144 | 9.845 | 492.25 | 18.8 | 0.09 | 9262 | 9262 | 062508recalc |
| 10SSK3 | 0 | 10SSK3-0 | 8.62 | 431 | 9.5 | 0.19 | 4096 | 4096 | 0718quant |
| 10SSK3 | 48 | 10SSK3-48 | 9.054 | 452.7 | 10.1 | 0.19 | 4577 | 4577 | 0718quant |
| 10SSK3 | 96 | 10SSK3-96 | 7.876 | 393.8 | 8.2 | 0.19 | 3230 | 3230 | 0718quant |
| 10SSK3 | 144 | 10SSK3-144 | 9.8478 | 492.39 | 19.9 | 0.09 | 9793 | 9793 | 062508recalc |

D1-3.4. SC 25 KILLED SELENIUM.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 25SCK1 | 0 | 25SCK1-0 | 9.448 | 472.4 | 9.98 | 0.25 | 4715 | 4715 | 0721quant |
| 25SCK1 | 48 | 25SCK1-48 | 8.272 | 413.6 | 7.99 | 0.25 | 3306 | 3306 | 0721quant |
| 25SCK1 | 96 | 25SCK1-96 | 7.916 | 395.8 | 8.35 | 0.25 | 3303 | 3303 | 0721quant |
| 25SCK1 | 144 | 25SCK1-144 | 9.836 | 491.8 | 18.31 | 0.09 | 9004 | 9004 | 062508recalc |
| 25SCK1 | 276 | 25SCK1-276 | 9.912 | 495.6 | 10.45 | 0.25 | 5179 | 5179 | 0721quant |
| 25SCK2 | 0 | 25SCK2-0 | 9.817 | 490.9 | 11.26 | 0.19 | 5527 | 5527 | 0718quant |
| 25SCK2 | 48 | 25SCK2-48 | 9.156 | 457.8 | 9.29 | 0.19 | 4253 | 4253 | 0718quant |
| 25SCK2 | 96 | 25SCK2-96 | 8.473 | 423.7 | 9.33 | 0.19 | 3953 | 3953 | 0718quant |
| 25SCK2 | 144 | 25SCK2-144 | 9.839 | 491.9 | 12.92 | 0.09 | 6354 | 6354 | 062508recalc |
| 25SCK2 | 276 | 25SCK2-276 | 10.400 | 520.0 | 10.36 | 0.25 | 5387 | 5387 | 0721quant |
| 25SCK3 | 0 | 25SCK3-0 | 9.274 | 463.7 | 9.92 | 0.19 | 4598 | 4598 | 0718quant |
| 25SCK3 | 48 | 25SCK3-48 | 9.272 | 463.6 | 9.99 | 0.19 | 4632 | 4632 | 0718quant |
| 25SCK3 | 96 | 25SCK3-96 | 7.268 | 363.4 | 7.54 | 0.19 | 2740 | 2740 | 0718quant |
| 25SCK3 | 144 | 25SCK3-144 | 9.849 | 492.4 | 19.85 | 0.09 | 9774 | 9774 | 062508recalc |
| 25SCK3 | 276 | 25SCK3-276 | 9.347 | 467.4 | 9.19 | 0.25 | 4293 | 4293 | 0721quant |
| 25SRK1 | 0 | 25SRK1-0 | 8.951 | 447.6 | 9.34 | 0.25 | 4181 | 4181 | 0721quant |
| 25SRK1 | 48 | 25SRK1-48 | 8.053 | 402.7 | 8.18 | 0.25 | 3295 | 3295 | 0721quant |
| 25SRK1 | 96 | 25SRK1-96 | 8.596 | 429.8 | 8.46 | 0.25 | 3636 | 3636 | 0721quant |
| 25SRK1 | 144 | 25SRK1-144 | 9.836 | 491.8 | 19.89 | 0.09 | 9780 | 9780 | 062508recalc |
| 25SRK1 | 276 | 25SRK1-276 | 9.525 | 476.3 | 10.20 | 0.25 | 4858 | 4858 | 0721quant |
| 25SRK2 | 0 | 25SRK2-0 | 8.668 | 433.4 | 9.23 | 0.19 | 3999 | 3999 | 0718quant |
| 25SRK2 | 48 | 25SRK2-48 | 7.938 | 396.9 | 8.50 | 0.19 | 3375 | 3375 | 0718quant |
| 25SRK2 | 96 | 25SRK2-96 | 8.626 | 431.3 | 8.70 | 0.19 | 3754 | 3754 | 0718quant |
| 25SRK2 | 144 | 25SRK2-144 | 9.846 | 492.3 | 12.15 | 0.09 | 5983 | 5983 | 062508recalc |
| 25SRK2 | 276 | 25SRK2-276 | 9.425 | 471.3 | 9.64 | 0.25 | 4541 | 4541 | 0721quant |
| 25SRK3 | 0 | 25SRK3-0 | 9.128 | 456.4 | 9.41 | 0.19 | 4293 | 4293 | 0718quant |
| 25SRK3 | 48 | 25SRK3-48 | 8.431 | 421.6 | 9.01 | 0.19 | 3796 | 3796 | 0718quant |
| 25SRK3 | 96 | 25SRK3-96 | 8.919 | 446.0 | 10.14 | 0.19 | 4522 | 4522 | 0718quant |
| 25SRK3 | 144 | 25SRK3-144 | 9.859 | 492.9 | 15.94 | 0.09 | 7857 | 7857 | 062508recalc |

D1-3.4. SC 25 KILLED SELENIUM, continued.

| Sample Information | | | | | SELENIUM | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Se, Measured** | **d.l.** | **Se, µg/L** | **Se µg/L, Reported** | **Se Source** |
| 25SRK3 | 276 | 25SRK3-276 | 9.859 | 493.0 | 10.05 | 0.25 | 4954 | 4954 | 0721quant |
| 25SSK1 | 0 | 25SSK1-0 | 7.853 | 392.7 | 8.37 | 0.25 | 3286 | 3286 | 0721quant |
| 25SSK1 | 48 | 25SSK1-48 | 7.819 | 391.0 | 7.89 | 0.25 | 3085 | 3085 | 0721quant |
| 25SSK1 | 96 | 25SSK1-96 | 7.814 | 390.7 | 7.56 | 0.25 | 2954 | 2954 | 0721quant |
| 25SSK1 | 144 | 25SSK1-144 | 9.778 | 488.9 | 16.27 | 0.09 | 7955 | 7955 | 062508recalc |
| 25SSK1 | 276 | 25SSK1-276 | 9.559 | 478.0 | 9.31 | 0.25 | 4448 | 4448 | 0721quant |
| 25SSK2 | 0 | 25SSK2-0 | 7.325 | 366.3 | 7.68 | 0.19 | 2812 | 2812 | 0718quant |
| 25SSK2 | 48 | 25SSK2-48 | 7.818 | 390.9 | 8.83 | 0.19 | 3452 | 3452 | 0718quant |
| 25SSK2 | 96 | 25SSK2-96 | 8.384 | 419.2 | 8.69 | 0.19 | 3643 | 3643 | 0718quant |
| 25SSK2 | 144 | 25SSK2-144 | 9.842 | 492.1 | 17.48 | 0.09 | 8600 | 8600 | 062508recalc |
| 25SSK2 | 276 | 25SSK2-276 | 9.453 | 472.7 | 10.21 | 0.25 | 4826 | 4826 | 0721quant |
| 25SSK3 | 0 | 25SSK3-0 | 7.675 | 383.8 | 8.41 | 0.19 | 3226 | 3226 | 0718quant |
| 25SSK3 | 48 | 25SSK3-48 | 7.987 | 399.4 | 8.77 | 0.19 | 3502 | 3502 | 0718quant |
| 25SSK3 | 96 | 25SSK3-96 | 9.226 | 461.3 | 9.85 | 0.19 | 4546 | 4546 | 0718quant |
| 25SSK3 | 144 | 25SSK3-144 | 9.800 | 490.0 | 14.82 | 0.09 | 7262 | 7262 | 062508recalc |
| 25SSK3 | 276 | 25SSK3-276 | 10.070 | 503.5 | 10.43 | 0.25 | 5252 | 5252 | 0721quant |

Table D1-4. Iron and Manganese ICP-MS data for Smoky Canyon Mine Saturated Rate Experiments.

D1-4.1. SC 10 LIVE IRON/MANGANESE.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 10SCL1 | 0 | 10SCL1-0 | 9.80 | 490.2 | -0.8 | 1.7 | -392 | 809 | 0.31 | 0.1 | 0.0 | 49 | 032309cpsrecalc |
| 10SCL1 | 12 | 10SCL1-12 | 9.89 | 494.6 | -0.7 | 1.7 | 816 | 816 | 0.24 | 0.1 | 49 | 49 | 032309cpsrecalc |
| 10SCL1 | 24 | 10SCL1-24 | 9.84 | 491.9 | -7.52 | 1.7 | 812 | 812 | -0.05 | 0.1 | 49 | 49 | 2008621_M_quant |
| 10SCL1 | 36 | 10SCL1-36 | 9.86 | 492.9 | -0.85 | 1.7 | 838 | 838 | -0.10 | 0.1 | 49 | 49 | 071408quant |
| 10SCL1 | 48 | 10SCL1-48 | 9.81 | 490.4 | -4.06 | 1.0 | 490 | 490 | 0.03 | 0.06 | 29 | 29 | 062008Aquant |
| 10SCL1 | 60 | 10SCL1-60 | 9.86 | 492.9 | -2.33 | 1.5 | 739 | 739 | 0.11 | 0.1 | 53 | 53 | 2008621_M_quant |
| 10SCL1 | 84 | 10SCL1-84 | 9.85 | 492.4 | -2.36 | 1.5 | 739 | 739 | 0.10 | 0.1 | 51 | 51 | 2008621_M_quant |
| 10SCL1 | 108 | 10SCL1-108 | 9.79 | 489.7 | -0.05 | 1.4 | 686 | 686 | -0.05 | 0.1 | 49 | 49 | 062508recalc |
| 10SCL1 | 132 | 10SCL1-132 | 9.85 | 492.3 | -0.05 | 1.4 | 689 | 689 | -0.05 | 0.1 | 49 | 49 | 062508recalc |
| 10SCL1 | 156 | 10SCL1-156 | 9.85 | 492.4 | 7.83 | 1.4 | 3855 | 3855 | 1.53 | 0.1 | 755 | 755 | 062508recalc |
| 10SCL1 | 246 | 10SCL1-246 | 9.82 | 490.9 | 0.08 | 1.7 | 835 | 835 | 0.01 | 0.1 | 49 | 49 | 071408quant |
| 10SCL2 | 0 | 10SCL2-0 | 9.85 | 492.6 | 0.6 | 1.7 | 813 | 813 | 0.17 | 0.1 | 86 | 86 | 032309cpsrecalc |
| 10SCL2 | 12 | 10SCL2-12 | 9.88 | 493.8 | -1.0 | 1.7 | 815 | 815 | 0.24 | 0.1 | 49 | 49 | 032309cpsrecalc |
| 10SCL2 | 24 | 10SCL1-24 | 9.85 | 492.4 | -7.677 | 1.7 | 813 | 813 | -0.03 | 0.1 | 49 | 49 | 2008621_M_quant |
| 10SCL2 | 36 | 10SCL2-36 | 9.85 | 492.7 | 0.17 | 1.7 | 838 | 838 | -0.12 | 0.1 | 49 | 49 | 071408quant |
| 10SCL2 | 48 | 10SCL2-48 | 9.81 | 490.7 | -4.92 | 1.0 | 491 | 491 | 0.02 | 0.06 | 29 | 29 | 062008Aquant |
| 10SCL2 | 60 | 10SCL2-60 | 9.86 | 492.9 | -2.55 | 1.5 | 739 | 739 | 0.10 | 0.1 | 49 | 49 | 062008Aquant |
| 10SCL2 | 84 | 10SCL2-84 | 9.86 | 492.8 | -2.67 | 1.5 | 739 | 739 | 0.12 | 0.1 | 60 | 60 | 2008612quantxls |
| 10SCL2 | 108 | 10SCL2-108 | 9.77 | 488.7 | 0.00 | 1.4 | 684 | 684 | 0.00 | 0.1 | 49 | 49 | 062508recalc |
| 10SCL2 | 132 | 10SCL2-132 | 9.83 | 491.6 | 0.00 | 1.4 | 688 | 688 | 0.00 | 0.1 | 49 | 49 | 062508recalc |
| 10SCL2 | 156 | 10SCL2-156 | 9.79 | 489.7 | 7.51 | 1.4 | 3679 | 3679 | 1.72 | 0.1 | 841 | 841 | 062508recalc |
| 10SCL2 | 246 | 10SCL2-246 | 9.80 | 490.1 | -0.27 | 1.7 | 833 | 833 | 0.02 | 0.1 | 49 | 49 | 071408quant |
| 10SCL3 | 0 | 10SCL3-0 | 9.85 | 492.5 | -0.9 | 1.7 | 813 | 813 | 0.14 | 0.3 | 173 | 148 | 032309cpsrecalc |
| 10SCL3 | 12 | 10SCL3-12 | 9.87 | 493.7 | -1.2 | 1.7 | 815 | 815 | 0.23 | 0.1 | 49 | 49 | 032309cpsrecalc |
| 10SCL3 | 24 | 10SCL1-24 | 9.84 | 491.8 | -6.966 | 1.7 | 811 | 811 | 0.01 | 0.1 | 49 | 49 | 2008621_M_quant |
| 10SCL3 | 36 | 10SCL3-36 | 9.86 | 492.8 | -0.35 | 1.7 | 838 | 838 | -0.12 | 0.1 | 49 | 49 | 071408quant |
| 10SCL3 | 48 | 10SCL3-48 | 9.79 | 489.6 | -5.27 | 1.0 | 490 | 490 | 0.02 | 0.06 | 29 | 29 | 062008Aquant |

D1-4.1. SC 10 LIVE IRON/MANGANESE, continued.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 10SCL3 | 60 | 10SCL3-60 | 9.86 | 492.8 | -2.64 | 1.5 | 739 | 739 | 0.11 | 0.1 | 56 | 56 | 062008Aquant |
| 10SCL3 | 84 | 10SCL3-84 | 9.85 | 492.4 | -1.57 | 1.5 | 739 | 739 | 0.19 | 0.1 | 91 | 91 | 2008612quantxls |
| 10SCL3 | 108 | 10SCL3-108 | 9.79 | 489.5 | 0.06 | 1.4 | 685 | 685 | 0.06 | 0.1 | 49 | 49 | 062508recalc |
| 10SCL3 | 132 | 10SCL3-132 | 9.84 | 492.2 | 0.06 | 1.4 | 689 | 689 | 0.06 | 0.1 | 49 | 49 | 062508recalc |
| 10SCL3 | 156 | 10SCL3-156 | 9.81 | 490.6 | 7.49 | 1.4 | 3676 | 3676 | 1.94 | 0.1 | 953 | 953 | 062508recalc |
| 10SCL3 | 246 | 10SCL3-246 | 9.84 | 491.8 | 0.03 | 1.7 | 836 | 836 | 0.05 | 0.1 | 49 | 49 | 071408quant |
| 10SRL1 | 0 | 10SRL1-0 | 9.85 | 492.4 | -0.8 | 1.7 | 812 | 812 | 0.20 | 0.3 | 173 | 148 | 032309cpsrecalc |
| 10SRL1 | 12 | 10SRL1-12 | 9.95 | 497.3 | -1.2 | 1.7 | 821 | 821 | 0.43 | 0.1 | 199 | 199 | 032309cpsrecalc |
| 10SRL1 | 24 | 10SRL1-24 | 9.80 | 490.0 | -6.967 | 1.7 | 808 | 808 | 0.13 | 0.1 | 63 | 63 | 2008621_M_quant |
| 10SRL1 | 36 | 10SRL1-36 | 9.86 | 493.0 | -0.38 | 1.7 | 838 | 838 | -0.06 | 0.1 | 49 | 49 | 071408quant |
| 10SRL1 | 48 | 10SRL1-48 | 9.80 | 490.0 | -5.41 | 1.0 | 490 | 490 | 0.25 | 0.06 | 121 | 121 | 062008Aquant |
| 10SRL1 | 60 | 10SRL1-60 | 9.85 | 492.6 | -1.48 | 1.5 | 739 | 739 | 0.35 | 0.1 | 172 | 172 | 062008Aquant |
| 10SRL1 | 84 | 10SRL1-84 | 9.85 | 492.5 | -0.05 | 1.5 | 739 | 739 | 0.46 | 0.1 | 225 | 225 | 2008612quantxls |
| 10SRL1 | 108 | 10SRL1-108 | 9.78 | 489.0 | 0.44 | 1.4 | 214 | 685 | 0.44 | 0.1 | 214 | 214 | 062508recalc |
| 10SRL1 | 132 | 10SRL1-132 | 9.83 | 491.7 | 0.44 | 1.4 | 216 | 688 | 0.44 | 0.1 | 216 | 216 | 062508recalc |
| 10SRL1 | 156 | 10SRL1-156 | 9.82 | 490.8 | 7.43 | 1.4 | 3647 | 3647 | 1.97 | 0.1 | 964 | 964 | 062508recalc |
| 10SRL1 | 246 | 10SRL1-246 | 9.82 | 490.8 | -0.23 | 1.7 | 834 | 834 | 0.03 | 0.1 | 49 | 49 | 071408quant |
| 10SRL2 | 0 | 10SRL2-0 | 9.87 | 493.5 | -1.1 | 1.7 | 814 | 814 | 0.21 | 0.3 | 174 | 148 | 032309cpsrecalc |
| 10SRL2 | 12 | 10SRL2-12 | 9.84 | 492.1 | -1.2 | 1.7 | 812 | 812 | 0.34 | 0.1 | 169 | 169 | 032309cpsrecalc |
| 10SRL2 | 24 | 10SRL2-24 | 9.85 | 492.5 | -7.282 | 1.7 | 813 | 813 | 0.06 | 0.1 | 49 | 49 | 2008621_M_quant |
| 10SRL2 | 36 | 10SRL2-36 | 9.83 | 491.6 | -0.35 | 1.7 | 836 | 836 | -0.08 | 0.1 | 49 | 49 | 071408quant |
| 10SRL2 | 48 | 10SRL2-48 | 9.82 | 490.8 | -3.26 | 1.0 | 491 | 491 | 0.16 | 0.06 | 78 | 78 | 062008Aquant |
| 10SRL2 | 60 | 10SRL2-60 | 9.85 | 492.6 | -2.54 | 1.5 | 739 | 739 | 0.29 | 0.1 | 144 | 144 | 062008Aquant |
| 10SRL2 | 84 | 10SRL2-84 | 9.86 | 492.9 | -1.71 | 1.5 | 739 | 739 | 0.37 | 0.1 | 185 | 185 | 2008612quantxls |
| 10SRL2 | 108 | 10SRL2-108 | 9.80 | 490.2 | 0.34 | 1.4 | 167 | 686 | 0.34 | 0.1 | 167 | 167 | 062508recalc |
| 10SRL2 | 132 | 10SRL2-132 | 9.83 | 491.5 | 0.34 | 1.4 | 167 | 688 | 0.34 | 0.1 | 167 | 167 | 062508recalc |
| 10SRL2 | 156 | 10SRL2-156 | 9.81 | 490.7 | 7.65 | 1.4 | 3754 | 3754 | 1.93 | 0.1 | 946 | 946 | 062508recalc |
| 10SRL2 | 246 | 10SRL2-246 | 9.81 | 490.3 | -0.27 | 1.7 | 834 | 834 | 0.03 | 0.1 | 49 | 49 | 071408quant |
| 10SRL3 | 0 | 10SRL3-0 | 9.86 | 493.1 | -1.0 | 1.7 | 814 | 814 | 0.29 | 0.3 | 174 | 148 | 032309cpsrecalc |
| 10SRL3 | 12 | 10SRL3-12 | 9.83 | 491.5 | 2.0 | 1.7 | 997 | 997 | 0.54 | 0.3 | 246 | 246 | 032309cpsrecalc |

D1-4.1. SC 10 LIVE IRON/MANGANESE, continued.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 10SRL3 | 24 | 10SRL1-24 | 9.78 | 488.9 | -5.968 | 1.7 | 807 | 807 | 0.16 | 0.1 | 78 | 78 | 2008621_M_quant |
| 10SRL3 | 36 | 10SRL3-36 | 9.85 | 492.6 | 0.91 | 1.7 | 837 | 837 | -0.07 | 0.1 | 49 | 49 | 071408quant |
| 10SRL3 | 48 | 10SRL3-48 | 9.82 | 491.2 | -4.99 | 1.0 | 491 | 491 | 0.19 | 0.06 | 95 | 95 | 062008Aquant |
| 10SRL3 | 60 | 10SRL3-60 | 9.85 | 492.7 | -2.79 | 1.5 | 739 | 739 | 0.38 | 0.1 | 188 | 188 | 062008Aquant |
| 10SRL3 | 84 | 10SRL3-84 | 9.85 | 492.6 | -1.26 | 1.5 | 739 | 739 | 0.53 | 0.1 | 263 | 263 | 2008612quantxls |
| 10SRL3 | 108 | 10SRL3-108 | 9.78 | 488.8 | 0.38 | 1.4 | 187 | 684 | 0.38 | 0.1 | 187 | 187 | 062508recalc |
| 10SRL3 | 132 | 10SRL3-132 | 9.83 | 491.5 | 0.38 | 1.4 | 188 | 688 | 0.38 | 0.1 | 188 | 188 | 062508recalc |
| 10SRL3 | 156 | 10SRL3-156 | 9.81 | 490.7 | 7.66 | 1.4 | 3756 | 3756 | 2.06 | 0.1 | 1011 | 1011 | 062508recalc |
| 10SRL3 | 246 | 10SRL3-246 | 9.82 | 491.2 | -0.24 | 1.7 | 835 | 835 | 0.04 | 0.1 | 49 | 49 | 071408quant |
| 10SSL1 | 0 | 10SSL1-0 | 9.86 | 492.8 | -1.0 | 1.7 | 813 | 813 | 0.25 | 0.3 | 173 | 148 | 032309cpsrecalc |
| 10SSL1 | 12 | 10SSL1-12 | 9.82 | 490.8 | -1.2 | 1.7 | 810 | 810 | 0.41 | 0.3 | 196 | 196 | 032309cpsrecalc |
| 10SSL1 | 24 | 10SSL1-24 | 9.86 | 492.9 | -5.877 | 1.7 | 813 | 813 | 0.14 | 0.1 | 69 | 69 | 2008621_M_quant |
| 10SSL1 | 36 | 10SSL1-36 | 9.85 | 492.6 | 0.48 | 1.7 | 837 | 837 | -0.07 | 0.1 | 49 | 49 | 071408quant |
| 10SSL1 | 48 | 10SSL1-48 | 9.81 | 490.4 | 0.16 | 1.0 | 490 | 490 | 1.79 | 0.06 | 877 | 877 | 062008Aquant |
| 10SSL1 | 60 | 10SSL1-60 | 9.86 | 492.8 | -2.24 | 1.5 | 739 | 739 | 0.38 | 0.1 | 185 | 185 | 062008Aquant |
| 10SSL1 | 84 | 10SSL1-84 | 9.87 | 493.3 | 1.12 | 1.5 | 740 | 740 | 0.46 | 0.1 | 229 | 229 | 2008612quantxls |
| 10SSL1 | 108 | 10SSL1-108 | 9.50 | 475.0 | 0.37 | 1.4 | 175 | 665 | 0.37 | 0.1 | 175 | 175 | 062508recalc |
| 10SSL1 | 132 | 10SSL1-132 | 9.83 | 491.6 | 0.37 | 1.4 | 181 | 688 | 0.37 | 0.1 | 181 | 181 | 062508recalc |
| 10SSL1 | 156 | 10SSL1-156 | 9.81 | 490.7 | 7.93 | 1.4 | 3889 | 3889 | 1.87 | 0.1 | 920 | 920 | 062508recalc |
| 10SSL1 | 246 | 10SSL1-246 | 9.80 | 489.8 | 0.11 | 1.7 | 833 | 833 | -0.03 | 0.1 | 49 | 49 | 071408quant |
| 10SSL2 | 0 | 10SSL2-0 | 9.84 | 491.8 | -1.0 | 1.7 | 812 | 812 | 0.31 | 0.3 | 173 | 173 | 032309cpsrecalc |
| 10SSL2 | 12 | 10SSL2-12 | 9.84 | 492.0 | -1.3 | 1.7 | 812 | 812 | 0.32 | 0.1 | 157 | 157 | 032309cpsrecalc |
| 10SSL2 | 24 | 10SSL1-24 | 9.85 | 492.3 | -3.144 | 1.7 | 812 | 812 | 0.17 | 0.1 | 83 | 83 | 2008621_M_quant |
| 10SSL2 | 36 | 10SSL2-36 | 9.85 | 492.7 | -0.13 | 1.7 | 838 | 838 | -0.07 | 0.1 | 49 | 49 | 071408quant |
| 10SSL2 | 48 | 10SSL2-48 | 9.87 | 493.7 | -0.14 | 1.0 | 494 | 494 | 1.68 | 0.06 | 829 | 829 | 062008Aquant |
| 10SSL2 | 60 | 10SSL2-60 | 9.82 | 490.9 | -3.27 | 1.5 | 736 | 736 | 0.36 | 0.1 | 177 | 177 | 062008Aquant |
| 10SSL2 | 84 | 10SSL2-84 | 9.85 | 492.5 | -1.98 | 1.5 | 739 | 739 | 0.45 | 0.1 | 221 | 221 | 2008612quantxls |
| 10SSL2 | 108 | 10SSL2-108 | 9.79 | 489.4 | 0.40 | 1.4 | 194 | 685 | 0.40 | 0.1 | 194 | 194 | 062508recalc |
| 10SSL2 | 132 | 10SSL2-132 | 9.85 | 492.5 | 0.40 | 1.4 | 195 | 690 | 0.40 | 0.1 | 195 | 195 | 062508recalc |
| 10SSL2 | 156 | 10SSL2-156 | 9.81 | 490.6 | 7.73 | 1.4 | 3794 | 3794 | 2.05 | 0.1 | 1007 | 1007 | 062508recalc |
| 10SSL2 | 246 | 10SSL2-246 | 9.83 | 491.3 | -0.19 | 1.7 | 835 | 835 | -0.01 | 0.1 | 49 | 49 | 071408quant |

D1-4.1. SC 10 LIVE IRON/MANGANESE, continued.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 10SSL3 | 0 | 10SSL3-0 | 9.85 | 492.3 | -1.2 | 1.7 | 812 | 812 | 0.24 | 0.3 | 173 | 148 | 032309cpsrecalc |
| 10SSL3 | 12 | 10SSL3-12 | 9.84 | 492.1 | -1.2 | 1.7 | 812 | 812 | 0.33 | 0.1 | 161 | 161 | 032309cpsrecalc |
| 10SSL3 | 24 | 10SSL1-24 | 9.84 | 491.8 | -5.823 | 1.7 | 811 | 811 | 0.09 | 49 | 49 | 24188 | 2008621_M_quant |
| 10SSL3 | 36 | 10SSL3-36 | 9.85 | 492.6 | -0.03 | 1.7 | 837 | 837 | -0.09 | 0.1 | 49 | 49 | 071408quant |
| 10SSL3 | 48 | 10SSL3-48 | 9.81 | 490.5 | -2.53 | 1.0 | 491 | 491 | 0.24 | 0.06 | 120 | 120 | 062008Aquant |
| 10SSL3 | 60 | 10SSL3-60 | 9.84 | 492.2 | -2.28 | 1.5 | 738 | 738 | 0.38 | 0.1 | 188 | 188 | 062008Aquant |
| 10SSL3 | 84 | 10SSL3-84 | 9.85 | 492.7 | -2.09 | 1.5 | 739 | 739 | 0.43 | 0.1 | 213 | 213 | 2008612quantxls |
| 10SSL3 | 108 | 10SSL3-108 | 9.78 | 489.0 | 0.37 | 1.4 | 179 | 685 | 0.37 | 0.1 | 179 | 179 | 062508recalc |
| 10SSL3 | 132 | 10SSL3-132 | 9.77 | 488.4 | 0.37 | 1.4 | 178 | 684 | 0.37 | 0.1 | 178 | 178 | 062508recalc |
| 10SSL3 | 156 | 10SSL3-156 | 9.81 | 490.6 | 8.00 | 1.4 | 3924 | 3924 | 2.05 | 0.1 | 1007 | 1007 | 062508recalc |
| 10SSL3 | 246 | 10SSL3-246 | 9.84 | 491.8 | -0.09 | 1.7 | 836 | 836 | -0.02 | 0.1 | 49 | 49 | 071408quant |

D1-4.2. SC 25 LIVE IRON/MANGANESE.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Experiment | Time | Name | Weight | Dilution | Fe, Measured | d.l. | Fe, µg/L | Fe µg/L, Reported | Mn, Measured | d.l. | Mn, µg/L | Mn, µg/L, Reported | Fe and Mn Source |
| 25SCL1 | 0 | 25SCL1-0 | 9.802 | 490.1 | -0.7 | 1.65 | -346 | 809 | 0.3 | 0.3 | 157 | 157 | 032309cpsrecalcFeMn |
| 25SCL1 | 12 | 25SCL1-12 | 9.834 | 491.7 | -0.8 | 1.65 | -385 | 811 | 0.3 | 0.3 | 170 | 170 | 032309cpsrecalcFeMn |
| 25SCL1 | 24 | 25SCL1-24 | 9.854 | 492.7 | -0.9 | 1.65 | -463 | 813 | 0.4 | 0.3 | 174 | 174 | 032309cpsrecalcFeMn |
| 25SCL1 | 36 | 25SCL1-36 | 9.825 | 491.3 | 1.6 | 1.65 | 771 | 811 | 1.3 | 0.3 | 641 | 641 | 032309cpsrecalcFeMn |
| 25SCL1 | 48 | 25SCL1-48 | 9.562 | 478.1 | -3.4 | 1.65 | -1616 | 789 | 0.3 | 0.3 | 142 | 143 | 032309cpsrecalcFeMn |
| 25SCL1 | 60 | 25SCL1-60 | 9.833 | 491.6 | -5.4 | 1.0 | -2650 | 492 | 0.0 | 0.06 | 23 | 29 | 062008Aquant |
| 25SCL1 | 72 | 25SCL1-72 | 9.793 | 489.7 | -5.7 | 0.7 | -2781 | 343 | 0.0 | 0.02 | 13 | 13 | 062008B25SC 60_72 |
| 25SCL1 | 96 | 25SCL1-96 | 9.836 | 491.8 | -0.4 | 1.7 | -178 | 836 | -0.4 | 0.10 | -178 | 49 | 071408quant |
| 25SCL1 | 120 | 25SCL1-120 | 9.780 | 489.0 | 6.5 | 1.4 | 3176 | 3176 | 0.3 | 0.1 | 146 | 146 | 062508recalc |
| 25SCL1 | 144 | 25SCL1-144 | 9.856 | 492.8 | 26.3 | 1.4 | 12967 | 12967 | 1.0 | 0.1 | 471 | 471 | 062508recalc |
| 25SCL1 | 168 | 25SCL1-168 | 9.798 | 489.9 | 7.7 | 1.4 | 3750 | 3750 | 2.0 | 0.1 | 1004 | 1004 | 062508recalc |
| 25SCL1 | 228 | 25SCL1-228 | 9.829 | 491.4 | -0.1 | 1.7 | -47 | 835 | 0.1 | 0.1 | 48 | 49 | 071408quant |
| 25SCL2 | 0 | 25SCL2-0 | 9.830 | 491.5 | -0.8 | 1.65 | -408 | 811 | 0.3 | 0.3 | 149 | 149 | 032309cpsrecalcFeMn |
| 25SCL2 | 12 | 25SCL2-12 | 9.838 | 491.9 | -1.0 | 1.65 | -516 | 812 | 0.3 | 0.3 | 151 | 151 | 032309cpsrecalcFeMn |
| 25SCL2 | 24 | 25SCL2-24 | 9.842 | 492.1 | -1.2 | 1.65 | -575 | 812 | 0.3 | 0.3 | 153 | 153 | 032309cpsrecalcFeMn |
| 25SCL2 | 36 | 25SCL2-36 | 9.847 | 492.3 | -1.2 | 1.65 | -591 | 812 | 0.3 | 0.3 | 134 | 148 | 032309cpsrecalcFeMn |
| 25SCL2 | 48 | 25SCL2-48 | 9.852 | 492.6 | -1.0 | 1.65 | -492 | 813 | 0.4 | 0.3 | 180 | 180 | 032309cpsrecalcFeMn |
| 25SCL2 | 60 | 25SCL2-60 | 9.841 | 492.0 | -1.8 | 1.0 | -896 | 492 | 0.7 | 0.06 | 331 | 331 | 062008Aquant |
| 25SCL2 | 72 | 25SCL2-72 | 9.774 | 488.7 | -5.8 | 0.7 | -2831 | 342 | 0.0 | 0.02 | 5 | 10 | 062008B25SC 60_72 |
| 25SCL2 | 96 | 25SCL2-96 | 9.812 | 490.6 | -0.3 | 1.7 | -161 | 834 | -0.3 | 0.10 | -161 | 49 | 071408quant |
| 25SCL2 | 120 | 25SCL2-120 | 9.771 | 488.5 | 6.3 | 1.4 | 3093 | 3093 | 0.2 | 0.1 | 83 | 83 | 062508recalc |
| 25SCL2 | 144 | 25SCL2-144 | 9.852 | 492.6 | 10.8 | 1.4 | 5299 | 5299 | 0.5 | 0.1 | 261 | 261 | 062508recalc |
| 25SCL2 | 168 | 25SCL2-168 | 9.812 | 490.6 | 7.6 | 1.4 | 3739 | 3739 | 1.9 | 0.1 | 946 | 946 | 062508recalc |
| 25SCL2 | 228 | 25SCL2-228 | 9.793 | 489.7 | -0.3 | 1.7 | -134 | 832 | 0.1 | 0.1 | 40 | 49 | 071408quant |
| 25SCL3 | 0 | 25SCL3-0 | 9.820 | 491.0 | 0.8 | 1.65 | 394 | 810 | 0.3 | 0.3 | 162 | 162 | 032309cpsrecalcFeMn |
| 25SCL3 | 12 | 25SCL3-12 | 9.841 | 492.1 | 0.4 | 1.65 | 202 | 812 | 0.4 | 0.3 | 185 | 185 | 032309cpsrecalcFeMn |
| 25SCL3 | 24 | 25SCL3-24 | 9.846 | 492.3 | 0.4 | 1.65 | 175 | 812 | 0.4 | 0.3 | 213 | 213 | 032309cpsrecalcFeMn |
| 25SCL3 | 36 | 25SCL3-36 | 9.840 | 492.0 | 0.7 | 1.65 | 366 | 812 | 0.4 | 0.3 | 179 | 179 | 032309cpsrecalcFeMn |

D1-4.2. SC 25 LIVE IRON/MANGANESE, continued.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Experiment | Time | Name | Weight | Dilution | Fe, Measured | d.l. | Fe, µg/L | Fe µg/L, Reported | Mn, Measured | d.l. | Mn, µg/L | Mn, µg/L, Reported | Fe and Mn Source |
| 25SCL3 | 48 | 25SCL3-48 | 9.805 | 490.3 | 0.7 | 1.65 | 330 | 809 | 0.6 | 0.3 | 312 | 312 | 032309cpsrecalcFeMn |
| 25SCL3 | 60 | 25SCL3-60 | 9.813 | 490.6 | -4.5 | 1.0 | -2187 | 491 | 0.1 | 0.06 | 52 | 52 | 062008Aquant |
| 25SCL3 | 72 | 25SCL3-72 | 9.790 | 489.5 | -5.5 | 0.7 | -2702 | 343 | 0.0 | 0.02 | 15 | 15 | 062008B25SC 60_72 |
| 25SCL3 | 96 | 25SCL3-96 | 9.829 | 491.5 | -0.3 | 1.7 | -156 | 835 | -0.3 | 0.10 | -156 | 49 | 071408quant |
| 25SCL3 | 120 | 25SCL3-120 | 9.771 | 488.6 | 7.1 | 1.4 | 3482 | 3482 | 0.2 | 0.1 | 75 | 75 | 062508recalc |
| 25SCL3 | 144 | 25SCL3-144 | 9.817 | 490.9 | 7.4 | 1.4 | 3642 | 3642 | 0.5 | 0.1 | 226 | 226 | 062508recalc |
| 25SCL3 | 168 | 25SCL3-168 | 9.813 | 490.6 | 8.1 | 1.4 | 3953 | 3953 | 2.0 | 0.1 | 963 | 963 | 062508recalc |
| 25SCL3 | 228 | 25SCL3-228 | 9.815 | 490.7 | -0.3 | 1.7 | -126 | 834 | 0.1 | 0.1 | 25 | 49 | 071408quant |
| 25SRL1 | 0 | 25SRL1-0 | 9.853 | 492.6 | -1.1 | 1.65 | -558 | 813 | 0.3 | 0.3 | 160 | 160 | 032309cpsrecalcFeMn |
| 25SRL1 | 12 | 25SRL1-12 | 9.837 | 491.9 | -1.1 | 1.65 | -561 | 812 | 0.4 | 0.3 | 220 | 220 | 032309cpsrecalcFeMn |
| 25SRL1 | 24 | 25SRL1-24 | 9.833 | 491.6 | -1.2 | 1.65 | -565 | 811 | 0.5 | 0.3 | 233 | 233 | 032309cpsrecalcFeMn |
| 25SRL1 | 36 | 25SRL1-36 | 9.838 | 491.9 | 0.2 | 1.65 | 102 | 812 | 0.9 | 0.3 | 445 | 445 | 032309cpsrecalcFeMn |
| 25SRL1 | 48 | 25SRL1-48 | 9.829 | 491.4 | -1.0 | 1.65 | -475 | 811 | 0.5 | 0.3 | 249 | 249 | 032309cpsrecalcFeMn |
| 25SRL1 | 60 | 25SRL1-60 | 9.845 | 492.2 | -5.5 | 1.0 | -2727 | 492 | 0.6 | 0.06 | 307 | 307 | 062008Aquant |
| 25SRL1 | 72 | 25SRL-1-72 | 9.780 | 489.0 | -6.6 | 0.7 | -3216 | 342 | 0.8 | 0.02 | 388 | 388 | 062008B25SC 60_72 |
| 25SRL1 | 96 | 25SRL1-96 | 9.799 | 489.9 | -0.2 | 1.7 | -89 | 833 | -0.2 | 0.10 | -89 | 49 | 071408quant |
| 25SRL1 | 120 | 25SRL1-120 | 9.796 | 489.8 | 6.7 | 1.4 | 3260 | 3260 | 1.5 | 0.1 | 736 | 736 | 062508recalc |
| 25SRL1 | 144 | 25SRL1-144 | 9.848 | 492.4 | 5.0 | 1.4 | 2438 | 2438 | 1.9 | 0.1 | 932 | 932 | 062508recalc |
| 25SRL1 | 168 | 25SRL1-168 | 9.816 | 490.8 | 8.1 | 1.4 | 3987 | 3987 | 3.2 | 0.1 | 1594 | 1594 | 062508recalc |
| 25SRL1 | 228 | 25SRL1-228 | 9.822 | 491.1 | -0.2 | 1.7 | -100 | 835 | 0.4 | 0.1 | 208 | 208 | 071408quant |
| 25SRL2 | 0 | 25SRL2-0 | 9.847 | 492.3 | -1.2 | 1.65 | -585 | 812 | 0.4 | 0.3 | 190 | 190 | 032309cpsrecalcFeMn |
| 25SRL2 | 12 | 25SRL2-12 | 9.861 | 493.1 | -1.1 | 1.65 | -554 | 814 | 0.4 | 0.3 | 195 | 195 | 032309cpsrecalcFeMn |
| 25SRL2 | 24 | 25SRL2-24 | 9.838 | 491.9 | -1.1 | 1.65 | -565 | 812 | 0.5 | 0.3 | 228 | 228 | 032309cpsrecalcFeMn |
| 25SRL2 | 36 | 25SRL2-36 | 9.838 | 491.9 | -1.3 | 1.65 | -621 | 812 | 0.4 | 0.3 | 185 | 185 | 032309cpsrecalcFeMn |
| 25SRL2 | 48 | 25SRL2-48 | 9.842 | 492.1 | 1.4 | 1.65 | 708 | 812 | 0.7 | 0.3 | 334 | 334 | 032309cpsrecalcFeMn |
| 25SRL2 | 60 | 25SRL2-60 | 9.829 | 491.4 | -3.5 | 1.0 | -1697 | 491 | 0.4 | 0.06 | 192 | 192 | 062008Aquant |
| 25SRL2 | 72 | 25SRL-2-72 | 9.804 | 490.2 | -6.4 | 0.7 | -3129 | 343 | 0.3 | 0.02 | 160 | 160 | 062008B25SC 60_72 |
| 25SRL2 | 96 | 25SRL2-96 | 9.814 | 490.7 | -0.3 | 1.7 | -140 | 834 | -0.3 | 0.10 | -140 | 49 | 071408quant |
| 25SRL2 | 120 | 25SRL2-120 | 9.784 | 489.2 | 6.8 | 1.4 | 3328 | 3328 | 1.1 | 0.1 | 517 | 517 | 062508recalc |
| 25SRL2 | 144 | 25SRL2-144 | 9.855 | 492.7 | 3.4 | 1.4 | 1667 | 1667 | 1.4 | 0.1 | 702 | 702 | 062508recalc |
| 25SRL2 | 168 | 25SRL2-168 | 9.818 | 490.9 | 8.1 | 1.4 | 3958 | 3958 | 2.9 | 0.1 | 1435 | 1435 | 062508recalc |

D1-4.2. SC 25 LIVE IRON/MANGANESE, continued.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 25SRL2 | 228 | 25SRL2-228 | 9.831 | 491.5 | -0.2 | 1.7 | -93 | 836 | 0.4 | 0.1 | 197 | 197 | 071408quant |
| 25SRL3 | 0 | 25SRL3-0 | 9.859 | 492.9 | 0.1 | 1.65 | 44 | 813 | 0.6 | 0.3 | 303 | 303 | 032309cpsrecalcFeMn |
| 25SRL3 | 12 | 25SRL3-12 | 9.841 | 492.1 | 0.2 | 1.65 | 94 | 812 | 0.5 | 0.3 | 259 | 259 | 032309cpsrecalcFeMn |
| 25SRL3 | 24 | 25SRL3-24 | 9.855 | 492.8 | 0.4 | 1.65 | 199 | 813 | 0.6 | 0.3 | 278 | 278 | 032309cpsrecalcFeMn |
| 25SRL3 | 36 | 25SRL3-36 | 9.853 | 492.7 | 0.7 | 1.65 | 325 | 813 | 0.5 | 0.3 | 265 | 265 | 032309cpsrecalcFeMn |
| 25SRL3 | 48 | 25SRL3-48 | 9.843 | 492.2 | 2.1 | 1.65 | 1025 | 1025 | 0.5 | 0.3 | 251 | 251 | 032309cpsrecalcFeMn |
| 25SRL3 | 60 | 25SRL3-60 | 9.835 | 491.8 | -4.2 | 1.0 | -2088 | 492 | 0.3 | 0.06 | 158 | 158 | 062008Aquant |
| 25SRL3 | 72 | 25SRL3-72 | 9.776 | 488.8 | -6.2 | 0.7 | -3008 | 342 | 0.5 | 0.02 | 231 | 231 | 062008B25SC 60_72 |
| 25SRL3 | 96 | 25SRL3-96 | 9.826 | 491.3 | -0.2 | 1.7 | -116 | 835 | -0.2 | 0.10 | -116 | 49 | 071408quant |
| 25SRL3 | 120 | 25SRL3-120 | 9.669 | 483.5 | 2.0 | 1.4 | 957 | 957 | 0.9 | 0.1 | 431 | 431 | 062508recalc |
| 25SRL3 | 144 | 25SRL3-144 | 9.807 | 490.3 | 3.3 | 1.4 | 1626 | 1626 | 0.8 | 0.1 | 403 | 403 | 062508recalc |
| 25SRL3 | 168 | 25SRL3-168 | 9.818 | 490.9 | 7.9 | 1.4 | 3864 | 3864 | 2.3 | 0.1 | 1110 | 1110 | 062508recalc |
| 25SRL3 | 228 | 25SRL3-228 | 9.826 | 491.3 | -0.2 | 1.7 | -121 | 835 | 0.2 | 0.1 | 101 | 101 | 071408quant |
| 25SSL1 | 0 | 25SSL1-0 | 9.772 | 488.6 | -1.3 | 1.65 | -619 | 806 | 0.3 | 0.3 | 158 | 158 | 032309cpsrecalcFeMn |
| 25SSL1 | 12 | 25SSL1-12 | 9.830 | 491.5 | -1.1 | 1.65 | -521 | 811 | 0.4 | 0.3 | 211 | 211 | 032309cpsrecalcFeMn |
| 25SSL1 | 24 | 25SSL1-24 | 9.829 | 491.4 | -1.2 | 1.65 | -612 | 811 | 0.4 | 0.3 | 213 | 213 | 032309cpsrecalcFeMn |
| 25SSL1 | 36 | 25SSL1-36 | 9.809 | 490.5 | 0.1 | 1.65 | 35 | 809 | 0.4 | 0.3 | 192 | 192 | 032309cpsrecalcFeMn |
| 25SSL1 | 48 | 25SSL1-48 | 9.857 | 492.9 | -1.2 | 1.65 | -573 | 813 | 0.4 | 0.3 | 200 | 200 | 032309cpsrecalcFeMn |
| 25SSL1 | 60 | 25SSL1-60 | 9.853 | 492.6 | -5.9 | 1.0 | -2900 | 493 | 0.3 | 0.06 | 171 | 171 | 062008Aquant |
| 25SSL1 | 72 | 25SSL-1-72 | 9.499 | 475.0 | -6.1 | 0.7 | -2914 | 332 | 0.3 | 0.02 | 162 | 162 | 062008B25SC 60_72 |
| 25SSL1 | 96 | 25SSL1-96 | 9.815 | 490.8 | -0.1 | 1.7 | -61 | 834 | -0.1 | 0.10 | -61 | 49 | 071408quant |
| 25SSL1 | 120 | 25SSL1-120 | 9.820 | 491.0 | 3.5 | 1.4 | 1722 | 1722 | 0.7 | 0.1 | 359 | 359 | 062508recalc |
| 25SSL1 | 144 | 25SSL1-144 | 10.130 | 506.5 | 5.7 | 1.4 | 2871 | 2871 | 0.9 | 0.1 | 432 | 432 | 062508recalc |
| 25SSL1 | 168 | 25SSL1-168 | 9.819 | 491.0 | 7.8 | 1.4 | 3836 | 3836 | 2.2 | 0.1 | 1095 | 1095 | 062508recalc |
| 25SSL1 | 228 | 25SSL1-228 | 9.819 | 491.0 | -0.2 | 1.7 | -122 | 835 | 0.0 | 0.1 | 23 | 49 | 071408quant |
| 25SSL2 | 0 | 25SSL2-0 | 9.888 | 494.4 | -1.1 | 1.65 | -565 | 816 | 0.4 | 0.3 | 178 | 178 | 032309cpsrecalcFeMn |
| 25SSL2 | 12 | 25SSL2-12 | 9.837 | 491.9 | -1.2 | 1.65 | -598 | 812 | 0.4 | 0.3 | 183 | 183 | 032309cpsrecalcFeMn |
| 25SSL2 | 24 | 25SSL2-24 | 9.839 | 492.0 | -1.3 | 1.65 | -630 | 812 | 0.4 | 0.3 | 204 | 204 | 032309cpsrecalcFeMn |
| 25SSL2 | 36 | 25SSL2-36 | 9.848 | 492.4 | -1.2 | 1.65 | -583 | 812 | 0.2 | 0.3 | 123 | 148 | 032309cpsrecalcFeMn |
| 25SSL2 | 48 | 25SSL2-48 | 9.855 | 492.8 | -1.2 | 1.65 | -608 | 813 | 0.5 | 0.3 | 255 | 255 | 032309cpsrecalcFeMn |

D1-4.2. SC 25 LIVE IRON/MANGANESE, continued.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 25SSL2 | 60 | 25SSL2-60 | 9.835 | 491.8 | -1.9 | 1.0 | -920 | 492 | 0.3 | 0.06 | 169 | 169 | 062008Aquant |
| 25SSL2 | 72 | 25SSL-2-72 | 9.788 | 489.4 | -6.1 | 0.7 | -2995 | 343 | 0.3 | 0.02 | 130 | 130 | 062008B25SC 60_72 |
| 25SSL2 | 96 | 25SSL2-96 | 9.820 | 491.0 | -0.2 | 1.7 | -122 | 835 | -0.2 | 0.10 | -122 | 49 | 071408quant |
| 25SSL2 | 120 | 25SSL2-120 | 9.763 | 488.1 | 13.5 | 1.4 | 6582 | 6582 | 1.0 | 0.1 | 484 | 484 | 062508recalc |
| 25SSL2 | 144 | 25SSL2-144 | 9.636 | 481.8 | 6.3 | 1.4 | 3020 | 3020 | 0.8 | 0.1 | 387 | 387 | 062508recalc |
| 25SSL2 | 168 | 25SSL2-168 | 9.763 | 488.1 | 7.8 | 1.4 | 3829 | 3829 | 2.1 | 0.1 | 1038 | 1038 | 062508recalc |
| 25SSL2 | 228 | 25SSL2-228 | 9.838 | 491.9 | -0.3 | 1.7 | -157 | 836 | 0.0 | 0.1 | 13 | 49 | 071408quant |
| 25SSL3 | 0 | 25SSL3-0 | 9.894 | 494.7 | 0.2 | 1.65 | 99 | 816 | 0.4 | 0.3 | 219 | 219 | 032309cpsrecalcFeMn |
| 25SSL3 | 12 | 25SSL3-12 | 9.845 | 492.3 | 0.5 | 1.65 | 230 | 812 | 0.6 | 0.3 | 293 | 293 | 032309cpsrecalcFeMn |
| 25SSL3 | 24 | 25SSL3-24 | 9.831 | 491.6 | 0.3 | 1.65 | 129 | 811 | 0.6 | 0.3 | 284 | 284 | 032309cpsrecalcFeMn |
| 25SSL3 | 36 | 25SSL3-36 | 9.857 | 492.8 | 0.8 | 1.65 | 375 | 813 | 0.6 | 0.3 | 314 | 314 | 032309cpsrecalcFeMn |
| 25SSL3 | 48 | 25SSL3-48 | 9.854 | 492.7 | 3.1 | 1.65 | 1549 | 1549 | 0.3 | 0.3 | 168 | 168 | 032309cpsrecalcFeMn |
| 25SSL3 | 60 | 25SSL3-60 | 9.844 | 492.2 | 1.2 | 1.0 | 589 | 589 | 0.4 | 0.06 | 214 | 214 | 062008Aquant |
| 25SSL3 | 72 | 25SSL-3-72 | 9.780 | 489.0 | -4.8 | 0.7 | -2358 | 342 | 0.4 | 0.02 | 182 | 182 | 062008B25SC 60_72 |
| 25SSL3 | 96 | 25SSL3-96 | 9.811 | 490.6 | -0.1 | 1.7 | -56 | 834 | -0.1 | 0.10 | -56 | 49 | 071408quant |
| 25SSL3 | 120 | 25SSL3-120 | 9.830 | 491.5 | 4.1 | 1.4 | 2034 | 2034 | 0.8 | 0.1 | 376 | 376 | 062508recalc |
| 25SSL3 | 144 | 25SSL3-144 | 9.776 | 488.8 | 6.7 | 1.4 | 3285 | 3285 | 0.9 | 0.1 | 423 | 423 | 062508recalc |
| 25SSL3 | 168 | 25SSL3-168 | 9.763 | 488.1 | 7.9 | 1.4 | 3857 | 3857 | 2.1 | 0.1 | 1046 | 1046 | 062508recalc |
| 25SSL3 | 228 | 25SSL3-228 | 9.807 | 490.4 | 5.3 | 1.7 | 2587 | 2587 | 0.1 | 0.1 | 37 | 49 | 071408quant |

D1-4.3. SC 10 KILLED IRON/MANGANESE.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Experiment | Time | Name | Weight | Dilution | Fe, Measured | d.l. | Fe, µg/L | Fe µg/L, Reported | Mn, Measured | d.l. | Mn, µg/L | Mn, µg/L, Reported | Fe and Mn Source |
| 10SCK1 | 0 | 10SCK1-0 | 9.852 | 492.6 | | | | | | | | | |
| 10SCK1 | 48 | 10SCK1-48 | 9.8278 | 491.39 | | | | | | | | | |
| 10SCK1 | 96 | 10SCK1-96 | 9.8638 | 493.19 | | | | | | | | | |
| 10SCK1 | 144 | 10SCK1-144 | 9.835 | 491.75 | 6.63 | 1.37 | 3262 | 3262 | 0.12 | 0.09 | 61.0 | 61 | 062508recalc |
| 10SCK2 | 0 | 10SCK2-0 | 8.721 | 436.05 | -1.11 | 2.41 | -484 | 1050 | 0.05 | 0.06 | 22.8 | 26 | 0718quant |
| 10SCK2 | 48 | 10SCK2-48 | 8.27 | 413.5 | -1.19 | 2.41 | -493 | 995 | 0.05 | 0.06 | 18.7 | 25 | 0718quant |
| 10SCK2 | 96 | 10SCK2-96 | 8.145 | 407.25 | -1.31 | 2.41 | -534 | 980 | 0.06 | 0.06 | 25.1 | 25 | 0718quant |
| 10SCK2 | 144 | 10SCK2-144 | 9.8342 | 491.71 | 7.20 | 1.37 | 3540 | 3540 | 0.31 | 0.09 | 150.7 | 151 | 062508recalc |
| 10SCK3 | 0 | 10SCK3-0 | 8.58 | 429 | -1.34 | 2.41 | -573 | 1033 | 0.06 | 0.06 | 26.4 | 26 | 0718quant |
| 10SCK3 | 48 | 10SCK3-48 | 8.62 | 431 | -1.39 | 2.41 | -597 | 1037 | 0.06 | 0.06 | 26.7 | 27 | 0718quant |
| 10SCK3 | 96 | 10SCK3-96 | 7.441 | 372.05 | -1.30 | 2.41 | -485 | 896 | 0.06 | 0.06 | 20.7 | 22 | 0718quant |
| 10SCK3 | 144 | 10SCK3-144 | 9.8343 | 491.715 | 6.14 | 1.37 | 3020 | 3020 | 0.08 | 0.09 | 37.2 | 44 | 062508recalc |
| 10SRK1 | 0 | 10SRK1-0 | 9.8471 | 492.355 | | | | | | | | | |
| 10SRK1 | 48 | 10SRK1-48 | 9.8667 | 493.335 | | | | | | | | | |
| 10SRK1 | 96 | 10SRK1-96 | 9.8649 | 493.245 | | | | | | | | | |
| 10SRK1 | 144 | 10SRK1-144 | 9.7213 | 486.065 | 7.42 | 1.37 | 3604 | 3604 | 0.34 | 0.09 | 166.3 | 166 | 062508recalc |
| 10SRK2 | 0 | 10SRK2-0 | 8.413 | 420.65 | -1.21 | 2.41 | -507 | 1013 | 0.11 | 0.06 | 45.1 | 45 | 0718quant |
| 10SRK2 | 48 | 10SRK2-48 | 8.046 | 402.3 | -1.35 | 2.41 | -543 | 968 | 0.11 | 0.06 | 45.5 | 46 | 0718quant |
| 10SRK2 | 96 | 10SRK2-96 | 7.989 | 399.45 | -0.99 | 2.41 | -396 | 961 | 0.11 | 0.06 | 44.9 | 45 | 0718quant |
| 10SRK2 | 144 | 10SRK2-144 | 9.8498 | 492.49 | 6.58 | 1.37 | 3241 | 3241 | 0.18 | 0.09 | 87.9 | 88 | 062508recalc |
| 10SRK3 | 0 | 10SRK3-0 | 8.751 | 437.55 | -1.31 | 2.41 | -574 | 1053 | 0.12 | 0.06 | 50.7 | 51 | 0718quant |
| 10SRK3 | 48 | 10SRK3-48 | 7.513 | 375.65 | -1.38 | 2.41 | -518 | 904 | 0.13 | 0.06 | 48.0 | 48 | 0718quant |
| 10SRK3 | 96 | 10SRK3-96 | 8.034 | 401.7 | -1.41 | 2.41 | -567 | 967 | 0.12 | 0.06 | 46.8 | 47 | 0718quant |
| 10SRK3 | 144 | 10SRK3-144 | 9.832 | 491.6 | 6.35 | 1.37 | 3120 | 3120 | 0.22 | 0.09 | 107.3 | 107 | 062508recalc |
| 10SSK1 | 0 | 10SSK1-0 | 9.8576 | 492.88 | | | | | | | | | |
| 10SSK1 | 48 | 10SSK1-48 | 9.8451 | 492.255 | | | | | | | | | |
| 10SSK1 | 96 | 10SSK1-96 | 9.851 | 492.55 | | | | | | | | | |
| 10SSK1 | 144 | 10SSK1-144 | 9.8572 | 492.86 | 6.53 | 1.37 | 3218 | 3218 | 0.25 | 0.09 | 121.0 | 121 | 062508recalc |
| 10SSK2 | 0 | 10SSK2-0 | 8.334 | 416.7 | -1.20 | 2.41 | -498 | 1003 | 0.12 | 0.06 | 51.8 | 52 | 0718quant |

D1-4.3. SC 10 KILLED IRON/MANGANESE, continued.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 10SSK2 | 48 | 10SSK2-48 | 8.664 | 433.2 | -1.25 | 2. 41 | -540 | 1043 | 0.13 | 0.06 | 58.2 | 58 | 0718quant |
| 10SSK2 | 96 | 10SSK2-96 | 7.899 | 394.95 | -0.78 | 2.41 | -310 | 951 | 0.17 | 0.06 | 65.2 | 65 | 0718quant |
| 10SSK2 | 144 | 10SSK2-144 | 9.845 | 492.25 | 10.00 | 1.37 | 4922 | 4922 | 0.44 | 0.09 | 218.0 | 218 | 062508recalc |
| 10SSK3 | 0 | 10SSK3-0 | 8.62 | 431 | -0.79 | 2.41 | -340 | 1037 | 0.15 | 0.06 | 65.5 | 66 | 0718quant |
| 10SSK3 | 48 | 10SSK3-48 | 9.054 | 452.7 | -0.88 | 2.41 | -400 | 1090 | 0.17 | 0.06 | 77.0 | 77 | 0718quant |
| 10SSK3 | 96 | 10SSK3-96 | 7.876 | 393.8 | -0.86 | 2.41 | -340 | 948 | 0.17 | 0.06 | 66.2 | 66 | 0718quant |
| 10SSK3 | 144 | 10SSK3-144 | 9.8478 | 492.39 | 6.36 | 1.37 | 3133 | 3133 | 0.37 | 0.09 | 181.0 | 181 | 062508recalc |

Shaded fields = data not collected for those samples.

D1-4.4. SC 25 KILLED IRON/MANGANESE.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 25SCK1 | 0 | 25SCK1-0 | 9.448 | 472.4 | -0.750 | 0.57 | 269.3 | 269 | 0.074 | 0.17 | 80.31 | 80 | 0721quant |
| 25SCK1 | 48 | 25SCK1-48 | 8.272 | 413.6 | -0.726 | 0.57 | 235.8 | n.d. | 0.070 | 0.17 | 70.31 | n.d. | 0721quant |
| 25SCK1 | 96 | 25SCK1-96 | 7.916 | 395.8 | -0.775 | 0.57 | 225.6 | n.d. | 0.071 | 0.17 | 67.29 | n.d. | 0721quant |
| 25SCK1 | 144 | 25SCK1-144 | 9.836 | 491.8 | 8.3 | 1.37 | 4083 | 4083 | 0.098 | 0.09 | 48.25 | 48.25 | 062508recalc |
| 25SCK1 | 276 | 25SCK1-276 | 9.912 | 495.6 | -0.640 | 0.57 | 282.5 | n.d. | 0.108 | 0.17 | 84.25 | n.d. | 0721quant |
| 25SCK2 | 0 | 25SCK2-0 | 9.817 | 490.9 | -0.818 | 2.4 | 1178.0 | n.d. | 0.129 | 0.06 | 63.22 | 63.22 | 0718quant |
| 25SCK2 | 48 | 25SCK2-48 | 9.156 | 457.8 | -0.694 | 2.4 | 1098.7 | n.d. | 0.136 | 0.06 | 62.31 | 62.31 | 0718quant |
| 25SCK2 | 96 | 25SCK2-96 | 8.473 | 423.7 | -0.835 | 2.4 | 1016.8 | n.d. | 0.144 | 0.06 | 60.88 | 60.88 | 0718quant |
| 25SCK2 | 144 | 25SCK2-144 | 9.839 | 491.9 | 6.6 | 1.37 | 3256 | 3256 | 0.081 | 0.09 | 44.27 | n.d. | 062508recalc |
| 25SCK2 | 276 | 25SCK2-276 | 10.400 | 520.0 | -0.765 | 0.57 | 296.4 | n.d. | 0.115 | 0.17 | 88.40 | n.d. | 0721quant |
| 25SCK3 | 0 | 25SCK3-0 | 9.274 | 463.7 | -0.861 | 2.4 | 1112.9 | n.d. | 0.126 | 0.06 | 58.29 | 58.29 | 0718quant |
| 25SCK3 | 48 | 25SCK3-48 | 9.272 | 463.6 | -0.799 | 2.4 | 1112.6 | n.d. | 0.126 | 0.06 | 58.60 | 58.60 | 0718quant |
| 25SCK3 | 96 | 25SCK3-96 | 7.268 | 363.4 | -0.909 | 2.4 | 872.2 | n.d. | 0.131 | 0.06 | 47.42 | 47.42 | 0718quant |
| 25SCK3 | 144 | 25SCK3-144 | 9.849 | 492.4 | 6.0 | 1.37 | 2954 | 2954 | 0.136 | 0.09 | 67.11 | 67.11 | 062508recalc |
| 25SCK3 | 276 | 25SCK3-276 | 9.347 | 467.4 | 0.194 | 0.57 | 266.4 | n.d. | 0.310 | 0.17 | 145.02 | 145.02 | 0721quant |
| 25SRK1 | 0 | 25SRK1-0 | 8.951 | 447.6 | -0.720 | 0.57 | 255.1 | n.d. | 0.069 | 0.17 | 76.08 | n.d. | 0721quant |
| 25SRK1 | 48 | 25SRK1-48 | 8.053 | 402.7 | -0.167 | 0.57 | 229.5 | n.d. | 0.103 | 0.17 | 68.45 | n.d. | 0721quant |
| 25SRK1 | 96 | 25SRK1-96 | 8.596 | 429.8 | -0.790 | 0.57 | 245.0 | n.d. | 0.091 | 0.17 | 73.07 | n.d. | 0721quant |
| 25SRK1 | 144 | 25SRK1-144 | 9.836 | 491.8 | 3.5 | 1.37 | 1729 | 1729 | -0.031 | 0.09 | 44.26 | n.d. | 062508recalc |
| 25SRK1 | 276 | 25SRK1-276 | 9.525 | 476.3 | -0.686 | 0.57 | 271.5 | n.d. | 0.129 | 0.17 | 80.96 | n.d. | 0721quant |
| 25SRK2 | 0 | 25SRK2-0 | 8.668 | 433.4 | -0.747 | 2.4 | 1040.2 | n.d. | 0.166 | 0.06 | 72.12 | 72.12 | 0718quant |
| 25SRK2 | 48 | 25SRK2-48 | 7.938 | 396.9 | -0.843 | 2.4 | 952.6 | n.d. | 0.167 | 0.06 | 66.16 | 66.16 | 0718quant |
| 25SRK2 | 96 | 25SRK2-96 | 8.626 | 431.3 | -0.771 | 2.4 | 1035.1 | n.d. | 0.182 | 0.06 | 78.45 | 78.45 | 0718quant |
| 25SRK2 | 144 | 25SRK2-144 | 9.846 | 492.3 | 7.0 | 1.37 | 3445 | 3445 | 0.236 | 0.09 | 116.25 | 116.25 | 062508recalc |
| 25SRK2 | 276 | 25SRK2-276 | 9.425 | 471.3 | 0.756 | 0.57 | 356.4 | 0.756 | 0.260 | 0.17 | 122.34 | 122.34 | 0721quant |
| 25SRK3 | 0 | 25SRK3-0 | 9.128 | 456.4 | -0.857 | 2.4 | 1095.4 | n.d. | 0.182 | 0.06 | 82.88 | 82.88 | 0718quant |
| 25SRK3 | 48 | 25SRK3-48 | 8.431 | 421.6 | -0.888 | 2.4 | 1011.7 | n.d. | 0.193 | 0.06 | 81.36 | 81.36 | 0718quant |
| 25SRK3 | 96 | 25SRK3-96 | 8.919 | 446.0 | -0.840 | 2.4 | 1070.3 | n.d. | 0.198 | 0.06 | 88.25 | 88.25 | 0718quant |
| 25SRK3 | 144 | 25SRK3-144 | 9.859 | 492.9 | 27.0 | 1.37 | 13293 | 13293 | 0.467 | 0.09 | 229.99 | 229.99 | 062508recalc |

D1-4.4. SC 25 KILLED IRON/MANGANESE, continued.

| Sample Information | | | | | IRON | | | | MANGANESE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Experiment** | **Time** | **Name** | **Weight** | **Dilution** | **Fe, Measured** | **d.l.** | **Fe, µg/L** | **Fe µg/L, Reported** | **Mn, Measured** | **d.l.** | **Mn, µg/L** | **Mn, µg/L, Reported** | **Fe and Mn Source** |
| 25SRK3 | 276 | 25SRK3-276 | 9.859 | 493.0 | 0.066 | 0.57 | 281.0 | n.d. | 0.231 | 0.17 | 113.97 | 113.97 | 0721quant |
| 25SSK1 | 0 | 25SSK1-0 | 7.853 | 392.7 | -0.764 | 0.57 | 223.8 | n.d. | 0.117 | 0.17 | 66.75 | n.d. | 0721quant |
| 25SSK1 | 48 | 25SSK1-48 | 7.819 | 391.0 | -0.815 | 0.57 | 222.8 | n.d. | 0.125 | 0.17 | 66.46 | n.d. | 0721quant |
| 25SSK1 | 96 | 25SSK1-96 | 7.814 | 390.7 | -0.610 | 0.57 | 222.7 | n.d. | 0.156 | 0.17 | 66.42 | n.d. | 0721quant |
| 25SSK1 | 144 | 25SSK1-144 | 9.778 | 488.9 | 7.2 | 1.37 | 3519 | 3519 | 0.372 | 0.09 | 181.78 | 181.78 | 062508recalc |
| 25SSK1 | 276 | 25SSK1-276 | 9.559 | 478.0 | -0.724 | 0.57 | 272.4 | n.d. | 0.221 | 0.17 | 105.53 | 105.53 | 0721quant |
| 25SSK2 | 0 | 25SSK2-0 | 7.325 | 366.3 | -0.814 | 2.4 | 879.0 | n.d. | 0.174 | 0.06 | 63.65 | 63.65 | 0718quant |
| 25SSK2 | 48 | 25SSK2-48 | 7.818 | 390.9 | -0.880 | 2.4 | 938.2 | n.d. | 0.206 | 0.06 | 80.33 | 80.33 | 0718quant |
| 25SSK2 | 96 | 25SSK2-96 | 8.384 | 419.2 | -0.892 | 2.4 | 1006.1 | n.d. | 0.230 | 0.06 | 96.25 | 96.25 | 0718quant |
| 25SSK2 | 144 | 25SSK2-144 | 9.842 | 492.1 | 3.8 | 1.37 | 1864 | 1864 | 0.153 | 0.09 | 75.53 | 75.53 | 062508recalc |
| 25SSK2 | 276 | 25SSK2-276 | 9.453 | 472.7 | -0.708 | 0.57 | 269.4 | n.d. | 0.208 | 0.17 | 98.22 | 98.22 | 0721quant |
| 25SSK3 | 0 | 25SSK3-0 | 7.675 | 383.8 | -0.583 | 2.4 | 921.0 | n.d. | 0.168 | 0.06 | 64.55 | 64.55 | 0718quant |
| 25SSK3 | 48 | 25SSK3-48 | 7.987 | 399.4 | -0.788 | 2.4 | 958.4 | n.d. | 0.186 | 0.06 | 74.20 | 74.20 | 0718quant |
| 25SSK3 | 96 | 25SSK3-96 | 9.226 | 461.3 | -0.858 | 2.4 | 1107.1 | n.d. | 0.202 | 0.06 | 93.18 | 93.18 | 0718quant |
| 25SSK3 | 144 | 25SSK3-144 | 9.800 | 490.0 | 7.3 | 1.37 | 3577 | 3577 | 1.707 | 0.09 | 836.29 | 836.29 | 062508recalc |
| 25SSK3 | 276 | 25SSK3-276 | 10.070 | 503.5 | -0.716 | 0.57 | 287.0 | n.d. | 0.224 | 0.17 | 112.99 | 112.99 | 0721quant |

n.d. = no data

Table D2-1. Dry Valley Mine Ion Chromotography Data.

| Experiment | Replicate | Time | pH | $O_2$ | $PO_4^{+}$ | $SO_4^{2-}$ | $SeO_4^{2-}$ | $SeO_3^{2-}$ | $NO_3^{-}$ | $O_2$ Ave | $NO_3^{-}$ Ave | $NO_3^{-}$ s.d. | Sample ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10DCL | 1 | 0 | 7.47 | 0.7 | nd | 343 | 13.6 | 3.0 | 4.7 | | | | |
| 10DCL | 2 | 0 | 7.33 | 0.3 | nd | 310 | 13.7 | nd | 3.0 | | | | |
| 10DCL | 3 | 0 | 7.36 | 0.22 | nd | 301 | 14.0 | nd | 4.9 | 0.41 | 4.2 | 1.0 | 10 DCL-1-0 |
| 10DCL | 1 | 53 | 7.32 | 0.18 | nd | 339 | 11.0 | nd | 2.4 | 0.18 | 2.4 | na | 10 DCL-1-53 |
| 10DCL | 1 | 80 | nr | 0.11 | nd | nr | nr | nr | nr | | | | |
| 10DCL | 2 | 80 | nr | 0.2 | nd | nr | nr | nr | nr | | | | |
| 10DCL | 3 | 80 | nr | 0.99* | nd | 340 | 5.5 | nd | 2.0 | 0.20 | 2.0 | na | 10 DCL-1-80 |
| 10DCL | 1 | 104 | nr | 0.1 | nd | 311 | 3.7 | 2.9 | 2.0 | | | | |
| 10DCL | 1 | 128 | 7.30 | 0.09 | nd | 320 | 5.5 | nd | 2.0 | | | | |
| 10DCL | 2 | 128 | 7.40 | 0.04 | nd | 345 | nd | nd | 2.3 | | | | |
| 10DCL | 3 | 128 | 7.38 | 0.21 | nd | 301 | 3.2 | nd | 2.0 | 0.11 | 2.1 | 0.2 | 10DCL-128 |
| 10DCL | 1 | 272 | nr | nr | *5.9* | 318 | nd | nd | 2.3 | | | | |
| 10DCL | 2 | 272 | nr | nr | nd | 345 | nd | nd | 2.0 | | | | |
| 10DCL | 3 | 272 | nr | nr | nd | 320 | nd | nd | 2.0 | | 2.1 | 0.2 | 10DCL-272 |
| | | | | | | | | | | | | | |
| 10DRL | 1 | 0 | 6.85 | 0.31 | nd | 1512 | 14.2 | nd | 4.4 | | | | |
| 10DRL | 2 | 0 | 6.81 | 0.26 | nd | 1348 | 12.2 | nd | 4.1 | | | | |
| 10DRL | 3 | 0 | 6.86 | 0.25 | nd | 1130 | 13.7 | nd | 4.2 | 0.27 | 4.2 | 0.2 | 10DRL-0 |
| 10DRL | 1 | 53 | 6.82 | 0.15 | nd | 1391 | 16.4 | nd | 2.5 | | | | |
| 10DRL | 1 | 80 | nr | 0.13 | nd | 1338 | 11.9 | nd | 2.0 | | | | |
| 10DRL | 2 | 80 | nr | 0.12 | nd | 1416 | 7.0 | 2.9 | 2.0 | 0.13 | 2.0 | 0.3 | 10DRL--80 |
| 10DRL | 3 | 80 | nr | 0.09 | nd | 1310 | nr | nr | nr | | | | |
| 10DRL | 1 | 104 | nr | 0.1 | nd | 1338 | 11.9 | nd | 2.0 | | | | |
| 10DRL | 1 | 128 | 6.86 | 0.05 | nd | 1544 | 7.0 | 2.8 | 2.0 | | | | |
| 10DRL | 2 | 128 | 6.92 | 0.07 | nd | 1130 | nd | nd | 2.0 | | | | |
| 10DRL | 3 | 128 | 6.64 | 0.05 | nd | 1416 | nd | nd | 2.3 | 0.06 | 2.1 | 0.2 | 10DRL-128 |
| 10DRL | 1 | 272 | nr | nr | nd | 1230 | 14.2 | nd | 4.4 | | | | |
| 10DRL | 2 | 272 | nr | nr | nd | 1272 | nd | nd | 2.0 | | | | |
| 10DRL | 3 | 272 | nr | nr | nd | 1131 | nd | nd | 2.3 | | 2.9 | 1.3 | 10DRL-272 |
| | | | | | | | | | | | | | |
| 10DSL | 1 | 0 | 6.75 | 0.32 | *5.5* | 1321 | 13.5 | nd | 3.1 | | | | |
| 10DSL | 2 | 0 | 6.66 | 0.16 | nd | 1169 | 11.5 | nd | 2.7 | | | | |

Table D2-1. Dry Valley Mine Ion Chromotography Data, continued.

| Experiment | Replicate | Time | pH | $O_2$ | $PO_4^{+}$ | $SO_4^{2-}$ | $SeO_4^{2-}$ | $SeO_3^{2-}$ | $NO_3^{-}$ | $O_2$ Ave | $NO_3^{-}$ Ave | $NO_3^{-}$ s.d. | Sample ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10DSL | 3 | 0 | 6.72 | 0.21 | nd | 1131 | 14.8 | nd | 2.9 | 0.23 | 2.9 | 0.2 | 10DSL-0 |
| 10DSL | 1 | 53 | 6.7 | 0.1 | *5.4* | 1255 | 14.9 | nd | 2.5 | 0.10 | 2.48 | na | 10DSL-53 |
| 10DSL | 1 | 80 | nr | 0.08 | nd | 1264 | 5.2 | nd | 2.0 | | | | |
| 10DSL | 2 | 80 | nr | 0.11 | *5.5* | 1205 | 6.2 | nd | 2.0 | | | | |
| 10DSL | 3 | 80 | nr | 0.3 | nd | 1228 | 5.8 | 2.9 | 2.0 | 0.16 | 2.0 | 0.0 | 10DSL-80 |
| 10DSL | 1 | 104 | nr | 0.1 | *5.4* | 1234 | 3.0 | nd | 2.4 | | | | |
| 10DSL | 1 | 128 | 6.76 | 0.07 | *5.5* | 1536 | nd | 2.9 | 2.0 | | | | |
| 10DSL | 2 | 128 | 6.76 | 0.84 | nd | 1542 | nd | nd | 2.0 | | | | |
| 10DSL | 3 | 128 | 6.82 | 0.06 | nd | 1205 | nd | nd | 2.0 | 0.32 | 2.0 | 0.0 | 10DSL-128 |
| 10DSL | 1 | 272 | nr | nr | nd | 1536 | nd | nd | 2.0 | | | | |
| 10DSL | 2 | 272 | nr | nr | nd | 1367 | nd | nd | 2.0 | | | | |
| 10DSL | 3 | 272 | nr | nr | *5.4* | 1036 | nd | 3.1 | 2.0 | 0.06 | 2.0 | 0.0 | 10DSL-272 |
| | | | | | | | | | | | | | |
| 25DCL | 1 | 0 | 7.45 | 0.18 | *5.7* | 300 | 14.4 | nd | 7.1 | | | | |
| 25DCL | 2 | 0 | 7.38 | 0.16 | nd | 323 | 5.3 | nd | 2.0 | | | | |
| 25DCL | 3 | 0 | 7.4 | 0.09 | nd | 392 | 8.2 | nd | 2.4 | 0.14 | 3.8 | 2.8 | 25DCL-0 |
| 25DCL | 1 | 20 | 7.39 | 0.11 | nr | 285 | 14.1 | nr | 2.6 | | | | |
| 25DCL | 2 | 20 | 7.35 | 0.12 | nr | 269 | nr | nr | 2.0 | 0.12 | 2.3 | 0.5 | 25DCL-20 |
| 25DCL | 1 | 54 | nr | nr | nd | 323 | 5.3 | nd | 2.0 | | | | |
| 25DCL | 1 | 66 | 7.35 | 0.1 | *6.2* | 392 | nd | nd | 2.5 | | | | |
| 25DCL | 2 | 66 | 7.49 | 0.1 | nd | 403 | nd | nd | 2.0 | | | | |
| 25DCL | 3 | 66 | 7.52 | 0.09 | nd | 305 | nd | nd | 2.0 | 0.10 | 2.2 | 0.3 | 25DCL-66 |
| 25DCL | 1 | 90 | nr | nr | nd | 301 | nd | nd | 2.0 | | | | |
| 25DCL | 2 | 90 | nr | nr | nd | 403 | nd | nd | 2.0 | | 2.0 | 0.0 | 25DCL-90 |
| 25DCL | 1 | 188 | nr | nr | nd | 299 | nd | nd | 2.5 | | | | |
| 25DCL | 2 | 188 | nr | nr | nd | 403 | nd | nd | 2.0 | | 2.2 | 0.3 | 25DCL-188 |
| | | | | | | | | | | | | | |
| 25DRL | 1 | 0 | 6.88 | 0.22 | nd | 991 | 15.9 | nd | 5.5 | | | | |
| 25DRL | 2 | 0 | 6.74 | 0.1 | nd | 1037 | 5.8 | 3.4 | 2.0 | 0.16 | 3.8 | 2.5 | 25DRL-0 |
| 25DRL | 1 | 20 | 6.82 | 0.07 | nd | 1108 | 14.7 | nd | 3.2 | | | | |
| 25DRL | 2 | 20 | 6.8 | 0.09 | nd | 1056 | 1.7 | nd | 2.0 | 0.08 | 2.6 | 0.9 | 25DRL-20 |
| 25DRL | 1 | 54 | 6.97 | 0.18 | nd | 1029 | 6.0 | 3.4 | 2.0 | 0.18 | 2 | na | 25DRL-54 |
| 25DRL | 1 | 66 | 7 | 0.07 | nd | 908 | nd | nd | 2.0 | | | | |

Table D2-1. Dry Valley Mine Ion Chromotography Data, continued.

| Experiment | Replicate | Time | pH | $O_2$ | $PO_4^{+}$ | $SO_4^{2-}$ | $SeO_4^{2-}$ | $SeO_3^{2-}$ | $NO_3^{-}$ | $O_2$ Ave | $NO_3^{-}$ Ave | $NO_3^{-}$ s.d. | Sample ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25DRL | 2 | 66 | 6.9 | 0.08 | nd | 893 | nd | nd | 2.5 | | | | |
| 25DRL | 3 | 66 | 6.95 | 0.08 | nd | 1222 | nd | nd | 2.0 | 0.08 | 2.2 | 0.3 | 25DRL-66 |
| 25DRL | 1 | 90 | 6.95 | 0.1 | nd | 908 | nd | nd | 2.0 | 0.10 | 2 | 0 | 25DRL-90 |
| 25DRL | 1 | 188 | 6.97 | 0.09 | nd | 1528 | nd | nd | 2.0 | | | | |
| 25DRL | 2 | 188 | 6.94 | 0.1 | nd | 897 | nd | nd | 2.0 | 0.10 | 2.0 | 0.0 | 25DRL-188 |
| | | | | | | | | | | | | | |
| 25DSL | 1 | 0 | 6.75 | 0.17 | nd | 1128 | 17.5 | nd | 3.4 | | | | |
| 25DSL | 2 | 0 | 6.91 | 0.14 | nd | 1216 | 14.4 | nd | 7.1 | | | | |
| 25DSL | 3 | 0 | 6.87 | 0.12 | nd | 1039 | 15.1 | nd | 5.8 | 0.14 | 5.4 | 1.8 | 25DSL-0 |
| 25DSL | 1 | 20 | 6.77 | 0.011 | nd | 991 | 15.0 | nd | 2.6 | | | | |
| 25DSL | 2 | 20 | 6.83 | 0.013 | nd | 1152 | 13.1 | nd | 2.0 | 0.01 | 2.3 | 0.4 | 25DSL-20 |
| 25DSL | 1 | 66 | nr | nr | nd | 1038 | 5.8 | 3.4 | 2.0 | | | | |
| 25DSL | 2 | 66 | 6.75 | 0.09 | nd | 1126 | 4.6 | nd | 2.0 | | | | |
| 25DSL | 3 | 66 | 6.84 | 0.07 | nd | 1039 | 4.9 | nd | 3.4 | 0.08 | 2.5 | 0.8 | 25DSL-66 |
| 25DSL | 1 | 90 | 6.81 | 0.08 | nd | 1113 | nd | nd | 2.0 | 0.08 | 2 | na | 25DSL-90 |
| 25DSL | 1 | 120 | nr | nr | nd | 726 | nd | nd | 2.0 | | | | |
| 25DSL | 2 | 120 | nr | nr | nd | nr | nr | nr | nr | | | | |
| 25DSL | 1 | 188 | nr | nr | nr | 1713 | nd | nd | 2.4 | | | | |
| 25DSL | 2 | 188 | nr | nr | nd | 1222 | nd | nd | 2.0 | | 2.2 | 0.3 | 25DSL-188 |
| | | | | | | | | | | | | | |
| D10CK | 1 | 0 | 7.05 | 0.57 | nd | 328 | 18.0 | nd | 7.7 | | | | |
| D10CK | 2 | 0 | 7.39 | 0.69 | nr | nr | nr | nr | nr | | | | |
| D10CK | 3 | 0 | 7.31 | 0.62 | nr | nr | nr | nr | nr | | | | |
| D10CK | 1 | 456 | 7.55 | 0.36 | nd | 341 | 18.0 | nd | 8.1 | | | | |
| D10CK | 2 | 456 | 7.57 | 0.47 | nr | nr | nr | nr | nr | | | | |
| D10CK | 3 | 456 | 6.94 | 0.07 | nr | nr | nr | nr | nr | | | | |
| D10RK | 1 | 0 | 6.72 | 0.35 | nd | 924 | 17.8 | nd | nr | | | | |
| D10RK | 2 | 0 | 6.93 | 0.39 | nr | nr | nr | nr | nr | | | | |
| D10RK | 3 | 0 | nr | nr | nr | nr | nr | nr | nr | | | | |
| D10RK | 1 | 456 | 6.94 | 0.37 | nd | 1349 | 18.2 | nd | 3.7 | | | | |
| D10RK | 2 | 456 | 7.12 | 0.59 | nr | nr | nr | nr | nr | | | | |
| D10SK | 1 | 0 | 6.74 | 0.45 | nd | 1238 | 18.1 | nd | 7.4 | | | | |
| D10SK | 2 | 0 | 6.64 | 0.44 | nr | nr | nr | nr | nr | | | | |

Table D2-1. Dry Valley Mine Ion Chromotography Data, continued.

| Experiment | Replicate | Time | pH | $O_2$ | $PO_4^{+}$ | $SO_4^{2-}$ | $SeO_4^{2-}$ | $SeO_3^{2-}$ | $NO_3^{-}$ | $O_2$ Ave | $NO_3^{-}$ Ave | $NO_3^{-}$ s.d. | Sample ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| D10SK | 3 | 0 | 6.64 | 0.29 | nr | nr | nr | nr | nr | | | | |
| D10SK | 1 | 456 | 6.9 | 0.59 | nr | nr | nr | nr | nr | | | | |
| D10SK | 2 | 456 | 6.82 | 0.91 | nr | nr | nr | nr | nr | | | | |
| D10SK | 3 | 456 | 6.86 | 0.43 | nr | nr | nr | nr | nr | | | | |
| | | | | | | | | | | | | | |
| DV25-CK1 | 1 | 0 | nr | nr | nd | 331 | 18.2 | nd | 11.6 | | | | |
| DV25-CK2 | 2 | 0 | 7.39 | 0.58 | nr | nr | nr | nr | nr | | | | |
| DV25-CK1 | 1 | 192 | 7.25 | 0.5 | nr | nr | nr | nr | nr | | | | |
| DV25-CK2 | 2 | 192 | 7.5 | 0.06 | nr | nr | nr | nr | nr | | | | |
| DV25-CK3 | 3 | 192 | 7.51 | 0.08 | nr | nr | nr | nr | nr | | | | |
| DV25-CK3 | 3 | 192 | 6.94 | 0.07 | nr | nr | nr | nr | nr | | | | |
| DV25-RK1 | 1 | 0 | nr | nr | nd | 1289 | 18.5 | nd | 8.3 | | | | |
| DV25-RK1 | 1 | 192 | 6.96 | 0.29 | nr | nr | nr | nr | nr | | | | |
| DV25-RK2 | 2 | 192 | 6.97 | 0.1 | nr | nr | nr | nr | nr | | | | |
| DV25-RK3 | 3 | 192 | 6.92 | 0.35 | nr | nr | nr | nr | nr | | | | |
| DV25-SK1 | 1 | 0 | 6.78 | 0.08 | nd | 1349 | 18.2 | nd | 3.7 | | | | |
| DV25-SK1 | 1 | 192 | 6.7 | 0.15 | nr | nr | nr | nr | nr | | | | |
| DV25-RK2 | 2 | 192 | 6.76 | 0.45* | *5.55* | 2 | nd | nd | 2.3 | | | | |
| DV25-RK3 | 3 | 192 | 6.96 | 0.35 | *5.33* | nd | nd | nd | nd | | | | |

Values are micrograms/liter (µg/L)

*= degassed

$NO_3^{-}$ detection limit= 2 µg/L

nr= not reported

nd= not detected

na= not applicable

+ $PO_4^{+}$ was detected only immediately following analysis of a standard containing $PO_4^{+}$

Table D2-2. Smoky Canyon Mine Ion Chromotography Data.

| Experiment | Replicate | Time | pH | $O_2$ | $PO_4^{+}$ | $SO_4^{2-}$ | $SeO_4^{2-}$ | $SeO_3^{2-}$ | $NO_3^{-}$ | $O_2$ Ave | $NO_3^{-}$ Ave | $NO_3^{-}$ s.d. | Sample ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10SCL | 1 | 0 | nr | 0.76 | nd | 419.3 | 13.2 | nd | 9.7 | | | | |
| 10SCL | 2 | 0 | nr | 0.71 | nd | 436.2 | 15.1 | 5.4 | 8.6 | 0.74 | 9.150 | 0.778 | 10SCCL0 |
| 10SCL | 1 | 12 | nr | 0.26 | nd | 511.9 | 16.1 | 2 | 5.1 | | | | |
| 10SCL | 2 | 12 | nr | 0.2 | nd | 528.7 | 17.6 | nd | 4.83 | 0.23 | 4.965 | 0.191 | 10SCCL12 |
| 10SCL | 1 | 24 | nr | 0.64 | nr | nr | nr | nr | nr | | | | |
| 10SCL | 1 | 36 | nr | 0.23 | nr | nr | nr | nr | nr | | | | |
| 10SCL | 1 | 48 | nr | 0.36 | nr | nr | nr | nr | nr | | | | |
| 10SCL | 1 | 60 | nr | 0.37 | nd | 467.8 | 11.1 | nd | 10.37 | | | | |
| 10SCL | 2 | 60 | nr | 0.35 | nd | 473.9 | 12.5 | nd | 12.41 | 0.36 | 11.390 | 1.442 | 10SCCL60 |
| 10SCL | 1 | 84 | nr | 0.32 | nd | 446.4 | 4.7 | nd | 6.89 | | | | |
| 10SCL | 2 | 84 | nr | 0.29 | nd | 420.0 | 3.8 | nd | 2 | 0.31 | 4.445 | 3.458 | 10SCCL84 |
| 10SCL | 1 | 108 | nr | 0.2 | nr | nr | nr | nr | nr | | | | |
| 10SCL | 1 | 120 | 7.21 | 0.61 | nr | nr | nr | nr | nr | | | | |
| 10SCL | 2 | 120 | 7.98 | 0.61 | nr | nr | nr | nr | nr | | | | |
| 10SCL | 3 | 120 | 7.68 | 0.76 | nr | nr | nr | nr | nr | | | | |
| 10SCL | 1 | 132 | nr | 0.23 | nr | nr | nr | nr | nr | | | | |
| 10SCL | 1 | 246 | nr | 0.11 | nd | 655.0 | nd | nd | 2 | | | | |
| 10SCL | 2 | 246 | nr | 0.09 | nd | 649.1 | nd | nd | 2 | 0.10 | 2.000 | 0.000 | 10SCCL246 |
| | | | | | | | | | | | | | |
| 10SRL | 1 | 0 | nr | 0.49 | nd | 612.1 | 11.1 | nd | 11.36 | | | | |
| 10SRL | 2 | 0 | nr | 0.64 | nd | 605.1 | 10.5 | nd | 9.2 | | | | |
| 10SRL | 3 | 0 | nr | 0.61 | nd | 744.0 | 6.2 | nd | 12.92 | 0.58 | 11.160 | 1.868 | 10SCRL0 |
| 10SRL | 1 | 12 | nr | 0.19 | nd | 703.3 | 14.2 | nd | 8.7 | | | | |
| 10SRL | 2 | 12 | nr | 1.01 | nd | 712.6 | 16.6 | nd | 6.3 | 0.60 | 7.500 | 1.697 | 10SCRL0 |
| 10SRL | 3 | 12 | nr | 0.38 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 1 | 24 | nr | 0.49 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 2 | 24 | nr | 0.45 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 3 | 24 | nr | 0.36 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 1 | 36 | nr | 0.07 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 2 | 36 | nr | 0.21 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 3 | 36 | nr | 0.12 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 1 | 48 | nr | 0.02 | nr | nr | nr | nr | nr | | | | |

Table D2-2. Smoky Canyon Mine Ion Chromotography Data, continued.

| Experiment | Replicate | Time | pH | $O_2$ | $PO_4^{+}$ | $SO_4^{2-}$ | $SeO_4^{2-}$ | $SeO_3^{2-}$ | $NO_3^{-}$ | $O_2$ Ave | $NO_3^{-}$ Ave | $NO_3^{-}$ s.d. | Sample ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10SRL | 2 | 48 | nr | 0.49 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 3 | 48 | nr | 0.25 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 1 | 60 | nr | 0.27 | nd | 724.1 | 4.0 | 2.31 | 4.2 | | | | |
| 10SRL | 2 | 60 | nr | 0.29 | nd | 718.6 | 5.6 | 3.5 | 2.27 | 0.28 | 3.235 | 1.365 | 10SCRL60 |
| 10SRL | 1 | 84 | nr | 0.29 | nd | 722.4 | nd | nd | 3.26 | | | | |
| 10SRL | 2 | 84 | nr | 0.32 | nd | 709.5 | nd | nd | 1.6 | 0.31 | 2.430 | 1.174 | 10SCRL84 |
| 10SRL | 3 | 84 | nr | 0.18 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 1 | 108 | nr | 0.32 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 2 | 108 | nr | 0.16 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 3 | 108 | nr | 0.1 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 1 | 120 | 7.78 | 1.11 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 2 | 120 | 7.73 | 0.77 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 3 | 120 | 7.66 | 0.79 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 1 | 132 | nr | 0.74 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 2 | 132 | nr | 0.17 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 3 | 132 | nr | 0.06 | nr | nr | nr | nr | nr | | | | |
| 10SRL | 1 | 246 | nr | 0.08 | nd | 708.9 | nd | nd | 2 | | | | |
| 10SRL | 2 | 246 | nr | 0.05 | nd | 710.1 | nd | nd | 2 | 0.07 | 2.000 | 0.000 | 10SCRL246 |
| | | | | | | | | | | | | | |
| 10SSL | 1 | 0 | nr | 0.36 | nd | 620.1 | 11.3 | nd | 8.1 | | | | |
| 10SSL | 2 | 0 | nr | 0.45 | nd | 631.3 | 12.6 | nd | 8.44 | 0.47 | 8.270 | 0.240 | 10SCSL0 |
| 10SSL | 3 | 0 | nr | 0.61 | nr | nr | nr | nr | 8.74 | | | | |
| 10SSL | 1 | 12 | nr | 0.46 | nd | 554.7 | 13.4 | nd | 7.19 | | | | |
| 10SSL | 2 | 12 | nr | 0.37 | nd | 521.9 | 14.6 | nd | 7.36 | 0.42 | 7.275 | 0.120 | 10SCSL12 |
| 10SSL | 1 | 24 | nr | 0.69 | nr | nr | nr | nr | nr | | | | |
| 10SSL | 2 | 24 | nr | 0.17 | nr | nr | nr | nr | nr | | | | |
| 10SSL | 3 | 24 | nr | 0.47 | nr | nr | nr | nr | nr | | | | |
| 10SSL | 1 | 36 | nr | 0.35 | nr | nr | nr | nr | nr | | | | |
| 10SSL | 2 | 36 | nr | 0.3 | nr | nr | nr | nr | nr | | | | |
| 10SSL | 3 | 36 | nr | 0.29 | nr | nr | nr | nr | nr | | | | |
| 10SSL | 1 | 48 | nr | 0.38 | nr | nr | nr | nr | nr | | | | |
| 10SSL | 2 | 48 | nr | 0.26 | nr | nr | nr | nr | nr | | | | |
| 10SSL | 3 | 48 | nr | 0.47 | nr | nr | nr | nr | nr | | | | |

Table D2-2. Smoky Canyon Mine Ion Chromotography Data, continued.

| Experiment | Replicate | Time | pH | $O_2$ | $PO_4^{+}$ | $SO_4^{2-}$ | $SeO_4^{2-}$ | $SeO_3^{2-}$ | $NO_3^{-}$ | $O_2$ Ave | $NO_3^{-}$ Ave | $NO_3^{-}$ s.d. | Sample ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 10SSL | 1 | 60 | nr | 0.16 | nd | 635.2 | 8.4 | 4.57 | 2.27 | | | | |
| 10SSL | 2 | 60 | nr | 0.11 | nd | 633.1 | 6.9 | 2.3 | 2 | 0.14 | 2.135 | 0.191 | 10SCSL60 |
| 10SSL | 3 | 60 | nr | 0.03 | nr | nr | nr | nr | nr | | | | |
| 10SSL | 1 | 84 | nr | 0.24 | nd | 820.1 | 2.1 | nd | 2 | | | | |
| 10SSL | 2 | 84 | nr | 0.46 | nd | 620.1 | 1.9 | nd | 2 | 0.35 | 2.000 | 0.000 | 10SCSL84 |
| 10SSL | 3 | 84 | nr | 0.06 | nr | nr | nr | nr | nr | | | | |
| 10SSL | 1 | 108 | nr | 0.01 | nr | nr | nr | nr | nr | | | | |
| 10SSL | 2 | 108 | nr | 0.2 | nr | nr | nr | nr | nr | | | | |
| 10SSL | 3 | 108 | nr | 0.15 | nr | nr | nr | nr | nr | | | | |
| 10SSL | 1 | 120 | 7.6 | 0.73 | nr | nr | nr | nr | nr | | | | |
| 10SSL | 2 | 120 | 7.76 | 0.65 | nr | nr | nr | nr | nr | | | | |
| 10SSL | 3 | 120 | 7.87 | 0.6 | nr | nr | nr | nr | nr | | | | |
| 10SSL | 2 | 132 | nr | 0.41 | nr | nr | nr | nr | nr | | | | |
| 10SSL | 3 | 132 | nr | 0.14 | nr | nr | nr | nr | nr | | | | |
| 10SSL | 1 | 246 | nr | 0.07 | nd | 679.5 | nd | nd | 2 | | | | |
| 10SSL | 2 | 246 | nr | 0.1 | nd | 668.3 | nd | nd | 2 | 0.09 | 2.000 | 0.000 | 10SCSL246 |
| | | | | | | | | | | | | | |
| 25SCL | 1 | 0 | nr | 0.81 | nd | 270.0 | 11.3 | nd | 11.2 | | | | |
| 25SCL | 2 | 0 | nr | 0.44 | nd | 283.98 | 9.0 | nd | 8.3 | | | | |
| 25SCL | 3 | 0 | nr | 1.2 | nd | 284.0 | 9.0 | nd | 5 | 0.82 | 8.167 | 3.102 | 25SCRL0 |
| 25SCL | 2 | 12 | nr | 0.37 | nd | 649.6 | 15.7 | 0 | 4.45 | | | | |
| 25SCL | 2 | 12 | nr | 0.03 | nd | 592.7 | 15.2 | nd | 7.98 | | | | |
| 25SCL | 3 | 12 | nr | 0.77 | nd | nr | 15.7 | nd | 4.45 | 0.39 | 5.627 | 2.038 | 25SCRL12 |
| 25SCL | 1 | 24 | nr | 0.13 | nr | nr | nr | nr | nr | | | | |
| 25SCL | 2 | 24 | nr | 1 | nr | nr | nr | nr | nr | | | | |
| 25SCL | 3 | 24 | nr | 0.08 | nr | nr | nr | nr | nr | | | | |
| 25SCL | 1 | 36 | nr | 0.42 | nr | nr | nr | nr | nr | | | | |
| 25SCL | 2 | 36 | nr | 0.23 | nr | nr | nr | nr | nr | | | | |
| 25SCL | 3 | 36 | nr | 0.88 | nr | nr | nr | nr | nr | | | | |
| 25SCL | 1 | 48 | nr | 0.56 | nd | 564.5 | 2.1 | nd | 1.1 | | | | |
| 25SCL | 2 | 48 | nr | 0.05 | nd | 625.7 | 3.4 | nd | 2.28 | | | | |
| 25SCL | 3 | 48 | nr | 0.37 | nd | 495.48 | 5.7 | 3.11 | 2.43 | 0.33 | 1.937 | 0.728 | 25SCRL48 |
| 25SCL | 2 | 53 | nr | nr | nr | nr | nr | nr | nr | | | | |

Table D2-2. Smoky Canyon Mine Ion Chromotography Data, continued.

| Experiment | Replicate | Time | pH | $O_2$ | $PO_4^{+}$ | $SO_4^{2-}$ | $SeO_4^{2-}$ | $SeO_3^{2-}$ | $NO_3^{-}$ | $O_2$ Ave | $NO_3^{-}$ Ave | $NO_3^{-}$ s.d. | Sample ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25SCL | 1 | 60 | nr | 0.51 | nd | 564.5 | nd | nd | 2 | | | | |
| 25SCL | 2 | 60 | nr | 0.46 | nd | 625.7 | nd | nd | 2 | | | | |
| 25SCL | 3 | 60 | nr | 0.35 | nd | 698.18 | 1.7 | nd | 7.16 | 0.44 | 3.720 | 2.979 | 25SCRL60 |
| 25SCL | 1 | 72 | nr | 0.46 | nd | 719 | 7.6 | nr | 6.78 | | | | |
| 25SCL | 2 | 72 | nr | 0.31 | nd | 506.96 | 6.8 | nr | 6.74 | | | | |
| 25SCL | 3 | 72 | nr | 0.13 | nd | 533.26 | 6.2 | nr | 6.51 | 0.30 | 6.677 | 0.146 | 25SCRL72 |
| 25SCL | 1 | 96 | nr | 0.63 | nr | nr | nr | nr | nr | | | | |
| 25SCL | 2 | 96 | nr | 0.84 | nr | nr | nr | nr | nr | | | | |
| 25SCL | 3 | 96 | nr | 0.18 | nr | nr | nr | nr | nr | | | | |
| 25SCL | 1 | 120 | 7.38 | 0.54 | nr | nr | nr | nr | nr | | | | |
| 25SCL | 2 | 120 | 7.41 | 1.6 | nr | nr | nr | nr | nr | | | | |
| 25SCL | 3 | 120 | 7.29 | 0.17 | nr | nr | nr | nr | nr | | | | |
| 25SCL | 1 | 144 | nr | 0.15 | nr | nr | nr | nr | nr | | | | |
| 25SCL | 2 | 144 | nr | 0.27 | nr | nr | nr | nr | nr | | | | |
| 25SCL | 3 | 144 | nr | 0.42 | nr | nr | nr | nr | nr | | | | |
| 25SCL | 1 | 168 | nr | 0.06 | nr | nr | nr | nr | nr | | | | |
| 25SCL | 2 | 168 | nr | 0.01 | nr | nr | nr | nr | nr | | | | |
| 25SCL | 3 | 168 | nr | 0.29 | nr | nr | nr | nr | nr | | | | |
| 25SCL | 1 | 228 | nr | 0.13 | nd | 506.96 | nd | nd | 2 | | | | |
| 25SCL | 2 | 228 | nr | 0.07 | nd | 533.26 | nd | nd | 2 | 0.10 | 2.000 | 0.000 | 25SCRL228 |
| | | | | | | | | | | | | | |
| 25SRL | 1 | 0 | nr | 1.8 | nd | 687.2 | 11.3 | nd | 8.63 | | | | |
| 25SRL | 2 | 0 | nr | 0.44 | nd | 683.0 | 10.8 | nd | 7.69 | | | | |
| 25SRL | 3 | 0 | nr | 1.2 | nd | 683.0 | 10.8 | nd | 7.69 | 1.15 | 8.003 | 0.543 | 25SCRL0 |
| 25SRL | 2 | 12 | nr | 0.37 | nd | 712.6 | 13.6 | nd | 4.12 | | | | |
| 25SRL | 2 | 12 | nr | 0.03 | nd | 649.61 | 14.9 | nd | 5.53 | 0.20 | 4.825 | 0.997 | 25SCRL12 |
| 25SRL | 3 | 12 | nr | 0.77 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 1 | 24 | nr | 0.13 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 2 | 24 | nr | 1 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 3 | 24 | nr | 0.08 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 1 | 36 | nr | 0.42 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 2 | 36 | nr | 0.23 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 3 | 36 | nr | 0.88 | nr | nr | nr | nr | nr | | | | |

Table D2-2. Smoky Canyon Mine Ion Chromotography Data, continued.

| Experiment | Replicate | Time | pH | $O_2$ | $PO_4^{+}$ | $SO_4^{2-}$ | $SeO_4^{2-}$ | $SeO_3^{2-}$ | $NO_3^{-}$ | $O_2$ Ave | $NO_3^{-}$ Ave | $NO_3^{-}$ s.d. | Sample ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25SRL | 3 | 48 | nr | 0.37 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 2 | 53 | nr | nr | nr | nr | nr | nr | nr | | | | |
| 25SRL | 1 | 60 | nr | 0.51 | nd | 799.3 | nd | nd | 2 | | | | |
| 25SRL | 2 | 60 | nr | 0.46 | nd | 805.3 | nd | nd | 2 | 0.49 | 2.000 | 0.000 | 25SCRL60 |
| 25SRL | 3 | 60 | nr | 0.35 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 1 | 72 | nr | 0.46 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 2 | 72 | nr | 0.31 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 3 | 72 | nr | 0.13 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 1 | 96 | nr | 0.63 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 2 | 96 | nr | 0.84 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 3 | 96 | nr | 0.18 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 1 | 120 | 7.38 | 0.54 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 2 | 120 | 7.41 | 1.6 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 3 | 120 | 7.29 | 0.17 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 1 | 144 | nr | 0.15 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 2 | 144 | nr | 0.27 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 3 | 144 | nr | 0.42 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 1 | 168 | nr | 0.06 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 2 | 168 | nr | 0.01 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 3 | 168 | nr | 0.29 | nr | nr | nr | nr | nr | | | | |
| 25SRL | 1 | 228 | nr | 0.06 | nd | 637.9 | nd | nd | 2 | | | | |
| 25SRL | 2 | 228 | nr | 0.13 | nd | 644.3 | nd | nd | 2 | 0.10 | 2.000 | 0.000 | 25SCRL228 |
| | | | | | | | | | | | | | |
| 25SSL | 1 | 0 | nr | 1.8 | nd | 1050.3 | 5.7 | nd | 10.56 | | | | |
| 25SSL | 2 | 0 | nr | 0.63 | nd | 1021.5 | 7.2 | nd | 10.43 | | | | |
| 25SSL | 3 | 0 | nr | 0.7 | nr | nr | nr | nr | nr | 1.04 | 10.495 | 0.092 | 25SCSL0 |
| 25SSL | 1 | 12 | nr | 0.37 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 1 | 12 | nr | 0.33 | nd | 799.3 | 2.1 | nd | 1.1 | | | | |
| 25SSL | 2 | 12 | nr | 0.75 | nd | 805.3 | 3.4 | nd | 2.28 | | | | |
| 25SSL | 3 | 12 | nr | 0.76 | nr | nr | nr | nr | nr | 0.55 | 1.690 | 0.834 | 25SCSL12 |
| 25SSL | 1 | 24 | nr | 0.62 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 2 | 24 | nr | 0.51 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 3 | 24 | nr | 0.32 | nr | nr | nr | nr | nr | | | | |

Table D2-2. Smoky Canyon Mine Ion Chromotography Data, continued.

| Experiment | Replicate | Time | pH | $O_2$ | $PO_4^{+}$ | $SO_4^{2-}$ | $SeO_4^{2-}$ | $SeO_3^{2-}$ | $NO_3^{-}$ | $O_2$ Ave | $NO_3^{-}$ Ave | $NO_3^{-}$ s.d. | Sample ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25SSL | 1 | 36 | nr | 0.24 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 1 | 36 | nr | 0.15 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 2 | 36 | nr | 0.28 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 3 | 36 | nr | 0.34 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 3 | 48 | nr | 0.3 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 2 | 48 | nr | 0.39 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 1 | 48 | nr | 0.89 | nd | 1118.9 | 5.7 | 3.11 | 2.43 | | | | |
| 25SSL | 3 | 48 | nr | 1.96 | nd | 830.9 | 8.3 | nd | 2.3 | 0.89 | 2.365 | 0.092 | 25SCSL48 |
| 25SSL | 1 | 60 | nr | 0.7 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 1 | 60 | nr | 0.35 | nd | 921.1 | 3.5 | nd | 2 | | | | |
| 25SSL | 2 | 60 | nr | 0.24 | 3.86 | 730.4 | nd | 3.92 | 2 | | | | |
| 25SSL | 3 | 60 | nr | 0.23 | nr | nr | nr | nr | nr | 0.38 | 2.000 | 0.000 | 25SCSL60 |
| 25SSL | 1 | 72 | nr | 0.52 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 1 | 72 | nr | 0.04 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 2 | 72 | nr | 0.05 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 3 | 72 | nr | 0.42 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 1 | 96 | nr | 0.17 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 1 | 96 | nr | 0.17 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 2 | 96 | nr | 0.01 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 3 | 96 | nr | 0.13 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 1 | 120 | 7.54 | 1.33 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 1 | 120 | 7.28 | 0.18 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 2 | 120 | 7.51 | 0.11 | nd | 810.3 | nd | nd | 2 | | | | |
| 25SSL | 2 | 120 | 7.35 | 0.17 | nd | 775.9 | nd | nd | 2 | | | | |
| 25SSL | 3 | 120 | 7.35 | 0.26 | nr | nr | nr | nr | nr | 0.35 | 2.000 | 0.000 | 25SCSL120 |
| 25SSL | 3 | 120 | 7.23 | 0.06 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 1 | 144 | nr | 0.38 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 1 | 144 | nr | 0.23 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 2 | 144 | nr | 0.22 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 3 | 144 | nr | 0.55 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 1 | 168 | nr | 0.23 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 1 | 168 | nr | 0.03 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 2 | 168 | nr | 0.14 | nr | nr | nr | nr | nr | | | | |

Table D2-2. Smoky Canyon Mine Ion Chromotography Data, continued.

| Experiment | Replicate | Time | pH | $O_2$ | $PO_4^{+}$ | $SO_4^{2-}$ | $SeO_4^{2-}$ | $SeO_3^{2-}$ | $NO_3^{-}$ | $O_2$ Ave | $NO_3^{-}$ Ave | $NO_3^{-}$ s.d. | Sample ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 25SSL | 3 | 168 | nr | 0.5 | nr | nr | nr | nr | nr | | | | |
| 25SSL | 1 | 228 | nr | nr | nr | nr | nr | nr | nr | | | | |
| 25SSL | 1 | 228 | nr | nr | nd | 831.8 | 1.1 | nd | 2 | | 2.000 | 0.000 | 25SCSL228 |
| | | | | | | | | | | | | | |
| SC10-CK1 | 1 | 0 | nr | 1.56 | nd | 213.3 | 7.8 | nd | nd | | | | |
| SC10-CK1 | 1 | 12 | nr | 1.09 | nd | 314.8 | 8.9 | nd | nr | | | | |
| SC10-CK1 | 1 | 24 | nr | 1.19 | nr | nr | nr | nr | nr | | | | |
| SC10-CK1 | 1 | 36 | nr | 0.87 | nr | nr | nr | nr | nr | | | | |
| SC10-CK1 | 1 | 48 | nr | 1.3 | nr | nr | nr | nr | nr | | | | |
| SC10-CK1 | 1 | 72 | nr | 0.61 | nr | nr | nr | nr | nr | | | | |
| SC10-CK1 | 1 | 96 | nr | 1.54 | nr | nr | nr | nr | nr | | | | |
| SC10-CK1 | 1 | 120 | nr | 1.12 | nr | nr | nr | nr | nr | | | | |
| SC10-CK1 | 1 | 144 | 7.29 | 0.83 | nr | nr | nr | nr | nr | | | | |
| SC10-CK1 | 1 | 168 | nr | 0.96 | nr | nr | nr | nr | nr | | | | |
| SC10-CK1 | 1 | 192 | nr | 1.84 | nr | nr | nr | nr | nr | | | | |
| SC10-CK2 | 2 | 0 | nr | 1.05 | nr | nr | nr | nr | nr | | | | |
| SC10-CK2 | 2 | 12 | nr | 0.12 | nr | nr | nr | nr | nr | | | | |
| SC10-CK2 | 2 | 24 | nr | 1.44 | nr | nr | nr | nr | nr | | | | |
| SC10-CK2 | 2 | 36 | nr | 0.62 | nr | nr | nr | nr | nr | | | | |
| SC10-CK2 | 2 | 48 | nr | 1.01 | nr | nr | nr | nr | nr | | | | |
| SC10-CK2 | 2 | 72 | nr | 0.34 | nr | nr | nr | nr | nr | | | | |
| SC10-CK2 | 2 | 96 | nr | 1.45 | nr | nr | nr | nr | nr | | | | |
| SC10-CK2 | 2 | 120 | nr | 0.61 | nr | nr | nr | nr | nr | | | | |
| SC10-CK2 | 2 | 144 | 7.66 | 1.16 | nr | nr | nr | nr | nr | | | | |
| SC10-CK2 | 2 | 168 | nr | 0.71 | nr | nr | nr | nr | nr | | | | |
| SC10-CK2 | 2 | 192 | nr | 0.72 | nr | nr | nr | nr | nr | | | | |
| SC10-CK3 | 3 | 0 | nr | 1.74 | nr | nr | nr | nr | nr | | | | |
| SC10-CK3 | 3 | 12 | nr | 0.81 | nr | nr | nr | nr | nr | | | | |
| SC10-CK3 | 3 | 24 | nr | 1.19 | nr | nr | nr | nr | nr | | | | |
| SC10-CK3 | 3 | 36 | nr | 0.82 | nr | nr | nr | nr | nr | | | | |
| SC10-CK3 | 3 | 48 | nr | 0.85 | nr | nr | nr | nr | nr | | | | |
| SC10-CK3 | 3 | 72 | nr | 0.26 | nr | nr | nr | nr | nr | | | | |
| SC10-CK3 | 3 | 96 | nr | 2.11 | nr | nr | nr | nr | nr | | | | |

Table D2-2. Smoky Canyon Mine Ion Chromotography Data, continued.

| Experiment | Replicate | Time | pH | $O_2$ | $PO_4^{+}$ | $SO_4^{2-}$ | $SeO_4^{2-}$ | $SeO_3^{2-}$ | $NO_3^{-}$ | $O_2$ Ave | $NO_3^{-}$ Ave | $NO_3^{-}$ s.d. | Sample ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SC10-CK3 | 3 | 120 | nr | 1.59 | nr | nr | nr | nr | nr | | | | |
| SC10-CK3 | 3 | 144 | 7.8 | 0.8 | nr | nr | nr | nr | nr | | | | |
| SC10-CK3 | 3 | 168 | nr | 1.69 | nr | nr | nr | nr | nr | | | | |
| SC10-CK3 | 3 | 192 | nr | 1.47 | nr | nr | nr | nr | nr | | | | |
| SC10-RK1 | 1 | 0 | nr | 0.61 | nd | 426.5 | 12.58 | nd | nd | | | | |
| SC10-RK1 | 1 | 12 | nr | 0.9 | nr | nr | nr | nr | nr | | | | |
| SC10-RK1 | 1 | 24 | nr | 0.71 | nr | nr | nr | nr | nr | | | | |
| SC10-RK1 | 1 | 36 | nr | 0.31 | nr | nr | nr | nr | nr | | | | |
| SC10-RK1 | 1 | 48 | nr | 1.05 | nr | nr | nr | nr | nr | | | | |
| SC10-RK1 | 1 | 72 | nr | 0.71 | nr | nr | nr | nr | nr | | | | |
| SC10-RK1 | 1 | 96 | nr | 0.79 | nr | nr | nr | nr | nr | | | | |
| SC10-RK1 | 1 | 120 | nr | 0.5 | nr | nr | nr | nr | nr | | | | |
| SC10-RK1 | 1 | 144 | 7.62 | 0.74 | nr | nr | nr | nr | nr | | | | |
| SC10-RK1 | 1 | 168 | nr | 0.64 | nr | nr | nr | nr | nr | | | | |
| SC10-RK1 | 1 | 192 | nr | 0.71 | nr | nr | nr | nr | nr | | | | |
| SC10-RK2 | 2 | 0 | nr | 0.67 | nr | nr | nr | nr | nr | | | | |
| SC10-RK2 | 2 | 12 | nr | 0.67 | nr | nr | nr | nr | nr | | | | |
| SC10-RK2 | 2 | 24 | nr | 0.87 | nr | nr | nr | nr | nr | | | | |
| SC10-RK2 | 2 | 36 | nr | 0.08 | nr | nr | nr | nr | nr | | | | |
| SC10-RK2 | 2 | 48 | nr | 0.64 | nr | nr | nr | nr | nr | | | | |
| SC10-RK2 | 2 | 72 | nr | 1.09 | nr | nr | nr | nr | nr | | | | |
| SC10-RK2 | 2 | 96 | nr | 0.77 | nr | nr | nr | nr | nr | | | | |
| SC10-RK2 | 2 | 120 | nr | 0.38 | nr | nr | nr | nr | nr | | | | |
| SC10-RK2 | 2 | 144 | 7.27 | 0.87 | nr | nr | nr | nr | nr | | | | |
| SC10-RK2 | 2 | 168 | nr | 0.36 | nr | nr | nr | nr | nr | | | | |
| SC10-RK2 | 2 | 192 | nr | 0.54 | nr | nr | nr | nr | nr | | | | |
| SC10-RK3 | 3 | 0 | nr | 1.7 | nr | nr | nr | nr | nr | | | | |
| SC10-RK3 | 3 | 12 | nr | 0.54 | nr | nr | nr | nr | nr | | | | |
| SC10-RK3 | 3 | 24 | nr | 1.29 | nr | nr | nr | nr | nr | | | | |
| SC10-RK3 | 3 | 36 | nr | 0.28 | nr | nr | nr | nr | nr | | | | |
| SC10-RK3 | 3 | 48 | nr | 0.22 | nr | nr | nr | nr | nr | | | | |
| SC10-RK3 | 3 | 72 | nr | 0.59 | nr | nr | nr | nr | nr | | | | |
| SC10-RK3 | 3 | 96 | nr | 0.23 | nr | nr | nr | nr | nr | | | | |

Table D2-2. Smoky Canyon Mine Ion Chromotography Data, continued.

| Experiment | Replicate | Time | pH | $O_2$ | $PO_4^{+}$ | $SO_4^{2-}$ | $SeO_4^{2-}$ | $SeO_3^{2-}$ | $NO_3^{-}$ | $O_2$ Ave | $NO_3^{-}$ Ave | $NO_3^{-}$ s.d. | Sample ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SC10-RK3 | 3 | 120 | nr | 0.31 | nr | nr | nr | nr | nr | | | | |
| SC10-RK3 | 3 | 144 | 7.46 | 0.84 | nr | nr | nr | nr | nr | | | | |
| SC10-RK3 | 3 | 168 | nr | 0.99 | nr | nr | nr | nr | nr | | | | |
| SC10-RK3 | 3 | 192 | nr | 0.54 | nr | nr | nr | nr | nr | | | | |
| SC10-SK1 | 1 | 0 | nr | 1.1 | nd | 719.1 | nd | nd | 2 | | | | |
| SC10-SK1 | 1 | 12 | nr | 0.2 | nr | nr | nr | nr | nr | | | | |
| SC10-SK1 | 1 | 24 | nr | 0.81 | nr | nr | nr | nr | nr | | | | |
| SC10-SK1 | 1 | 36 | nr | 0.53 | nr | nr | nr | nr | nr | | | | |
| SC10-SK1 | 1 | 48 | nr | 0.69 | nr | nr | nr | nr | nr | | | | |
| SC10-SK1 | 1 | 72 | nr | 0.68 | nr | nr | nr | nr | nr | | | | |
| SC10-SK1 | 1 | 96 | nr | 0.3 | nr | nr | nr | nr | nr | | | | |
| SC10-SK1 | 1 | 120 | nr | 0.55 | nr | nr | nr | nr | nr | | | | |
| SC10-SK1 | 1 | 144 | 7.29 | 0.48 | nr | nr | nr | nr | nr | | | | |
| SC10-SK1 | 1 | 168 | nr | 0.81 | nr | nr | nr | nr | nr | | | | |
| SC10-SK1 | 1 | 192 | nr | 0.45 | nr | nr | nr | nr | nr | | | | |
| SC10-SK2 | 2 | 0 | nr | 0.92 | nr | nr | nr | nr | nr | | | | |
| SC10-SK2 | 2 | 12 | nr | 0.44 | nr | nr | nr | nr | nr | | | | |
| SC10-SK2 | 2 | 24 | nr | 0.87 | nr | nr | nr | nr | nr | | | | |
| SC10-SK2 | 2 | 36 | nr | 0.36 | nr | nr | nr | nr | nr | | | | |
| SC10-SK2 | 2 | 48 | nr | 0.33 | nr | nr | nr | nr | nr | | | | |
| SC10-SK2 | 2 | 72 | nr | 0.46 | nr | nr | nr | nr | nr | | | | |
| SC10-SK2 | 2 | 96 | nr | 0.73 | nr | nr | nr | nr | nr | | | | |
| SC10-SK2 | 2 | 120 | nr | 0.65 | nr | nr | nr | nr | nr | | | | |
| SC10-SK2 | 2 | 144 | 7.25 | 0.95 | nr | nr | nr | nr | nr | | | | |
| SC10-SK2 | 2 | 168 | nr | 0.75 | nr | nr | nr | nr | nr | | | | |
| SC10-SK2 | 2 | 192 | nr | 0.49 | nr | nr | nr | nr | nr | | | | |
| SC10-SK3 | 3 | 0 | nr | 0.61 | nr | nr | nr | nr | nr | | | | |
| SC10-SK3 | 3 | 12 | nr | 0.35 | nd | 318.3 | 8.9 | nd | nd | | | | |
| SC10-SK3 | 3 | 24 | nr | 0.71 | nr | nr | nr | nr | nr | | | | |
| SC10-SK3 | 3 | 36 | nr | 0.62 | nr | nr | nr | nr | nr | | | | |
| SC10-SK3 | 3 | 48 | nr | 1.21 | nr | nr | nr | nr | nr | | | | |
| SC10-SK3 | 3 | 72 | nr | 0.69 | nr | nr | nr | nr | nr | | | | |
| SC10-SK3 | 3 | 96 | nr | 1.44 | nr | nr | nr | nr | nr | | | | |

Table D2-2. Smoky Canyon Mine Ion Chromotography Data, continued.

| Experiment | Replicate | Time | pH | $O_2$ | $PO_4^{+}$ | $SO_4^{2-}$ | $SeO_4^{2-}$ | $SeO_3^{2-}$ | $NO_3^{-}$ | $O_2$ Ave | $NO_3^{-}$ Ave | $NO_3^{-}$ s.d. | Sample ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SC10-SK3 | 3 | 120 | nr | 0.8 | nr | nr | nr | nr | nr | | | | |
| SC10-SK3 | 3 | 144 | 7.57 | 0.77 | nr | nr | nr | nr | nr | | | | |
| SC10-SK3 | 3 | 168 | nr | 0.67 | nr | nr | nr | nr | nr | | | | |
| SC10-SK3 | 3 | 192 | nr | 0.78 | nr | nr | nr | nr | nr | | | | |
| | | | | | | | | | | | | | |
| SC25-CK1 | 1 | 0 | nr | 0.65 | nr | nr | nr | nr | nr | | | | |
| SC25-CK1 | 1 | 12 | nr | 1.28 | nr | nr | nr | nr | nr | | | | |
| SC25-CK1 | 1 | 24 | nr | 0.92 | nr | nr | nr | nr | nr | | | | |
| SC25-CK1 | 1 | 36 | nr | 1.29 | nr | nr | nr | nr | nr | | | | |
| SC25-CK1 | 1 | 48 | nr | 0.27 | nr | nr | nr | nr | nr | | | | |
| SC25-CK1 | 1 | 72 | nr | 1.03 | nr | nr | nr | nr | nr | | | | |
| SC25-CK1 | 1 | 96 | nr | 0.6 | nr | nr | nr | nr | nr | | | | |
| SC25-CK1 | 1 | 120 | nr | 0.42 | nr | nr | nr | nr | nr | | | | |
| SC25-CK1 | 1 | 144 | 7.71 | 0.89 | nr | nr | nr | nr | nr | | | | |
| SC25-CK1 | 1 | 168 | nr | 1.17 | nr | nr | nr | nr | nr | | | | |
| SC25-CK1 | 1 | 192 | nr | 0.32 | nr | nr | nr | nr | nr | | | | |
| SC25-CK2 | 2 | 0 | nr | 1.33 | nr | nr | nr | nr | nr | | | | |
| SC25-CK2 | 2 | 12 | nr | 1.6 | nr | nr | nr | nr | nr | | | | |
| SC25-CK2 | 2 | 24 | nr | 1.27 | nr | nr | nr | nr | nr | | | | |
| SC25-CK2 | 2 | 36 | nr | 0.66 | nr | nr | nr | nr | nr | | | | |
| SC25-CK2 | 2 | 48 | nr | 0.76 | nr | nr | nr | nr | nr | | | | |
| SC25-CK2 | 2 | 72 | nr | 1.39 | nr | nr | nr | nr | nr | | | | |
| SC25-CK2 | 2 | 96 | nr | 0.58 | nr | nr | nr | nr | nr | | | | |
| SC25-CK2 | 2 | 120 | nr | 0.36 | nr | nr | nr | nr | nr | | | | |
| SC25-CK2 | 2 | 144 | 7.77 | 1.06 | nr | nr | nr | nr | nr | | | | |
| SC25-CK2 | 2 | 168 | nr | 0.27 | nr | nr | nr | nr | nr | | | | |
| SC25-CK2 | 2 | 192 | nr | 0.36 | nr | nr | nr | nr | nr | | | | |
| SC25-CK3 | 3 | 0 | nr | 1.03 | nr | nr | nr | nr | nr | | | | |
| SC25-CK3 | 3 | 12 | nr | 1.44 | nr | nr | nr | nr | nr | | | | |
| SC25-CK3 | 3 | 24 | nr | 0.73 | nr | nr | nr | nr | nr | | | | |
| SC25-CK3 | 3 | 36 | nr | 0.91 | nr | nr | nr | nr | nr | | | | |
| SC25-CK3 | 3 | 48 | nr | 1.43 | nr | nr | nr | nr | nr | | | | |
| SC25-CK3 | 3 | 72 | nr | 2.32 | nr | nr | nr | nr | nr | | | | |

Table D2-2. Smoky Canyon Mine Ion Chromotography Data, continued.

| Experiment | Replicate | Time | pH | $O_2$ | $PO_4^{+}$ | $SO_4^{2-}$ | $SeO_4^{2-}$ | $SeO_3^{2-}$ | $NO_3^{-}$ | $O_2$ Ave | $NO_3^{-}$ Ave | $NO_3^{-}$ s.d. | Sample ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SC25-CK3 | 3 | 96 | nr | 0.45 | nr | nr | nr | nr | nr | | | | |
| SC25-CK3 | 3 | 120 | nr | 0 | nr | nr | nr | nr | nr | | | | |
| SC25-CK3 | 3 | 144 | 7.79 | 1.05 | nr | nr | nr | nr | nr | | | | |
| SC25-CK3 | 3 | 168 | nr | 0.88 | nr | nr | nr | nr | nr | | | | |
| SC25-CK3 | 3 | 192 | nr | 0.33 | nr | nr | nr | nr | nr | | | | |
| SC25-RK1 | 1 | 0 | nr | 1.35 | nd | 353.5 | 8.3 | nd | nd | | | | |
| SC25-RK1 | 1 | 12 | nr | 0.74 | nr | nr | nr | nr | nr | | | | |
| SC25-RK1 | 1 | 24 | nr | 1.05 | nr | nr | nr | nr | nr | | | | |
| SC25-RK1 | 1 | 36 | nr | 0.51 | nr | nr | nr | nr | nr | | | | |
| SC25-RK1 | 1 | 48 | nr | 0.45 | nr | nr | nr | nr | nr | | | | |
| SC25-RK1 | 1 | 72 | nr | 0.95 | nr | nr | nr | nr | nr | | | | |
| SC25-RK1 | 1 | 96 | nr | 0.29 | nr | nr | nr | nr | nr | | | | |
| SC25-RK1 | 1 | 120 | nr | 0.12 | nr | nr | nr | nr | nr | | | | |
| SC25-RK1 | 1 | 144 | 7.76 | 0.95 | nr | nr | nr | nr | nr | | | | |
| SC25-RK1 | 1 | 168 | nr | 0.04 | nr | nr | nr | nr | nr | | | | |
| SC25-RK1 | 1 | 192 | nr | 0.12 | nr | nr | nr | nr | nr | | | | |
| SC25-RK2 | 2 | 0 | nr | 0.68 | nr | nr | nr | nr | nr | | | | |
| SC25-RK2 | 2 | 12 | nr | 0.85 | nr | nr | nr | nr | nr | | | | |
| SC25-RK2 | 2 | 24 | nr | 0.93 | nr | nr | nr | nr | nr | | | | |
| SC25-RK2 | 2 | 36 | nr | 0.31 | nr | nr | nr | nr | nr | | | | |
| SC25-RK2 | 2 | 48 | nr | 0.56 | nr | nr | nr | nr | nr | | | | |
| SC25-RK2 | 2 | 72 | nr | 1.16 | nr | nr | nr | nr | nr | | | | |
| SC25-RK2 | 2 | 96 | nr | 0.12 | nr | nr | nr | nr | nr | | | | |
| SC25-RK2 | 2 | 120 | nr | 0.12 | nr | nr | nr | nr | nr | | | | |
| SC25-RK2 | 2 | 144 | 7.56 | 0.12 | nr | nr | nr | nr | nr | | | | |
| SC25-RK2 | 2 | 168 | nr | -0.17 | nr | nr | nr | nr | nr | | | | |
| SC25-RK2 | 2 | 192 | nr | 0.01 | nr | nr | nr | nr | nr | | | | |
| SC25-RK3 | 3 | 0 | nr | 1.74 | nr | nr | nr | nr | nr | | | | |
| SC25-RK3 | 3 | 12 | nr | 1.07 | nr | nr | nr | nr | nr | | | | |
| SC25-RK3 | 3 | 24 | nr | 1.39 | nr | nr | nr | nr | nr | | | | |
| SC25-RK3 | 3 | 36 | nr | 0.74 | nr | nr | nr | nr | nr | | | | |
| SC25-RK3 | 3 | 48 | nr | 0.91 | nr | nr | nr | nr | nr | | | | |
| SC25-RK3 | 3 | 72 | nr | 0.38 | nr | nr | nr | nr | nr | | | | |

Table D2-2. Smoky Canyon Mine Ion Chromotography Data, continued.

| Experiment | Replicate | Time | pH | $O_2$ | $PO_4^{+}$ | $SO_4^{2-}$ | $SeO_4^{2-}$ | $SeO_3^{2-}$ | $NO_3^{-}$ | $O_2$ Ave | $NO_3^{-}$ Ave | $NO_3^{-}$ s.d. | Sample ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SC25-RK3 | 3 | 96 | nr | 0.68 | nr | nr | nr | nr | nr | | | | |
| SC25-RK3 | 3 | 120 | nr | 0.12 | nr | nr | nr | nr | nr | | | | |
| SC25-RK3 | 3 | 144 | 7.57 | 0.12 | nr | nr | nr | nr | nr | | | | |
| SC25-RK3 | 3 | 168 | nr | 0.55 | nr | nr | nr | nr | nr | | | | |
| SC25-RK3 | 3 | 192 | nr | 0.55 | nr | nr | nr | nr | nr | | | | |
| SC25-SK1 | 1 | 0 | nr | 1 | nr | nr | nr | nr | nr | | | | |
| SC25-SK1 | 1 | 12 | nr | 1.22 | nd | 540.5 | 9.17 | nd | nd | | | | |
| SC25-SK1 | 1 | 24 | nr | 0.43 | nr | nr | nr | nr | nr | | | | |
| SC25-SK1 | 1 | 36 | nr | 0.74 | nr | nr | nr | nr | nr | | | | |
| SC25-SK1 | 1 | 48 | nr | 0.74 | nr | nr | nr | nr | nr | | | | |
| SC25-SK1 | 1 | 72 | nr | 0.84 | nr | nr | nr | nr | nr | | | | |
| SC25-SK1 | 1 | 96 | nr | 0.35 | nr | nr | nr | nr | nr | | | | |
| SC25-SK1 | 1 | 120 | nr | 0.41 | nr | nr | nr | nr | nr | | | | |
| SC25-SK1 | 1 | 144 | 7.72 | 0.65 | nr | nr | nr | nr | nr | | | | |
| SC25-SK1 | 1 | 168 | nr | 0.11 | nr | nr | nr | nr | nr | | | | |
| SC25-SK1 | 1 | 192 | nr | 0.16 | nr | nr | nr | nr | nr | | | | |
| SC25-SK2 | 2 | 0 | nr | 1.14 | nr | nr | nr | nr | nr | | | | |
| SC25-SK2 | 2 | 12 | nr | 1.53 | nr | nr | nr | nr | nr | | | | |
| SC25-SK2 | 2 | 24 | nr | 0.65 | nr | nr | nr | nr | nr | | | | |
| SC25-SK2 | 2 | 36 | nr | 0.42 | nr | nr | nr | nr | nr | | | | |
| SC25-SK2 | 2 | 48 | nr | 0.49 | nr | nr | nr | nr | nr | | | | |
| SC25-SK2 | 2 | 72 | nr | 1.38 | nr | nr | nr | nr | nr | | | | |
| SC25-SK2 | 2 | 96 | nr | 0.39 | nr | nr | nr | nr | nr | | | | |
| SC25-SK2 | 2 | 120 | nr | 0.01 | nr | nr | nr | nr | nr | | | | |
| SC25-SK2 | 2 | 144 | 7.55 | 0.82 | nr | nr | nr | nr | nr | | | | |
| SC25-SK2 | 2 | 168 | nr | 0.23 | nr | nr | nr | nr | nr | | | | |
| SC25-SK2 | 2 | 192 | nr | 0.05 | nr | nr | nr | nr | nr | | | | |
| SC25-SK3 | 3 | 0 | nr | 0.54 | nr | nr | nr | nr | nr | | | | |
| SC25-SK3 | 3 | 12 | nr | 1.08 | nr | nr | nr | nr | nr | | | | |
| SC25-SK3 | 3 | 24 | nr | 0.39 | nr | nr | nr | nr | nr | | | | |
| SC25-SK3 | 3 | 36 | nr | 0.58 | nr | nr | nr | nr | nr | | | | |
| SC25-SK3 | 3 | 48 | nr | 0.95 | nr | nr | nr | nr | nr | | | | |
| SC25-SK3 | 3 | 72 | nr | 1.38 | nr | nr | nr | nr | nr | | | | |

Table D2-2. Smoky Canyon Mine Ion Chromotography Data, continued.

| Experiment | Replicate | Time | pH | $O_2$ | $PO_4^{+}$ | $SO_4^{2-}$ | $SeO_4^{2-}$ | $SeO_3^{2-}$ | $NO_3^{-}$ | $O_2$ Ave | $NO_3^{-}$ Ave | $NO_3^{-}$ s.d. | Sample ID |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SC25-SK3 | 3 | 96 | nr | 0.72 | nr | nr | nr | nr | nr | | | | |
| SC25-SK3 | 3 | 120 | nr | 0.26 | nr | nr | nr | nr | nr | | | | |
| SC25-SK3 | 3 | 144 | 7.59 | 1.05 | nr | nr | nr | nr | nr | | | | |
| SC25-SK3 | 3 | 168 | nr | 0.2 | nr | nr | nr | nr | nr | | | | |
| SC25-SK3 | 3 | 192 | nr | 0.36 | nr | nr | nr | nr | nr | | | | |

Values are micrograms/liter (µg/L)

*= degassed

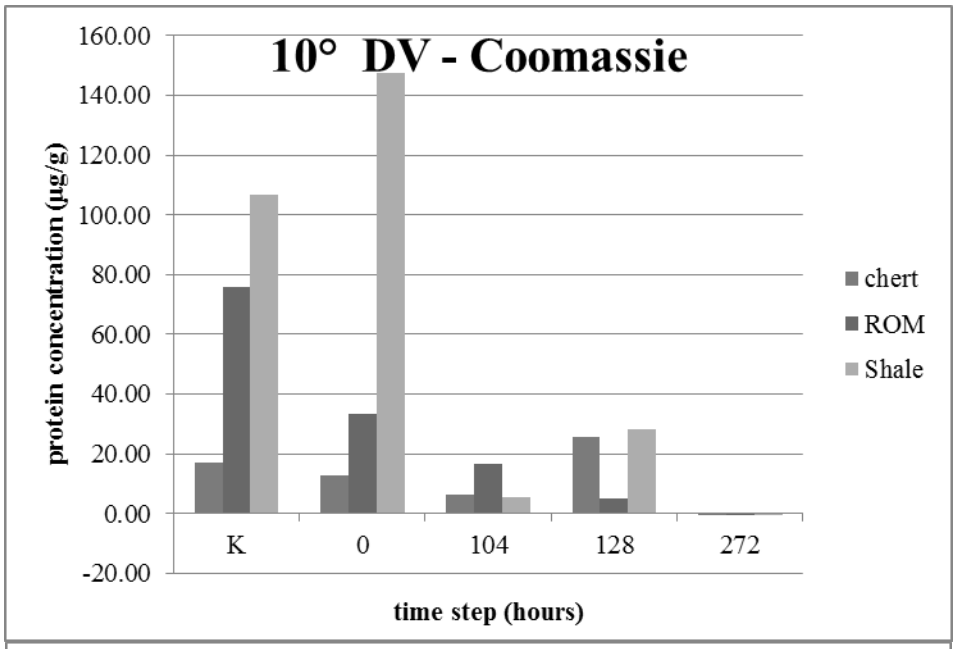
$NO_3^{-}$ detection limit= 2 µg/L

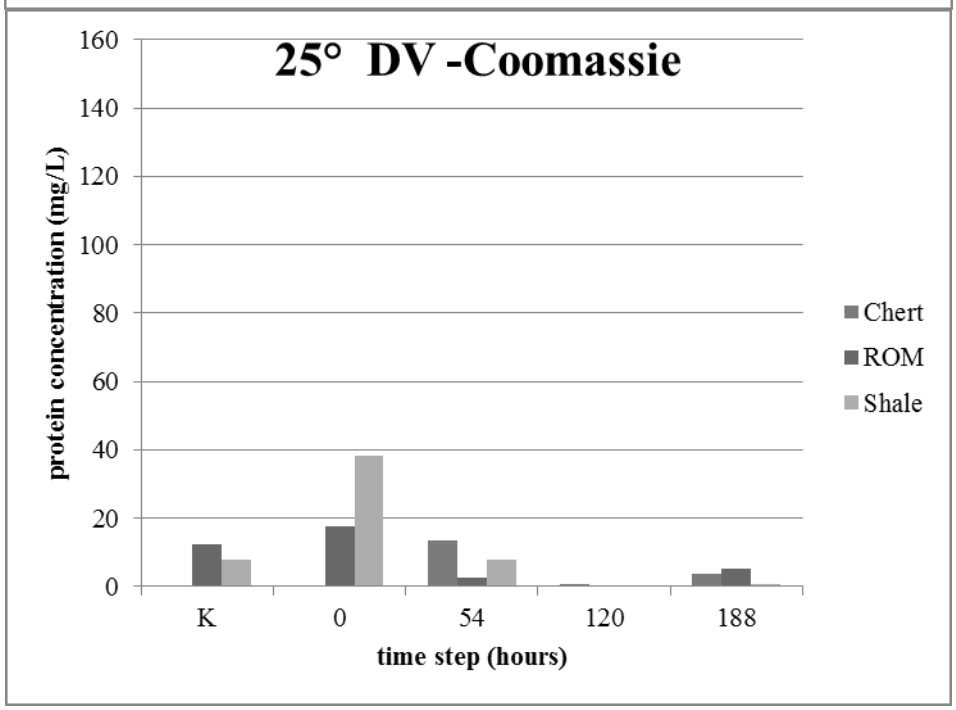
nr= not reported

nd= not detected

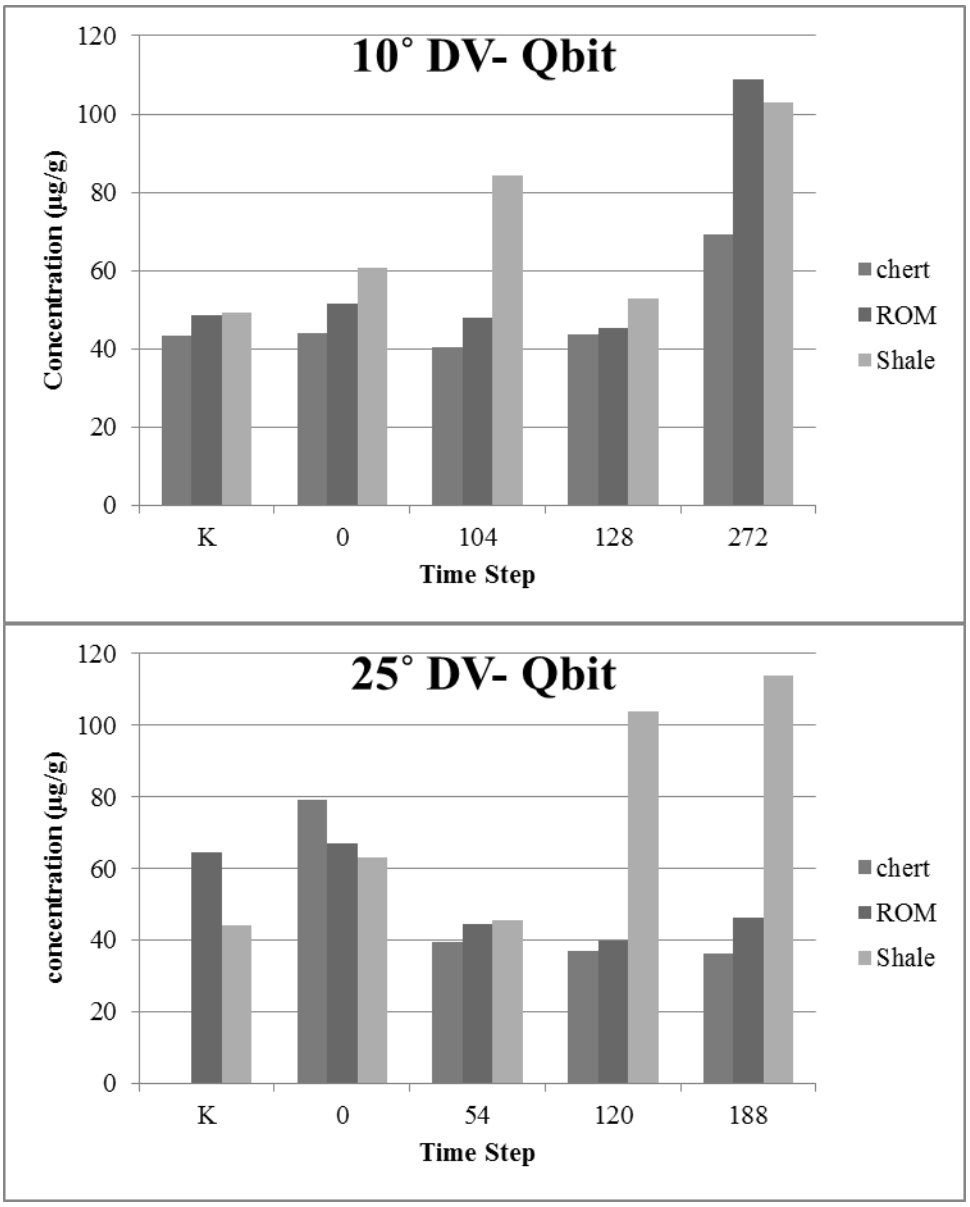
na= not applicable

+ $PO_4^{+}$ was detected only immediately following analysis of a standard containing $PO_4^{+}$

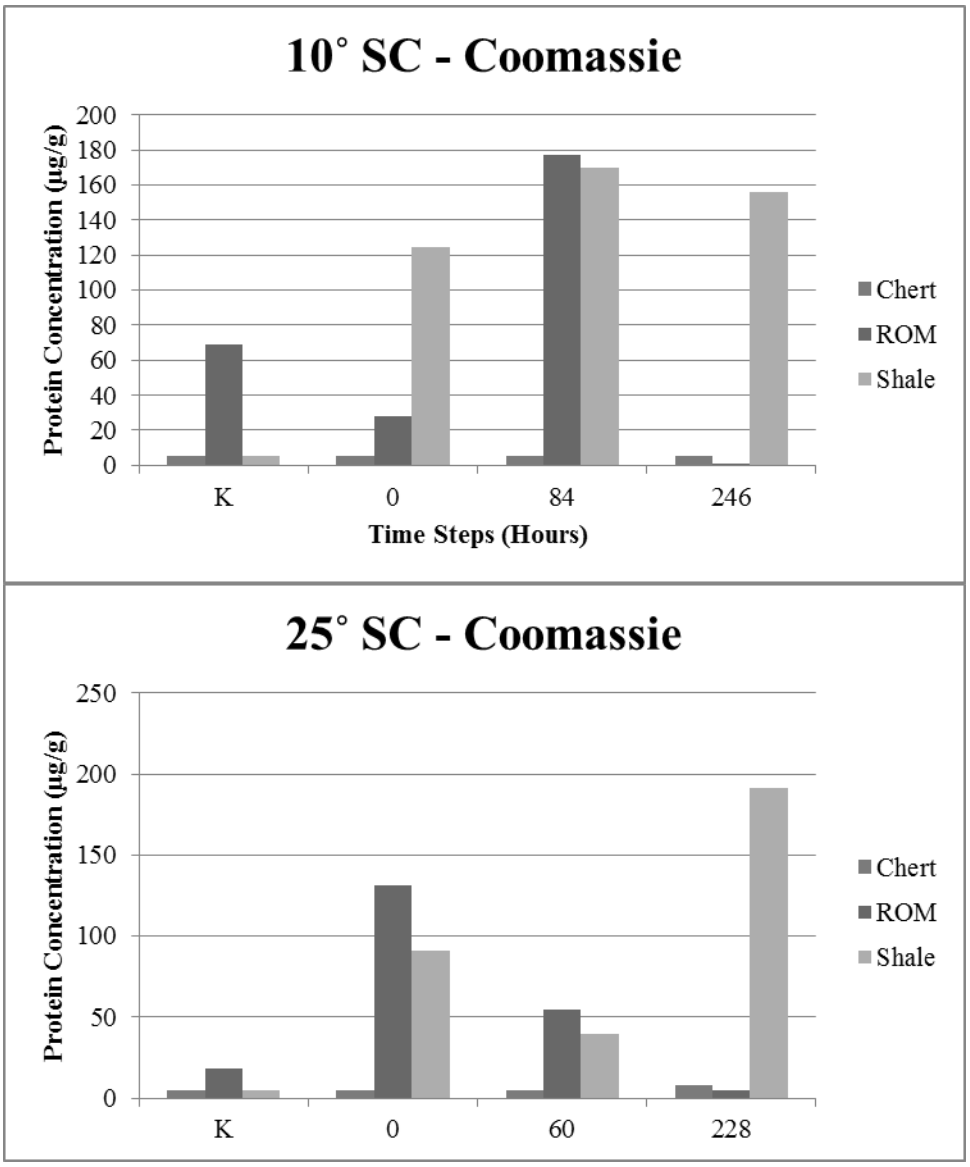



Appendix D3-1. Dry Valley Protein Assey – Coomassie Method




Appendix D3-1. Dry Valley Protein Assey – Qbit Nano-Orange Method




Appendix D3-2. Smoky Canyon Protein Assay – Coomassie Method

# APPENDIX E

## SPME HYDROCARBON ANALYSIS DATA

# APPENDIX E

## SPME HYDROCARBON ANALYSIS DATA

E1: Table E1-1. Dissolved Organic Carbon Speciation by HS-SPME-GCMS for Select Samples

Table E1. Dissolved Organic Carbon Speciation by HS-SPME-GCMS for Select Samples.

| | | | | | GWDV7D2A | ROM mix media | SCD Chert 5 | SCD shale 75 | 10 C con-trol | 10 C live end | 10 S con-trol | 10 S live end |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Example Compound** | **# Carbons** | **# Nitrogens** | **Chemical Formula** | **ng in vapor** | | | | | | | |
| Alkane | Ethanol | C2 | 0 | $C_2H_6O$ | | | | 14.20 | | | | |
| Alkane | ethane, 1,1 -oxybis- | C4 | 0 | $C_2H_6$ | | | | 40.02 | | | | |
| Alkane | Pentanal | C5 | 0 | $C_5H_{10}O$ | | | | | 36.36 | 13.31 | | |
| Alkane | cyclopentane, methyl- | C6 | 0 | $C_6H_{12}$ | 1.27 | 7.51 | 14.68 | 39.62 | 49.73 | 12.63 | 36.53 | 28.48 |
| Alkane | Pentane, 3-methyl- | 6 | | | | | | 7.71 | | 11.45 | | 10.60 |
| Alkane | 2-pentanone, 4-methyl- | 6 | | | | | 2.77 | 1.75 | 9.16 | | | |
| Alkane | Hexane | 6 | | | | | 3.60 | 23.44 | 17.46 | | 32.00 | 13.10 |
| Alkane | 2-butanone, 3-hydroxy-3-methyl- | 6 | | | 1.27 | 1.70 | | | 18.17 | | | |
| Alkane | Acetic acid, butyl ester | 6 | | | | | | 2.09 | | | | |
| Alkane | Acetic acid, 1,1-dimethylethyl e | 6 | | | | | 3.58 | | | | | |
| Alkane | 2-Pentanone, 3-methyl- | 6 | | | | | | 2.17 | | | | |
| Alkane | 3- hexanone | 6 | | | | | | | 4.94 | | 4.53 | 4.78 |
| Alkane | Hexane, 3-iodo | 6 | | | | 5.81 | 2.39 | 2.46 | | 1.18 | | |
| Alkane | Propane, 2-ethoxy-2-methyl- | 6 | | | | | 2.34 | | | | | |
| Alkane | 3-pentanone, 2,4-dimethyl- | C7 | 0 | $C_5H_{10}O$ | | 5.40 | | | 31.80 | | | |
| Alkane | 1-pentanol, 2,2-dimethyl- | 7 | 0 | | | | | | | | | |
| Alkane | 2-heptanol, 2-methyl- | C8 | 0 | $C_8H_{18}O$ | | | | | 26.11 | | | |
| Alkane | 2-heptanone, 4-methyl- | 8 | 0 | | | | | | 17.98 | | | |
| Alkane | Nonanal | C9 | 0 | $C_9H_{18}O$ | | 3.60 | 7.87 | | 21.63 | 12.28 | 7.09 | |
| Alkane | heptane, 4,4-dimethyl- | 9 | | | | 1.45 | | | 9.74 | 1.32 | | |
| Alkane | Undecane, 2,4, -dimethyl- | 9 | 0 | $C_{11}H_{24}$ | | | | | | | | |
| Alkane | 3-heptanone, 2,4-dimethyl- | 9 | 0 | $C_9H_{18}O$ | | | | | | | | |
| Alkane | octanal, 7-hydroxy-3,7- | 10 | 0 | $C_{10}H_{20}O_2$ | | | | | 17.27 | | | |

Table E1. Dissolved Organic Carbon Speciation by HS-SPME-GCMS for Select Samples, continued.

| | | | | | GWDV7D2A | ROM mix media | SCD Chert 5 | SCD shale 75 | 10 C con-trol | 10 C live end | 10 S con-trol | 10 S live end |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | dimethyl- | | | | | | | | | | | |
| Alkane | decanal | C10 | 0 | $C_{10}H_{20}O$ | | 4.61 | 7.97 | | 27.09 | 1.38 | 7.40 | 7.49 |
| Alkane | Dodecane | C12 | 0 | $C_{12}H_{26}$ | | | | 2.32 | 5.71 | 0.90 | 24.21 | |
| Alkane | Dodecane, 1-iodo- | 12 | 0 | | | | | | | | 12.29 | |
| Alkane | 3-Dodecanol | 12 | 0 | | | | | | 5.71 | | | |
| Alkane | 3,6- dimethyldecane | 12 | 0 | | | | | | | | 11.92 | |
| Alkane | Propanic acid, 2-methyl-, 2-methyl | 12 | 0 | | | | | 2.32 | | | | |
| Alkane | 9-Undecen-2-one, 6,10-dimethyl- | C13 | 0 | $C_{13}H_{24}O$ | | 3.63 | | | | 1.05 | 4.85 | |
| Alkane | Undecane, 4,6-dimethyl- | 13 | 0 | | | | | | | | 4.85 | |
| Alkane | hexane, 1,1-[ehtylidenebis(oxy) | C14 | 0 | $C_6H_{14}$ | | 6.43 | | 2.14 | 5.27 | | 20.67 | |
| Alkane | Decane, 2,3,5,8-tetramethyl- | 14 | 0 | | | | | | | | 7.32 | |
| Alkane | Tetradecane | 14 | 0 | | | | | | | | 4.66 | |
| Alkane | Tridecane, 5-methyl- | 14 | 0 | | | 6.43 | | | | | | |
| Alkane | 2-nonanone,9-[(tetrahydro-2h-py | 14 | 0 | | | | | | 5.27 | | | |
| Alkane | Dodecane, 4,6-dimethyl- | 14 | 0 | | | | | | | | 8.69 | |
| Alkane | Pentadecane | C15 | 0 | $C_{15}H_{32}$ | | | | | | | 10.79 | |
| Alkane | hexadecane | C16 | 0 | $C_{16}H_{34}$ | 1.21 | | | 13.39 | | | | |
| Alkane | Tridecane, 6-propyl- | 16 | 0 | | | | | 1.88 | | | | |
| Alkane | propanic acid, 2-methyl-, 1-(1 | 16 | 0 | | | | | 11.51 | | | | |
| Alkane | octadecane | C18 | 0 | $C_{18}H_{38}$ | | | | | | | 4.89 | |
| Alkane | Tricosane | C23 | 0 | $C_{23}H_{48}$ | | | | | | | 5.09 | |
| Alkane | Octosane | C28 | 0 | $C_{28}H_{48}$ | | | | | | | 7.97 | |
| Alkane | triacontane | C30 | 0 | $C_{30}H_{62}$ | | | | | | | 27.31 | |
| Alkane | hexatriacontane | C36 | 0 | $C_{36}H_{74}$ | | | | | | | 4.64 | |
| **Alkane** | | | | | **2.48** | **31.18** | **30.52** | **111.69** | **203.70** | **41.55** | **161.44** | **35.97** |
| Alkene | 1-Pentene, 2,4,4 trimethyl- | C8 | 0 | $C_5H_{10}$ | | | | | | 0.77 | | |

Table E1. Dissolved Organic Carbon Speciation by HS-SPME-GCMS for Select Samples, continued.

| | | | | | GWDV7D2A | ROM mix media | SCD Chert 5 | SCD shale 75 | 10 C con-trol | 10 C live end | 10 S con-trol | 10 S live end |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alkene | 2-Pentene, 3-ethyl-4,4-dimethyl- | C9 | 0 | $C_5H_{10}$ | | | | 1.68 | | | | |
| Alkene | 1,2,6 Heptatriene, 2,5,5-trimethyl | C10 | 0 | C9H12 | | | | 1.89 | | | | |
| Alkene | 4,4,7,7 -Tetramethyldeca-1,9-dien | C14 | 0 | C14H24O2 | | | | | 7.47 | | | |
| **Alkene** | | | | | | | | **3.57** | **7.47** | **0.77** | | |
| Aromatic | Pyrimidine, 5-bromo- | C4 | 2 | $C_4H3BrN_2$; | | 1.25 | | | | | | |
| Aromatic | 1-H-Pyrazole, 4,5-dihydro-3,5,5-t | C6 | 3 | C29H22F3N3O2 | | | | | 20.66 | | | |
| Aromatic | benzene, methyl- | C7 | | C6H6 | 0.81 | | | 1.77 | | | | 2.94 |
| Aromatic | Furan, tetrahydro-2,2,5,5-tetram | C8 | | C4H4O | 0.79 | | | | 17.00 | | 4.36 | |
| Aromatic | Benzenemethanol, 4-hydroxy-.al | C9 | | C8H10O | | 1.20 | 7.27 | | | | | |
| Aromatic | Benzenedicarboxylic acid, di | C12 | | $C_8H_6O_4$ | | | | 2.54 | | | | |
| Aromatic | Benzenedicarboxylic acid, butyl | C20 | | $C_7H_6O_2$ | | | | 2.48 | | | | |
| **Aromatic** | | | | | **1.60** | **2.45** | **7.27** | **6.79** | **37.66** | **0.00** | **4.36** | **2.94** |
| Cyclic | cyclopentane, methyl- | C6 | | $C_5H_{10}$ | | 1.33 | 6.32 | 7.26 | | 1.83 | | 9.59 |
| Cyclic | 1,2 cyclopentadiol, 1-(1-methyl | C8 | | | | | | | | | | 7.51 |
| Cyclic | 1,8- cineole | C10 | | $C_{10}H_{18}O$ | | | 3.68 | | 22.53 | | | |
| Cyclic | fenchone | 10 | | | | | | | 8.83 | | | |
| Cyclic | (+)- isomethol | 10 | | | | | | | 4.87 | | | |
| Cyclic | Cyclohexane, 1,2-diethyl-3-methy | C11 | | $C_6H_{12}$ | | | | | | | 16.27 | |
| Cyclic | Cyclohexane, 1-ethyl-2-propyl | 11 | | | | | | | | | 7.29 | |
| Cyclic | Cyclooctane,butyl- | C12 | | $C_6H_{12}$ | | | | | | | 6.98 | |
| Cyclic | cyclohexane, octyl- | C14 | | $C_{14}H_{28}$ | | | | | 4.90 | | | |
| **Cyclic** | | | | | | **1.33** | **10.00** | **7.26** | **41.13** | **1.83** | **30.54** | **17.10** |
| Amine | 2-Propanamine | C3 | 1 | $C_3H_9N$ | | | 9.38 | | | | | |

Table E1. Dissolved Organic Carbon Speciation by HS-SPME-GCMS for Select Samples, continued.

| | | | | | GWDV7D2A | ROM mix media | SCD Chert 5 | SCD shale 75 | 10 C con-trol | 10 C live end | 10 S con-trol | 10 S live end |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ketone | 2-Propanone | 3 | | | | | 2.09 | | | | | |
| | Plumbane, tetramethyl- | 4 | | | | | | | 6.45 | | | 2.04 |
| | 4-Nitro-1-methylimidazole | 4 | | | | | | | 6.45 | | | |
| heterocyclic | 2,2,3,3-Tetramethyl-1-d1-aziridine | 4 | | | | | | | 13.40 | | | 13.82 |
| | 1,2,3- Oxazaborolane, 2-butyl- | 6 | | | | | | | | | | 13.82 |
| ketone | Camphor | C10 | | | | | | 2.04 | 14.54 | | | |
| | Hydroxylamine, o-decyl- | 10 | | | | | | 2.04 | | | | |
| terpene | Farnesol | C15 | | | | | | | | | 4.58 | |

These analyses represent GCMS speciation of the aqueous hydrocarbons using SPME methods - volatiles stirred out of aqueous phase, collected on siloxane fiber that is destructively sampled in GCMS.

This table summarizes total concentration by #C compounds within each major class of hydrocarbon (alkane, alkene, aromatic, cyclic, misc)

Samples are groundwater GWDV7D2a and aqueous GWTOC extract (bottle roll);two of the bottle roll extracts used for the MPN work, DMSo and DC5; starting (killed) and ending (live) solutions from the 10 Chert and 10 shale (Dry Valley).

# APPENDIX F

## SYNCHROTRON MINERALOGY DATA

# APPENDIX F

## SYNCHROTRON MINERALOGY DATA

Data on CD.
To request DVD copies contact your local public or university library to place an interlibrary loan request to Montana State University. Questions call 406-994-3161.