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AUGMENTING THE APPLICABILITY AND EFFICIENCY OF ECOLOGICAL ENGINEERING SOLUTIONS FOR WATER QUALITY IMPROVEMENT

A DISSERTATION APPROVED FOR THE
SCHOOL OF CIVIL ENGINEERING AND ENVIRONMENTAL SCIENCE

BY

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Abstract

The design options available to the ecological engineer were expanded by exploring new sustainable water quality improvement techniques, specifically the applicability of passive treatment of acid mine drainage (AMD) in high-altitude, arid environments, passive co-treatment of AMD and municipal wastewater (MWW), and ecologically engineered floating vegetation mats (EFVM).

Field studies at Cerro Rico de Potosí, Bolivia demonstrated that AMD must be addressed to render local waters safe for agricultural use. AMD discharges from both operating and abandoned portals as well as tailings-related deposits displayed a high degree of heterogeneity with total metal concentrations ranging from 0.11-7,48l, <0.022-889, <0.0006-65.3, <0.001-310, 0.12-72,100, 0.3-402, <0.012-34.8, and 0.24-19,600 mg/L of Al, As, Cd, Cu, Fe, Mn, Pb and Zn, respectively. Net acidity and pH ranged from -10 to 246,000 mg/L as $CaCO_3$ equivalent and 0.90-6.94 standard units, respectively. In-stream waters contained total metals concentrations of up to 16 mg/L As, 4.9 mg/L Cd, 0.97 mg/L Co, 1100 mg/L Fe, 110 mg/L Mn, 4.1 mg/L Pb, and 1500 mg/L Zn with pH ranging from 2.8-9.5. AMD-impacted streams contained elevated concentrations of the same major ecotoxic constituents present in AMD discharges at concentrations statistically greater than in those stream unimpacted by AMD. The data indicate that historic and current mining activities have transformed these key natural resources into potential human and environmental health hazards.

To assess the viability of passive water quality improvement approaches for treating AMD from Cerro Rico, alkalinity production, acidity neutralization and metals removal were tracked for incubations of AMD in the presence of limestone (LS), a 1:1 mix of

AMD and raw MWW, and a 1:1 mix of AMD and WW in the presence of LS. Three AMD sources from abandoned adits on Cerro Rico, raw WW from the city of Potosí and locally available LS were incubated in-situ for 72 hr in 1-L cubitainers. Although locally sourced LS can increase final alkalinity up to 397 mg/L as $CaCO_3$, it is a prospective source for Mn and a few other potentially undesirable elements. Relevant to the prospects of AMD and WW passive co-treatment, mixing AMD with WW had relatively little effect on the final alkalinity achieved by LS dissolution. Accounting for dilution, dissolved concentrations of Ag, Al, As, Cd, Cr, Fe, Pb, Sb, Se, Sn, V and Zn decreased with AMD and WW incubation.

In laboratory studies, passive co-treatment of AMD and MWW was further explored, resulting in a system that efficiently removed key constituents of both effluents. A laboratory-scale, four-stage continuous-flow reactor system was constructed to test the viability of simulated Cerro Rico high-strength AMD and MWW passive co-treatment. The synthetic AMD had pH 2.6 and 1860 mg/L acidity as $CaCO_3$ equivalent and with 46, 0.25, 2, 290, 55, 1.2 and 390 mg/L of Al, As, Cd, Fe, Mn, Pb and Zn, respectively. The AMD was mixed at a 1:2 ratio with raw MWW from the City of Norman, Oklahoma containing 265 ± 94 mg/L BOD_5, 11.5 ± 5.3 mg/L PO_4^{-3}, and 20.8 ± 1.8 mg/L NH_4^+-N and introduced to the system which had a total residence time of 6.6 d. During the 135 d experiment, dissolved Al, As, Cd, Fe, Mn, Pb and Zn concentrations were consistently decreased by 99.8, 87.8, 97.7, 99.8, 13.9, 87.9 and 73.4%, respectively, pH increased to 6.8 ± 0.1, and net acidic influent was converted to net alkaline effluent. PO_4^{-3} and NH_4^+-N were decreased to <0.75 and 7.4 ± 1.8 mg/L, respectively. BOD_5 was generally decreased to below detection limits. Nitrification increased NO_3^- to 4.9 ± 3.5 mg/L NO_3^--

N, however relatively little denitrification occurred. Sulfate reducing bacteria were able to maintain a relatively high level of sulfate reduction (0.56 mol/m^3-d). A 100% reduction of all fecal indicator bacteria was observed. Results indicated that passive AMD and MWW co-treatment is a viable ecological engineering approach for the developed and developing world that can be optimized and applied to improve water quality with minimal use of fossil fuels and refined materials.

Field studies of EFVM illustrated that these systems could encourage water quality and temperature changes conducive to the passive treatment of various constituents. Four EFVM designs were constructed of drainpipe, burlap, mulch, utility netting, and reused polyethylene bottles then planted with *Typha* spp. and *Juncus effusus*. The water column beneath the EFVM in two test ponds was compared to that in an open water control pond. Dissolved oxygen concentrations and pH were lower, diurnal temperature range was dampened, and sulfate/nitrate reduction was greater under the EFVM with respect to the control. Alkalinity was also greater under EFVM. Although plant propagation was limited, results suggest that EFVM may be applied to encourage reducing, thermally insulated conditions for passive treatment of AMD and a wide range of other pollutants. Specifically, they may be employed to improve immediate and long-term performance of vertical flow bioreactors for AMD treatment by lowering dissolved oxygen concentrations in the water column and providing a continual source of organic carbon to the underlying substrate.

CHAPTER I

Introduction

This dissertation addresses augmenting and expanding the design options available to the ecological engineer by exploring new sustainable water quality improvement techniques. Ecological engineering entails “the design of sustainable ecosystems that integrate human society with its natural environment for the benefit of both” (Mitsch, 1996) and this dissertation specifically addresses several novel applications of this field: (i) the efficacy of passive treatment of AMD in high-altitude, low-productivity environments in the developing world, (ii) the co-treatment of acid mine drainage (AMD) and municipal wastewater (MWW), and (iii) the utility of ecologically engineered floating vegetation mats (EFVM) to impact water quality.

The passive treatment of single waste sources, e.g, AMD and MWW alone, is a common proven ecological engineering application (Mitsch and Jorgensen, 2004; Kadlec and Wallace, 2009). However, adoption of ecological engineering practices has lagged in the developing world (Rusong et al., 1998) despite the relative advantages that ecological engineering solutions hold over conventional engineering approaches in these settings (Rusong et al., 1998; Nelson et al., 2001). This first chapter provides an outline for the following chapters, which present sustainable approaches for increasing the applicability, longevity and efficiency of passive AMD and MWW treatment systems for water quality improvement in the developed and developing world.

High mountain environments often are the location of economically recoverable mineral deposits. The Andes of South America host some of the world’s great mineral deposits, many of which have been mined for centuries (Miller and Singewald, 1919) and will be mined far into the future (Rice and Steele, 2005). Chapter II characterizes AMD discharges on the world's largest silver deposit, Cerro Rico (Rich Hill) of Potosí, Bolivia, which is situated on the eastern flank of the Andes. Cerro Rico has been intensively mined since the mid 16th century using a variety of technologies, creating a diversity of environmental problems primarily centered around mineral processing effluent and AMD. Local residents are subject to increased trace metals exposure and degraded irrigation water (Choque, 2007; Strosnider et al., 2007, 2008). Although downstream impacts have been documented (Hudson-Edwards et al., 2001; Miller et al., 2002, 2004; Smolders et al., 2002, 2003, 2004; Archer et al., 2005), there exists no published study of specific AMD sources on Cerro Rico. Chapter II provides that study.

Chapter III illuminates the need to address Cerro Rico AMD by explicitly linking contamination from AMD sources to downstream water quality degradation that places impacted waters below Bolivian and international standards for irrigation water. Many of the first- through fifth-order streams and rivers near Cerro Rico are used extensively for irrigation of edible root crops which may pose a human health risk to the indigenous population. Chapter III documents considerable environmental impacts from AMD, demonstrating the need for treatment technologies appropriate to the developing world.

Chapter IV describes microcosm field studies that demonstrate the feasibility of passively treating Cerro Rico AMD via limestone exposure or incubation with MWW. Passive treatment, which relies on natural biogeochemical and microbiological processes to improve water quality, is well-established in the developed world (Younger et al., 2002; Watzlaf et al., 2004). However, few passive treatment studies have been conducted in the extreme environment of the Andes (Younger, 2007) and none of these focused on the possibility of using untreated MWW as an organic carbon source. The study described in Chapter IV was designed to explore these promising ecological engineering options.

Chapter V builds upon chapter IV by extensively exploring the passive co-treatment of high strength AMD (such as that from Cerro Rico) and raw MWW. AMD and MWW are prevalent across the globe and their treatment is central to maintaining global water resource quality (e.g., Gadgil, 1998; Kivaisi, 2001; Nelson et al., 2001; Wolkersdorfer and Bowell, 2004a, 2004b, 2004c). Although Roetman (1932) first suggested mixing AMD with MWW for pathogen removal, very few systems have been intentionally constructed to simultaneously treat these effluents and performance data are sparse (Rose et al., 1998; Van Hille et al., 1999; Johnson and Younger, 2006). The author is not aware of any high-strength AMD and raw MWW co-treatment investigation, which is essential to addressing the approach's feasibility and applicability. Chapter V describes the results of a laboratory, flow-through, multi-stage, treatment mesocosm experiment and reveals that passive co-treatment of high-strength AMD and MWW is a highly efficient ecological engineering approach to address primary constituents of interest in each waste stream. If AMD and MWW could be treated passively and simultaneously within the same system, many locales not currently treating AMD or MWW could inexpensively and sustainably improve local water quality.

Chapter VI describes a field study which evaluates a new sustainable ecological engineering method of designing an ecosystem, in this case an EFVM, to optimize conditions in the underlying water column for the treatment of AMD and other effluents. Although tests and/or full-scale applications have been few, EFVM have been applied to treat meat processing effluent (Van Oostrom, 1995), improve lake water quality (Boutwell, 2001), treat dilute de-icers from airport runoff (Revitt et al., 2001; Richter et al., 2003), and treat AMD (Smith and Kalin, 2000; Kalin and Caetano Chaves, 2003; Kalin, 2004; Kalin et al., 2006). In addition, EFVM design specifications and performance are lacking in the literature. Results presented in Chapter VI suggest that EFVM may be applied to encourage reducing, thermally-insulated conditions for sustainable passive treatment of AMD and a wide range of other pollutants. EFVM may be especially valuable in cold climates, such as the Andean highlands, where temperatures limit treatment rates.

Overall, this dissertation illuminates novel ecological engineering approaches to address water quality issues in the developed and developing world. The goals to augment the applicability and efficiency of ecologically engineered water quality improvement were realized. Applicability was expanded by demonstrating that passive AMD treatment or co-treatment with MWW is viable in highland Bolivia, AMD and MWW co-treatment is a feasible approach for effluents formerly thought to be passively untreatable, and EFVM may be able to create the setting for more sustainable and effective passive treatment in colder climates. The following chapters also describe that AMD and MWW co-treatment can be more efficient than active or passive methods for AMD or MWW treatment alone and EFVM incorporated into conventional passive AMD treatment unit processes would likely increase treatment efficiency. Ecological engineering applications hold promise for sustainably addressing water quality degradation in the developed and developing world, but expansion of their applicability and efficiency, as described in this dissertation, is key to their successful adoption.

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Chapter II

The Legacy of Five Centuries of Intensive Mining Cerro Rico de Potosí, Bolivia: Extreme Acid Mine Drainage Source Identification and Characterization

Portions of this chapter are included in:

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2.1 Abstract

Intensive mining and processing of Ag, Sn, Pb and Zn ores have occurred in various locations within and around the city of Potosí, Bolivia since 1545. Surface and subsurface waters, stream sediments and soils are contaminated with various ecotoxic metals. Acid mine drainage (AMD) is an important contamination source in the headwaters of the economically vital, yet highly impacted, Rio Pilcomayo watershed. Previous studies have documented downstream heavy metal contamination however not addressed their specific sources. The AMD discharges identified in this study help link

downstream pollution to primary origins. The majority of AMD would be considered high-strength due to metals and acidity concentrations orders of magnitude greater than typical AMD. Discharges from both operating and abandoned portals as well as tailings-related deposits displayed a high degree of heterogeneity with total metal concentrations ranging from 0.11-7,48l, <0.022-889, <0.0006-65.3, <0.001-310, 0.12-72,100, 0.3-402, <0.012-34.8, and 0.24-19,600 mg/L of Al, As, Cd, Cu, Fe, Mn, Pb and Zn, respectively. Net acidity and pH ranged from -10 to 246,000 mg/L as $CaCO_3$ equivalent and 0.90-6.94 standard units, respectively. Data were gathered during two sampling events centered around the most extreme periods of the dry and wet seasons of one water-year. Loadings to local streams were marginally greater for most metals in the wet season. If observed loadings are historically representative, AMD has contributed thousands of tonnes of ecotoxic metals to the upper Rio Pilcomayo over the last five centuries. Metals and hydrogen ion concentrations in the majority of AMD sampled were several orders of magnitude above discharge limits set by the Bolivian government, yet no action has historically or currently been taken.

2.2 Introduction

2.2.1 Historical Background

Twelve years after Pizarro dethroned Atahualpa and conquered the Inca, the largest Ag deposit in the world was discovered at Cerro Rico (Rich Hill) by the indigenous nobleman Diego Huallpa. The next year the Spanish founded the city of Potosí at Cerro Rico's base with Huallpa's confidant, the Spaniard Juan de Villarroel, registering the first claim in 1545 (Wilson and Petrov, 1999). Within a hundred years Potosí became one of the world's richest and most populous cities during a boom fueled by Cerro Rico veins of up to 25% pure Ag (Wilson and Petrov, 1999; Bartos, 2000; Abbot and Wolfe, 2003; Waltham, 2005). Mining has proceeded nearly continuously over the last five centuries and it is estimated that between 20,000 and 40,000 tonnes of Ag were produced from 1545 to 1824 and over 10,000 tonnes from 1824 to present (Lindgren, 1928; Zartman and Cunningham, 1995; Pretes, 2002; Abbot and Wolfe, 2003). Abbot and Wolfe (2003) also postulate that thousands of tonnes of Ag were produced from Cerro Rico and nearby deposits in Pre-Colombian times before and after the Incan conquest from the 10th to the 15th centuries. Ores from Potosí subsidized Spanish wars in Europe while millions of forced indigenous and slave African laborers died premature deaths mining the depths of Cerro Rico and processing the ores found within (Galeano, 1971; Tandeter, 1981; Bakewell, 1984). Ag, Sn, Pb and Zn have been the primary metals mined and processed in and around the slopes of Cerro Rico, sustaining the city economically for centuries. However, the environmental cost of Potosí's good fortune has been steep. Terrestrial zones have experienced extreme deforestation and associated soil loss (Godoy, 1990).

Local watercourses have been impacted by mineral processing effluent and unmitigated acid mine drainage (AMD).

2.2.2 Acid Mine Drainage

Economically-valuable geologic deposits such as coal and metal ores are normally chemically stable under undisturbed in-situ conditions. AMD forms when isolated sulfide minerals, such as pyrite (FeS_2), sphalerite (ZnS) and galena (PbS), are exposed to oxygen and water (Younger et al., 2002). Microbes such as *Acidithiobaccillus ferrooxidans* increase the rate of AMD evolution by catalyzing mineral oxidation (Younger et al., 2002). This mineral oxidation creates and mobilizes free metal, sulfate and hydrogen ions into solution (equations 1-5):

$$2FeS_2(s) + 7O_2(aq) + 2H_2O \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+ \quad (1)$$

$$2Fe^{2+} + \tfrac{1}{2}O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \quad (2)$$

$$2Fe^{3+} + 6H_2O \rightarrow 2Fe(OH)_3(s) + 6H^+ \quad (3)$$

$$14Fe^{3+} + FeS_2(s) + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (4)$$

$$ZnS(s) + 2O_2(aq) \rightarrow Zn^{2+} + SO_4^{2-} \quad (5)$$

Pyrite oxidation (Equations 1-4) is the primary agent of AMD formation. The acidity generated by pyrite oxidation lowers the pH, increasing the solubility of many metals and allowing faster weathering of other metal sulfides. Weathering of other metal sulfides will not necessarily produce acidity, but will release metal ions to solution (Younger et al., 2002). Sphalerite oxidation (Equation 5) is an example of these reactions which may release Zn, Pb, Ni, Cd, Cu, As and other ecotoxic metals to solution often to be transported to discharge points such as seeps, adits or boreholes which then impact downstream environments (Younger et al., 2002).

The environmental cost of AMD has been known for centuries. The man considered to be the founder of geology as a discipline, Georgius Agricola, stated in the 16th century that "…when the ores are washed, the water which has been used poisons the brooks and streams, and either destroys the fish or drives them away" (Agricola, 1556). The ecotoxic metal ions, acidity and resultant precipitates (such as iron oxyhydroxide often referred to as *ochre*) associated with AMD are a significant threat to freshwater resources and can cause fish-kills and lasting degradation of aquatic habitats (Adams and Younger, 2001; Younger et al., 2002). AMD also often renders receiving watercourses unfit for use as water resources (Adams and Younger, 2001).

2.2.3 Geological Setting

Cerro Rico de Potosí was created by volcanic eruptions of the Tertiary Age. It lies within a Neogene-Quaternary volcanic-plutonic complex stretching for approximately 800 km along the Eastern Cordillera of the Andes (Zartman and Cunningham, 1995; Kamenov et al., 2002). Ore occurs throughout systems of veins in a conical dacitic volcanic dome rising 700 m above the city of Potosí (Zartman and Cunningham, 1995). Argentiferous magma crystallized into cassiterite-rich veins formed in Ordovician slate, dacitic tuff and tuff breccia, and other dacitic stock (Griess, 1951; Brading and Cross, 1972; Rice and Steele, 2005). The veins are enclosed in zones of metal sulfides, oxides and gangue minerals such as quartz, tourmaline, siderite and kaolinite. Silver oxides predominated in the upper altitudes of Cerro Rico while Ag sulfide ores dominate in the lower reaches (Bartos, 2000). Host rock is pyritized near the veins and pyrite is the predominant associated mineral with the sulfide ores and country rock (Lindgren, 1928; Lindgren and Creveling, 1928; Petersen, 1945; Wilson and Petrov, 1999). Pyrite is the prime source of AMD formation and its pervasiveness indicates that AMD will be released to the surrounding areas for decades or centuries unless remedial actions are undertaken. The polymetallic nature of the orebody indicates that this AMD would likely contain elevated concentrations of multiple metals of concern.

2.2.4 AMD Legacy

The history of Potosí, like many historic mining centers, is one of cyclical boom and bust that has likely maximized AMD evolution. Many local mines have been abandoned, flooded, dewatered and mined again multiple times (Hillman, 1984), following the rise and fall of ore prices. There is evidence from Pb-contaminated lake sediments that mining and smelting for Ag production may have begun at Cerro Rico around 1000 A.D (Abbot and Wolfe, 2003). However, intensive mining did not begin until Spanish conquest. Cerro Rico is considered the world's largest Ag deposit and Potosí led the world in production during the 16th and 17th centuries (Lofstrom, 1970; Zartman and Cunningham, 1995; Bartos, 2000; Rice and Steele, 2005). After an initial boom fueled by Ag oxide ores of up to 25% purity, mining lulled from approximately 1555-1575 until the introduction of the mercury amalgamation process (Serrano et al., 1996; Wilson and Petrov, 1999). Ag production peaked in the late 16th century when there were over 600 mines on the mountain working a vertical interval of approximately 250 m (Serrano et al., 1996; Waltham, 2005). Production lulled yet again from approximately 1700 to 1745

(Serrano et al., 1996). After a resurgence from approximately 1745 to 1805, production fell precipitously so that by 1825 Cerro Rico was home to more than 5,000 open mine shafts and adits, most of which were abandoned, flooded or collapsed (Lofstrom, 1970). Of those shafts, only 50-60 were in use and the lower majority of the mountain was flooded (Lofstrom, 1970). In the 1800s Potosí's population had fallen from a maximum of approximately 160,000 during the Ag boom of the 1600s to about 10,000 (Pretes, 2002).

Resurgence of the Ag industry from 1850-73 enabled by lower Hg prices (Hg was used in the amalgamation technique for processing Ag sulfides) caused the short-lived dewatering and re-start of many mines (Hillman, 1984). In the latter quarter of the 1800s, Potosí's fortunes rebounded yet again with the emergence of the Sn industry as the Ag industry declined (Hillman, 1984). An indication of the rich polymetallic nature of the Cerro Rico ore body, another metal, Cu, was mined and processed in the early 1900s (Miller and Singewald, 1919; Cunningham et al., 1996). As of 1928, many veins in Potosí had been worked over a vertical interval of approximately 600 m (Lindgren, 1928). The pinnacle of the Sn industry was in the first half of the 20th century when Bolivia was one of the top three worldwide Sn producers during World War II (Griess, 1951). Although the Sn industry was first established by the Incas, Sn only surpassed Ag in economic importance around the turn of the 20th century (Hillman, 1984; Godoy, 1985). At this time Sn miners dewatered and worked former Ag mines as well as alluvial deposits (*pallacos*) at the base of Cerro Rico (Bartos, 2000; Waltham, 2005). However, the 1985 Sn price collapse caused the closure and flooding of many Potosí mines (Waltham, 2005; Younger, 2007). Mineral prices have risen in recent years, leading to yet another boom cycle of dewatering and ore exploitation. In 1996, Serrano et al. (1996) reported that over 500 shafts and adits were in operation, worked by about 5,000 miners. As of 2000, mine workings had extended to a vertical interval of 1,150 m (Bartos, 2000). Currently, it is estimated that approximately 20,000 miners are either reworking old tailings and mine workings or opening new workings in deeper zones of Cerro Rico mining primarily Pb and Zn ores, and to a lesser extent, Ag and Sn ores. The repeated flooding, dewatering and mining of Cerro Rico has likely led to near-continuous production of high-strength AMD for centuries as freshly exposed sulfide minerals contact water during non-operational periods. The subsequent dewatering allows for oxygen ingress and fresh mineral exposure as the cycle is repeated.

Broad and progressive Bolivian environmental regulations became law in 1992 (BMSDP, 2000). The law (Number 1333) regulates pollutant discharges of nearly all industries, sets water quality standards for receiving bodies and establishes limits for liquid discharges. However, it appears to have been largely ignored by the mining industry (Garcia-Guinea and Harffy, 1998). Article 45 of the Bolivian Mining Code states that mining operations should use systems and technology compatible with environmental protection (Bocangel, 2001). The Bolivian government issued and widely publicized Supreme Decree 25419, requiring all mining operations to obtain an environmental license (Bocangel, 2001). Supreme Decree 25877 extended the deadline for compliance and has also met very limited success (Bocangel, 2001). It is likely that, in addition to discharging AMD and mineral processing effluent out of compliance with Bolivian law, many mining facilities have not acquired an environmental license.

2.2.5 Previous Environmental Studies

Intensive mining over several centuries has devastated the aquatic environment around Potosí. Hudson-Edwards et al. (2001), Smolders et al. (2002, 2003, 2004), Miller et al. (2002, 2004) and Archer et al. (2005) documented dissolved and total ecotoxic metals concentrations orders of magnitude greater than natural background levels in Rio Tarapaya from the western edge of Potosí's city limits to ~500 km downstream in Rio Pilcomayo. Miller et al. (2002) linked this contamination to mining activity via isotopic analysis of Pb in river sediment, however the specific contaminant sources (i.e., AMD, mineral processing effluent, tailings dam erosion, etc.) could not be identified.

Although downstream heavy metals contamination has been fairly well-documented, contamination sources have not. No peer-reviewed studies of Cerro Rico AMD or the other probable ecotoxic metal sources have been encountered in the literature. The following study was conducted to characterize one of the major sources of ecotoxic metals pollution from Cerro Rico mining operations to the upper Rio Pilcomayo to better understand the relationship between downstream pollution and upstream sources.

2.3 Methods

2.3.1 Study Area

The study centered around Cerro Rico, approximately 1 km south of the city of Potosí, Bolivia. Potosí (19.585°S 65.754°W) lies in the Eastern Cordillera range of the Central Andes in the upper reaches of the Rio Pilcomayo watershed. As the entirety of Cerro Rico and immediate environs can essentially be considered a mine, all groundwater discharges noted were classified as AMD. AMD identified and characterized were from active and abandoned mine portals of Cerro Rico and seeps influenced by tailings deposits and/or the mineral-rich *pallacos* at the northwest base of Cerro Rico (Figures 2.1-3). The AMD documented in this study are those which were flowing during Potosí's dry season of July/August 2006 and wet season of March 2007 and those not intercepted for mineral processing use. Innumerable working and abandoned mine portals dot Cerro Rico, however the majority were observed to not produce effluent. Portals likely to be producing drainage near the base of Cerro Rico were visited in this field study. The majority of those portals were not flowing at the time. Active mine portal, abandoned portal, and tailings-related discharges were assigned the prefixes "C", "A" and "T", respectively. Of the thirteen AMD sources identified and characterized on Cerro Rico proper, seven were from active mine portals, five were from abandoned portals and one was from a tailings deposit. Three tailings-related AMD sources were located at the northwest base of Cerro Rico.

Discharges 1A, 3A, 4T and 1-4C (Figure 2.1) drain to Rio Huayna Mayu which empties into the highly polluted Ribera de la Vera Cruz, which also contains residual tailings from past mineral processing plant discharges and raw sewage from the city. Discharges 2A, 4-5A and 6C drain to Quebrada Chimborazo which shortly thereafter confluences with Rio Villacollu Mayu. Discharge 5C drains to the highly impacted Rio Jayac Mayu ("spicy river" in Quechua, the language of the Inca) which also may receive diffuse acid rock drainage (ARD) from numerous waste rock piles near its source. Discharge 7C drains to Rio Sucu / Kori Mayu which also receives AMD from the *pallacos* zones (discharges 1-3T). The *pallacos* discharges are associated with natural and anthropogenic erosion of mineral-rich material from Cerro Rico and the tailings from

Sn mining of that material that ceased decades ago (Bartos, 2000; Waltham, 2005). These sources are also likely influenced by groundwater seepage from the San Miguel tailings dump, which lies between Rio Sucu / Kori Mayu and the Ribera de la Vera Cruz. The Pailaviri tailings deposit (discharge 4T) on the slope of Cerro Rico drains to Huayna Mayu (Figure 2.3). A few AMD sources (7C, 4A, 5A and 4T) were only sampled in the wet season because they were not flowing during the dry season.

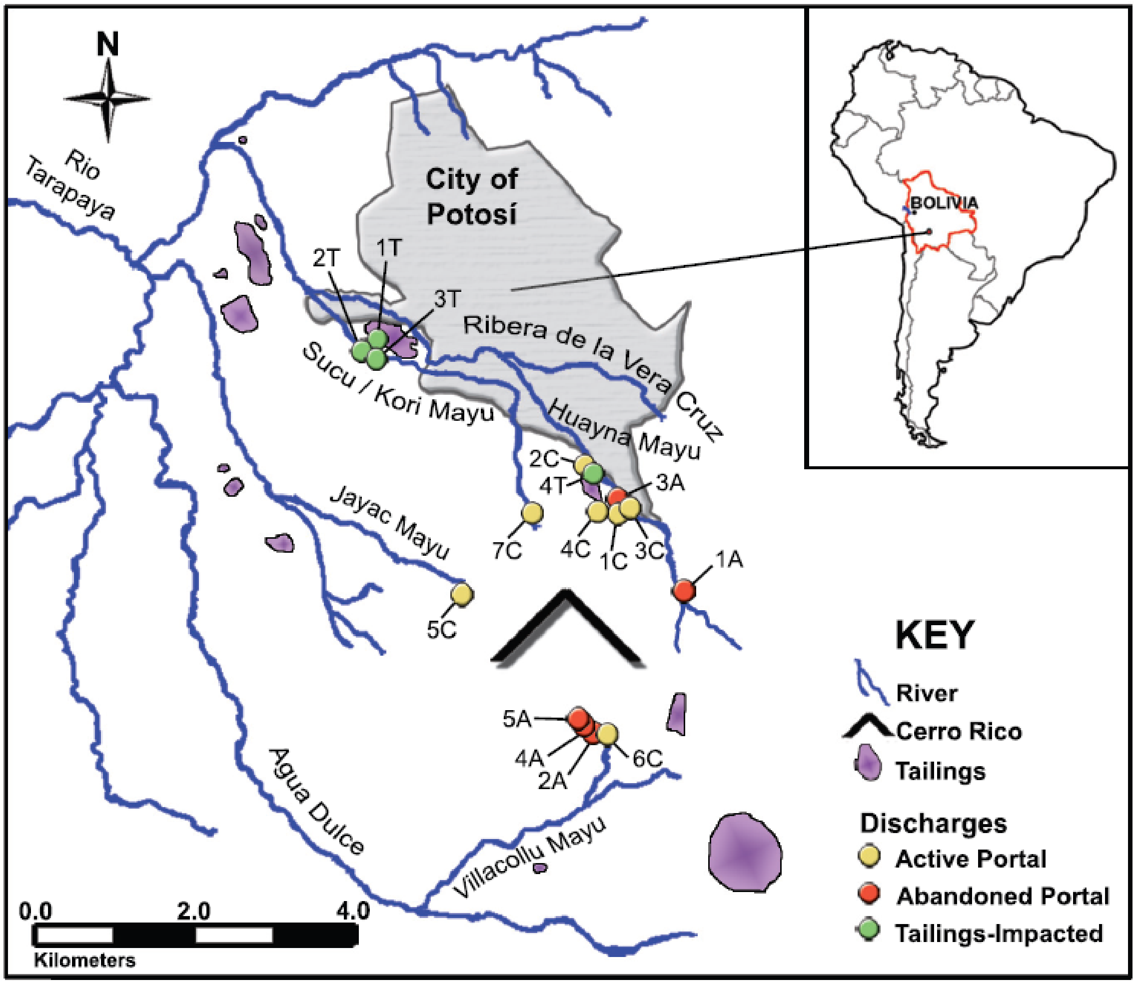

Figure 2.1 Study area and AMD discharges with respect to receiving streams, tailings deposits, greater Bolivia and South America.

Figure 2.2 AMD sources 1A (left) and 1C (right). Source 1A is an abandoned portal near the eastern base of Cerro Rico. Source 1C is a functioning mine portal on the north slope of Cerro Rico.

Figure 2.3 A portion of the Pailaviri tailings deposit which lies less than 100 m from Huayna Mayu, which confluences with the Ribera de la Vera Cruz less than 2 km from the location pictured.

2.3.2 Data Collection

Water quality parameters and grab samples were obtained at the points indicated on Figure. 1 during the dry (July-August 2006) and wet (March 2007) seasons of one water-year (Figure 2.4). These intervals were chosen in an attempt to capture the groundwater level/efflux and surface water flow extremes of both seasons, when are near their minima (dry) and maxima (wet). Sampling periods were offset from the minima and maxima of the monthly average precipitation because of the common response lag of groundwater level/efflux and surface water flows to precipitation events (Eltahir and Yeh, 1999).

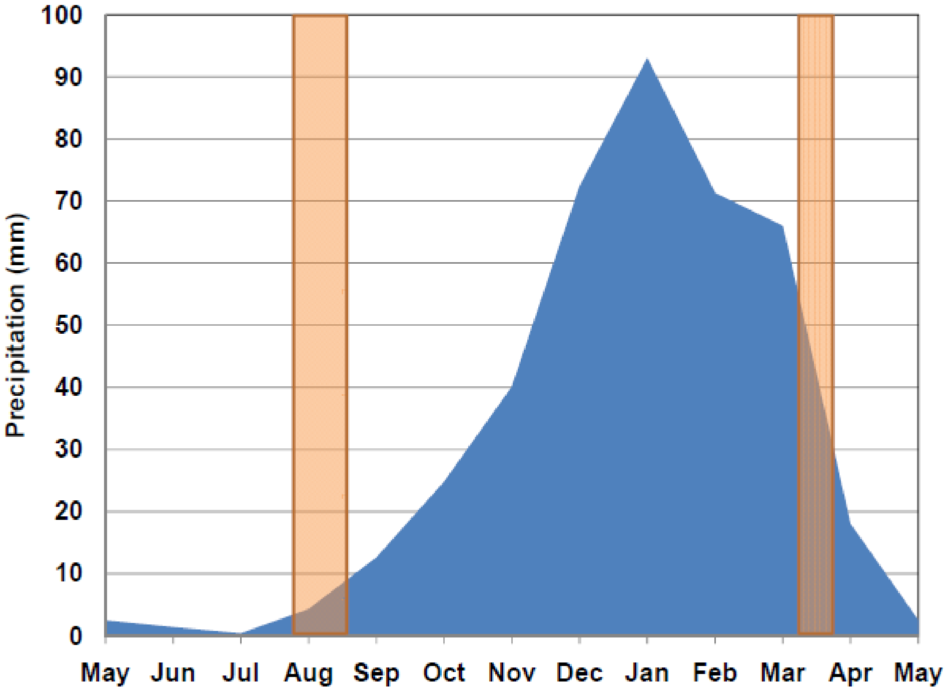

Figure 2.4 Sampling periods (orange) with respect to monthly average precipitation in the City of Potosí from 1958-2002 (BSNMH, 2003).

The location of each AMD discharge was recorded with a Garmin® GPS unit. Alkalinity titrations were conducted in the field following standard methods (APHA, 1998). Temperature, pH, dissolved oxygen (DO) and specific conductance (SC) were determined using a properly calibrated Orion 1230 multimeter. All grab samples were taken using 125-mL HDPE containers for subsequent analysis at the University of Oklahoma Center for Restoration of Ecosystems and Watersheds (CREW) laboratories. Samples for anion analyses were stored at 4 °C until filtered through 0.2 µm nylon filters and Dionex OnGuard® II H cartridges. A MetrOhm® 761 Compact ion chromatograph unit was used to quantify SO_4 concentrations following EPA method 300. Total metals samples were immediately preserved with concentrated nitric acid and stored at 4 °C until microwave acid digestion. Dissolved metals samples were first filtered through 0.45 µm nylon filters then preserved with concentrated nitric acid and stored at 4 °C until microwave acid digestion. All microwave acid digestions followed EPA method 3015. Digested metals samples were filtered through 0.45 µm nylon filters then analyzed via a Varian Vista-Pro® simultaneous inductively coupled plasma-optical emission spectrometer (ICP-OES) following EPA method 6010. Acidity was calculated following a modified version of that presented by Younger et al. (2002), which accounted for the acidity from the substantial Cd^{2+}, Cu^{2+} and Zn^{2+} concentrations present in these unique waters. For acidity calculations, all Fe was assumed to be Fe(II) when $pH > 3$ and Fe(III) when $pH < 3$, because Fe(III) predominates for most solutions with $pH < 3$ (Kirby and Cravotta, 2005). This assumption generally follows empirical observations (Younger et al., 2002; Watzlaf et al., 2004; Kirby and Cravotta, 2005). Hedin (2006) demonstrated an excellent relationship between calculated and measured net acidity for a diverse data set of 1,484 AMD sources.

Flow rates (Q) were obtained via two methods. When possible, flow rates were obtained by building temporary weirs and determining time to gather a known volume in a bucket or graduated cylinder. When flow rates were greater than this method would allow, discharge was estimated by determining channel cross-section, depth and velocity as approximated by floating a partially submersible object a given distance. Wet and dry season loadings were determined by multiplying total metals concentrations by their respective flow rates. Where flow rates were too low or diffuse to determine using the aforementioned methods, a conservative flow rate of 0.01 L/s based on comparative observation was assigned to enable the prudent estimation of metal loadings.

2.4 Results and Discussion

Physiochemical parameters, total and dissolved metal concentrations, and SO_4 concentrations for sampled AMD are presented in Tables 2.1 and 2.2. These results are compared to various water quality criteria set by the Bolivian government in Table 2.3. Generally, dissolved metal concentrations were similar to total metal concentrations, indicating that the bulk of metals discharged to receiving streams from mine water discharges are in the more mobile, dissolved form. Metals, SO_4 and acidity concentrations in AMD varied by several orders of magnitude, indicating a high degree of heterogeneity within the groundwaters of Cerro Rico. Metals are not uniformly disseminated throughout Cerro Rico which is reflected by these data. The majority of AMD would be considered high-strength AMD due to metals and acidity concentrations orders of magnitude greater than typical AMD (e.g., Younger et al., 2002; Watzlaf et al., 2004). Concentrations of contaminants in most sampled AMD were orders of magnitude out of compliance with Bolivian discharge limits and likely contributed to the aforementioned pollution downstream.

Table 2.1 Mean physical parameter measurements, net acidity and SO_4 concentrations, and flow rates of Cerro Rico AMD in the dry and wet season. Dry season data are unshaded and wet season data are shaded grey. N=1 except where otherwise noted. Net acidity calculations used total and (dissolved) metal concentrations. pH is bolded where exceeding the monthly Bolivian discharge limit.

Site	pH s.u.	DO mg/L	SC µS/cm	Net Acidity[a] mg/L as $CaCO_3$ eq.	SO_4 mg/L	Q L/s
1C	**2.97**	3.5	10440	9870		0.03
1C	**3.24**	3.6	6370	7280(6860)	6480	0.05
2C	**3.30**	0.8	9470	19300		0.01
2C	**3.10**	2.5	6800	24400	19300	0.10
3C	**3.02**	4.08	19070	25400		0.01
3C	**3.20**	3.80	22000	47800	34000	0.05
4C	**3.15**	4.40	8690	16300		0.13
4C	**3.60**	2.70	4290	8370(8850)	8830	0.18
5C	**2.46**	6.10	18640	39900		0.28
5C	**3.39**	3.75	9290	11200	8720	0.28
6C[ε]	**3.25**	2.15	3160	2190	3400	0.17
6C	**3.56**	7.20	3780	3350(3190)	3270	0.08
7C	**2.11**	3.1	23200	31600(28800)	23000	0.05
1A[η]	**3.56**	6.63	1888	1150	1320	0.86
1A[ε]	**4.52**	6.39	1457	870(455)	631	0.86
2A[ε]	**2.90**	1.33	7530	11100	9180	0.02
2A	**2.30**	0.10	11300	12500(12200)	8740	0.18
3A	**3.02**	2.91	14900	25200		0.07
3A	**3.60**	3.00	9230	12300(11500)	10000	0.02
4A	6.94	5.70	345	-6.61(-6.51)	232	0.03
5A	6.9	5.55	272	-10.0(-37.3)	257	0.06
1T	**2.96**	2.50	2820	1400		0.26
1T	**2.98**	3.75	2710	1050(1020)	1730	0.72
2T	**4.20**	4.65	1115	78.4		4.6
2T	**4.08**	1.00	1174	83.1(82.1)	498	1.1
3T	6.49	6.60	893	-46.9		0.10
3T	6.40	3.79	960	-30.9(-32.0)	322	0.33
4T	**0.90**[π]	ND	54600	246000(239000)	175000	0.03

ND = Not determined; [η] n = 3; [ε] n = 2; [a]Calculated net acidity = acidity – alkalinity
[π]Value is suspect due to the extreme pH

Table 2.2 Mean total and (dissolved) metal concentrations determined for grab samples of Cerro Rico AMD. Dry season data are unshaded and wet season data are shaded grey. As, Cd, Cr, Cu, Fe, Pb and Zn concentrations are bolded where exceeding the monthly Bolivian discharge limit. Total Cr concentrations were compared to more conservative Cr(III) discharge limits because it is the more commonly prevalent species at the pH conditions noted (Stumm and Morgan, 1996).

Site	Al	As	Cd	Co	Cr	Cu
			mg/L			
1C	273	**0.57**	**12.4**	2.28	0.030	**1.76**
1C	211(196)	**1.35**(0.65)	**10.7**(11.2)	2.08(2.02)	0.034(0.032)	**2.83**(2.75)
2C	461	**29.6**	**7.19**	4.05	0.09	**10.8**
2C	420	**85.2**	**12.2**	2.68	0.12	**30.7**
3C	752	**34.4**	**35.9**	3.93	0.13	**12.9**
3C	1110	**125**	**65.3**	7.22	**0.63**	**39.6**
4C	648	**40.7**	**21.2**	2.51	0.22	**84.9**
4C	306(258)	**30.6**(4.66)	**10.3**(11.0)	1.35(1.84)	0.29(0.17)	**49.5**(44.0)
5C	1120	**180**	**36.2**	4.03	0.43	**152**
5C	440	**8.46**	**6.94**	2.21	0.16	**5.91**
6C	110	**13.6**	**0.57**	1.05	0.031	0.39
6C	64.1(64.1)	**15.0**(8.31)	**4.73**(4.99)	0.70(0.75)	0.049(0.042)	0.32(0.28)
7C	1300(1170)	**138**(125)	**10.6**(11.1)	2.79(2.66)	**1.10**(1.10)	**227**(232)
1A	5.76	0.030	**0.17**	0.14	0.002	0.037
1A	25.7(8.97)	**0.94**(0.022)	**0.43**(0.38)	0.079(0.057)	0.011(<0.001)	0.13(0.040)
2A	85.0	**21.5**	**7.79**	0.59	0.031	0.047
2A	77.1(80.5)	**41.5**(38.8)	**9.42**(9.84)	0.73(0.74)	0.042(0.043)	0.13(0.12)
3A	810	**7.35**	**48.5**	5.58	0.15	**10.5**
3A	446(437)	**8.44**(7.15)	**16.5**(17.3)	2.02(2.46)	0.14(0.14)	**29.9**(29.9)
4A	0.11(0.023)	<0.022(<0.022)	<0.0006(0.001)	<0.001(<0.001)	<0.001(<0.001)	<0.001(0.003)
5A	3.82(0.052)	<0.022(<0.022)	0.0008(<0.0006)	<0.001(<0.001)	0.001(<0.001)	<0.001(0.002)
1T	167	<0.022	**0.72**	0.33	0.006	**23.6**
1T	123(115)	<0.022(<0.022)	**0.55**(0.64)	0.27(0.30)	0.005(0.007)	**20.0**(20.3)
2T	8.12	<0.022	0.087	0.018	0.004	**0.60**
2T	8.27(8.06)	<0.022(<0.022)	0.10(0.10)	0.022(0.021)	<0.001(<0.001)	**0.68**(0.68)
3T	0.28	<0.022	0.025	<0.001	0.002	0.030
3T	0.48(0.27)	<0.022(<0.022)	0.035(0.035)	<0.001(0.002)	<0.001(<0.001)	<0.001(0.044)
4T	7480(7390)	**889**(821)	**16.6**(15.1)	14.8(8.3)	**2.51**(2.56)	**310**(301)

[η] n = 3

[ε] n = 2

Table 2.2 (cont.)

Site	Fe	Mn	Ni	Pb	Zn
			mg/L		
1C	**796**	18.3	2.92	0.211	**4000**
1C	**896**(795)	18.8(17.0)	3.08(2.86)	**0.50**(0.29)	**2880**(2780)
2C	**6320**	402	4.26	**1.40**	**3050**
2C	**6390**	221	4.20	**2.82**	**6630**
3C	**2580**	33.6	6.36	**2.53**	**10800**
3C	**6310**	56.0	11.2	**34.8**	**19600**
4C	**3050**	84.6	1.87	**1.07**	**4500**
4C	**1750**(1710)	74.8(73.7)	2.01(1.71)	**13.1**(0.61)	**3460**(2690)
5C	**6410**	51.7	2.55	**17.5**	**10500**
5C	**2052**	114	2.15	**5.41**	**3180**
6C	**512**	54.2	0.56	**7.36**	**351**
6C	**637**(587)	43.4(42.2)	2.26(0.55)	**6.05**(1.21)	**1140**(1100)
7C	**8130**(7410)	9.60(6.31)	2.83(2.56)	**5.65**(1.28)	**1170**(1120)
1A	**111**	13.2	0.16	0.065	**574**
1A	**105**(14.3)	14.1(9.94)	0.087(0.067)	**3.23**(0.22)	**271**(226)
2A	**2000**	135	0.82	**0.50**	**3240**
2A	**2410**(2410)	122(125)	0.92(0.96)	**1.46**(1.67)	**3360**(3130)
3A	**3240**	44.9	5.92	**0.64**	**9620**
3A	**2140**(1920)	45.8(46.5)	2.91(2.60)	**0.65**(0.60)	**3810**(3050)
4A	0.12(0.15)	3.95(4.09)	<0.004(<0.004)	<0.012(<0.012)	0.24(0.43)
5A	**3.12**(0.072)	0.42(0.060)	<0.004(<0.004)	0.043(<0.012)	0.26(0.11)
1T	**49.1**	48.5	0.29	0.17	**103**
1T	**27.8**(29.0)	39.4(40.4)	0.23(0.25)	0.036(0.049)	**87.0**(90.3)
2T	0.36	3.65	0.078	0.042	**14.3**
2T	0.14(0.14)	4.23(4.08)	0.058(0.058)	<0.012(<0.012)	**15.5**(15.9)
3T	0.15	0.30	0.026	0.025	**4.84**
3T	0.13(0.028)	0.79(0.80)	0.022(0.023)	<0.012(<0.012)	**6.32**(6.46)
4T	**72100**(70500)	96.1(91.5)	11.4(11.6)	**24.8**(26.1)	**1660**(1630)

Table 2.3 Bolivian discharge and receiving water body criteria

Standard	pH	Al	As	Cd	Co	Cr $^{\alpha}$	Cu	Fe	Mn	Ni	Pb	Zn	SO_4
	s.u.						mg/L						
Daily discharge	6-9		1.0	0.3		1.0/0.1	1.0	1.0			0.6	3.0	
Monthly discharge	6-9		0.5	0.15		0.5/0.05	0.5	0.5			0.3	1.5	
Class "A"	6.0-8.5	0.2	0.05	0.005	0.1	0.05	0.05	0.3	0.5	0.05	0.05	0.2	300
Class "B"	6-9	0.5	0.05	0.005	0.2	0.6/0.05	1.0	0.3	1.0	0.05	0.05	0.2	300
Class "C"	6-9	1.0	0.05	0.005	0.2	0.5/0.05	1.0	1.0	1.0	0.5	0.05	0.2	300
Class "D"	6-9	1.0	0.1	0.005	0.2	1.1/0.05	1.0	1.0	1.0	0.5	0.05	0.2	400

$^{\alpha}$Cr (III) and Cr (VI) limits respectively

The active mine portals generally had higher metals concentrations than the abandoned and tailings-related sources (Table 2.2). The tailings-impacted *pallacos* discharges (1-3T) generally exhibited lower metals concentrations than the other abandoned and active mine sources. This is likely due to the increased weathering that the alluvial deposits have experienced. These deposits were created over millennia as surface rock of Cerro Rico weathered and was transported downhill. Much of the sulfides on the exposed surfaces of the alluvial deposits have likely been weathered, leaving more inert material that produces AMD or ARD of lower metals concentrations and acidity. The freshly exposed sulfides within Cerro Rico are likely leading to higher metals concentrations in the drainage of the active mines. Cerro Rico has been mined for centuries at irregular unsystematic intervals and many workings are interconnected due to the degree of exploration and common collapse features (Petersen, 1945; Cunningham et al., 1996). The active mine portals shared the same general pH and metals concentrations as three of the abandoned portals (1-3A). The abandoned mine portals sampled in this study are likely connected to and receive waters from active workings, thus sharing in the effect of the ongoing fresh sulfide mineral exposure.

The seep from the Pailaviri tailings deposit (4T) produced the most acidic and metal-laden AMD identified. The pH and metals concentrations of this seep place it among the most extreme examples of AMD in the world. Dissolved As, Fe and SO_4 concentrations rival those documented at Iron Mountain, which as of 1999 was reported to have the greatest concentrations of these constituents ever recorded in groundwater (Nordstrom and Alpers, 1999; Nordstrom et al., 2000). Another site with acute AMD, the Iron Duke mine in Zimbabwe, demonstrated similar Al and Fe concentrations to the Pailaviri seep

(Williams and Smith, 2000). The Iron Mountain and Iron Duke mines are examples of the most ideal geologic settings for AMD production in the world. The Pailaviri tailings deposit must present a similar geologic setting, and thus a major environmental liability.

The AMD documented in this study are contributing to the downstream violations of Bolivian receiving body water quality limits noted in the following chapter. Class “D” is the lowest designation of Bolivian receiving water bodies in which industrial applications and navigation are the only suitable uses except in extreme circumstances (BMSDP, 2000). Bolivian law states that class “D” waters must be “coagulated, flocculated, filtered and disinfected” prior to domestic use (BMSDP, 2000). It is not known how or if the water bodies downstream of Potosí are designated. However, they are used for agriculture and therefore should be rated at a minimum above class D. The introduction of AMD with concentrations of Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn and SO_4 orders of magnitude greater than class “D” limits is contributing to non-compliance downstream.

All but one of the AMD discharges (4A) exceeded Bolivian monthly discharge limits to some degree and most by orders of magnitude for multiple metals. Half of the sixteen AMD discharges had higher than permissible concentrations of As, Cd, Cu, Fe, Pb and Zn. Fourteen AMD discharges had higher than permissible Zn concentrations, often by three or four orders of magnitude. Thirteen had lower than permissible pH. The data support the assertion of Garcia-Guinea and Harffy (1998) that Bolivian environmental law “has been sadly ignored where mining is concerned.”

Local streams were considerably loaded with ecotoxic metals from Cerro Rico (Table 2.4). Active mine portals contributed the majority of metal loadings in both seasons. All metal loadings except Cd, Cu and Zn were at least marginally greater during the wet season. Combined Al, As, Fe, Mn, Ni and Pb loadings were 26, 12, 88, 76, 45 and 57% greater, respectively, in the wet season. Tailings related discharge loadings were much greater in the wet season when 4T was flowing. The majority of AMD discharges had concentrations of As, Cd, Cu, Pb and Zn orders of magnitude higher than those found downstream in Rio Tarapaya and Rio Pilcomayo by earlier studies (e.g., Hudson-Edwards et al., 2001; Smolders et al., 2003). Overall, the data indicate that these AMD discharges are contributing to some degree to downstream heavy metals pollution in the upper Rio Pilcomayo basin. If observed loadings are historically representative, AMD has contributed thousands of tonnes of ecotoxic metals to the upper Rio Pilcomayo over the last five centuries. Nonpoint source ephemeral ARD sources also exist, due to the prevalence of innumerable ancient and recent ore and tailings dumps upon and around Cerro Rico (Miller and Singewald, 1919; Lindgren and Creveling, 1928; Francis et al., 1981), exposure of ores near the peak with recent and ongoing surface mining, as well as the highly mineralized nature of Cerro Rico's slopes. Despite efforts to locate all AMD sources around Cerro Rico, other point sources likely exist because of the size of Cerro Rico and dynamic nature of ongoing mining. The likelihood of other AMD sources render downstream loadings documented in this study a probable underestimate.

Table 2.4 Seasonal AMD total metal loadings to streams draining Cerro Rico

	Dry Season				Wet Season			
	Operating	Abandoned	Tailings	**Total**	Operating	Abandoned	Tailings	**Total**
				kg/d				
Al	38	5.6	6.9	**50**	31	3.7	28	**63**
As	5.1	0.08	Δ	**5.2**	2.7	0.73	2.4	**5.8**
Cd	1.2	0.33	0.05	**1.6**	0.84	0.20	0.09	**1.1**
Co	0.15	0.5	0.01	**0.21**	0.15	0.02	0.06	**0.23**
Cr	0.01	0.001	0.002	**0.02**	0.02	0.002	0.007	**0.03**
Cu	4.7	0.07	0.76	**5.5**	2.3	0.05	2.1	**4.5**
Fe	210	32	1.2	**240**	200	48	200	**450**
Mn	3.4	1.5	2.5	**7.4**	6.5	3.0	3.1	**13**
Ni	0.11	0.05	0.04	**0.20**	0.21	0.02	0.05	**0.29**
Pb	0.55	0.01	0.02	**0.58**	0.58	0.26	0.07	**0.91**
Zn	330	110	8.0	**450**	280	77	11	**370**

Δ Concentrations below detection limits, loadings inestimable

The relative importance of different pollution sources to the Rio Pilcomayo is unknown. Hudson-Edwards et al. (2001) and Smolders et al. (2003) stress the significance of mineral processing effluent in downstream contamination. However, these studies were undertaken when the tailings load to the upper Rio Pilcomayo was much higher, before the construction of the Laguna Pampa and San Antonio tailings dams which are now the destination of most mineral processing effluent. In addition, no published peer-reviewed studies have documented the mineral processing effluent characteristics and loading rates. This study is the first to characterize AMD sources. However, the data presented in this study were the bare minimum required to extrapolate annual loading to downstream watercourses, and are likely an underestimate of the total loading from point AMD sources because of the probable existence of other sources. Further research, including mineral processing effluent discharge and nonpoint ARD characterization will help solidify the relative importance of the upper Rio Pilcomayo's pollution sources. This information would help establish priorities for future remediation efforts.

2.5 Conclusions and Recommendations

The extraction and export of mineral wealth has dominated the political economy of Bolivia for centuries, however, the importance of mining has declined somewhat in recent decades (Griess, 1951; Hillman, 1984). However, Potosí's economy and roughly 150,000 residents are still heavily dependent upon mineral extraction and processing. Therefore, environmental law enforcement should be carefully applied and fitting solutions presented to lessen the impact of mining operations. Measured by multiple metrics, Bolivia is one of the poorest nations in the Western Hemisphere (World Bank, 2009). Therefore, solutions attempted to address to the mine water pollution problems in Potosí can not be capital-intensive. A labor-intensive solution may be desirable because of high unemployment and underemployment in Potosí, as well as the low cost of local labor.

Passive systems may be more suitable than active systems for AMD treatment in Potosí. Passive treatment uses unrefined natural materials to promote natural chemical and biological processes to improve water quality (Younger et al., 2002). Active treatment, the improvement of water quality by methods that require ongoing inputs of energy and chemical reagents, generally has higher operational costs than passive systems (Younger et al., 2002). Highly mechanized construction or operational activities are not desirable in Potosí because of logistical and supply issues. In developed nations, passive treatment systems have higher up-front costs because of greater land and construction expenses (Younger et al., 2002). However in Bolivia depressed land and low construction costs may make passive treatment a logical solution from both long- and short-term perspectives. The chemicals, electricity and equipment needed for active treatment may make passive treatment by default the most applicable solution. In addition, passive treatment system construction and metal reclamation could provide needed employment for Potosínos.

In 2000, Younger's preliminary experiments indicated passive treatment system feasibility on the slopes of Chacaltaya and Huayna Potosí, near La Paz, a setting similar to the Cerro Rico de Potosí (Younger, 2007). Limestone gravel and llama dung were set in a series of buckets receiving a continuous flow of AMD from the abandoned Milluni mine. The average pH rose from 3.2 to 6.3 and metals were taken out of solution even though the experiment ran through the coldest time of year when reaction rates are lowest (Younger, 2007). There are documented limestone and dolomite deposits around Potosí (Zartman and Cunningham, 1995; Deconinck et al., 2000; Kamenov et al., 2002). The Cayara® lime plant on the outskirts of Potosi currently accepts limestone of 85-92% calcite from local sources, which indicates that high quality limestone for passive treatment is available. Llama dung is readily available around Potosí as domesticated llama herds roam nearby valleys and mountainsides. Other carbon sources for SO_4 reducing bacteria are also available, including domestic sewage, cow dung, brewery waste and waste sugar cane from lowland regions northeast of Potosí. However, the ubiquity of Al and likely prevalence of Fe(III) in high concentrations within the AMD around Potosí precludes the application of anoxic limestone drains and may limit the useful life of other traditional passive treatment systems, such as reducing and alkalinity producing systems. Hybrid active-passive treatment systems or a new type of passive treatment system may need to be developed and applied to sustainably address the situation.

Action is necessary to address the non-compliant AMD sources documented in this study. They are contributing to previously-documented downstream pollution. If environmental laws are to be enforced, care should be taken to ensure that the fragile mineral extraction and processing industries vital to Potosí can continue operation. Passive treatment may prove a suitable solution to the unique circumstances presented in the "Villa Imperial" of Potosí.

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Chapter III

The Legacy of Five Centuries of Intensive Mining Cerro Rico de Potosí, Bolivia: Extreme Acid Mine Drainage Contaminates the Headwaters of the Rio Pilcomayo

Portions of this chapter are included in:

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3.1 Abstract

Ag, Pb, Sn and Zn ores have been intensively mined and processed at Cerro Rico de Potosí, Bolivia since 1545. Acid mine drainage (AMD) and mineral processing plant effluent are prime sources of water contamination in the headwaters of the economically and ecologically vital, yet highly impacted, Rio Pilcomayo watershed. Streams receiving drainage from the slopes of Cerro Rico and surrounding landscapes were sampled during the dry (July-August 2006) and wet (March 2007) seasons of one water-year. In-stream

waters contained total metals concentrations of up to 16 mg/L As, 4.9 mg/L Cd, 0.97 mg/L Co, 1100 mg/L Fe, 110 mg/L Mn, 4.1 mg/L Pb, and 1500 mg/L Zn with pH ranging from 2.8-9.5. AMD-impacted streams contained elevated concentrations of the same major ecotoxic constituents present in AMD discharges at concentrations statistically greater than in those streams unimpacted by AMD. Many of the AMD impacted water bodies are more degraded than class "D" of the Bolivian receiving water body criteria, rendering them unfit for domestic or agricultural use. Natural attenuation is insufficient to render waters safe for use, however some of these waters are currently being utilized for irrigation and livestock watering. The data indicate that historic and current mining activities have transformed these key natural resources into potential human and environmental health hazards.

3.2 Introduction

3.2.1 Historical Background

Cerro Rico de Potosí lies near the headwaters of a major Bolivian watershed, the Rio Pilcomayo, which flows from central Bolivia east to Argentina. Mining joins grazing pressure, farmland expansion, road construction, and urbanization as serious causes of long-term environmental degradation in highland southern Bolivia (Brandt and Townsend, 2006). Potosí has endured water shortages throughout its history and local streams are key resources in the arid, high-altitude, low-productivity landscape (Rudolf, 1936). Contamination limits the uses of the Rio Pilcomayo. Water is precious in the arid Eastern Cordillera and Chaco regions of southern Bolivia (USACE, 2004), one of the poorest nations in the Western Hemisphere (World Bank, 2009).

Aside from tremendous anthropogenic alterations in local groundwater hydrology from intensive mining, surface waters in Potosí bear no physical semblance to the pre-mining landscape. Water resources in and around the city of Potosí (estimated population 150,000) have been dramatically and continuously impacted geomorphologically, geochemically, and ecologically by human activities since the initiation of mining. First, a system of artificial lakes was constructed in the Kari-Kari region east of the city to provide drinking water and hydropower for ore processing (Rudolf, 1936). Concurrently, the Ribera de la Vera Cruz was constructed through the city with a system of 32 dams to power the waterwheels of over a hundred ore processers or *ingenios* (Rudolf, 1936; García-Guinea, 1995). *Ingenios* continuously discharged tailings to the Ribera de la Vera Cruz until 2004. AMD (acid mine drainage) and ARD (acid rock drainage) has likely continuously impacted local waterways since the onset of mining. ARD differs from

AMD in that it is a broader term that can include the diffuse acidic runoff of acid-generating from both disturbed and undisturbed surface mineral deposits (Younger et al., 2002).

Wasteful mineral processing methods used in Potosí have led to the release of ecotoxic heavy metals to the local landscape and waterways. Initially, Incan smelting technology was applied to process the Ag oxide cap of Cerro Rico with small charcoal-fueled clay furnaces (*huayras*) (García-Guinea, 1995; Abbott and Wolfe, 2003). During this period, thousands of active huayras illuminated the slopes of Cerro Rico (Bakewell, 1984; García-Guinea, 1995). The silver oxide minerals were mixed with galena (PbS) in the huayras to decrease the melting point (García-Guinea, 1995; García-Guinea and Harffy, 1998). Abbott and Wolfe (2003) documented the diffuse Pb deposition from these operations in nearby lake sediments. It is likely that other ecotoxic heavy metals common within Cerro Rico ores, such as As and Cd, were dispersed into the local environment during this period as well. In 1572, hydraulically powered Hg amalgamation replaced smelting as the primary Ag extraction process as mine workings moved deeper into Cerro Rico where Ag sulfide ores predominate (Serrano et al., 1996; Bartos, 2000; Abbot and Wolfe, 2003). Wasteful use of Hg in amalgamation processes has been documented and estimates suggest that 0.85-4.1 kg of Hg was lost to the environment for every kg of Ag produced (Lofstrom, 1970; Nriagu, 1993). A dam burst in 1626, causing extreme destruction over two hours as an increasingly polluted torrent of ore, tailings, amalgam and Hg rushed down the Ribera de la Vera Cruz through Rio Tarapaya (Serrano et al. 1996). Approximately 4,000 residents died as hundreds of homes and over 125 of the 132 ingenios were destroyed (Rudolf, 1936; Serrano et al.

1996). However, the ingenios were soon reconstructed so by 1636 there were more ingenious than before the disaster (Serrano et al., 1996) and the gangue from the amalgamation process continued to be washed out of the mills and into local waterways (García-Guinea, 1995).

Ingenios used amalgamation until the late 19th century, at which time Sn processing by gravimetric concentration became the prime source of mineral processing effluent to local streams. Gravimetric concentration is a water-intensive process that involves the turbulent mixing of crushed ores with water to separate the denser Sn-related particles. This process weathers ores, releasing metals and contributing acidity to solution. Flotation for Zn, Pb and Ag recovery became the primary mineral processing technique after 1985 and up to the present day.

Until 2004, local streams received froth flotation effluent and pyrite-rich tailings from local mills as well as AMD from the extensive mine shafts within Cerro Rico and large tailings piles around the mountain. Waters traversing Potosí are used by multiple ore processing facilities concentrating Pb, Ag, Zn, and Sn. Most ore processing facilities within Potosí are outdated flotation systems using disproportionate amounts of reagents discharging sulfurous solids, cyanide, xanthate and elevated concentrations of metals in their highly alkaline (pH 10-13) effluent (Bocangel, 2001). In 1997, the owners of 35 active operations in the department of Potosí announced their commitment to pay for rehabilitating the Pilcomayo (García-Guinea and Harffy, 1998). However, there was little follow-through until the construction of the tailings dams and canals to shunt ore processing effluent west of the city into the Laguna Pampa and the San Antonio tailings dams. Over the centuries and until the last few years, nearly all tailings were discharged directly into local streams. Mining activities before the tailings dams, were estimated to annually release approximately 360,000 tonnes of mining-related sludge to Ribera de la Vera Cruz (Smolders et al., 2002). Despite the new tailings canal and tailings impoundment, some processing effluent still regularly enters the Ribera de la Vera Cruz, often washed in during rain events from an overflowing tailings canal and/or poorly designed floatation ponds or purposely released to avoid costs associated with pumping tailings to the dams. However, the recent controls on tailings discharges leave AMD as the primary unabated ecotoxic metal source to local streams.

3.2.2 Previous Studies

Intensive mining over several centuries has severely degraded the aquatic environment downstream of Potosí. Previous studies have documented the general downstream geochemical and ecological impacts of mining and ore processing. Downstream of Cerro Rico near the western edge of Potosí's city limits, Hudson-Edwards et al. (2001) found dissolved metals concentrations orders of magnitude above background levels (Table 3.1). However, the pH was 10.3, which limited the solubility of metals species and indicates that local mineral processing plants were discharging directly to local streams at the time. Near the same location, Smolders et al. (2003) found dissolved metals concentrations similar to that found by Hudson-Edwards et al., (2001). Miller et al. (2002 and 2004) documented severe contamination of water and sediments up to 200 km downstream of Cerro Rico. Smolders et al. (2002 and 2004) sampled dissolved ions and suspended solids over 500 km downstream at Villa Montes to find vast differences in pollution levels between the rainy (December – March) and dry (May

– September) seasons. Smolders et al. (2002) found that in the rainy season, when heavy erosion from uncontaminated sources dilutes the metals concentrations of suspended solids, suspended solids averaged 23.6 g/L with Zn, Cu, Pb and Cd levels of 139, 23.9, 35.3 and 0.55 mg/kg dry weight, respectively. In the dry season, when mining related effluent contributes a higher proportion of flow, suspended solids averaged 0.011 g/L with Zn, Cu, Pb and Cd levels of 19327, 1107, 1495 and 12.4 mg/kg dry weight, respectively (Smolders et al., 2002). This ecotoxic metals pollution dramatically reduced the diversity of benthic macroinvertebrate communities downstream of the mines, with a gradient of increasing diversity noted with distance from Cerro Rico (Smolders et al., 2003). The environmental effects of mining on the lower Rio Pilcomayo are diminished by the dilution of both dissolved and suspended heavy metals by uncontaminated waters and sediment (Smolders et al., 2002). Miller et al. (2002) linked the aforementioned contamination to mining activity via isotopic analysis of Pb in river sediment, however the specific contaminant sources (i.e., AMD, mineral processing effluent, tailings dam discharges/erosion, etc.) could not be identified.

Table 3.1 Aqueous metals concentrations in stream grab samples taken in previous studies downstream of Potosí

Site	Type	pH	As	Cd	Cu	Pb	Zn	Season	Source
			- - - - - - - - - - - - -	- - - - - - - -	mg/L	- - - - - - - -	- - - - - - - - - - - - -		
Potosí	dissolved	10.3	0.065	0.01	0.3	0.041	0.035	dry-1998	HE
Potosí	dissolved	NR	NR	0.00076	0.014	0.0285	0.238	dry-1999	S
Potosí	total	NR	NR	0.0592	0.304	1.399	6.021	dry-1999	S
El Molino	total	NR	12.8	NR	NR	NR	NR	dry-2003	A
El Molino	total	NR	2.74	NR	NR	NR	NR	wet-2004	A
Rio Tarapaya	dissolved	NR	NR	0.005	0.013	0.056	0.601	dry-1999	S
Rio Tarapaya	total	NR	NR	0.315	1.709	2.291	12.416	dry-1999	S
Tasapampa	total	NR	0.272	NR	NR	NR	NR	dry-2003	A
Tasapampa	total	NR	0.113	NR	NR	NR	NR	wet-2004	A
Tuero Chico	total	NR	0.154	NR	NR	NR	NR	dry-2003	A
Tuero Chico	total	NR	0.199	NR	NR	NR	NR	wet-2003	A
Sotomayor	total	NR	1.213	NR	NR	NR	NR	dry-2003	A
Sotomayor	total	NR	0.421	NR	NR	NR	NR	wet-2003	A
Villa Montes	dissolved	8.77	0.03	<0.01	<0.02	0.025	0.016	dry-1998	HE
Villa Montes	dissolved	NR	NR	0.00039	0.0022	0.0007	0.017	dry-1999	S
Villa Montes	total	NR	NR	0.00077	0.017	0.0198	0.186	dry-1999	S

HE = Hudson-Edwards et al., 2001
S = Smolders et al., 2003
A = Archer et al., 2005
NR = Not reported

Primary human exposure pathways in the region are likely through inhalation of airborne particulates and ingestion of contaminated water, agricultural produce and fish (Smolders et al., 2002; Miller et al., 2004). Downstream communities rely on river water for irrigation, washing and occasional cooking and drinking (Garcia-Guinea and Harffy, 1998; Archer et al., 2005). Little study has been undertaken to quantify contaminant risk to humans. However, As concentrations in human hair and urine from downstream communities exceed published values for non-occupationally exposed subjects (Archer et al., 2005). Concentrations of Cd, Pb and Zn in some local agricultural soils exceed recommended guidelines for agricultural usage (Miller et al., 2004). Increased local human exposure risks are present because rural communities in the region have been found to consume 56 plant species for medicinal purposes (Fernandez et al., 2003). Distant consumers could be at risk as well because contaminated irrigation water is used extensively to grow vegetables for sale in greater Bolivia (Miller et al., 2004). The lower Rio Pilcomayo is also a major fishery. The sábalo (*Prochilodus platensis*) fishery in the lower Pilcomayo is the most important commercial fishery in Bolivia according to Payne and Harvey (1989). By addressing pollution sources to the upper Rio Pilcomayo, the region's ecosystem and human health would be improved.

No peer-reviewed studies have been encountered in the literature regarding the degradation of stream water quality within the city of Potosí and on the slopes of Cerro Rico or its links to specific contamination sources, such as AMD and mineral processing effluent. The study presented in this paper characterizes the current water quality of the streams that drain Cerro Rico and Potosí to form Rio Tarapaya, a chief tributary of the Rio Pilcomayo system. The purpose of this study was to determine the extent of water quality degradation and its relationship to unabated AMD from Cerro Rico.

3.3 Methods

3.3.1 Study Area

The study centered around Cerro Rico, approximately 1 km south of Potosí, Bolivia (Figure 3.1). Potosí (19.585°S 65.754°W) lies in the Eastern Cordillera range of the Central Andes in the upper reaches of the Rio Pilcomayo watershed. The Rio Pilcomayo is a chief tributary of the Rio de la Plata system, a crucial water resource for south-central and southeastern South America. The sampled streams and rivers drain to Rio Tarapaya, which later combines with Rio Yocalla approximately 30 km downstream to become Rio

Pilcomayo. The Rio Pilcomayo flows in a general southeasterly direction down the Eastern Cordillera range and through the semi-arid Chaco Plains. Eventually, the Rio Pilcomayo forms Argentina's northern border with Paraguay before it diffuses, and partially disappears beneath the surface, into a wide alluvial fan that empties into the Rio Paraguay at Asunción, Paraguay.

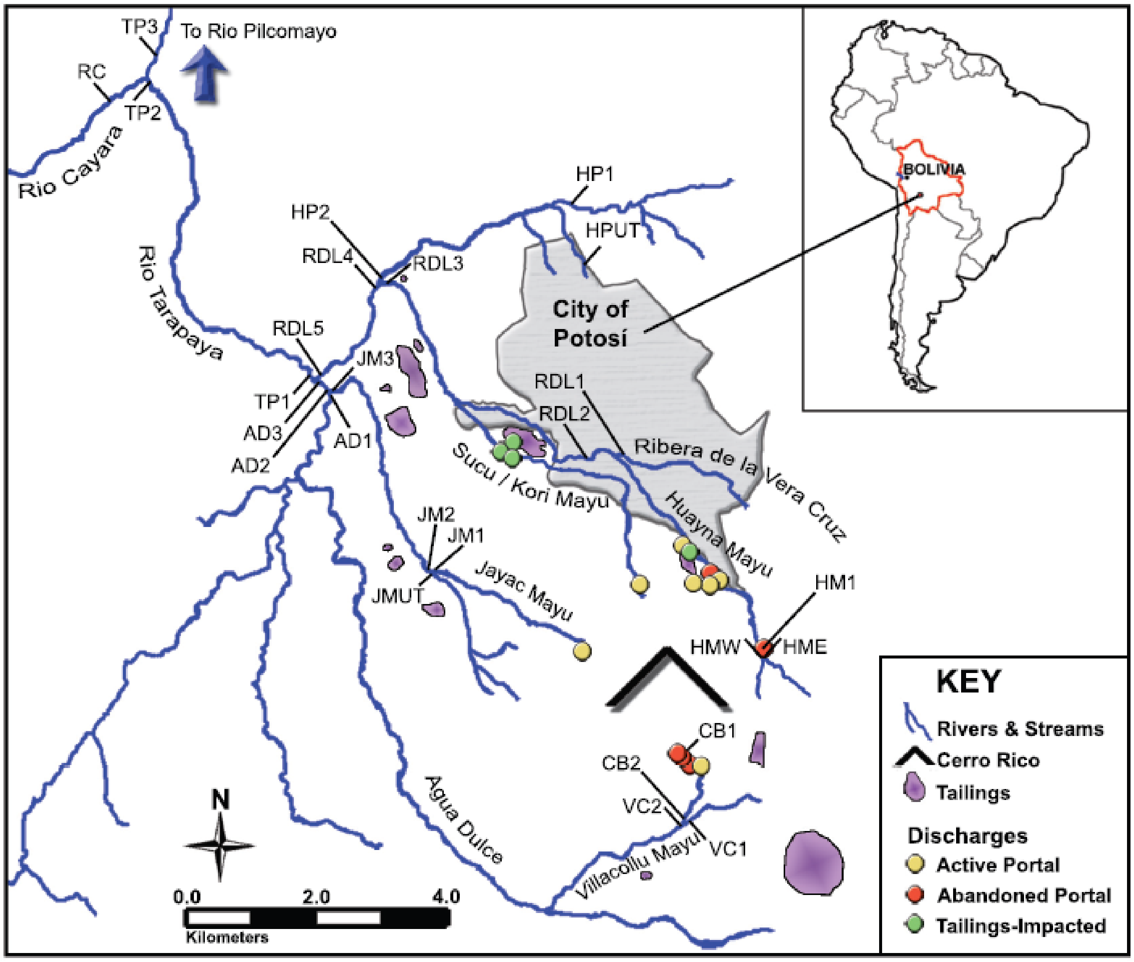

Figure 3.1 Study area and stream sample points with respect to AMD sources, tailings deposits, greater Bolivia and South America

3.3.2 Sample Sites

The streams and rivers sampled in this study vary widely in the types of effluent they receive and the watersheds they drain, ranging from highly urbanized to low-intensity agriculture to barren mining-impacted landscapes. Each water body and watershed is described below.

Huayna Mayu (sample sites HM1, HME, and HMW on Figure 3.1), "young river" in Quechua, is born near an abandoned mine discharge at the eastern base of Cerro Rico. Huayna Mayu runs north past abandoned brick foundries then turns west to collect drainage from AMD discharges and runoff from the Pailaviri tailings deposit on the north face of Cerro Rico. As Huayna Mayu flows through the city it also receives stormwater and wastewater until emptying into the Ribera de la Vera Cruz. During the dry season sampling period, Huayna Mayu was formed solely by an abandoned mine discharge (HM1). HM1 is equivalent to discharge 1A in the previous chapter. In the wet season, two ephemeral tributaries to Huayna Mayu, (HMW) and (HME), joined HM1 to form Huayna Mayu.

The headwaters of Rio Agua Dulce (sample sites AD1, AD2 and AD3), "sweet water river" in Spanish, lie in a sparsely populated agricultural area with little to no mining. Vilacollu Mayu (VC1 and VC2) is a tributary of Rio Agua Dulce that collects drainage from the southern slopes of Cerro Rico and Cerro Chico in the Canta Canta valley. The ephemeral stream Quebrada Chimborazo (CB1 and CB2) is impacted by several AMD sources in the Chimborazo region of Cerro Rico and impacts Vilacollu Mayu before it is impounded and diverted for agricultural use in the village of Vilacollu (Choque, 2007). Rio Agua Dulce borders many farms and is used for agricultural purposes along its length.

Jayac Mayu (JM1, JM2 and JM3), “spicy river” in Quechua, drains the southwestern mining district of Cerro Rico and impacts an unnamed tributary before joining with Rio Agua Dulce. An unnamed tributary to Jayac Mayu (JMUT) is a key water resource to a few farms upstream of the confluence. A cistern has been recently constructed approximately 10 m upstream of the confluence to capture and store water from the unnamed tributary before it is impacted by Jayac Mayu. Downstream of the confluence, Jayac Mayu borders small agricultural plots (Figure 3.2), but does not appear to be used for irrigation.

Figure 3.2 Llama and sheep grazing aside a parched farm plot that has been prepared for planting aside a highly impacted reach of Jayac Mayu between JM2 and JM3

Rio Huarampaya (HP1 and HP2) accepts wastewater and stormwater from unnamed tributaries that drain the north side of the City. It neither receives AMD nor has received mineral processing effluent. An unnamed tributary of Rio Huarampaya (sample site HPUT) drains wastewater and stormwater from a residential district. Rio Huarampaya runs through a small agricultural area before emptying into the Ribera de la Vera Cruz.

The Ribera de la Vera Cruz (RDL1, RDL2, RDL3, RDL4, and RDL5) runs east to west through the city past multiple active and defunct mineral processing plants. Since its construction in the 1570's, tailings and other mineral processing waste had been dumped directly into this stream. However, after the construction of the Laguna Pampa tailings dam and tailings diversion chute in 2004, tailings introduction has dramatically decreased. The tailings diversion chute runs parallel to Ribera de la Vera Cruz until it veers towards the San Antonio tailings dam, near the Laguna Pampa tailings dam. Tailings still periodically enter the stream during rain events when the chute overflows and when mineral processing plants fail to pump their tailings to the chute. Ribera de la Vera Cruz also accepts stormwater and wastewater from the city. Despite the recent measures to limit tailings introduction, the substrate of Ribera de la Vera Cruz remains "tailings" grey. The telltale red staining from weathering pyrite is evident along the banks and in stagnant pools in the stream. In addition, the Ribera de la Vera Cruz runs less than 100 m from the San Miguel tailings deposit, a serious potential nonpoint pollutant threat, and accepts AMD from springs in abandoned alluvial deposit mines.

Rio Tarapaya (TP1, TP2 and TP3) is formed by the combined flows of the Ribera de la Vera Cruz and Rio Agua Dulce. The substrate of Rio Tarapaya is visually similar to that of Ribera de la Vera Cruz and red staining from weathering pyrite is evident in stagnant portions of the river. Rio Tarapaya meets Rio Cayara at a shuttered Sn smelting facility where slag piles line the southern bank. Tailings have accumulated over the centuries in the Rio Tarapaya valley because its bed slope is much less than its tributaries.

Rio Cayara (RC) is an important water resource in a productive agricultural watershed, which is nearly the same size as that of Rio Tarapaya, in which no evidence of mining was observed. However, past or present mining activity cannot be ruled out due to the near ubiquity of mining in the region.

Reference points (JMUT, HP1, VC1 and RC) were designated in reaches without in-stream tailings deposits that were expected to not be considerably impacted by historical or current mining or mineral processing. The only reference site sampled in the dry season was JMUT, however all four were sampled in the wet season. In all, 10 locations were sampled in the dry season and 16 sites were added to those in the wet season. Sampling points (HMW, HME, VC1, VC2, CB1 and CB2) were added in the wet season because some of the streams sampled are ephemeral and were not flowing or imperceptibly flowing during the dry season. Other points of interest were added in the wet season to expand the scope of the study (AD3, RDL1, RDL3, RDL4, HP1, HPUT, HP2, TP2, TP3 and RC).

The AMD sources characterized in the previous chapter are represented on Figure 3.1. Tailings deposits, innumerable ore piles that dot the slopes of Cerro Rico, tailings discharges by ore processors and tailings within stream sediments are other probable ephemeral and permanent point and non-point sources of water quality degradation.

3.3.3 Data Collection

Water quality parameters and grab samples were obtained at the points indicated on Figure 3.1 during the dry (July-August 2006) and wet (March 2007) seasons of one water-year. These intervals were chosen in an attempt to capture the groundwater level/efflux and surface water flow extremes of both seasons, when are near their minima (dry) and maxima (wet). Sampling periods were offset from the minima and maxima of the monthly average precipitation because of the common response lag of groundwater level/efflux and surface water flows to precipitation events (Eltahir and Yeh, 1999). No rain events occurred during the dry season sampling. However, two rain events influenced wet season sampling of AD3, TP1, HME and HMW.

The location of each sampling point was recorded with a Garmin® GPS unit. Acidity and alkalinity titrations were conducted in the field following standard methods (APHA, 1998). Temperature, pH, dissolved oxygen (DO), and specific conductance (SC) were determined using an Orion 1230 multi-meter. All grab samples were taken using 125-mL HDPE containers for later analyses at the University of Oklahoma Center for Restoration of Ecosystems and Watersheds (CREW) laboratories. Samples for anion analyses were stored at 4-°C until filtered through 0.2 μm nylon filters and Dionex OnGuard® II H cartridges. A MetrOhm® 761 compact ion chromatograph (IC) unit was used to quantify SO_4 concentrations following EPA method 300. As a field backup, SO_4 was also quantified on-site using EM QUANT® 200-1600 ppm test strips. Samples were diluted when concentrations were greater than 1600 ppm. Total metals samples were preserved with concentrated trace metals grade nitric acid and stored at approximately 4-°C until microwave acid digestion following EPA method 3015. The digested metals samples were filtered through 0.45 μm nylon filters then analyzed via a Varian Vista-Pro® simultaneous inductively coupled plasma-optical emission spectrometer following EPA method 6010. Acidity was calculated following a modified version of that presented by Younger et al. (2002), which accounted for the acidity from the substantial Cd^{2+}, Cu^{2+} and Zn^{2+} concentrations present in these unique waters. For acidity calculations, all Fe was assumed to be Fe(II) when $pH > 3$ and Fe(III) when $pH < 3$, because Fe(III) predominates for most solutions with $pH < 3$ (Kirby and Cravotta, 2005). This assumption generally follows empirical observations (Younger et al., 2002; Watzlaf et al., 2004; Kirby and Cravotta, 2005). Hedin (2006) demonstrated an excellent relationship between calculated and measured net acidity for a diverse data set of 1,484 AMD.

3.3.4 Data Analysis

Water quality parameters were primarily compared to Bolivian and international water quality standards. Wet season mean concentrations of SO_4 and total metals of streams unimpacted by AMD or ARD (HPUT, HP1-2, VC1, RC), streams impacted by AMD (JM1-3, AD1-3, RDL1-5, HME, HMW, TP1-3, CB2, VC2), and source AMD (discharges 1-7C, 1-3A, 1-2T from the previous chapter) were compared using two-tailed heteroscedastic Student's *t*-tests ($\alpha = 0.05$). HM1 was excluded from the impacted group because it is also an AMD source. JMUT and CB1 were excluded from the unimpacted stream group because they may receive diffuse ARD from the slopes of Cerro Rico. Discharges 4-5A and 3T were excluded from the AMD group because they did not display the typical characteristics of AMD (low pH and elevated SO_4, Fe and other metals). Discharge 4T was excluded from the AMD group because it had concentrations of Al, As, Fe and SO_4 over three standard deviations greater than the mean of the AMD group. Dry season data were not statistically tested because no suitable reference location was sampled.

3.4 Results and Discussion

Physical parameters, SO_4, and total metals concentrations for sampled streams are presented in Tables 3.2-4. These results may be compared to various water quality criteria set by the Bolivian government and irrigation standards set by the United Nations Food and Agriculture Organization (UNFAO) and, for reference purposes, drinking water standards set by the United States Environmental Protection Agency (USEPA) and World Health Organization (WHO) as shown in Table 3.5. UNFAO irrigation water guidelines are established at contaminant concentrations above which water can degrade agricultural soils and decrease crop productivity (Ayers and Westcot, 1994). UNFAO livestock drinking water guidelines are set at contaminant concentrations above which water can be toxic or unpalatable to livestock or lead to bioaccumulation, rendering livestock products unsuitable for human consumption (Ayers and Westcot, 1994). WHO drinking water guidelines and USEPA primary drinking water standards have been established to protect public health (WHO, 2006; USEPA, 2007). The secondary drinking water standards determined by the USEPA are guidelines for contaminants that can cause adverse cosmetic or aesthetic effects, such as teeth staining, odor, and bad taste (USEPA, 2007). All mining-impacted reaches had concentrations of the ecotoxic metals that place them well above WHO, USEPA and UNFAO standards.

Table 3.2 Physiochemical parameters. pH is bolded if it exceeds Bolivian Class "D" requirements. SC is underlined if it exceeds the 700 µS/cm ceiling recommended for UNFAO long term irrigation use. Sites indented by watershed

Site	pH (s.u)	Net Acidity^a (mg/L as CaCO$_3$ eq.)	DO (mg/L)	SC (µS/cm)	Temp. (°C)
Dry Season					
JM1	**3.4**	5570	13.4	5070	2.9
JMUT	7.3	-	9.7	534	6.1
JM2	**3.5**	5690	10.6	4870	4.9
JM3	**9.5**	-	8.5	2100	9.5
AD1	8.0	-	10.0	1010	10.0
AD2	**9.4**	-	7.7	2010	10.6
HM1*	**3.6**	910	6.6	1890	8.5
RDL2	**5.0**	-	10.8	1420	7.4
RDL5	7.5	-	9.0	1160	11.7
TP1	7.7	-	8.7	1170	11.0
Wet Season					
VC1	7.7	-23	7.5	593	14.0
CB1	**4.7**	30	6.1	216	13.3
CB2	**3.1**	1620	9.4	2510	13.4
VC2	**3.6**	525	8.0	1210	12.8
JM1	**2.8**	1250	8.3	2820	13.0
JMUT	8.1	82	8.8	160	11.8
JM2	**4.8**	521	9.4	870	12.3
JM3	**5.0**	529	6.5	1140	16.5
AD1	7.5	562	9.5	290	14.8
AD2	6.9	652	6.2	613	14.9
AD3	7.7	5320	5.8	438	16.7
HP1	8.3	-196	5.2	1460	19.8
HPUT**	8.5	-329	4.5	1280	14.3
HP2	8.4	-295	5.7	1020	16.3
HM1**	**4.5**	625	6.4	1460	9.6
HMW	**5.4**	1910	6.0	181	6.0
HME	**5.5**	2360	6.0	718	5.0
RDL1	6.3	151	7.5	1290	11.3
RDL2	**3.2**	1320	9.2	2430	10.6
RDL3	**3.4**	1140	6.2	2310	14.0
RDL4	**5.4**	319	6.3	1380	17.0
RDL5	6.3	351	6.1	1390	18.0
TP1	7.4	4250	1.7	548	15.3
TP2	**4.9**	569	5.5	1620	18.5
RC	7.1	-80	6.4	374	15.8
TP3	**5.9**	351	5.9	1040	17.9

*n = 3; ** n = 2 (n =1 for all other sites); ^{a}Net acidity = acidity – alkalinity; - = no data available

Table 3.3 Dry season mean SO_4 and total metal concentrations. Concentrations are bolded if exceeding Bolivian Class "D" requirements and underlined if exceeding UNFAO long term use irrigation standards. Sites indented by watershed

Site	Al	As	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Na	Ni	Pb	Zn	SO_4^{2-}	SO_4^{2-}‡
	mg/L															
JM1	**210**	**14**	140	**4.9**	**0.90**	0.065	**19**	**1100**	110	**82**	24	**0.81**	**3.2**	**1500**	-	**4000-8000**
JMUT	0.21	<0.022	46	0.0007	<0.001	<0.001	0.007	0.44	36	0.044	23	0.009	0.040	**0.29**	-	< 200
JM2	**220**	**16**	140	**4.9**	**0.97**	0.071	**20**	**1100**	110	**79**	22	**0.84**	**4.1**	**1500**	-	**4800-6400**
JM3	**19**	**1.1**	**540**	**0.41**	0.11	0.008	**2.6**	**44**	19	**10**	52	0.10	**1.2**	**110**	-	**400-800**
AD1	0.44	<0.022	90	0.002	<0.001	0.003	0.009	0.52	41	0.23	57	0.017	0.041	**0.76**	-	< 200
AD2	**17**	**0.86**	**530**	**0.38**	0.10	0.008	**2.4**	**37**	19	**9.3**	52	0.094	**1.1**	**100**	-	**1200-1600**
HM1*	**5.8**	0.03	100	**0.17**	0.14	0.002	0.037	**110**	16	**13**	15	0.16	**0.065**	**570**	**1300**	**1200-1600**
RDL2	**38**	**1.2**	51	**0.12**	0.066	0.015	**1.2**	**140**	11	**2.9**	57	0.073	**0.26**	**29**	-	**400-800**
RDL5	**14**	**1.7**	100	**0.10**	0.038	0.014	0.84	**67**	14	**2.2**	65	0.041	**1.1**	**32**	-	**400-800**
TP1	**13**	**1.4**	140	**0.12**	0.042	0.012	0.82	**59**	13	**2.9**	63	0.065	**0.92**	**34**	-	**400-800**

‡ SO_4 ranges obtained in the field with EM QUANT® test strips; bdl = Below detection limits; * n = 3 (n =1 for all other sites); - = no data available

Table 3.4 Wet season mean SO_4 and total metal concentrations. Bolded concentrations exceed Bolivian Class "D" requirements. Underlined concentrations exceed UNFAO long term use irrigation standards. Sites indented by watershed

Site	Al	As	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Na	Ni	Pb	Zn	SO_4	SO_4‡
	mg/L															
VC1	**1.3**	<0.022	74	0.002	<0.001	<0.001	0.005	1.0	13	**3.0**	31	0.008	<0.012	**1.5**	200	**400-800**
CB1	**3.5**	0.091	18	**0.018**	<0.001	0.0020	0.014	**2.7**	3.3	0.79	3.5	0.011	**0.33**	**7.3**	74	**400-800**
CB2	**41**	**1.1**	110	**1.2**	**0.27**	0.014	0.50	**210**	27	**61**	6.7	0.26	**0.33**	**560**	**1400**	**> 1600**
VC2	**16**	**0.38**	86	**0.39**	0.095	0.008	0.17	**66**	18	**21**	24	0.095	**0.13**	**170**	**720**	**800-1200**
JM1	**71**	**0.25**	92	**0.24**	**0.81**	0.020	**6.7**	**180**	78	**110**	13	**0.80**	**0.24**	**64**	**2200**	**>1600**
JMUT	**15**	<0.022	22	0.002	0.035	0.015	0.015	0.030	9.2	**16**	3.2	**7.2**	0.046	**0.37**	23	<200
JM2	**21**	**0.11**	38	**0.63**	**0.22**	0.009	**1.7**	**63**	28	**25**	8.3	0.21	**0.17**	**160**	**440**	**400-800**
JM3	**24**	**0.31**	120	**0.58**	0.20	0.020	**1.3**	**79**	45	**27**	19	0.23	**0.37**	**140**	**640**	**800-1200**
AD1	**81**	0.088	97	**0.018**	0.053	0.054	0.076	**100**	37	**2.5**	17	0.065	**0.18**	**2.3**	66	**400-800**
AD2	**71**	**0.13**	97	**0.16**	0.10	0.049	0.40	**100**	41	**9.5**	17	0.12	**0.26**	**44**	240	**400-800**
AD3	**630**	**0.45**	930	**0.082**	**0.38**	0.47	0.91	**950**	**310**	**27**	47	**0.57**	**0.95**	**25**	110	**400-800**
HP1	**8.1**	0.034	190	0.002	<0.001	0.004	0.015	**7.3**	51	0.30	100	0.010	0.046	0.16	6	< 200
HPUT*	**5.9**	0.053	66	**0.006**	<0.001	0.007	0.065	**6.7**	12	0.34	78	0.011	**0.22**	**1.6**	10	< 200
HP2	**2.6**	0.032	72	0.002	<0.001	0.006	0.033	**4.7**	35	0.55	57	0.005	**0.069**	0.47	230	< 200
HM1*	**21**	**0.37**	71	**0.27**	0.065	0.007	0.064	**73**	14	**7.7**	8.7	0.073	**1.2**	**220**	**630**	**800-1200**
HMW	**190**	**1.8**	65	**0.34**	0.12	0.074	0.35	**370**	34	**11**	9.8	0.095	**6.1**	**120**	100	-
HME	**240**	**2.2**	43	**0.14**	0.13	0.11	0.69	**530**	41	**17**	6.9	0.10	**9.2**	**38**	330	-
RDL1	**16**	**0.84**	150	**0.23**	0.051	0.009	**1.9**	**40**	14	**5.2**	55	0.046	**0.66**	**38**	**420**	**400-800**
RDL2	**71**	**5.0**	110	**0.37**	0.16	0.026	**3.6**	**410**	19	**6.7**	55	0.16	**0.52**	**93**	**1400**	**> 1600**
RDL3	**60**	**4.6**	150	**0.39**	0.17	0.022	**3.5**	**330**	24	**10**	53	0.15	**0.62**	**100**	**1300**	**800-1200**
RDL4	**20**	**1.6**	97	**0.12**	0.059	0.010	**1.1**	**100**	18	**3.7**	58	0.056	**0.35**	**35**	**490**	**400-800**
RDL5	**19**	**2.1**	180	**0.11**	0.061	0.010	**1.2**	**110**	19	**4.2**	58	0.079	**0.57**	**33**	**600**	**1200-1600**
TP1	**550**	**0.43**	450	**0.12**	**0.27**	0.29	0.60	**650**	**200**	**18**	47	0.25	**0.95**	**10**	160	**400-800**
TP2	**36**	**1.8**	180	**0.18**	0.099	0.019	**1.4**	**150**	30	**7.2**	50	0.097	**0.98**	**53**	**720**	**800-1200**
RC	**14**	<0.022	70	0.002	<0.001	0.009	0.011	**12**	11	0.38	16	0.023	0.024	0.15	110	< 200
TP3	**24**	**1.2**	130	**0.11**	0.060	0.013	0.93	**92**	20	**4.1**	32	0.065	**1.2**	**30**	**420**	**800-1200**

SO_4 ranges obtained in the field with EM QUANT® test strips; * n = 2 (n =1 for all other sites); bdl = Below detection limits; - = no data available

Table 3.5 Bolivian receiving water body criteria, UNFAO irrigation and livestock drinking water guidelines, USEPA drinking water standards, and WHO drinking water guidelines

Standard	pH	Al	As	Ca	Cd	Co	Cr	Cu	Fe	Mg	Mn	Na	Ni	Pb	Zn	SO_4^{2-}
		mg/L														
Bolivian class “A”	6.0-8.5	0.2	0.05	200	0.005	0.1	0.05	0.05	0.3	100	0.5	200	0.05	0.05	0.2	300
Bolivian class “B”	6-9	0.5	0.05	300	0.005	0.2	0.6/0.05$^{\alpha}$	1.0	0.3	100	1.0	200	0.05	0.05	0.2	300
Bolivian class “C”	6-9	1.0	0.05	300	0.005	0.2	0.5/0.05$^{\alpha}$	1.0	1.0	150	1.0	200	0.5	0.05	0.2	300
Bolivian class “D”	6-9	1.0	0.1	400	0.005	0.2	1.1/0.05$^{\alpha}$	1.0	1.0	150	1.0	200	0.5	0.05	0.2	400
UNFAO$^{\mu}$	6.5-8.4	20	2.0		0.05	5.0	1.0	5.0	20		10		2.0	10	10	
UNFAO$^{\delta}$	6.5-8.4	5.0	0.1		0.01	0.05	0.1	0.2	5.0		0.2		0.2	5.0	2.0	
UNFAO$^{\tau}$		5.0	0.2		0.05	1.0	1.0	0.5	2.0		0.2		0.2	5.0	2.0	
WHO			0.01		0.003		0.05	2.0			0.4		0.07	0.01		
USEPA primary			0.01		0.005		0.1	1.3						0.015		
USEPA secondary	6.5-8.5	0.2						1.0	0.3		0.05				5	250

$^{\alpha}$ Cr (III) and Cr (VI) limits respectively

$^{\mu}$ Short term (< 20 years) of continuous irrigation usage

$^{\delta}$ Long term (> 20 years) of continuous irrigation usage

$^{\tau}$ Livestock drinking water standard

Bolivian water quality standards are stringent, progressive and comparable with those of most industrial nations. However, non-compliance with pollutant discharge criteria is rarely punished and little regulation is in effect to assure that water bodies meet their class designations. Class “A” receiving water bodies, those which are suitable for drinking without treatment or only with bacterial disinfection, are suitable for most uses, including livestock watering and irrigation (BMSDP, 2000). Class “B” waters only differ from Class “A” in that they require filtration and disinfection to be suitable for drinking. Class “C” waters differ from Class “B” in that they are unsuitable for the irrigation of fruits or vegetables. Class “D” is the lowest designation of Bolivian receiving water body in which industrial application and navigation are the only suitable uses except in extreme circumstances. Bolivian law states that class “D” waters must be “coagulated, flocculated, filtered and disinfected” prior to domestic use and that they are unsuitable for agricultural usage or animal watering (BMSDP, 2000). There is no class designation lower than “D” and it is not known how the water bodies downstream of Potosí are designated, nor if they have a designation at all. However, some are used for agriculture and therefore should be rated at a minimum above class “D”. All of the AMD-impacted streams sampled did not meet class “D” requirements.

AMD impacted streams contained elevated concentrations of the same major ecotoxic constituents present in AMD and at concentrations statistically greater than those unimpacted by AMD. Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn and SO_4, which were major components of Cerro Rico AMD, were all significantly greater in the AMD impacted streams, often by multiple orders of magnitude (Table 3.6). Only the base cations (Ca, K, Mg and Na), were not significantly different in the AMD impacted streams versus the unimpacted streams, because they were not major components of the Cerro Rico AMD. Results indicate dramatic transformative impacts from AMD discharges upon receiving stream water quality.

Table 3.6 Mean AMD, AMD impacted stream, and AMD unimpacted stream total metals and SO_4 concentrations for the wet season

	AMD	AMD Impacted	AMD Unimpacted
		mg/L	
Al	378	121	6.39
As	37.8	1.36	0.029
Cd	12.1	0.30	0.003
Co	1.84	0.18	<0.001
Cr	0.41	0.068	0.005
Cu	33.8	1.50	0.026
Fe	2470	253	6.28
Mn	63.7	20.4	0.91
Ni	2.67	0.19	0.011
Pb	6.13	1.35	0.073
Zn	3690	96.1	0.78
SO_4	10400	654	110

All streams sampled failed to meet Bolivian class “D” water quality standards in the wet and the dry seasons. The introduction of AMD orders of magnitude over class “D” limits was likely leading to non-compliance downstream. However, in some cases the failure to meet class “D” standards could be indicative of overly stringent water quality standards applied in a mineral-rich landscape where background concentrations of many of the metals tracked are naturally elevated. In fact, Cerro Rico was dubbed “The Red Mountain” because of its color, which is due to the oxidation of Fe on its slopes, and mining abounds in the region due to the naturally elevated metals concentrations. The chosen reference reaches (JMUT, HP1, VC1 and RC) did not meet class “D” requirements and were unsuitable for irrigation and livestock despite the minimal influence that mining likely had on these systems. Generally, elevated concentrations of the less ecotoxic metals (Fe and/or Mn) place these water bodies above the various limits. However, the reference reaches were the least contaminated in the region and it is likely that these reaches could be representative of background metals concentrations.

Conversely, all mining-impacted reaches had concentrations of the ecotoxic metals (Al, As, Cd, Cr, Pb and/or Zn), in addition to the aforementioned less ecotoxic metals, that place them well above WHO, USEPA, UNFAO, and Bolivian class "D" standards. Nearly all of the waters sampled were unsuitable for irrigation according to the UNFAO, save the unnamed tributary to Jayac Mayu in the dry season. However this tributary does not adequately dilute Jayac Mayu, which after the confluence retained concentrations of ecotoxic elements orders of magnitude greater than are acceptable for irrigation or livestock use. In addition, Jensen et al. (2001) argue that the UNFAO limits likely must be more stringent to avoid unsafe accumulation of metals in agricultural soils, especially in water-stressed developing nations. SC of many stream reaches was also greater than limits set by the UNFAO for unrestricted irrigation use. Overall, SC and pH combine with metals, acidity and SO_4 concentrations to rendered receiving waters unusable for multiple reasons.

In general, there is a trend of AMD-impacted waters intercepting and degrading waters that were formerly much more suitable for irrigation and livestock consumption, such as was observed when Jayac Mayu intercepted its unnamed tributary then Rio Agua Dulce, Quebrada Chimborazo intercepted Vilacollu Mayu, Ribera de la Vera Cruz intercepted Rio Huarampaya, and Rio Tarapaya intercepted Rio Cayara. Some of these streams may have been impacted by previously deposited in-stream tailings or current tailings discharges. However, AMD alone was sufficient to severely degrade receiving waters, as was demonstrated in the cases of the degradation of Jayac Mayu, Quebrada Chimborazo and upstream Huayna Mayu as well as the continuing impacts noted when Jayac Mayu intercepted its unnamed tributary and Quebrada Chimborazo intercepted Vilacollu Mayu.

Oporto et al. (2007) found that Cd concentrations of 0.065-0.24 mg/L in a mining-impacted river used for irrigation 150 km northwest of Cerro Rico caused a human health risk via Cd uptake in potatoes, a crucial Andean staple. Many of the reaches sampled have higher Cd concentrations than those noted by Oporto et al. (2007), indicating that they could present a yet greater risk if their waters were used for crop irrigation. The presence of 0.39 mg/L Cd in Vilacollu Mayu at VC2, which is diverted for agricultural use shortly thereafter, is of concern. Application of AMD-impacted waters, such as those from Vilacollu Mayu, could increase metals soil concentrations to unsafe levels (e.g., Jensen et al., 2001). Because some of the waters sampled are used for agriculture, locally grown beans, potatoes, barley, and maize (Choque, 2006) likely pose a human health risk. Indigenous populations in the region make use of tens of other plant species for natural remedies (Fernandez et al., 2003), another possible bioaccumulation route.

The rain events during the wet season sampling of AD3, TP1, HME and HMW likely led to an increase in suspended sediment, which could explain the elevated metals concentrations that accompany uncharacteristically low SO_4 and SC within the streams at that time. However, the data from these events indicate that large quantities of metals from non-point sources may enter streams with runoff, increasing rather than diluting contaminants. These data indicate the opposite of the trend observed by Smolders et al. (2002) downstream in the Rio Pilcomayo where metals concentrations in suspended sediment were diluted by erosion from uncontaminated tributary watersheds. The highly mineralized nature of the study area (e.g., Cunningham et al., 1996; Deconinck et al., 2000; Rice and Steele, 2005) likely lead to elevated concentrations of metals in suspended sediment from erosion.

Findings indicate that not all *ingenios* were diverting their tailings to the recently constructed tailings dams, continuing their past behavior. Between sampling points JM2 and JM3, an *ingenio* was likely discharging tailings to Jayac Mayu. Elevated pH is necessary for ore floatation processing and this is typically achieved by the addition of quicklime (CaO). The great increase in pH and Ca concentrations observed in the dry season in a locality where there is little natural alkalinity generating capacity and the telltale grey froth observed indicate that this tailings discharge likely exists. Tailings discharges are often grey and frothy due to the mix of fine grey ore particles and floatation agents. Wet season pH was essentially the same between JM2 and JM3, however, Ca concentrations dramatically increased, indicating a continuing tailings discharge. Similar total Ca of 397 mg/L and pH 11.40, which are well above reference conditions, were recorded along with the grey froth approximately 400 m downstream from JM3 at AD3 in the dry season of August 2008 during unrelated studies performed with the same equipment. One or more of the multiple *ingenios* bordering this reach appeared to be contributing yet more tailings to local streams.

The decrease in pH from TP1 to TP2 is likely partially due to weathering of sulfide minerals from tailings present within the bed of Rio Tarapaya. No other mining influences were noted in this reach. The inefficient floatation methods applied in Potosí lead to high concentrations of unrecovered metals (metal sulfides) in tailings (Smolders et al., 2003). Rio Tarapaya has collected tailings for centuries through direct discharges from bordering *ingenios* and tailings discharges to the Ribera de la Vera Cruz within the city of Potosí. Despite the dramatic recent decrease in tailings introduction to Rio Tarapaya and the Ribera de la Vera Cruz, tailings are still evident within Rio Tarapaya and appear to be negatively influencing water quality.

3.5 Conclusions

Mining and ore processing are considerable contributors to water quality degradation downstream of the Cerro Rico mines. Streams that directly receive AMD and the streams and rivers with which they confluence contain concentrations of ecotoxic metals (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn), SC, and acidity that render them unusable and environmental hazards. Dilution and natural passive biogeochemical attenuation are the likely mechanisms by which metals and hydrogen ion concentrations decrease with distance from AMD sources within the streams not heavily contaminated with tailings. However, the weathering of tailings within the bed of Rio Tarapaya appears to present a strong enough signal to override some of the effects of the natural attenuation that occur in its tributaries.

The dynamic nature of Potosí's surface hydrology and contamination sources solicit further study. The water quality conditions related in this study represent a limited snapshot of one water-year. The previous chapter demonstrated that unabated AMD was a noteworthy contributor of trace metals and acidity to local streams. The findings presented here solidify the link between Cerro Rico AMD and downstream contamination by demonstrating 1) significantly elevated concentrations of the same ecotoxic elements discharged by AMD in receiving stream waters and 2) extreme water quality degradation in those streams in which AMD was the only impacting factor. Findings confirm that AMD must be addressed in any attempt to restore the upper Rio Pilcomayo watershed. However, results suggest that tailings in stream sediments and ongoing tailings discharges are also sources of water quality degradation. Due to the inherent natural dynamic hydrology of the region with the extremes of the wet and dry seasons overlying the El Niño / La Niña cycle, further study is recommended to better characterize contamination sources and downstream impacts. Nonpoint ARD, in-stream tailings, and tailings discharges are likely contributors to water quality degradation and should be further studied to determine remediation priorities.

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Chapter IV

Effective Passive Treatment of High Strength Acid Mine Drainage and Raw Municipal Wastewater in Potosí, Bolivia using Simple Mutual Incubations and Limestone

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4.1 Abstract

To assess the viability of passive water quality improvement approaches for treating acid mine drainage (AMD) at Cerro Rico de Potosí, Bolivia, alkalinity production, acidity neutralization and metals removal were tracked for incubations of AMD in the presence of limestone (LS), a 1:1 mix of AMD and raw municipal wastewater (WW), and a 1:1 mix of AMD and WW in the presence of LS. Three AMD sources from abandoned adits on Cerro Rico, raw WW from the city of Potosí and locally available LS were incubated in-situ for 72 hr in 1-L cubitainers. Although locally sourced LS can increase final alkalinity up to 397 mg/L as $CaCO_3$, it is a prospective source for Mn and a few other potentially undesirable elements, indicating that in lieu of substrate analysis, AMD and LS cubitainer incubations reveal the quality and chemical composition of potential calcareous passive treatment substrate. Relevant to the prospects of AMD and WW passive co-treatment, mixing AMD with WW had relatively little effect on the final alkalinity achieved by LS dissolution. Accounting

for dilution, dissolved concentrations of Ag, Al, As, Cd, Cr, Fe, Pb, Sb, Se, Sn, V and Zn decreased with AMD and WW incubation. Especially efficient As removal was noted, with WW incubation driving mixed concentrations from 19.4 to 0.34 and 3.58 to 0.28 mg/L with the two higher strength AMD source waters. Rare earth element (REE) results were varied. Although AMD mixed and unmixed with WW then incubated with LS generally decreased REE concentrations, Pr and Nd concentrations increased under some LS exposures. Incubation with WW alone generally decreased dissolved concentrations of REE, however La, Pr, Eu and Nd concentrations increased with WW exposure. Overall, results indicate that cubitainer incubations have broader utility than that for which has been previously taken advantage, passive treatment can be expanded to target more constituents of interest than it has been traditionally applied, and the co-treatment of AMD with WW is a promising new application of passive treatment that should be further investigated.

4.2 Introduction

Acid mine drainage (AMD) and municipal wastewater (WW) are common environmental liabilities worldwide and their treatment is central to maintaining global water resource quality. Untreated AMD causes water quality degradation in coal and metal mining regions worldwide (e.g., Salomons, 1995; Bell and Donnelly, 2006). Discharges of untreated WW degrade water resources in many developing nations (e.g., Gadgil, 1998; Kivaisi, 2001, Nelson et al., 2001). In developed nations, where WW is generally treated actively, treatment consumes considerable financial, material and energy resources. With respect to passive methods, conventional active WW and AMD treatment are less sustainable and more energy-intensive with higher operational and maintenance costs (Nelson et al., 2001; Younger et al., 2002; Muga and Mihelcic, 2008).

AMD is formed by the weathering of metal sulfide minerals, principally pyrite (FeS_2). Generally, AMD contains elevated concentrations of hydrogen ion, trace metals, sulfate and acidity (Watzlaf et al., 2004). Because AMD is often net acidic and low pH, passive treatment usually necessitates alkalinity generation and pH augmentation, which is often realized via limestone (LS) dissolution. The dissolution of calcite, the principle component of LS, occurs via a combination of the subsequent reactions (Stumm and Morgan, 1996):

$$1)\ \ CaCO_3(s) + 2H^+ \leftrightarrow Ca^{2+} + H_2CO_3^*$$

$$2)\ \ CaCO_3(s) + H_2CO_3^* \leftrightarrow Ca^{2+} + HCO_3^-$$

$$3)\ \ CaCO_3(s) + H_2O \leftrightarrow Ca^{2+} + HCO_3^- + OH^-$$

where $[H_2CO_3^*] = [CO_{2(aq)}] + [H_2CO_3]$. The three reactions affect pH and alkalinity via changes in free hydrogen ion concentration and the generation of HCO_3^- and OH^-. Dissolved Ca concentrations also increase, creating a signature that can be used to track dissolution efficiencies. The rate of calcite dissolution, and hence alkalinity generation, depends on the pH, partial pressure of CO_2 (Pco_2), and the activities of Ca^{2+}, HCO_3^- and H_2O, which ionic strength and temperature substantially impact (Cravotta and Trahan, 1999; LaBar et al., 2008).

Cubitainer experiments, initially developed by Watzlaf and Hedin (1993), have been used to determine the viability of passive treatment options by demonstrating the alkalinity generating capacity of calcareous substrate when exposed to AMD (Watzlaf et al., 2004). A cubitainer is a collapsible, cubic, sealable LDPE container in which in-situ or ex-situ incubations can be rapidly initiated and undertaken. Anoxic LS drains (ALD), reducing and alkalinity producing systems (RAPS) and oxic LS drains/channels (OLD/C) are the primary applications of calcareous substrates in passive treatment systems. The alkalinity produced by calcareous substrate can vary by several hundred mg/L as $CaCO_3$ equivalents, depending upon substrate quality, AMD chemical composition and exposure conditions (Watzlaf and Hedin, 1993; Cravotta and Trahan, 1999; Watzlaf et al., 2000, 2004; Cravotta, 2003). Results of cubitainer tests can be directly applied via a set of sizing equations to design and predict the performance of passive alkalinity generating unit processes, such as RAPS and ALDs (Cravotta and Watzlaf, 2002; Cravotta, 2003) and OLD/Cs (Mukhopadhyay et al., 2007; Cravotta, 2008). Because calcareous substrate and AMD vary widely in chemical composition between regions, site-specific cubitainer tests are necessary to help determine the overall regional viability of passive treatment. As of the writing of this manuscript, no Andean cubitainer test studies were encountered in the literature.

The passive co-treatment of AMD and WW is a promising remediation approach. However to the authors knowledge, only two published studies (Johnson and Younger, 2006; McCullough et al., 2008) have investigated the simultaneous synergistic water quality improvement possible by mixing AMD with WW. McCullough et al. (2008) documented fortuitous water quality improvement and bacterial sulfate reduction (BSR) in an evaporation pond in which high-strength AMD was introduced to secondary WW. The Johnson and Younger (2006) single-stage constructed wetland treatment system successfully improved the water quality of relatively weak secondary WW effluent and net-alkaline AMD.

Aside from AMD and WW tending to be relatively low in specific pollutants which are relatively elevated in the other, allowing for contaminant dilution, beneficial reactions are likely to occur when mixing these two environmental liabilities. The dilution of AMD hydrogen ion concentrations is important because the solubilities of many metals of concern decrease with increasing pH. Dissolved Al and Fe in AMD can react with phosphate and precipitate (Omoike and Vanloon, 1999; Johnson and Younger, 2006). Many metals of concern can bind to the organic ligands present in WW (Fristoe and Nelson, 1983; Fletcher and Beckett, 1987; Omoike and Vanloon, 1999; Norton et al., 2004; Gibert et al., 2005). The organic matter present in WW is also a carbon source that sulfate reducing bacteria (SRB) can utilize to generate alkalinity and precipitate metal sulfides. Studies have documented the effectiveness of sewage sludge as an SRB medium (Waybrant et al., 1998; Harris and Ragusa, 2000; McCullough et al., 2006).

AMD passive treatment systems have generally been limited to the treatment of the more prevalent metal contaminants (i.e., Al, As, Cd, Fe, Mn, Pb, Zn, etc.) (Younger et al., 2002; Watzlaf et al., 2004). However, recent research has been pushing the boundaries of passive treatment to enable the treatment of less common elements of concern, such as Sb, Se, U and others (Simmons et al., 2002; Merten et al., 2004; Luo et al., 2008). It is likely that passive treatment can be expanded to target yet more elements, including rare earth elements (REE).

REE are a relatively chemically uniform group that includes Y, La and the Lanthanide series (Tyler et al., 2004). REE typically form trivalent cations and behave similarly in the environment. Until recently, the REE have neither been characterized as supportive for nor toxic to life. Although REE are generally thought to be relatively benign (Hirano and Suzuki, 1996; Tyler, 2004), relatively few toxicity studies have yet been undertaken because REE are often in such low concentrations as to be difficult to detect (Tyler, 2004). REE concentrations are highly elevated in some AMD (Verplank et al., 2004; Gilchrist et al., 2009) and could eventually prove to be of importance. Hirano and Suzuki (1996) document that extremely elevated concentrations (ppm) of REE can be toxic to mammals. However, a few studies indicate that REE can be preferentially taken up by plants (Stille et al., 2006) and slightly elevated concentrations of REE may be preferential to plant growth (Tyler, 2004) and thus removal within passive treatment systems may be undesirable. As of the writing of this manuscript, no studies have yet been encountered that document the dynamics of REE within any types of AMD passive treatment systems.

This experiment was executed to determine the suitability of AMD from Cerro Rico de Potosí, Bolivia and local LS and WW for conventional passive treatment or co-treatment. The overall goals of the experiment were to 1) increase the understanding of conventional passive treatment of high strength AMD, 2) explore the removal of elements usually not tracked or addressed with passive treatment, such as REE, to better understand the full utility of the approach, and 3) investigate the feasibility of AMD and WW co-treatment.

4.3 Methods

4.3.1 Experimental Design

The experimental setup involved three AMD incubation regimes in triplicate: AMD mixed at a 1:1 ratio with WW (AMD+WW), unadulterated AMD incubated in the presence of LS (AMD+LS), and AMD mixed at a 1:1 ratio with WW and incubated in the presence of LS (AMD+WW+LS). The WW and AMD for all incubation regimes were collected the same day as the incubation initiation to minimize biochemical changes to the effluents. Unmixed AMD and WW initial field pH and alkalinity determination, as well as initial dissolved metals and sulfate sampling, were performed prior to cubitainer introduction. AMD+WW initial field pH and alkalinity were determined at the exposure initiation immediately upon mixing. However, to eliminate sampling error from ongoing reactions, initial AMD+WW dissolved metals concentrations were calculated by averaging concentrations in the pre-exposure AMD and WW. All cubitainers were initially sealed with zero headspace then incubated throughout the 72-hr exposure March 19-22nd 2007 outside in ambient conditions approximately 4 km from the peak of Cerro Rico at the Universidad Autónoma de "Tomás Frías" (UATF) (3950 m amsl, S 19.58148°, W 65.75111°). At the conclusion of the incubation, field pH and alkalinity were measured and dissolved metals samples were taken.

4.3.2 Effluent Collection Areas

Raw WW was collected from an unnamed tributary to Rio Huarampaya that drains WW from the north-central sector of the city of Potosí previously characterized by Strosnider et al. (2008) (Figure 4.1). AMD was collected from three abandoned adits that drain active mining zones of Cerro Rico de Potosí: Esperanza, Guadalupe and Real Socavón, also previously characterized by Strosnider et al. (2007). Cerro Rico is a highly mineralized extinct volcano located adjacent to Potosí, with mine portals situated less than 100 m from populated areas of the city. Cerro Rico is the world's largest Ag deposit and has been intensively mined for nearly five centuries, one environmental liability of which is AMD (Cunningham et al., 1999; Strosnider et al., 2007). Discharge Esperanza emanates from an abandoned mine portal that is likely draining an active mining operation. Discharge Guadalupe drains the abandoned Guadalupe mine in the Chimborazo sector of Cerro Rico which is also likely hydraulically connected to active mining operations. Real Socavón was constructed from 1779 to 1790 to lower the water table within Cerro Rico and is situated near the heart of its most active mining sector. Real Socavón and Discharge Esperanza drain to Rio Huayna Mayu and Discharge Guadalupe drains to Rio Villacollu Mayu, both heavily contaminated tributaries at the headwaters of the ecologically and economically vital Rio Pilcomayo (Strosnider et al., 2007, 2008). LS of approximately 3-cm diameter was donated by the Estuquera Cayara, a local quicklime producer approximately 10 km from Potosí that sources LS from nearby deposits.

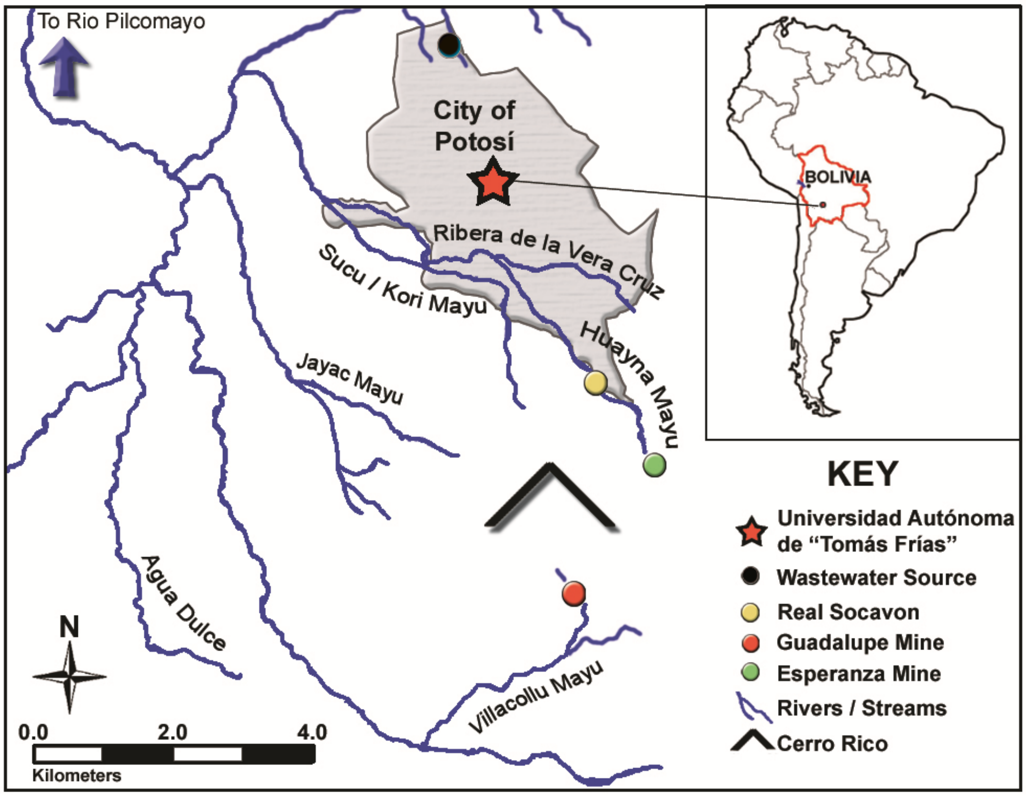

Figure 4.1 Location of each effluent collected with respect to the urbanized areas of Potosí, the incubation location and receiving streams.

4.3.3 Sample analysis

An Orion 1230 multimeter was used to determine pH and temperature. Alkalinity titrations were conducted in accordance with standard methods (APHA, 1998) and Hach Method 8203 (Hach, 2006). A MetrOhm® 761 compact ion chromatograph unit was used to quantify initial sulfate concentrations following EPA method 300. Dissolved metals samples were filtered through 0.45-µm nylon filters prior to preservation with concentrated trace metal grade nitric acid then stored at 4°C until microwave acid digestion following EPA method 3015. Digested metals samples were analyzed via a Varian Vista-Pro® simultaneous inductively coupled plasma-optical emission spectrometer following EPA method 6010.

4.3.4 Data Analysis

An estimate of initial ionic strength was calculated with PHREEQC version 2.16.02 (Parkhurst and Appelo, 2008) using initial sulfate, [H^+] and dissolved metals concentrations. Net acidity and acidity were calculated following a modified version of that presented by Younger et al. (2002), which accounted for the acidity from the substantial Cd^{2+}, Cu^{2+} and Zn^{2+} concentrations present in these waters. For acidity calculations, all Fe was assumed to be Fe(II) when $pH > 3$ and Fe(III) when $pH < 3$, because Fe(III) predominates for most solutions with $pH < 3$ (Kirby and Cravotta, 2005). This assumption generally follows empirical observations (Younger et al., 2002; Watzlaf et al., 2004; Kirby and Cravotta, 2005). PHREEQC was also employed to estimate which species of the major constituents may be precipitating at the incubation endpoint, assuming final DO to be zero, temperature to be 10°C and sulfate to be unchanged because the short incubation period would likely not allow for SRB to acclimate and reduce significant concentrations of sulfate (e.g., Gilbert et al., 1999; Pruden et al., 2007). To enable various calculations, when concentrations were below practical quantification limits (PQL), they were assigned values one half of the PQL. All AMD+WW and AMD+WW+LS results and statistical comparisons account for dilution.

Prior to statistical testing with Microsoft Excel®, variances were calculated and found to be similar. Assuming normality, two-tailed two sample homoscedastic Student's *t*-tests ($\alpha = 0.05$) were performed to determine statistical difference between all final results. Single sample homoscedastic Student's *t*-tests ($\alpha = 0.05$) were used to determine if the initial conditions of each exposure were not equal to the mean of the final conditions. Correlation was tested with Minitab® version 15 by calculating

Pearson's product moment correlation coefficients. All mean comparisons presented in the following paragraphs are based upon statistical testing ($p < 0.05$).

4.4. Results and Discussion

4.4.1 Influent Characteristics

Relatively extremely polluted waters were employed for this study. Two of the AMD discharges, Real Socavón and Guadalupe, are high-strength AMD with metals and acidity concentrations orders of magnitude greater than typical AMD (e.g., Younger et al., 2002; Watzlaf et al., 2004). The Esperanza mine AMD had more typical metals concentrations and acidity, except for highly elevated Zn. The Potosí WW alkalinity places it in the "strong" designation of WW established by Metcalf and Eddy (1991).

4.4.2 pH, Alkalinity and Acidity

The final alkalinity produced by the LS was representative of real-world ALD or RAPS performance (Watzlaf et al., 2000, 2004; Cravotta, 2003), indicating that the locally sourced LS is of high quality and suitable for passive treatment. Each AMD+LS and AMD+WW+LS treatment produced substantial alkalinity and raised pH (Table 4.1). Higher pH resulted from LS exposures of the weaker AMD (Esperanza). Relevant to passive treatment, all LS exposures sufficiently produced alkalinity and raised pH for aerobic Fe oxidation to subsequently proceed efficiently, which requires circumneutral pH and sufficient buffering capacity to counteract the release of protons that accompanies Fe hydrolysis (Younger et al., 2002).

Table 4.1 Alkalinity generation and pH results (mean ± standard deviation). N=1 for all initial ($_i$) measurements. N=3 for all final ($_f$) measurements except where standard deviation is omitted because N=2. Negative net acidity is net alkalinity.

	pH	Alkalinity	Acidity	Net Acidity
	s.u.		mg/L as CaCO$_3$	
WW$_i$	8.62	338	3	-335
Esperanza$_i$	4.10	0	454	454
+LS$_f$	7.56 ± 0.08	149 ± 4.0	93 ± 8.3	-56 ± 12
+WW$_i$	6.25	152	227	75
+WW$_f$	7.57 ± 0.53	152 ± 13	144	-1.0
+WW+LS$_f$	7.92 ± 0.35	232 ± 7.6	20	-212
Guadalupe$_i$	2.30	0	12745	12745
+LS$_f$	6.78 ± 0.16	313 ± 13	7750 ± 129	7438 ± 119
+WW$_i$	3.05	0	5127	5127
+WW$_f$	3.15 ± 0.05	0 ± 0	5149	5149
+WW+LS$_f$	6.50 ± 0.53	299 ± 12	3112 ± 168	2923 ± 162
R. Socavon$_i$	2.70	0	12499	12499
+LS$_f$	6.05 ± 0.22	397 ± 13	4889 ± 167	4502 ± 176
+WW$_i$	2.85	0	6272	6272
+WW$_f$	3.00 ± 0.07	0 ± 0	5011	5011
+WW+LS$_f$	6.61 ± 0.16	370 ± 10	1696	1326

Final alkalinity values were significantly different for all AMD+WW+LS and AMD+LS exposures. Given that waters were exposed to the same LS under identical conditions, differences in final alkalinity and pH were dependent upon AMD chemical compositions. The final alkalinity produced by LS exposure is dependent upon various physiochemical factors of the AMD (Cravotta and Trahan, 1999; Cravotta, 2003; Watzlaf et al., 2004). For example, calcite dissolution is slowed by increased pH and concentrations of Ca^{2+} and HCO$_3^-$ and decreased Pco$_2$ (Cravotta and Trahan, 1999). LaBar et al. (2008) also noted a highly significant trend of increasing alkalinity generation with increasing ionic strength in a modified ALD design, which is reinforced by theory (Stumm and Morgan, 1996).

Guadalupe and Real Socavón had much higher final alkalinities than Esperanza, which had higher initial pH than both waters, lower initial ionic strength than both waters and similar initial dissolved Ca concentrations as Guadalupe. Real Socavón had a higher initial pH and contained 1.86 times more initial dissolved Ca than Guadalupe, however Real Socavón had slightly greater initial ionic strength and produced 84 mg/L more final alkalinity. Linear regression of initial AMD and AMD+WW initial dissolved Ca concentration versus final alkalinity resulted in a poor relationship (Pearson's correlation coefficient of 0.635 and p = 0.175). However, a relationship was found between final alkalinity and initial ionic strength as well as initial pH (Figures 4.2 and 4.3). These contrasts were statistically significant (Pearson's correlation coefficients of 0.814 and -0.833, p = 0.049 and 0.039, respectively). Overall, results indicate these waters' initial ionic strength and pH are better predictors of final alkalinity than initial Ca concentration.

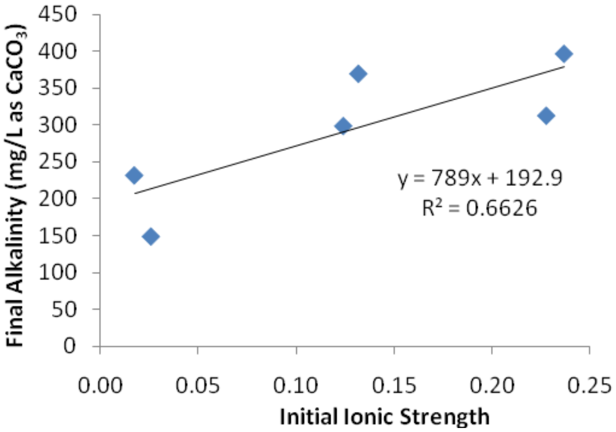

Figure 4.2 Relationship between mean initial solution ionic strength calculated with PHREEQC and final alkalinity for all LS incubations of AMD and AMD+WW.

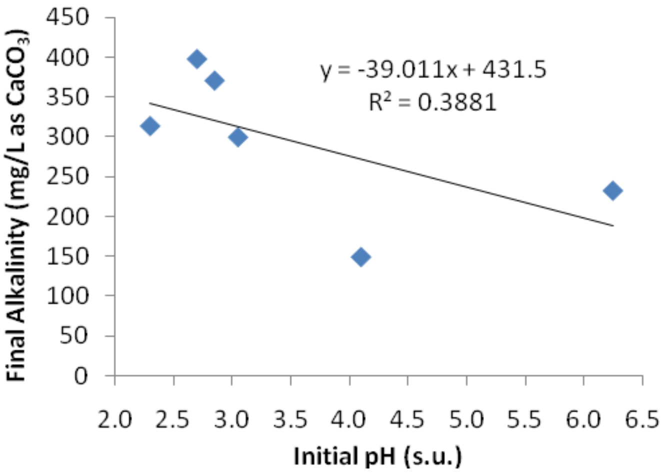

Figure 4.3 Relationship between mean initial pH and final alkalinity for all LS incubations of AMD and AMD+WW.

Relative to future co-treatment efforts, mixing AMD with WW before exposure to LS has mixed impacts on final alkalinity and pH. Higher final alkalinity and pH are preferred because they enable greater and faster Fe or Mn oxidation in subsequent unit processes. Although mixing AMD with WW prior to LS exposure raised initial pH and decreased initial ionic strength, it had relatively little effect on the final alkalinity of the two higher strength AMD. Compared to unmixed AMD+LS, the final alkalinity of the AMD+WW+LS was only 4.3 and 6.7% less for Guadalupe and Real Socavón, respectively. In the case of Esperanza, the addition of WW imparted alkalinity which was further enhanced by the LS exposure, resulting in final alkalinity 55% greater than the unmixed LS exposure. The impact of decreased ionic strength on final alkalinity is likely offset by the alkalinity imparted by the WW. Regarding the influence on pH of mixing AMD with WW before LS exposure, the final pH of the unmixed Real Socavón was higher while that of Esperanza and Guadalupe were not statistically different than their unmixed respective AMD+LS.

Despite the dilution involved, adding WW to AMD raised pH for only Esperanza. Although the net acidity of Guadalupe did not significantly decrease over the incubation period, the net acidity of Real Socavón and Esperanza decreased. These results indicate that the full advantages of mixing AMD with WW may not be immediately realized and further acidity can be buffered during incubation. It is unknown if the primary mechanism for acid neutralization is abiotic (i.e., sorption to organic ligands) or biotic (i.e., BSR) because final sulfate concentrations were not determined. However, following Neculita et al. (2007) significant BSR is unlikely given the short incubation duration and Zn concentrations orders of magnitude greater than those determined to be severely inhibitive to SRB. Therefore, it is assumed that the bulk of acid neutralization noted when incubating AMD+WW was via abiotic means. In the case of the weaker AMD (Esperanza), the acid neutralization capacity provided by the WW was sufficient to produce marginally net-alkaline effluent. This has implications for treatment because it may be possible to co-treat these net-acidic waters without LS and/or other organic substrate, which could result in significant cost savings. In addition, further alkalinity may be generated by SRB using the substantial labile organic material in WW.

Regarding the LS exposures of the two higher strength AMD sources, it does not appear that calcite dissolution can account for all the acidity neutralization of Real Socavón and Guadalupe with or without WW. Alkalinity generation from calcite dissolution was estimated by tracking the change in Ca concentrations, pre- and post-LS exposure. However, there are some drawbacks with this approach. There appears to be some release of Ca by WW during incubation with AMD, evidenced by the increase in Ca during all WW+AMD incubations. Cation exchange between WW solids and more electronegative elements in the AMD may explain this phenomenon.

The likelihood of gypsum ($CaSO_4$) precipitation, which was indicated by PHREEQC modelling, would also detract from the accuracy of this approach. In addition, the formation of compounds that do not result in the release of protons, such as metal carbonates that were modelled with PHREEQC to be oversaturated ($ZnCO_3$, $FeCO_3$ and $CdCO_3$), would decrease calculated acidity without consuming alkalinity. The sorption of Zn or Cd to Fe hydroxides would also decrease calculated acidity without consuming alkalinity.

4.4.3 Metals Behavior

As expected, dissolved concentrations of major elements (those generally in the ppm range) of interest in the mixed and unmixed AMD decreased with LS exposure (Table 4.2). The AMD+WW incubations resulted in decreased dissolved Al, Fe, As and Zn. The AMD+WW+LS exposures resulted in more complete removal of some metals of interest such as Al, Cd, Fe, Ni and Zn. Overall, results indicate that passive mixing and incubation of AMD with WW can remove significant quantities of metals from solution.

Table 4.2 Mean dissolved major constituents (mg/L). Standard deviation is italicized. N=1 for initial values, N=2 when standard deviation is not displayed (-), and N=3 when standard deviation is presented.

	Al	As	Ca	Cd	Cu	Fe
WW_i	0.26	<0.022	44.4	<0.0006	0.029	0.51
Esperanza_i	10.3	0.025	122	0.46	0.046	6.9
$+\text{LS}_f$	0.025	<0.022	397	0.029	0.015	0.034
	0.005	-	*75.0*	*0.004*	*0.001*	*0.01*
$+\text{WW}_i$	5.30	0.018	83.2	0.23	0.038	3.70
$+\text{WW}_f$	0.020	<0.022	98.7	0.178	0.028	0.042
$+\text{WW+LS}_f$	0.095	<0.022	216	0.006	0.11	0.14
Guadalupe_i	80.5	38.8	121	9.84	0.12	2612
$+\text{LS}_f$	0.11	<0.022	702	3.21	0.10	1826
	0.003	-	*6.6*	*0.21*	*0.013*	*24.7*
$+\text{WW}_i$	40.4	19.4	82.7	4.92	0.074	1306
$+\text{WW}_f$	37.4	0.34	105	4.80	0.092	1162
$+\text{WW+LS}_f$	0.20	0.10	674	1.62	0.055	612
	0.026	*0.009*	*26.6*	*0.050*	*0.005*	*50.2*
R. Socavon_i	437	7.15	225	17.3	29.9	1916
$+\text{LS}_f$	0.53	<0.022	686	7.28	1.03	381
	0.18	-	*8.9*	*0.34*	*0.049*	*4.49*
$+\text{WW}_i$	218	3.58	135	8.64	15.0	958
$+\text{WW}_f$	203	0.28	156	8.16	13.6	609
$+\text{WW+LS}_f$	0.093	<0.022	760	2.26	0.72	66.2

Table 4.2 (cont.)

	K	Mg	Mn	Na	Ni	Zn
WW$_i$	44.1	9.67	0.12	68.8	<0.004	0.35
Esperanza$_i$	12.0	18.0	11.6	13.1	0.072	233
+LS$_f$	23.6	37.9	5.33	18.4	0.029	53.9
	1.15	*0.33*	*0.17*	*0.76*	*0.002*	*5.21*
+WW$_i$	28.1	13.8	5.84	41.0	0.037	117
+WW$_f$	28.5	13.8	5.87	41.2	0.331	86.4
+WW+LS$_f$	40.9	42.3	1.62	46.5	0.015	10.6
Guadalupe$_i$	10.5	31.7	125	9.14	0.96	3129
+LS$_f$	29.2	80.0	162	16.1	1.12	2724
	1.50	*3.08*	*1.22*	*0.20*	*0.007*	*54.2*
+WW$_i$	27.3	20.7	62.4	39.0	0.48	1565
+WW$_f$	30.8	23.7	68.0	40.9	0.48	1755
+WW+LS$_f$	49.4	70.0	80.4	49.2	0.605	1222
	2.37	*19.0*	*2.02*	*1.72*	*0.005*	*55.2*
R. Socavon$_i$	3.89	178	46.5	14.3	2.60	3051
+LS$_f$	20.4	236	70.1	19.8	2.92	2652
	0.71	*4.23*	*0.93*	*0.20*	*0.098*	*104*
+WW$_i$	24.0	93.9	23.3	41.5	1.30	1526
+WW$_f$	30.3	98.5	22.9	43.9	1.28	1569
+WW+LS$_f$	47.6	126	37.4	53.5	1.09	979

Concentrations of minor elements (those generally in the ppb range) tracked were also significantly affected by the treatments. Cr, In, Pb, Sb, Sn and V were removed from solution by LS incubation of mixed and unmixed AMD (Table 4.3). Some minor elements (Ag, Cr, Pb, Sb, Se, Sn and V) were removed from solution by WW incubation alone. Although Ag was removed from solution in two of the three WW incubations, it was released by the LS. The presence of leachable Ag in the local LS may be explained by the source's proximity to Cerro Rico, the world largest Ag deposit, which is situated in a highly mineralized Ag-rich province (Cunningham et al., 1996). Cr was removed more completely by mixed and unmixed LS exposure. Indium (In) was consistently removed to below the PQL during unmixed and mixed LS exposures. Cerussite ($PbCO_3$) formation, as these waters were modelled to be supersaturated by PHREEQC, was a likely Pb removal mechanism. However, Pb was also removed in the absence of LS and could have complexed with organic matter (e.g., Fletcher and Beckett, 1987) or sorbed to Fe oxyhydroxides (e.g., Carroll et al., 1998). Multiple minor elemental constituents (Ag, B, Ba, Co, Li, and Sr) were contributed to the water column by the LS.

Table 4.3 Mean dissolved minor elemental constituents (µg/L). Standard deviation is italicized. N=1 for initial values, N=2 when standard deviation is not displayed (-), and N=3 when standard deviation is presented.

	Ag	B	Ba	Co	Cr	In	Li
WW$_i$	<1.6	293	41	<1	3.2	<24	29
Esperanza$_i$	2.6	171	41	61	<1	<24	166
+LS$_f$	5.9	1254	81	31	1.3	<24	203
	0.2	*69*	*2.1*	*0.7*	*0.2*	-	*20*
+WW$_i$	1.7	232	41	31	2.2	<24	98
+WW$_f$	<1.6	214	36	28	<1	<24	96
+WW+LS$_f$	2.7	1301	88	11	<1	<24	115
Guadalupe$_i$	10	33	12	745	43	408	348
+LS$_f$	12	1446	73	2133	7.4	<24	430
	0.5	*55*	*4.1*	*147*	*0.6*	-	*4.5*
+WW$_i$	5.6	163	27	373	23	205	189
+WW$_f$	3.0	125	31	405	18	208	195
+WW+LS$_f$	7.5	1248	81	1179	3.2	<24	246
	0.5	*130*	*3.8*	*22*	*0.2*	-	*9.6*
R. Socavon$_i$	3.4	364	8.6	246	135	3847	753
+LS$_f$	7.5	1581	78	3086	2.9	<24	836
	1.8	*74*	*7.2*	*69*	*0.1*	-	*13*
+WW$_i$	2.1	329	25	123	69	1924	391
+WW$_f$	<1.6	317	31	1053	64	1788	375
+WW+LS$_f$	7.8	1402	87	1534	1.4	<24	442

Table 4.3 (cont.)

	Pb	Sb	Se	Sn	Sr	V
WW$_i$	19	<12	<42	<18	373	<1.5
Esperanza$_i$	288	<12	<42	<18	1245	<1.5
+LS$_f$	<12	25	<42	<18	1693	<1.5
	-	*1.5*	-	-	*183*	-
+WW$_i$	153	<12	<42	<18	809	<1.5
+WW$_f$	<12	<12	<42	<18	792	<1.5
+WW+LS$_f$	23	<12	<42	<18	1086	1.7
Guadalupe$_i$	1669	27	54	18	591	60
+LS$_f$	452	21	63	<18	1524	<1.5
	1.7	*2.4*	*4.0*	-	*29*	-
+WW$_i$	844	16	37	14	482	30
+WW$_f$	660	<12	<42	<18	505	8.0
+WW+LS$_f$	294	<12	<42	<18	1273	<1.5
	6.7	-	-	-	*42*	-
R. Socavon$_i$	604	18	<42	59	320	90
+LS$_f$	137	<12	<42	<18	1037	<1.5
	10	-	-	-	*37*	-
+WW$_i$	312	12	<42	34	346	46
+WW$_f$	301	<12	<42	<18	359	10
+WW+LS$_f$	39	<12	<42	<18	1117	<1.5

Generally, it is disadvantageous in passive treatment systems to remove Al or Fe in the presence of LS due to armoring and clogging issues (Watzlaf et al., 2000, 2004). As expected, the high Al and Fe removal rates noted during the Real Socavón and Guadalupe LS exposures indicate that some form of pretreatment would be necessary for higher strength AMD (Table 4.4). The pH increase created by LS dissolution likely formed insoluble Al and Fe oxyhydroxides which over the long-term will decrease LS dissolution efficiency (Younger et al., 2002). Orders of magnitude greater than ALD design guidance (Watzlaf et al., 2004), Al and Fe concentrations of the AMD may be too high for conventional RAPS designs as well.

Table 4.4 Volumetric removal rates calculated using initial and final dissolved concentrations. Negative values denote an increase in dissolved concentrations. Values are omitted (-) where there was no significant difference between initial and final concentrations. Mo is not included because concentrations were consistently below the PQL (<48 μg/L).

	Esperanza			Guadalupe			Real Socavón		
	+LS	+WW	+WW +LS	+LS	+WW	+WW +LS	+LS	+WW	+WW +LS
Major Constituents (g m^{-3} d^{-1})									
Al	3.4	1.8	1.7	27	-	13	145	-	73
As	0.005	0.002	0.002	13	6.4	6.4	2.4	1.1	1.2
Ca	-92	-5.2	-	-194	-7.5	-197	-153	-7.1	-208
Cd	0.14	0.02	0.074	2.2	-	1.1	3.3	-	2.1
Cu	0.010	-	-0.025	-	-0.006	0.006	9.6	-	4.8
Fe	2.3	1.2	1.2	262	-	231	512	116	297
K	-3.9	-	-4.3	-6.2	-	-7.4	-5.5	-2.1	-7.9
Mg	-6.6	-	-	-16	-	-16	-19	-	-
Mn	2.1	-	-	-13	-	-6.0	-7.9	-	-4.7
Na	-1.8	-	-1.8	-2.3	-	-3.4	-1.8	-	-4.0
Ni	0.014	-0.098	-	-0.054	-	-0.042	-0.11	-	0.071
Zn	60	10	35	135	-	114	133	-	182
Minor Constituents (mg m^{-3} d^{-1})									
Ag	-1.1	0.30	-	-0.53	-	-0.64	-	0.44	-1.9
B	-361	-	-356	-471	13	-362	-406	-	-358
Ba	-13	-	-	-20	-1.4	-18	-23	-	-21
Co	9.9	-	-	-463	-	-269	-947	-310	-470
Cr	-	0.44	0.44	12	1.7	6.7	44	-	23
In	-	-	-	132	-	66	1280	-	639
Li	-	-	-5.9	-27	-	-19	-27	-	-
Pb	94	49	-	406	61	183	156	-	91
Sb	-	-	-	-	3.4	3.4	4.1	2.0	2.0
Se	-	-	-	-	21	5.5	-	-	-
Sn	-	-	-	3.0	1.5	1.5	17	8.4	8.4
Sr	-	-	-	-311	-	-264	-239	-	-257
V	-	-	-	20	7.5	9.9	30	12	15

Experimental results indicate that it could be beneficial to remove Al and Fe by pretreatment with WW. Real Socavón and Esperanza dissolved Fe concentrations decreased significantly with WW incubation. However, only Esperanza dissolved Al decreased significantly under the same treatment regime, likely due to the increase in pH which favored the formation of the insoluble amorphous $Al(OH)_3$. In addition to combining with phosphate to form a relatively stable solid, Al and Fe can complex with living and dead organic ligands (Fletcher and Beckett, 1987; Omoike and Vanloon, 1999). In future studies the ratio of AMD to WW and incubation duration may be manipulated to encourage further Al and Fe removal.

Other major constituents of concern were removed by the three treatment regimes. Elevated concentrations of dissolved As were greatly diminished by all three treatment regimes, often to below the PQL. The high rate of As removal from AMD when incubated with WW alone indicates that mixing with WW can effectively remove As from solution. The mechanism of removal is unknown. However, extensive research has documented the affinity of As to Fe hydroxides (Mohan and Pittman, 2007), which likely formed under all treatment regimes. Cd concentrations of all AMD and AMD+WW treatments dramatically decreased in the presence of LS. It is likely that otavite ($CdCO_3$) formed, which was predicted to be supersaturated by PHREEQC. In addition, Cd removal was more complete with the AMD+WW+LS incubations, which may indicate Cd complexation with organic ligands and/or adsorption to bacterial solids (e.g., Fristoe and Nelson, 1983). Only Real Socavón had highly elevated concentrations of Cu, which were decreased during mixed and unmixed exposures to LS. Various solid Cu compounds were found by PHREEQC modelling to be supersaturated, such as $CuFeO_2$, $CuFeO_4$ and $CuCr_2O_4$. Zn was removed at a high rate in the mixed and unmixed LS exposures. PHREEQC modelling indicated that various Zn carbonates, such as smithsonite ($ZnCO_3$), were supersaturated in the final solution. Some Zn removal was likely due to sorption to Fe oxyhydroxides which Zn has a high affinity for at circumneutral pH (Carroll et al., 1998). Zn removal was also observed without the presence of LS, in the Esperanza AMD+WW incubation. Living and dead organic ligands present in the WW likely served as Zn sorption sites (e.g., Fletcher and Beckett, 1987; Norton et al., 2004).

Concentrations of some major constituents increased during LS exposures. Ca, K, Mg and Na concentrations increased due to dissolution of the LS. Unexpectedly, Mn concentrations increased during the LS exposures of the mixed and unmixed, more acidic (Real Socavón and Guadalupe) AMD. However, Mn was removed from solution during mixed and unmixed LS exposure of the weaker Esperanza AMD. These mixed results may be due to increased attack of the LS matrix by the higher ionic strength, lower pH, more acidic AMD. The generation of substantial Mn indicates that other LS sources ought to be explored for more suitable passive treatment substrate. In the unfortunate case that a more suitable source is not available, the Mn generated may be removed in the final stages of passive treatment.

Many of the minor constituent metals either contributed to (Ag, Ba, Co and Li) or removed from solution (Ag, Cr, Pb, Sb, Se, Sn and V) are of human or ecological health concern (Nordberg et al., 2007). Of these metals, Pb is the only minor constituent typically targeted for passive treatment and tracked throughout systems. The results above indicate that LS passive treatment substrate can be a source or sink for some minor constituents of concern. However, conventional passive treatment unit processes with LS (i.e., ALDs, OLD/Cs, RAPS) can be applied to remove a much broader range of metals from solution. The wide range of minor constituent metals removed from solution during the two co-treatment regimes indicates that passive co-treatment may be a viable approach for remediation of waters with a broad range of contamination.

4.4.4 Rare Earth Elements

The REE tracked in this study generally decreased in concentration under all three incubation regimes (Tables 4.5 and 4.6). Generally higher REE removal rates were noted during mixed and unmixed LS exposures than those without LS. Four REE did not follow these general patterns. Eu concentrations decreased during mixed and unmixed LS exposures, yet increased during the Real Socavón AMD+WW incubation. Pr appeared to be released by the WW and LS. La was released by the WW exposures of Real Socavón and Guadalupe, but not the less acidic Esperanza AMD. However, all mixed and unmixed LS exposures resulted in decreased La. Nd results were mixed with some treatments increasing dissolved concentrations and others decreasing.

Table 4.5 Mean dissolved REE constituents (µg/L). Standard deviation is italicized. N=1 for initial values, N=2 when standard deviation is not displayed (-), and N=3 when standard deviation is presented. Er, Lu and Tb are not included because concentrations were consistently below the PQLs, at < 7.6, 4.3 and 10 µg/L, respectively.

	Ce	Dy	Er	Eu	Gd	Ho	La	Nd	Pr	Sc	Sm	Tm
WW_i	<2.8	<1.2	<6	<4.6	<2.8	<2	3.1	25	87	<0.8	<3.8	<2.8
$\mathrm{Esperanza}_i$	67	<1.2	7.5	5.8	11	<2	21	66	139	<0.8	13	<2.8
$+\mathrm{LS}_f$	6.3	<1.2	<6	<4.6	<2.8	<2	5.2	61	167	<0.8	<3.8	<2.8
	0.4	-	-	-	-	-	*0.1*	*2.4*	*9.6*	-	-	-
$+\mathrm{WW}_i$	34	<1.2	5.2	4.0	6.3	<2	12	46	*113*	<0.8	*7.4*	<2.8
$+\mathrm{WW}_f$	<2.8	<1.2	<6	5.0	<2.8	<2	4.0	37	*125*	<0.8	<3.8	<2.8
$+\mathrm{WW}+\mathrm{LS}_f$	4.3	<1.2	<6	<4.6	<2.8	<2	5.1	54	*168*	<0.8	<3.8	<2.8
$\mathrm{Guadalupe}_i$	65	6.6	<6	6.6	70	3.3	10	59	44	23	62	7.9
$+\mathrm{LS}_f$	42	<1.2	<6	<4.6	33	<2	4.1	69	84	<0.8	<3.8	6.8
	0.5	-	-	-	*0.5*	-	*0.1*	*1.1*	*15*	-	*1.0*	*0.4*
$+\mathrm{WW}_i$	33	<1.2	<6	4.4	36	2.4	6.5	42	66	12	32	<2.8
$+\mathrm{WW}_f$	30	<1.2	<6	5.9	32	<2	7.0	49	75	10.1	31	3.4
$+\mathrm{WW}+\mathrm{LS}_f$	25	<1.2	<6	<4.6	14	<2	4.4	68	128	<0.8	11	<2.8
	0.6	-	-	-	*0.4*	-	*0.2*	*0.2*	*4.4*	-	*1.2*	-
R. $\mathrm{Socavón}_i$	71	5.8	<6	6.4	66	4.1	22	84	60	87	34	6.7
$+\mathrm{LS}_f$	21	<1.2	<6	<4.6	10	<2	4.7	69	135	<0.8	11	3.1
	0.7	-	-	-	*0.8*	-	*0.2*	*0.6*	*4.9*	-	*0.8*	*0.2*
$+\mathrm{WW}_i$	36	<1.2	<6	4.4	34	2.7	13	55	74	44	18	<2.8
$+\mathrm{WW}_f$	34	<1.2	<6	6.2	30	2.2	13	64	109	41	16	<2.8
$+\mathrm{WW}+\mathrm{LS}_f$	13	<1.2	<6	<4.6	4.6	<2	4.8	71	148	<0.8	4.0	<2.8

Table 4.6 Volumetric removal rates of REE calculated using initial and final dissolved concentrations (mg $m^{-3}d^{-1}$). Negative values denote an increase in dissolved concentrations. Values are omitted (-) where there was no significant difference between initial and final concentrations.

	Esperanza			Guadalupe			Real Socavón		
	+LS	+WW	+WW +LS	+LS	+WW	+WW +LS	+LS	+WW	+WW +LS
Ce	20	11	10	7.4	0.86	2.7	17	-	7.8
Dy	-	-	-	2.0	1.0	1.0	1.7	0.86	0.86
Er	1.5	0.75	0.75	-	-	-	-	-	-
Eu	1.2	-	0.58	-	-0.49	0.71	1.4	-	0.68
Gd	3.2	1.6	1.6	12	1.3	7.1	19	1.4	9.7
Ho	-	-	-	0.78	0.39	0.39	1.0	-	0.51
La	5.4	2.7	2.3	2.0	-0.15	0.72	5.8	-0.26	2.6
Nd	1.6	2.8	-	-3.5	-	-8.7	4.9	-3.2	-
Pr	-	-4.0	-18	-13	-	-21	-25	-12	-25
Sc	-	-	-	7.4	0.46	3.7	29	-	14
Sm	3.7	1.8	1.8	11	-	6.9	7.7	-	4.7
Tm	-	-	-	0.38	0.44	-	1.4	0.89	0.89

Generally, similar behavior among REE was observed under the three incubation regimes. Consistent behavior was expected because REE have similar properties. REE solubility decreases with increasing pH (Verplank et al., 2004; Gilchrist et al., 2009), REE can sorb to Fe and Al oxyhydroxides (Verplank et al., 2004; Centeno et al., 2004; Marmolejo-Rodríguez et al., 2007) and various microorganisms may sorb or uptake REE (Tyler, 2004; Merten et al., 2004, 2005). Relative to co-treatment, Merten et al. (2004) found that REE can sorb to the fecal indicator bacteria, *E. coli*. It is likely that the aforementioned removal mechanisms contributed to the general decrease in aqueous REE concentrations during the three incubation regimes. Our results combine with the previously discussed findings to indicate that passive treatment systems are likely removing REE from their AMD influent. Enough is not yet known about the positive or negative environmental effects of REE to determine whether this removal is advantageous.

4.5 Conclusions and Recommendations

Results indicate that cubitainer tests have broader utility than has been previously taken advantage of, passive treatment can be expanded to target more constituents of interest than it has been traditionally applied, and that the co-treatment of AMD with WW is a promising new application of passive treatment that should be further investigated. In lieu of substrate analysis, AMD exposure can reveal the quality and chemical composition of potential calcareous passive treatment substrate. Cubitainer tests are not only valuable for predicting alkalinity generation, but determining if substantial concentrations of potentially undesirable elements may be released from calcareous substrate during passive treatment. Regarding the prospects of co-treatment, AMD exposure to WW can remove significant quantities of key metals, such as tens of mg/L of As, from solution as well as imbue sufficient alkalinity to AMD to allow for subsequent aerobic Fe removal. In addition, mixing AMD with WW before exposure to LS has relatively little effect on final alkalinity. Locally, results indicate that although regionally-sourced LS can produce relatively high final alkalinity, it is a possible source for Mn and a few other potentially undesirable elements.

This study solicits further exploration along a few avenues. Supplementary experimentation should be undertaken to explore the feasibility of high strength AMD and WW passive co-treatment. Specifically, the ability of WW to decrease DO, remove Al and reduce Fe(III) to Fe(II) before the mixed effluent's introduction to LS should be investigated. The experiment illuminated possible passive treatment removal options for elements not often targeted (i.e., Ag, Sb, Sn, V, REE). Further exploration is recommended to determine the potential for removal of these elements as our understanding of their ecotoxicity increases. Expanded elemental tracking within existing passive treatment systems is a logical step. In addition, the ability of SRB to utilize the buffering capacity and carbon substrate provided by WW to produce alkalinity and metal sulfides could be determined with longer duration incubations.

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CHAPTER V

Performance of an Ecologically-Engineered Multi-Stage Acid Mine Drainage and Municipal Wastewater Passive Co-Treatment System

Portions of this chapter are included in:

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Winfrey, B.K., Strosnider, W.H., Nairn, R.W. 2009. Reduction of fecal indicator bacteria counts in an ecologically-engineered acid mine drainage and municipal wastewater passive co-treatment system. Proceedings, 2009 National Meeting of the American Society of Mining and Reclamation, Billings, MT. p. 1611-1626.

Winfrey, B.K., Strosnider, W.H., Nairn, R.W., Strevett, K.A. *In Review*. Highly effective reduction of fecal indicator bacteria counts in an ecologically-engineered acid mine drainage and municipal wastewater passive co-treatment system. Ecological Engineering.

Strosnider, W.H., Winfrey, B.K., Nairn, R.W. *In Review*. Efficient metals removal and alkalinity generation in a novel ecologically-engineered multi-stage acid mine drainage and municipal wastewater passive co-treatment system. Water Research.

5.1 Abstract

A laboratory-scale, four-stage continuous-flow reactor system was constructed to test the viability of the passive co-treatment of high-strength acid mine drainage (AMD) and municipal wastewater (MWW). The synthetic AMD had pH 2.6 and 1860 mg/L acidity as $CaCO_3$ equivalent and with 46, 0.25, 2, 290, 55, 1.2 and 390 mg/L of Al, As, Cd, Fe, Mn, Pb and Zn, respectively. The AMD was mixed at a 1:2 ratio with raw MWW from the City of Norman, Oklahoma containing 265 ± 94 mg/L BOD_5, 11.5 ± 5.3 mg/L PO_4^{-3}, and 20.8 ± 1.8 mg/L NH_4^+-N and introduced to the system which had a total residence time of 6.6 d. During the 135 d experiment, dissolved Al, As, Cd, Fe, Mn, Pb and Zn concentrations were consistently decreased by 99.8, 87.8, 97.7, 99.8, 13.9, 87.9 and 73.4%, respectively, pH increased to 6.8 ± 0.1, and net acidic influent was converted to net alkaline effluent. PO_4^{-3} and NH_4^+-N were decreased to <0.75 and 7.4 ± 1.8 mg/L, respectively. BOD_5 was generally decreased to below detection limits. Nitrification increased NO_3^- to 4.9 ± 3.5 mg/L NO_3^--N, however relatively little denitrification occurred. Sulfate reducing bacteria were able to maintain a relatively high level of sulfate reduction (0.56 mol/m^3-d) despite inhibitory pH and metals concentrations. A 100% reduction of all fecal indicator bacteria (FIB) was observed. However, FIB exhibited evidence of sub-lethal injury with slower colony formation rates on standard growth media. At a wasting rate of 0.69% of total influent flow, the system produced sludge with total Al, As, Cd, Cr, Cu, Fe, Pb and Zn concentrations at least an order of magnitude greater than the theoretical influent mix, which presents a possible environmental liability if not sustainably recovered or disposed. Results suggest that the nitrogen processing community may require an extended period to mature and reach full

efficiency. Overall, results indicate that passive AMD and MWW co-treatment is a viable ecological engineering approach for the developed and developing world that can be optimized and applied to improve water quality with minimal use of fossil fuels and refined materials.

5.2 Introduction

Acid mine drainage (AMD) and municipal wastewater (MWW) are common environmental liabilities for which sustainable treatment is central to maintaining global water resource quality and conserving energy resources. Untreated AMD degrades water resources in coal and metal mining regions globally (Salomons, 1995; Wolkersdorfer and Bowell, 2004a, 2004b, 2004c; Bell and Donnelly, 2006). Discharges of untreated MWW degrade water resources in many developing nations (Gadgil, 1998; Kivaisi, 2001, Nelson et al. 2001). In developed nations, where MWW is generally addressed actively, treatment consumes substantial fiscal, material and energy resources (Muga and Mihelcic, 2008). Compared to passive methods, conventional active MWW and AMD treatment are energy-intensive with higher operational and maintenance costs (Nelson et al. 2001; Younger et al. 2002; Mannino et al. 2008; Muga and Mihelcic, 2008). Passive methods can be considered an application of ecological engineering, which entails "the design of sustainable ecosystems that integrate human society with its natural environment for the benefit of both" (Mitsch, 1996). Renewable energy and natural unprocessed material flows must outweigh those of fossil fuel and refined material in ecological engineering applications (Mitsch and Jorgensen, 2004).

The passive co-treatment of AMD and MWW is a nascent application of ecological engineering that blends aspects of passive AMD treatment and conventional active MWW treatment. The passive treatment of AMD often requires suitable organic substrate electron donors for dissolved oxygen (DO) stripping, bacterial sulfate reduction (BSR) and the bacterially-mediated reduction of metals, such as Fe. Conventional active MWW treatment can require electron acceptors for bacterial oxidation of carbon substrate, chemicals for pathogen removal, and physical or chemical filtration or flocculation for solids or P removal. In theory, the requirements of both AMD and MWW treatment can be met within the same system, as each effluent possesses properties and constituents that can be passively utilized by the other.

The possibility of using sulfate reducing bacteria (SRB) to treat AMD by BSR to generate alkalinity via bicarbonate production and remove metals from solution via sulfide precipitation was first suggested by Tuttle et al. (1969). The reactions can be generally expressed as follows (Neculita et al. 2007):

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^{-} + H_2S \quad (1)$$

$$H_2S + M^{2+} \rightarrow MS + 2H^{+} \quad (2)$$

where CH_2O is a simple sugar and M^{2+} is a metal ion such as Pb or Zn.

Numerous carbon sources, including horse and cow manure, chicken litter, ethanol, methanol, municipal sewage sludge, and municipal compost, have been successfully applied to encourage BSR (Waybrant et al., 1998; Tsukamoto and Miller, 1999; Younger et al., 2002; Benner et al., 2002; Cocos et al., 2002; Watzlaf et al., 2000; McCullough et al., 2006; Zamzow et al., 2006; Kaksonen and Puhakka, 2007; Luo et al., 2008). MWW contains a wide variety of organic compounds ranging from simple sugars to more recalcitrant compounds, such as cellulose (Metcalf and Eddy, 1991). Generally, BSR is coupled with limestone dissolution in passive treatment cells such as vertical flow bioreactors (VFB) to maximize alkalinity generation and metals retention. However, BSR alone can be effective as Benner et al. (2002) and Cocos et al. (2002) have demonstrated.

Sewage sludge has been used as a BSR carbon source to treat AMD. Waybrant et al. (1998) observed sewage sludge to encourage higher levels of sulfate reduction than seven other commonly used organic carbon sources. However, several studies have noted that mixtures of sludge with multiple organic carbon sources generally promote higher sulfate reduction rates than single sources (Waybrant et al., 1998; Harris and Ragusa, 2000; McCullough et al., 2006). MWW contains a mix of labile and recalcitrant organic substrates (Metcalf and Eddy, 1991) which indicates that it should be able to support a rich and diverse community of preferential microbes.

Metals and acidity can also be abiotically removed from solution by sorption to the organic substrates in AMD passive treatment system unit processes (Neculita et al. 2007). Organic substrates such as peat (Champagne et al. 2008), algae (Rose et al. 1998; Van Hille et al. 1999), dead algae and duckweed (Darnall et al. 1989; Jeffers et al. 1989), fungi (Subudhi and Kar, 1996; Delgado et al. 1998), seaweed (Aderhold et al. 1996), biosolids (Norton et al. 2004) and many of the aforementioned SRB carbon sources have demonstrated promise for abiotic AMD treatment via metals sorption. Relative to co-treatment, elevated concentrations of bacteria in raw MWW are present within activated sludge. Utgikar et al. (2000) demonstrated that non-viable activated sludge from conventional MWW treatment could be an effective abiotic biosorbent for metals in low pH AMD.

Effective MWW treatment is required to safeguard receiving water bodies from eutrophication and subsequent environmental degradation. Generally, MWW treatment must address suspended solids, P, N, oxygen demand and fecal indicator bacteria (FIB) concentrations. Suspended solids can be removed by biodegradation, flocculation, settling or filtration (Rebhun and Streit, 1974; Metcalf and Eddy, 1991). P and suspended solids concentrations can be decreased by flocculation with free Al(III) and Fe(III) ions (Metcalf and Eddy, 1991; Omoike and Vanloon, 1999; Parsons and Smith, 2008). In addition, Adler and Sibrell (2003) and Wei et al. (2008) demonstrated that soluble phosphorus will sorb to pre-existing AMD floc. MWW nitrogen processing generally requires sequential nitrification and denitrification (Metcalf and Eddy, 1991). Bacterial populations central to AMD treatment, such as aerobic heterotrophs, iron reducing bacteria (IRB), and sulfate reducing bacteria (SRB) as well as their associated supporting communities require sufficient nutrients for optimum operation (Neculita et al. 2007). Often, BSR substrate is supplemented with N and/or P to encourage greater SRB activity (Kaksonen and Puhakka, 2007). Oxygen demand is a function of the concentration of biodegradable organic matter, nutrients and readily oxidized constituents. Oxygen demand can be lowered by bacterial respiration or reaction of labile organic matter and nutrients. For example, aerobic heterotrophs, SRB, IRB and denitrifying bacteria utilize short-chain labile organic carbon thus lowering oxygen demand.

Pathogen removal is a key aspect of MWW treatment. Total coliform (TC), fecal coliform (FC), fecal streptococci (FS) and *E. coli* are typical FIB monitored to detect pathogenic risk. TC, FC and FS concentrations in untreated MWW are generally 10^5-10^6, 10^4-10^5 and 10^3-10^4 CFUs/mL respectively (Metcalf and Eddy, 1991). Pathogens are typically removed from MWW by chemical agents (e.g., chlorination), physical processes (e.g., heating), mechanical means (e.g., sedimentation), or radiation (e.g., ultraviolet disinfection) (Metcalf and Eddy, 1991). However, pathogens can be removed by exposure to other unsuitable growth circumstances, such as elevated concentrations of dissolved metals and extreme pH (Hackney and Bissonnette, 1978; Wortman and Bissonnette, 1985; Wortman et al., 1986; Wortman and Bissonnette, 1988).

The disinfection of domestic sewage by AMD was first documented in the 1930s. Roetman (1932) noted this phenomenon, specifically suggesting that wastewater treatment facilities take advantage of the observed deleterious effects of AMD on fecal indicator bacteria (FIB). Joseph and Shay (1952) found that populations of *E. coli* were rapidly decreased when exposed to AMD. Rogers and Wilson (1966) manipulated pH of water samples from the Monongahela River in West Virginia containing domestic sewage-related microorganisms, finding a marked decrease in microbial concentrations in low pH samples. More recent studies have noted that exposure to AMD causes death or widespread structural damage to *E. coli* and that extended incubation periods in specialized enriched medium are necessary to repair surviving cells (Hackney and Bissonnette, 1978; Wortman and Bissonnette, 1985; Wortman et al., 1986; Wortman and Bissonnette, 1988). Carlson-Gunnoe et al. (1983) determined TC, FC, and FS counts to be decreased by orders of magnitude within two hours following in-stream exposure of sewage to AMD. Keating et al. (1996) noted all strains of enteric bacteria tracked in a sewage-contaminated stream to be significantly and rapidly decreased to varying degrees during in-situ and laboratory bioassay exposure to AMD.

Although Roetman (1932) first suggested mixing AMD with MWW for pathogen removal, and despite the amount of aforementioned peripheral research, only a few systems have been intentionally constructed to simultaneously co-treat these effluents. McCullough et al. (2008) documented fortuitous water quality improvement and BSR in an evaporation pond in which high-strength AMD was accidentally introduced to secondary MWW. Johnson and Younger (2006) built a field scale single-stage constructed wetland treatment system that successfully improved the water quality of weak secondary MWW effluent (5-day biochemical oxygen demand (BOD_5) of ~14 mg/L) and relatively weak (net-alkaline with ~3 mg/L Fe) AMD. Rose et al. (1998) developed algal based systems in waste stabilization ponds that treated synthetic AMD and organic-rich tannery effluent to achieve relatively high metal removal efficiencies. Despite promising short term performance, these mixed effluent systems failed after a few months due to algal metal toxicity. In promising laboratory microcosm studies, Van Hille et al. (1999) initially separated the waste streams, using MWW to create algae-rich high pH effluent before mixing with AMD. However, the author is not aware of any high-strength AMD and raw MWW co-treatment investigation, which is essential to addressing the approach's feasibility and applicability. To ascertain the promise of co-treatment, the objectives of this study were to determine removal efficiencies of constituents of interest and concentrations of metals in the sludge produced.

5.3 Methods

5.3.1 Experimental Design

The experimental setup involved four serial unit processes in quadruplicate (Figure 5.1). The first unit processes were primary clarifiers for MWW and AMD mixing to raise AMD pH to that less inhibitory to BSR, complex metals with organic ligands, flocculate Fe and Al with P and suspended solids, reduce Fe(III) and SO_4, strip DO, and process BOD via heterotrophic bacterial activity and solids settling. The second and third unit processes together emulated a vertical flow bioreactor (VFB), which are common unit processes in AMD treatment. The upper column sections of the VFB emulation columns, which were filled with inert biomedia, were designed to encourage further DO stripping, BOD_5 processing, Fe(III) reduction and BSR. The bottom of the columns was filled with limestone for abiotic alkalinity generation via calcite dissolution and to encourage further BSR. The final unit processes were aerobic wetland mesocosms for further BOD processing via the activity of aerobic heterotrophic and denitrifying bacteria, sequential Fe then Mn oxidation and precipitation, as well as the removal of remaining As, Cd, Pb and Zn via sorption to Fe oxyhydroxides. Each unit process was connected to the next via clear vinyl tubing and sampled at its outflow.

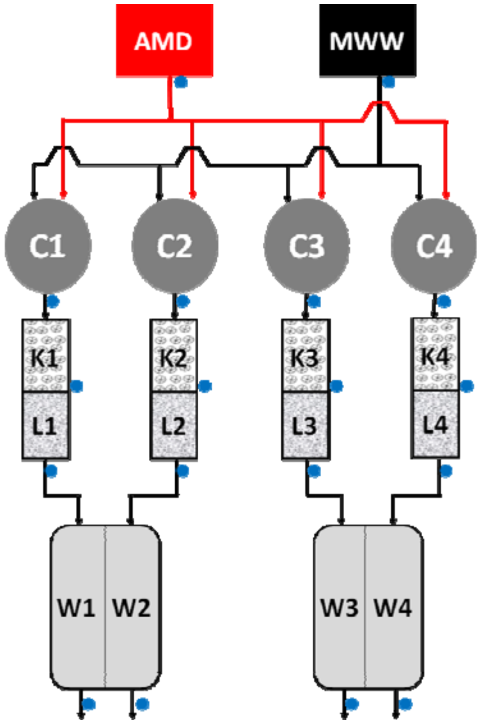

Figure 5.1 Conceptual experimental layout. Blue dots indicate sampling points. C1-4, K1-4, L1-4 and W1-4 represent the clarifiers, Kaldnes, limestone and wetlands of the four treatment trains, respectively.

The primary clarifier unit process was sized for a relatively high retention time of 32 hr for more complete mixing through passive diffusion, and to encourage bacterial activity and thorough solids settling of the light flocculant created. Retention times of 1.5 – 2.5 hr are typical for MWW primary clarification systems (Metcalf and Eddy, 1991; Frigon et al. 2006). However, retention times of around 6 hr or greater commonly exist where further sedimentation or biological activity is desired (Anderson, 1981; Gernaey et al. 2001). Four-cm deep single transverse baffles and 2.5-cm radius semi-circular weirs served as the physical structures in the LDPE basins that comprised this unit process. Sludge was wasted from the bottom of the clarifiers under gravity flow with a barbed HDPE T-connector attached to a HDPE valve and clear vinyl tubing.

The VFB emulation columns were 91.5 cm in height and 12.5 cm in diameter. The bottom 38 cm of the columns were filled with high quality (>90% $CaCO_3$) limestone washed of all fines and separated by sieve analysis adapted from ASTM D422 with the fraction passing a 2.54-cm sieve yet retained by a 1.27-cm sieve. The remaining top 53.5 cm of the columns were packed with Kaldnes K3 biofilm media to provide bacterial biofilm attachment surface. Kaldnes K3 media are polyethylene high surface area (500 m^2/m^3) components that are typically used in moving bed biofilm wastewater and drinking water treatment (Rusten et al., 2006). Following Pruden et al.'s (2007) findings of the importance of inoculation to sulfate reducing bioreactor performance, the Kaldnes zone was inoculated with 100 mL of VFB substrate from two mature passive coal mine AMD treatment systems in Pittsburg and Latimer Counties, OK. Each column was wrapped in aluminum foil to emulate the lightless conditions in VFB substrate.

The aerobic constructed treatment wetland mesocosms were two shallow LDPE basins. Each basin was bisected longitudinally with plastic to create the necessary four treatment trains. Wetland soil was collected from an existing constructed mitigation wetland at the Midwest City, Oklahoma MWW Treatment Plant. The surface flow mesocosms were planted with *Hydrocotyle verticillata* and *Nasturtium officinale* from nursery stock. The wetlands were placed under timed grow-lights on a 12 hr/d cycle.

5.3.2 Data Collection

Temperature, pH, and specific conductance (SC) were determined using an Orion 1230 multimeter. An Accumet AR60 multimeter was used to determine temperature and DO concentrations. BOD was determined using the 5-day BOD test following standard methods (APHA, 1998). Alkalinity titrations were conducted in accordance with standard methods (APHA, 1998) and Hach Method 8203 (Hach, 2002). Dissolved metals samples were filtered through 0.45-µm nylon filters prior to preservation. Total and dissolved metals samples were preserved with concentrated trace metal grade nitric acid and stored at 4°C until microwave acid digestion following EPA method 3015. Digested total metals samples were filtered through 0.45-µm nylon filters prior to analysis. Total and dissolved metals samples were analyzed via a Varian Vista-Pro® simultaneous inductively coupled plasma-optical emission spectrometer following EPA method 6010. Samples for anion (Cl^-, F^-, NO_2^-, NO_3^-, PO_4^{3-} and SO_4^{2-}) analyses were stored at 4°C until filtered through Dionex OnGuard® II H cartridges and 0.2-µm nylon filters. NH_4 samples were immediately processed using the Hach® Company high range Test 'N Tube® salicylate method (Hach, 2002). A MetrOhm® 761 compact ion chromatograph unit was used to quantify anion concentrations following EPA method 300. Following Sharp et al. (1995), water samples for dissolved organic carbon (DOC) and dissolved total nitrogen (DTN) were immediately filtered through 0.45-µm nylon filters and stored at <0 °C in 40-mL amber glass EPA vials with polypropylene caps and Teflon septa until quantification with an Analytik Jena multi N/C 2100 Analyzer.

Biological activity reaction tests (BART™) by Droycon Bioconcepts Inc. (DBI) and the MPN (most probable number) technique were applied to estimate non-indicator bacterial populations. BART™ tests were conducted to determine estimates of fermentative, sulfate reducing, Fe-related, nitrifying and denitrifying bacteria concentrations. BART™ tubes were incubated at ~22 ˚C and read every 12 or 24 hr. DBI QuickPop software was used to estimate populations. The MPN technique for enumeration of fermentative bacteria followed Pepper et al. (1995) using test tubes with Durham tubes containing phenol red broth with lactose from Becton Dickinson Diagnostic Systems. The MPN technique for enumeration of Fe reducing bacteria used test tubes with Durham tubes following Standard Methods (APHA, 2005) with a slight growth media modification following Viswanathan (2007). For fermentative bacteria, triplicate test tubes were inoculated in an anaerobic nitrogen-filled chamber for a dilution range of 10^{-1} to 10^{-8} and incubated for 24 hr at 37°C. For Fe reducing bacteria, triplicate test tubes were inoculated in an anaerobic nitrogen atmosphere for a dilution range of $1:10^2$ to $1:10^6$ and incubated at 25°C for 14 d. MPN results were interpreted using guidance and tables from Woomer (1994).

Raw MWW collected after grit screening at the City of Norman, Oklahoma Water Pollution Control Facility and synthetic high-strength AMD approximating that found at

Cerro Rico de Potosí, Bolivia were introduced to the system at a 1:2 ratio (AMD:MWW). The system was gravity flow from the first (clarifier) to the last (wetland) unit processes. MWW was collected weekly, homogenized during pumping and refrigerated at 4°C before introduction to the system. AMD was prepared weekly and stored at room temperature (20°C) until use. The AMD and MWW were introduced with peristaltic pumps at a combined flow rate (3.8 L/d) to produce an 18-hr residence time in the limestone stage, which is greater than the recommended minimum 15-hr design residence time suggested for anoxic limestone drains and the limestone drain components of VFBs (Younger et al., 2002; Table 5.1). All unit processes were maintained at room temperature throughout the experiment. Percent coverage of each plant species in the wetland mesocosms was estimated at days 29, 75, 94, 99, 112, 130 and 133 using digital photography. Sludge was wasted from the clarifiers in varying amounts at irregular intervals to average 0.69% of the combined inflow over the duration of the experiment.

Table 5.1 Design details and residence times for each unit process

Unit Process	**Surface Area**	**Total Volume**	**Porosity**	**Void Volume**	**Residence Time**
	cm^2	cm^3		cm^3	hr
Clarifier	405	5,020	1	5,020	32
Kaldnes	154	8,230	0.82*	6,750	42
Limestone	154	5,850	0.5^{α}	2,920	18
Wetland	5,100	10,600	1	10,600	67
Overall	***5,813***	***29,700***		***25,290***	***159***

* Value obtained from Saliling et al., 2007

$^{\alpha}$ Value obtained from Younger et al., 2002

The membrane filter technique (APHA, 1998) was employed to estimate TC, FC, FS, and *E. coli* concentrations in water samples. Sterilized lab-grade glassware was used to obtain samples that were analyzed upon collection. Samples from each location were replicated once for FIB analyses and examined at varying dilutions during each sampling event. Difco^{TM} growth media was prepared according to the manufacturer's instructions in sterile, 47-mm Millipore® disposable culture dishes prior to filtration. MI agar was used in culture dishes for TC and *E. coli*. FS and FC culture dishes used m-Enterococcus and m-FC media, respectively. As per manufacturer's instructions, rosolic acid salt reagent was mixed into the FC growth media. Water samples were filtered through sterile, 47-mm Millipore® 0.45-µm membrane filters. Following the designated incubation period, colony forming units were counted on each culture dish and recorded. In addition, culture dishes were incubated past their prescribed incubation periods. Rompré et al. (2002) suggest traditional membrane filter technique methods may not accurately assess FIB counts in the prescribed incubation period. Because sub-lethal damage to bacteria cells was suspected, colony forming units were counted and recorded after the prescribed incubation period every 24 or 48 hours for up to 244 hours. Since FIB in most treatment stages were exposed to extremely stressful conditions, some bacteria may have been non-culturable, yet viable in the environment (Maier et al., 2000).

The experiment consisted of two treatment regimens. First, during Treatment Regimen 1 (TR1) each treatment train continuously handled influent for 135 d. Sampling generally occurred bi-monthly during this standard operational run for a total of ten sampling events. BOD_5, dissolved metals and anion samples as well as pH, SC, DO, and alkalinity measurements were taken throughout the entire experiment. Total metals samples were only taken during the final two sampling events. Sludge metal samples were taken each time sludge was wasted. NH_4 samples were taken for the last five sampling events. FC and FS were examined throughout the experiment. TC and *E. coli* were examined for the last five sampling events. DOC and DTN samples were only taken during the final two sampling events. Water samples for MPN tests were taken from the columns on the final sampling event (day 133). BART tests were initiated on days 85, 112, 123, 130. At the conclusion of the standard operational run the VFB emulation column ports were sealed to begin Treatment Regimen 2 (TR2). For TR2, the columns remained sealed at room temperature for 91 d until sampling for dissolved and total metals, anions, FIB, pH, SC, DO, and alkalinity.

5.3.3 Data Analysis

Because direct sampling of the clarifier influent mix was impossible due to the experimental design, the theoretical influent mix (TMix) chemical composition was calculated using the ratio of AMD to MWW. To account for dilution, Tmix concentrations were used to calculate processing efficiencies and rates. Net acidity and acidity were calculated following a modified version of that presented by Younger et al. (2002), which accounted for the acidity from the substantial Cd^{2+}, $Cu^{2+}$and Zn^{2+} concentrations present in these unique waters. For acidity calculations, all Fe was assumed to be Fe(II) when $pH > 3$ and Fe(III) when $pH < 3$, because Fe(III) predominates for most solutions with $pH < 3$ (Kirby and Cravotta, 2005). This assumption generally follows empirical observations (Younger et al. 2002; Watzlaf et al. 2004; Kirby and Cravotta, 2005).

To facilitate the calculation of means and application of statistical tests, metal and anion concentrations below detection limits were assigned one half the value of the detection limit. All statistical testing was completed with Minitab® version 15 or with Microsoft Excel employing a type I error rate of $\alpha = 0.05$. Prior to means or median testing, all data sets were tested for normality with the Anderson-Darling test and similarity of variance. Due to the prevalence of normality and unequal variances, two-tailed, unpaired, heteroscedastic Student's *t*-tests were applied for total dissolved metal, clarifier sludge total metal, DTN, DOC, standard incubation period FIB and all TR2 data comparisons. FIB concentration means after extended incubation periods were compared between treatment stages at each colony enumeration. One-tailed unpaired heteroscedastic Student's t-tests were applied to test FIB means between treatment stages after each incubation period because only the FIB concentrations in a treatment stage that decreased from the previous stage were of interest. The nonparametric Kruskal-Wallis Multiple Comparisons test was used to determine statistical difference between the medians of the remaining data sets due to the prevalence of unequal variances and non-normality. Because estimated plant coverage and NO_3^- were not measured on the same days, a second order polynomial mathematical model of percent estimated coverage was created for each wetland to enable Pearson product moment correlation tests.

5.4 Results and Discussion

5.4.1 Influent Characteristics

The high strength synthetic AMD was similar in composition to that generated in the base/precious metal mining district of Cerro Rico de Potosí, Bolivia where untreated high-strength AMD and raw MWW pollute the headwaters of the Rio Pilcomayo (Strosnider et al. 2007, 2008; Tables 5.2 and 5.3). The AMD had 0.41 mg/L NO_3^--N, <0.4 mg/L NH_4^+-N, 0.29 mg/L DTN, and 0.0 mg/L DOC. The mean alkalinity, BOD_5 (265 ± 94 mg/L) (mean ± standard deviation), Cl^- (69 ± 3.8 mg/L) and SO_4^{2-} concentrations place the MWW used between the "medium" and "strong" designations of MWW established by Metcalf and Eddy (1991). MWW DOC and DTN were 42 and 30 mg/L, respectively. Although the AMD influent characteristics were consistent, some MWW influent characteristics, such as BOD_5 and PO_4^{3-} (11.5 ± 5.3 mg/L), varied somewhat throughout the experiment. This variance is to be expected and is typical of loadings experienced by conventional wastewater treatment plants (Metcalf and Eddy, 1991). However, MWW alkalinity, Cl^-, F^- (1.0 ± 0.09 mg/L), NH_4^+-N (20.8 ± 1.8 mg/L), NO_2^--N (<0.08 mg/L), and NO_3^--N (<0.11 mg/L) were relatively consistent. MWW FC, FS, *E. coli* and TC concentrations were $10^{6.37}$, $10^{5.67}$, $10^{6.58}$ and $10^{6.97}$ cfu/100mL. AMD FIB concentrations were consistently 0 cfu/100mL.

Table 5.2 Mean influent AMD and MWW physiochemical properties and sulfate concentrations; n=10 for all except where noted.

	pH	DO	SC	Alkalinity	Net Acidity$^{\delta}$	Net Acidity$^{\tau}$	SO_4^{2-}
	s.u.	mg/L	uS/cm	mg/L as CaCO3 equivalent			mg/L
MWW	7.67	0.98	951	288	-287	-268	70
s.d.	*0.12*	*0.49*	*66*	*20*	*20*	-	*16*
AMD	2.60	7.69	3010	0	1,870	1,810	1,920
s.d.	*0.04*	*0.64*	*112*	*0*	*91*	-	*140*

$^{\delta}$ Calculated with dissolved metal concentrations
$^{\tau}$ Calculated with total metal concentrations (n = 2)

Table 5.3 Mean influent AMD and MWW dissolved$^{\delta}$ (n=10) and total$^{\tau}$ (n=2) metal concentrations.

	MWW$^{\delta}$		MWW$^{\tau}$	AMD$^{\delta}$		AMD$^{\tau}$
	mean	s.d.	mean	mean	s.d.	mean
			mg/L			
Al	0.108	0.018	0.692	45.8	3.2	46.3
As	<0.022	-	<0.022	0.25	0.14	0.38
Ca	39.6	4.5	40.5	82.8	3.5	90.6
Cd	0.0010	0.0003	0.0009	2.02	0.08	2.29
Cr	0.0036	0.0045	0.0066	0.027	0.034	0.012
Cu	0.0067	0.0021	0.029	0.0052	0.0028	0.088
Fe	0.315	0.091	0.719	292	23.8	268
K	16.3	0.58	16.6	0.46	0.62	0.02
Mg	21.1	2.99	18.2	26.1	1.3	26.5
Mn	0.056	0.010	0.063	54.6	3.0	54.4
Na	73.7	3.25	66.9	<0.0006	-	<0.0006
Ni	0.0103	0.0017	0.0048	0.145	0.026	0.192
Pb	0.015	0.0051	0.014	1.21	0.10	1.25
Zn	0.045	0.0402	0.526	391	21.6	388

5.4.2 MWW Constituents

5.4.2.1 Phosphorus

PO$_4^{-3}$ was decreased from the theoretical influent mix 7.7 ± 3.5 mg/L to <0.75 mg/L by the clarifier outflow, producing a removal rate of 5.6 g m^{-3}d^{-1}. Flocculation with Al(III) and Fe(III) (e.g., Omoike and Vanloon, 1999; Parsons and Smith, 2008) or sorption to pre-existing AMD floc (e.g., Adler and Sibrell, 2003; Wei et al. 2008) were the likely primary removal mechanisms. Stoichiometrically, more than enough Fe and Al were removed from solution in the clarifier to account for the observed PO$_4^{-3}$ removal. The thorough and rapid PO$_4^{-3}$ removal observed indicates that higher removal rates may be possible in an optimized system.

The multi-stage co-treatment system removed PO_4^{-3} as well as conventional MWW treatment plants and better than conventional treatment wetlands and the single-stage co-treatment system described by Johnson and Younger (2006). The extent and rate of PO_4^{-3} removal noted in the clarifier is similar to that observed in conventional MWW treatment plants that incur significant costs via additional intensive treatment steps or flocculant dosing (Metcalf and Eddy, 1991; Parsons and Smith, 2008). Assuming that influent P was primarily in the form of PO_4^{-3}, the PO_4^{-3} removal rate in the clarifier was greater than 90% of FWS wetlands (Kadlec and Wallace, 2009). The Johnson and Younger (2006) single stage co-treatment system treated MWW with 39% less PO_4^{-3} yet removed 10-50% of PO_4^{-3}, a much lower removal efficiency and rate than was observed in the multi-stage co-treatment system clarifier. Results indicate that PO_4^{-3} removal is enhanced by co-treatment with higher strength AMD that contains greater concentrations of Al and Fe.

5.4.2.2 Nitrogen

The multi-stage co-treatment system demonstrated promising nitrification rates yet underperformed with regards to denitrification. NH_4^+ and NO_3^- concentrations were statistically unchanged until the wetlands, where nitrification occurred (Figure 5.2). NO_3^- concentrations increased to a mean 4.94 ± 3.49 mg/L NO_3^--N in the wetlands, for a mean rate of 0.036 g N $m^{-2}d^{-1}$. Extended column residence time (TR2) NO_3^- concentrations were driven to below detection limits (< 0.11 NO_3^--N) in the Kaldnes and limestone stages, indicating that denitrification could occur in this setting. TR2 NH_4^+ was not significantly different. For the final five sampling events, mean wetland NH_4^+-N removal (7.76 mg/L) compared well with NO_3^--N increase (6.95 mg/L), indicating that minimal denitrification occurred for this period on average. However, the DTN data taken for the final two sampling events indicated that N-removal, and hence denitrification, eventually occurred (Table 5.4). DTN was significantly unchanged until it decreased within the wetlands. BART results indicated that nitrifying bacteria were not aggressive until the wetlands as well, where 10^3 cfu/mL were detected. Denitrifying bacteria concentrations were 10^{1-6} cfu/mL throughout the first three unit processes, however there was little NO_3^- available. Denitrifying bacteria ranged from 10^{2-5} cfu/mL in the wetlands. Overall, data indicate that the denitrifying bacterial community was still developing by day 133 and that greater denitrification, and hence overall N removal, efficiency would eventually be better in a mature system.

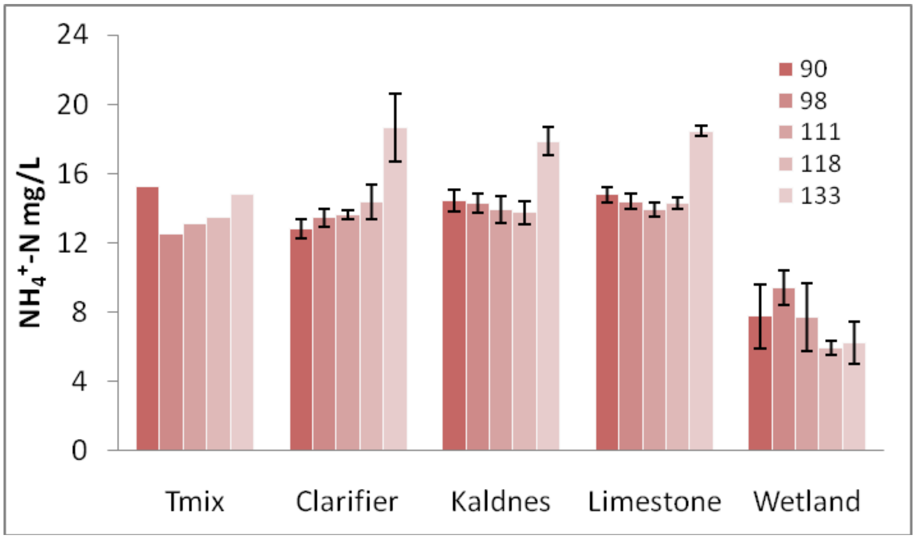

Figure 5.2 Mean NH_4^+-N concentrations from the TMix to the system outflow for each sampling period. Error bars represent one standard deviation above and below the mean.

Table 5.4 Mean DTN and DOC concentrations for the TMix and throughout the system

Site	DTN mg/L	DOC mg/L
Tmix	20	28
C	16	5.1
K	18	3.6
L	18	6.2
W	12	2.5

Conventional MWWTPs (municipal wastewater treatment plants) and treatment wetlands generally process N better than the multi-stage co-treatment system. Conventional MWWTPs generally nitrify and denitrify more effectively with less retention time than the multi-stage co-treatment system (e.g., Koivunen et al., 2003; Tandukar et al., 2007). FWS (free water surface) and HSSF (horizontal subsurface flow) treatment wetlands have been found to process TN and NO_3^- effectively (Kadlec and Wallace, 2009), unlike the multi-stage co-treatment system. The wetland unit process nitrification rate was an order of magnitude less than the mean FWS and HSSF wetland rates (Kadlec and Wallace, 2009) indicating that nitrification may have been substantially inhibited. Because AMD generally limits bacterial activity (Niyogi et al., 2003), microbial nitrification and denitrification as well as nitrogen assimilation could be relatively decreased in populations exposed to AMD. Stone et al. (2006) found that elevated Zn can inhibit denitrification. Although pH was circumneutral and the other AMD metals were decreased to below concentrations of concern, Zn was orders of magnitude above typical background concentrations from the wetland inflow to outflow. In addition, denitrification may have been limited by DO concentrations which averaged 4.4 mg/L and/or the lack of labile carbon substrate because BOD_5 was often driven to below detection limits by the wetland outflow. Johnson and Younger (2006) documented nitrification and denitrification within their single-stage co-treatment wetland where Zn concentrations were less, another indicator that Zn concentrations were likely an inhibiting factor for nitrification and/or denitrification. However, the multi-stage co-treatment system did not appear to have reached N treatment equilibrium.

The nitrification rate increased over the duration of the experiment from 0.012 g NO_3^--N $m^{-2}d^{-1}$ at day 20 to 0.091 g NO_3^--N $m^{-2}d^{-1}$ at day 133, indicating that the nitrifying community was maturing throughout the experiment and likely not reached maximum efficiency (Figures 5.3 and 5.4). Wetland outflow NO_3^- concentrations were positively correlated with time after experiment initiation (Pearson's correlation coefficient 0.859, $p < 0.001$) as well as modelled estimated plant coverage (Pearson's correlation coefficient 0.636, $p = 0.001$) (Figure 4). In a FWS wetland treating NH_4^+ and Fe-rich AMD, Demin et al. (2002) noted no NH_4^+ treatment for the first three years and that six years were necessary for removal optimization. Demin et al. (2002) posit that the buildup of Fe oxyhydroxide floc in their wetland created more suitable nitrifying community substrate and approximately 0.3 cm of Fe oxyhydroxide floc was noted to cover the multi-stage co-treatment wetland substrate by the conclusion of the experiment. However, nitrification can be enhanced by aquatic macrophyte oxygen transfer to substrate (Faulwetter et al. 2009; Kadlec and Wallace, 2009). The lag in nitrification performance may also be attributed to the time necessary for natural selection and horizontal gene transfer (e.g., Baker-Austin et al. 2006) to shape an efficient nitrifying community suited to the unique setting and stresses of the wetland. Regardless, the findings of Demin et al. (2002) buttress the supposition that the multi-stage co-treatment nitrification performance had not reached optimum efficiency by the termination of the experiment.

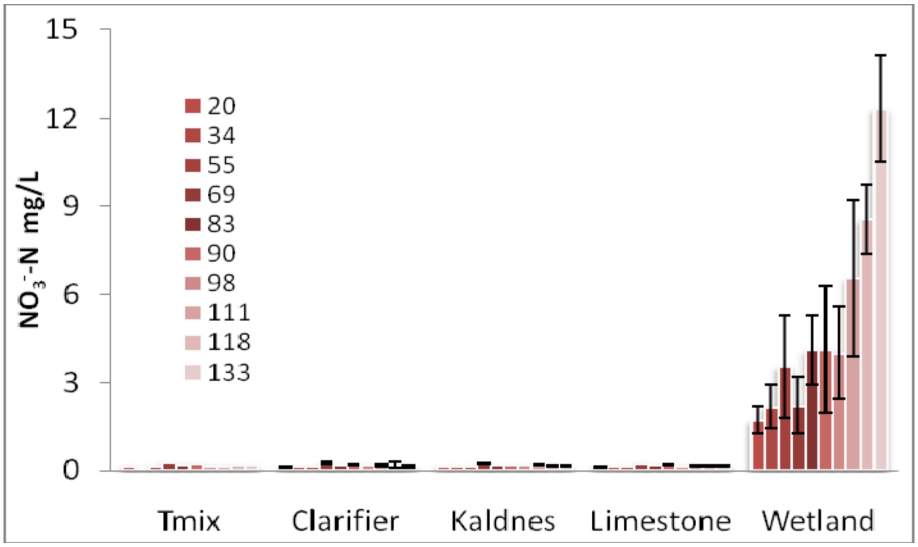

Figure 5.3 Mean NO_3^--N concentrations from the TMix to the system outflow for each sampling period. Bars are shaded according to the days elapsed from experiment initiation to the sampling event. Error bars represent one standard deviation above and below the mean.

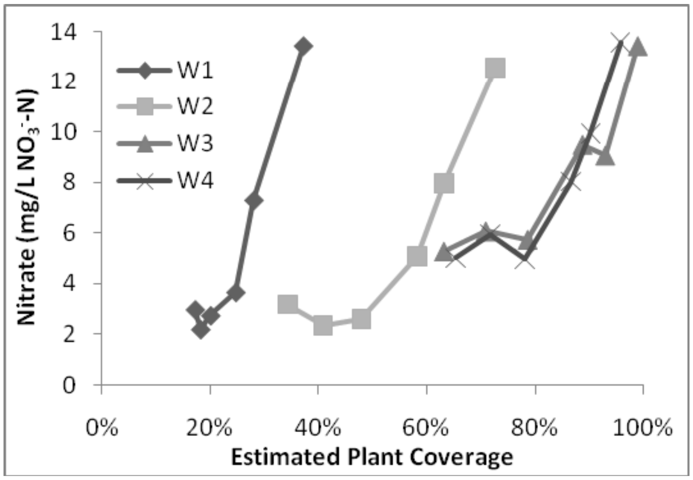

Figure 5.4 NO_3^--N concentrations with respect to modelled estimated plant coverage days after experiment initiation for each wetland in the treatment train

5.4.2.3 BOD$_5$

The system demonstrated consistently highly efficient BOD$_5$ processing (Table 5.5). Although BOD$_5$ could not be tracked throughout the entire system due to highly elevated concentrations of chemical oxygen demand unrelated to the MWW, DOC can be a suitable proxy for tracking BOD$_5$ (Khan et al., 1998; Servais et al., 1999). DOC was most dramatically and significantly decreased in the clarifier (Table 2). A further significant DOC decrease was noted in the wetlands. Sedimentation aided by flocculation with Al(III) or Fe(III) likely decreased BOD$_5$ and DOC in the clarifiers (e.g., Rebhun and Streit, 1974; Metcalf and Eddy, 1991; Omoike and Vanloon, 1999). The activity of various heterotrophic microbes (i.e., SRB, denitrifiers, fermenters, IRB, and aerobic heterotrophs) likely served to decrease BOD$_5$ and DOC throughout the system. BART results indicated that gram-negative fermenting bacteria were present in concentrations of $10^{2\text{-}3}$ cfu/mL in the limestone and wetlands. MPN results were lower, with $10^{1.83}$ and $10^{1.76}$ cells/mL of fermentative bacteria present in the Kaldnes and limestone unit processes, respectively. The aforementioned mechanisms resulted in BOD$_5$ processing to below detection limits and an overall systemic removal rate of 1.14 g $m^{-2}d^{-1}$ or 22.3 g $m^{-3}d^{-1}$.

Table 5.5 BOD$_5$ concentrations in the influent MWW, TMix, and each treatment train's wetland outflow (W_{1-4}).

	MWW	TMix	W_1	W_2	W_3	W_4
Day	mg/L					
20	472	311	3.49	1.92	1.50	3.24
34	296	195	1.24	<1.24	<1.24	1.63
55	236	156	2.10	2.30	2.40	2.50
69	148	97	<1.42	2.06	3.10	2.11
83	227	150	<1.35	<1.35	<1.35	<1.35
90	251	166	<1.16	<1.16	<1.16	<1.16
98	256	169	<1.04	<1.04	<1.04	<1.04
111	320	211	2.38	2.22	<0.98	1.15
118	302	199	<1.03	1.31	1.27	1.47
133	141	93	<1.47	<1.47	<1.47	<1.47

However, BOD$_5$ exertion can be decreased by elevated concentrations of heavy metals. Mittal and Ratra (2000) noted that Zn in concentrations of 20 and 50 mg/L decreased BOD$_5$ exertion by 28.7 and 37.5%, respectively. Because the wetland outflow BOD$_5$ was below or near detection limits, the mean 34.3 mg/L Zn present would not substantially impact overall removal performance. It appears as though BOD$_5$ was successfully processed despite concentrations of Al in the clarifiers and Kaldnes as well as Zn throughout the system that Mittal and Ratra (2000) found to be inhibitory to BOD$_5$ exertion.

The multi-stage co-treatment system processed BOD$_5$ as well or better than conventional MWWTPs, treatment wetlands and the single-stage wetland co-treatment wetland. BOD$_5$ processing was more complete than is often documented in conventional MWWTPs, which often produce effluent with BOD$_5$ of 5-30 mg/L (e.g., Koivunen et al. 2003; Tandukar et al. 2007; Jamwal et al. 2008). However, conventional MWWTPs often have much lower residence times (Metcalf and Eddy, 1991). The BOD$_5$ processing of the multi-stage co-treatment system also outperformed the three primary types of MWW treatment wetlands; FWS, HSSF and vertical flow (VF). Compared to the annual mean treatment performance of 136 FWS wetlands, the system produces influent with BOD_5 less than the vast majority treating tertiary MWW (influent BOD 0-30 mg/L) and all FWS treating primary and secondary MWW (Kadlec and Wallace, 2009). Compared to the annual mean treatment performance of 202 HSSF wetlands, the system produces influent with BOD_5 less than the vast majority treating tertiary MWW (influent BOD_5 0-30 mg/L) and all HSSF wetlands treating primary and secondary MWW (Kadlec and Wallace, 2009). The system produces influent with BOD_5 less than the annual mean treatment performance of the vast majority of 62 VF wetlands, (Kadlec and Wallace, 2009). The system actually drove BOD_5 to below typical background concentrations in natural or treatment wetlands (Kadlec and Wallace, 2009). Johnson and Younger (2006) reported 20-75% removal in their single-stage co-treatment wetland with a mean residence time of 14 hr receiving secondary MWW with only ~14 mg/L BOD_5. Although the residence time of the multi-stage co-treatment system was 10 times that of the Johnson and Younger (2006) system, the multi-stage system demonstrated much more complete BOD_5 removal while handling MWW with a BOD_5 concentration approximately 19 times greater.

5.4.2.4 FIB

5.4.2.4.1 Treatment Regimen 1

Co-treatment resulted in 100% removal of FIB in the last two stages of treatment (Figure 5.5). There was no significant difference between the limestone zone in the column (L) and wetland (W), for FC, FS, or *E. coli*. A significant difference between the treatment means of the L and W stages occurred for TC. Significant differences existed between all other stages of each FIB as exponential decreases in concentrations were observed through each stage prior to stage W.

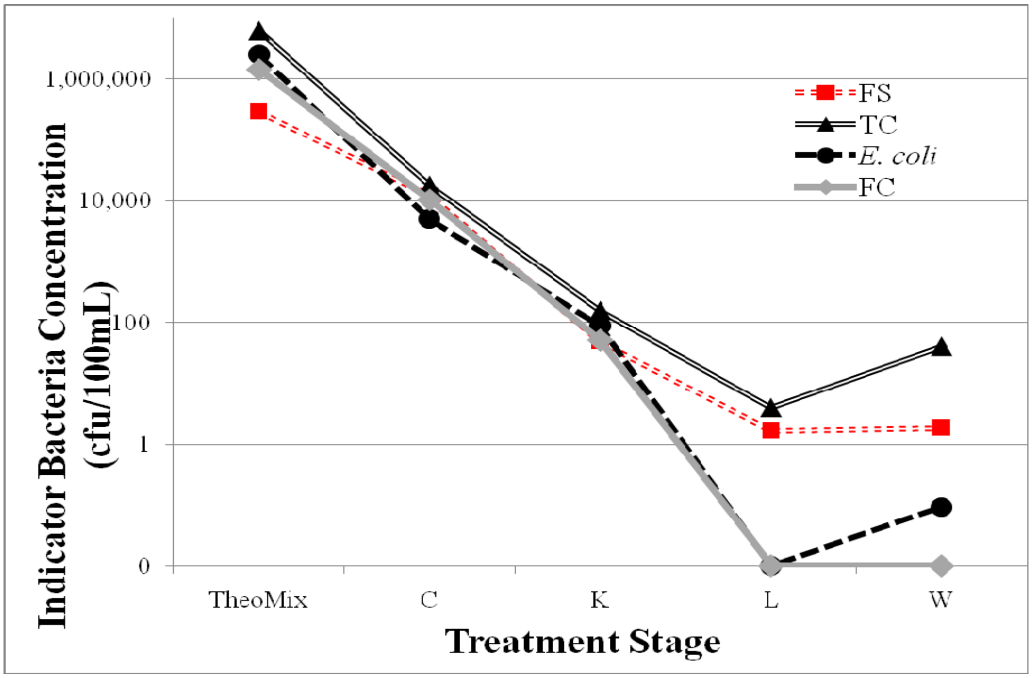

Figure 5.5 Indicator bacteria concentrations means in each treatment stage. TheoMix refers to the 2:1 ratio of MWW to AMD in the system influent. Standard errors of the means were too small to be perceptible on this figure and thus excluded. The number of samples for each treatment stage and indicator bacteria was highly variable.

The observed removal efficacy shows that this system had the ability to decrease FIB concentration by 100% during the first three treatment stages of this system. This reduction occurred within 81 hours of the introduction of AMD and MWW to the treatment system, prior to the wetland (W) treatment stage. The significant increase in

TC from stage L to the outflow of W indicated coliforms may proliferate in the wetland, despite close proximity to UV light from the grow lights, which has been shown to destroy FIB (Metcalf and Eddy, 1991; Chrtek and Popp, 1991). However, the other FIB tracked did not exhibit a significant increase in the wetland during standard incubation in culture dishes.

Most FIB removal occurred in treatment stage C. FIB were exposed to low pH and high metals concentrations (Figure 5.6). These stressful conditions can cause death in bacteria lacking proper adaptations (Maier et al., 2000). Oxidized iron bound to organic material settled in treatment stage C. It is likely FIB were bound to this flocculated material and removed by wasting the sludge. Vigneswaran and Visvanathan (1995) note the effectiveness of FIB concentration decrease via flocculation. In subsequent treatment stages, microorganisms present in the inoculants in the filter media could have outcompeted FIB for dwindling resources (Almasi and Pescod, 1996). In treatment stage K, low pH could have aided in the removal of FIB. Zn toxicity (Zn > 65 mg/L) could have also contributed to the death of FIB in treatment stages C and K (Nies, 1999). In treatment stage L, resource competition is likely the main removal mechanism. However, low biochemical oxygen demand (BOD) can affect FIB removal as well (James, 1987). Although the BOD at the effluent of treatment stage L was not determined, BOD in the effluent of treatment stage W was consistently less than 3 mg/L. James (1987) found that BOD concentrations less than 20 mg/L could starve FIB to the point of rapid die-off. In addition to this starvation, ultraviolet light irradiation was likely the main FIB removal mechanism in treatment stage W, but this treatment stage primarily served to keep concentrations of FIB low.

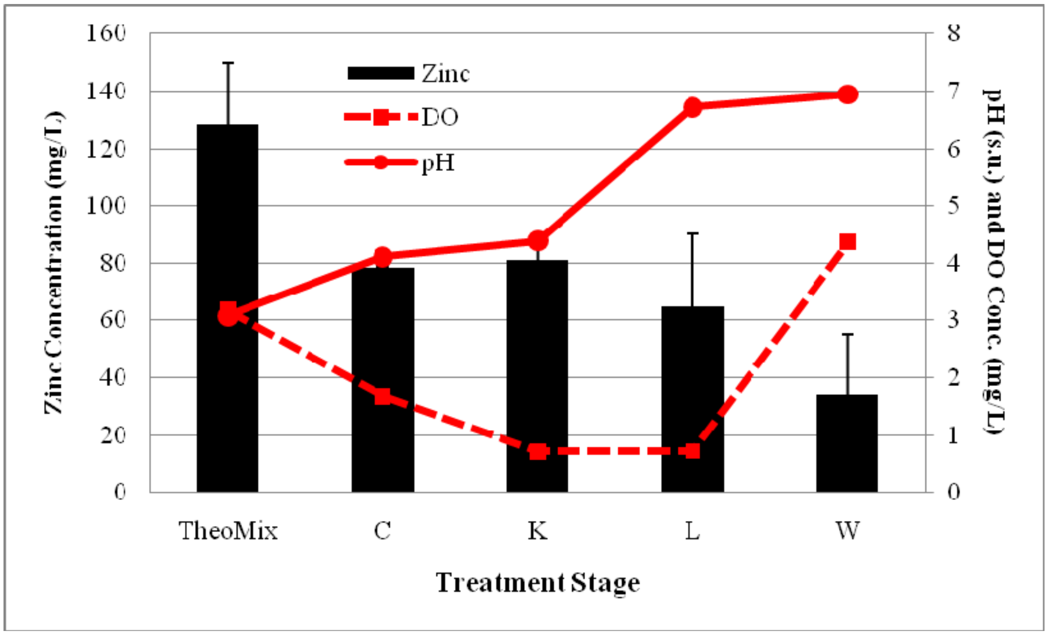

Figure 5.6 Zn and dissolved oxygen concentrations and pH for all treatment stages. Error bars represent standard errors of the means.

Many MWWTPs discharge treated effluent with substantial concentrations of FIB (e.g., Rose et al., 1996; Koivunen et al., 2003; Zhang and Farahbakhsh, 2007; Kay et al., 2008; Suh et al., 2009). In a study of 12 WWTPs across the United Kingdom, Kay et al. (2008) document no statistically significant elimination of FC or TC in primary clarification. However, Kay et al. (2008) noted FC decreases from $10^{7.23}$ to $10^{5.63}$, $10^{5.45}$, and $10^{5.20}$ and TC decreases from $10^{7.59}$ to $10^{6.15}$, $10^{5.89}$ and $10^{5.83}$ cfu/100mL as MWW flows through primary clarification and trickling filters, activated sludge, or rotating biological contactors, respectively. Ultraviolet light irradiation decreased FC and TC concentrations to $10^{2.45}$ and $10^{3.18}$, respectively. This study shows FIB removal in passive co-treatment systems may be higher than removal in active municipal wastewater treatment systems.

This co-treatment system outperformed other passive MWW treatment systems, such as free-water surface (FWS), subsurface flow (SSF) constructed wetlands, and soil filters (Table 5.6). In a compilation of FWS constructed wetland treatment data from several systems across the globe treating secondary and tertiary MWW, many of which have residence times days greater than the co-treatment system, Kadlec and Wallace (2009) document FC, FS, *E. coli*, and TC reductions in the approximate range of 10^5 to 10^3, 10^4 to 10^2, 10^5 to 10^1, and 10^7 to 10^5 cfu/100mL, respectively. Co-treatment achieves more complete and rapid removal of FIB than that documented in SSF constructed wetlands (Ottová et al., 1997; Green et al., 1997; Vymazal et al., 2005; Meuleman et al., 2003, Garcia et al., 2008; Kadlec and Wallace, 2009). The constructed soil filters examined by Kadam et al. (2008) removed FIB more rapidly, yet less completely than co-treatment.

Table 5.6 Passive MWW treatment system mean influent (I) and effluent (E) indicator bacteria concentrations (in $\log_{10}$ cfu/100mL) for systems handling primary, secondary and/or tertiary MWW.

	Primary/Secondary/Tertiary		Secondary/Tertiary					
	Co-Treatment		FWS		SSF		Soil Filter	
	I	E	I	E	I	E	I	E
FC	6.19	-[A]	5.21[B]	3.28[B]	6.04[B]	3.72[B]	7.70[F]	4.76[F]
FS	5.49	-	5.01[C]	3.62[C]	5.27[D]	2.27[D]		
E. coli	6.40	-	5.14[C]	3.72[C]	7.67[E]	1.27[E]		
TC	6.79	1.60	6.76[C]	4.91[C]	6.35[B]	4.23[B]	8.44[F]	6.41[F]

[A] “-” indicates 0 cfu/100mL detected.
[B] Kadlec and Wallace (2009) (7.7- and 5.8-d residence time for FC and TC, respectively)
[C] Molleda et al. (2008) (312-hr residence time)
[D] Garcia et al. (2008) (3-d residence time)
[E] Meuleman et al. (2003)
[F] Kadam et al. (2008) (0.5-2.0 hr residence time)

Extended incubation of culture dishes may have given evidence of sub-lethal damage to FIB cells (Figure 5.7). Although average concentrations of FS in AMD, MWW, and treatment stages C and K did not exhibit significant differences ($p > 0.05$) between incubation periods of 48, 72, or 120 hours, FS concentration in treatment stage L significantly increased from 3.2 ± 1.4 to 11 ± 2.5 cfu/100mL (mean and S.E.) in the standard incubation period of 48 hours to an extended incubation period of 120 hours. Although increasing incubation periods in treatment stage K did not increase FIB counts, significantly higher average concentrations of FS were observed in the K stage than in the L stage at all incubation period enumerations. Because a layer of biofilm was present on the Kaldnes K3 media in the K stage, it is possible this stage promoted higher microbial activity and higher tolerance of FS to metals. *Staphylococcus epidermidis* has been observed proliferating in biofilms in industrial, clinical, and environmental settings (Baker-Austin et al., 2006). Additionally, Baker-Austin et al. (2006) indicate that biofilms may encourage the co-selection of resistance to antibiotics and tolerance of metals. It is possible these biofilm communities amplified the resistance of FS. When allowed to mature in treatment stage L, FS cells were able to recover from the stressful environment on the culture plates in extended incubation.

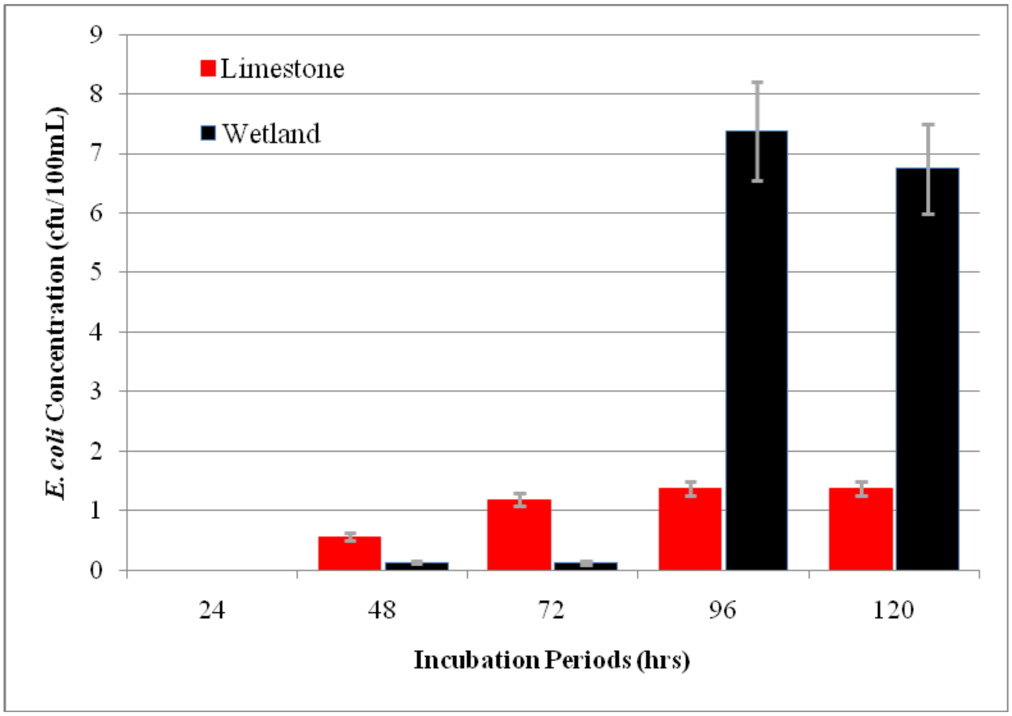

Figure 5.7 Average concentrations of *E. coli* in treatment stages L and W after extended incubations. Error bars show standard error of the means. Average concentrations after standard incubation were significantly different in both treatment stages after 72 hours of incubation.

Extended incubation of *E. coli* culture dishes also resulted in increased concentrations. Average *E. coli* concentrations in treatment stages L and W significantly increased after extended incubation from 0 cfu/100mL each to 1.4 and 6.8 cfu/100mL, respectively (1:1 and 1:2 dilution for treatment stages L and W, respectively; Figure 5.4). Because the residence time is over 4 times as long in treatment stage W than stage L, there may be greater potential for metal tolerant bacteria to recover in the wetland through increased exposure to resistance genes. Metal tolerant *E. coli* has been found to proliferate in municipal wastewater at circumneutral pH (Gikas, 2008). Perhaps, after the limestone treatment increased the pH of the system, *E. coli* cells were impeded in growth rather than destroyed. *E. coli* is typically suppressed by aerobic conditions, since it is accustomed to growth in the gastrointestinal tracts of warm-blooded animals (Maier et al., 2000). Treatment stage W exhibited higher dissolved oxygen concentration than treatment stage L, yet *E. coli* colonies increased in number after extended incubation in treatment stage W. Increased *E. coli* concentrations at extended incubations periods in the W treatment stage may be due to horizontal transfer of metal tolerance genes (Baker-Austin et al., 2006; Abskharon et al., 2008).

Nies (1999) presented minimal inhibitory concentrations (MIC) of metals that depress the growth of *E. coli*. Only Zn exceeded the MIC of *E. coli* (65.4 mg/L) in this system and did so in all treatment stages except L and W. Although the MICs from the Nies (1999) study were determined for single metals (i.e., not in combination), it is possible the lower Zn concentrations in the L and W treatment stages allowed sub-lethally damaged *E. coli* to survive. All ecotoxic metal concentrations decreased from the influent to treatment stages L and W. In the presence of metals, *E. coli* has been shown to produce metallothionein, a protein that has a high affinity for some metals and tends to detoxify them with regard to the bacteria cell (Maier et al., 2000). Additionally, plasmid-encoded mechanisms, such as metal efflux systems, can physically remove metals from the bacteria cell through an efflux pump (Maier et al., 2000; Baker-Austin et al., 2006). Co-selection of metal tolerance and antibiotic resistance genes may explain the observed metal tolerance of FIB in this study. Because many of these bacteria may have been exposed to antibiotics in the wastewater conveyance system, they could be inclined to tolerate metals. The same genes that promote antibiotic resistance may also promote metal tolerance and may be passed to cells via horizontal gene transfer prior to introduction to the co-treatment system (Baker-Austin et al., 2006).

5.4.2.4.2 Treatment Regimen 2

In Treatment Regimen 2, FIB concentrations were determined at days 1 and 91 in the column. No significant changes occurred in the L treatment stage for any FIB during the 91-day incubation period in the columns. Coliform concentrations were not affected to a large extent by extended residence time in the sealed columns. Only TC exhibited significantly higher average concentrations over the extended residence time in the column (in treatment stage K). *E. coli* and FC concentrations showed no significant difference at days 1 and 91 in K and L treatment stages after standard incubation in culture dishes. TC concentrations increased significantly, yet marginally, from an average of 0 ± 0 to 0.75 ± 0.1 cfu/100mL in the K treatment stage over the 91-day incubation period in the columns. Although *E. coli* concentrations did not significantly differ under standard incubation periods (24 hrs), significant increases in bacterial counts were seen after 120 hours of incubation on culture dishes (Figure 5.8). This delayed growth of *E. coli* could have been caused by sub-lethal damage done to cells exposed to metals in the column.

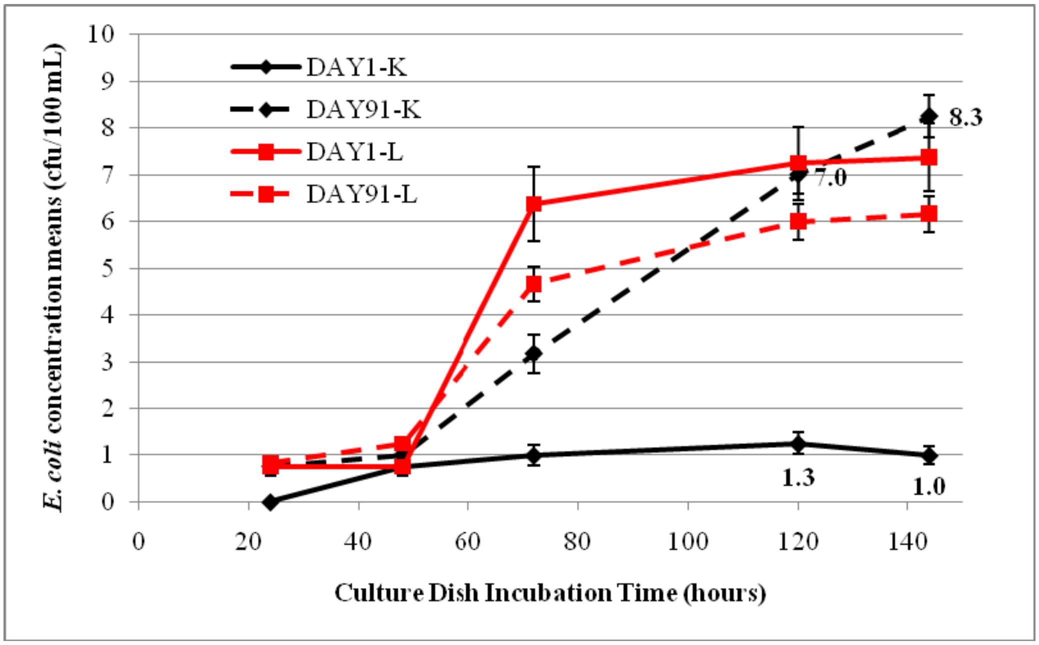

Figure 5.8 Average *E. coli* concentrations at treatment stages K and L at days 1 and 91 in extended incubation of culture dishes from the extended column incubation period. Data points with values labeled indicate a significant difference existed between *E. coli* concentrations at days 1 and 91. P-values for the difference in *E. coli* concentrations in the K treatment stage between days 1 and 91 for extended incubation in culture dishes at 120 and 144 hrs were 0.042 and 0.020, respectively. Error bars represent standard error of the means.

In treatment stage K, FS decreased from 21 to 0 cfu/100mL. Previous studies have documented greater AMD resistance by FS than by coliform bacteria (Hackney and Bissonnette, 1978; Carlson-Gunnoe et al., 1983; Keating et al., 1996). This suggests FS was less impacted by continuous exposure to AMD in the column. However, when the columns stopped receiving effluent on day 1, perhaps TC and *E. coli* were able to grow in lieu of FS as pH and alkalinity increased and Al, Mn, and Zn decreased in the columns over time. TC and *E. coli* increased over the extended residence time, which may indicate sub-lethal cell damage by the high metals concentrations and low pH described in the following pages. Over time, without perpetual exposure to the waste stream from the clarifiers, some coliform bacteria were able to proliferate in the columns, perhaps by outcompeting FS in the absence of high-strength AMD. Although this system removed FIB more efficiently than other passive treatment systems, the impacts of sub-lethal damage to these cells should be further investigated.

5.4.2 Alkalinity Generation

Alkalinity was generated via abiotic and biotic processes from the clarifier to the limestone stages (Figure 5.9). Dissolved Ca remained statistically unchanged until the limestone stage where it increased from a mean 57.4 to 183 mg/L which was calculated to correspond to generation of 314 mg/L of alkalinity as $CaCO_3$. This corresponded relatively well with the mean 285 mg/L drop in net acidity in the limestone. The pH in the clarifier and Kaldnes stages was sufficiently high to permit bacterial activity. This pH allowed for DO stripping via bacterial activity, as well as other desirable abiotic and bacterially-mediated reactions, such as metals sorption and BSR. BSR was the primary biotic alkalinity generating process. The alkalinity produced by the limestone stage was representative of real-world ALD performance (Cravotta, 2003; Watzlaf et al., 2004). The alkalinity provided by the MWW and generated by BSR and limestone dissolution was sufficient to produce net-alkaline effluent and buffer pH in the wetland, where H^+ liberating reactions such as Fe, Mn and NH_4^+ oxidation occurred.

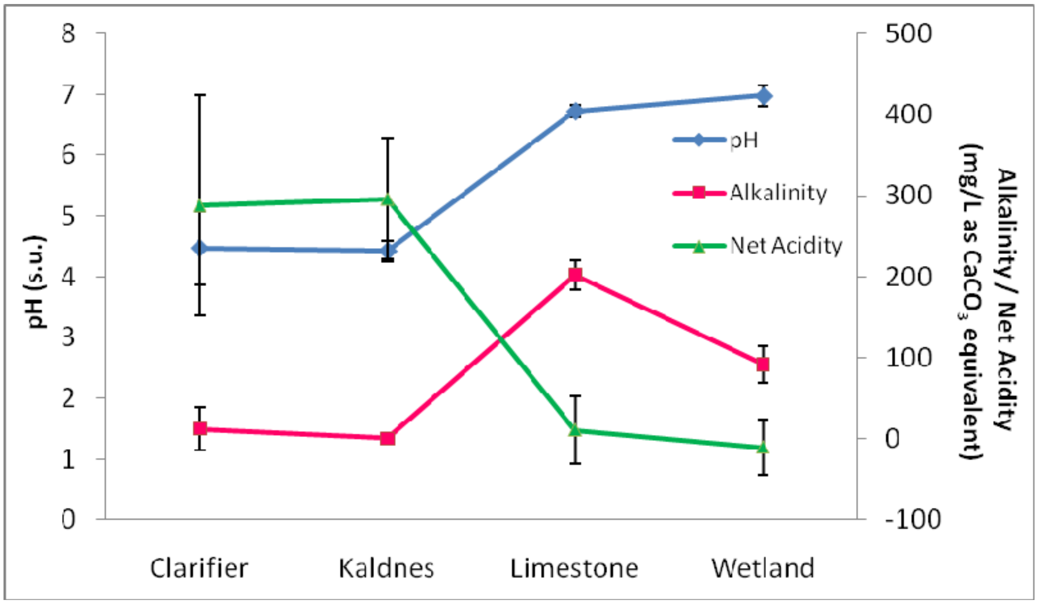

Figure 5.9 Mean of all sampling events (n=40) for pH, alkalinity and net acidity throughout the system. Error bars represent one standard deviation above and below the mean and are not visible when bars are smaller than the symbol.

5.4.3 AMD Constituents

5.4.3.1 Aluminum

Al was primarily removed from solution in the clarifier and limestone stages of the VFB emulation unit process (Figure 5.10). At the pH in the clarifiers, Al can combine with phosphate to form a relatively stable solid, complex with particulate organic matter, precipitate as alunite ($KAl_3(OH)_6(SO_4)_2$), and react with dissolved organic carbon (DOC) and precipitate (Omoike and Vanloon, 1999; Munk et al. 2002; Wilkin, 2008; Parsons and Smith, 2008). It is likely that all of these removal mechanisms were occurring to varying extents in the clarifier. However, the mean 46.3 mg/L total vs. 7.66 mg/L dissolved Al in the clarifier effluent was indicative of inefficient sedimentation. The Al floc did not pass through the Kaldnes stage as there was no statistical difference between dissolved and total Al in the Kaldnes effluent. The lack of Al, K, or SO_4 removal in the Kaldnes stage indicates that alunite formation was likely an insignificant removal mechanism in the clarifier because the bulk of particulate organic matter settled and phosphate was decreased to below detection limits (<0.75 mg/L) in the clarifier, leaving alunite formation the most plausible removal mechanism in the Kaldnes. In addition, dissolved K concentrations were unchanged in the clarifier and K was not concentrated in the clarifier sludge, where aluminum-related precipitates settled. In the limestone stage pH increased to 6.72, which dramatically decreased Al solubility, likely forming insoluble amorphous $Al(OH)_3$ (e.g., Munk et al. 2002; Wilkin, 2008). This solid was flushed from the limestone during standard sampling events, resulting in a mean 77 mg/L total vs. 0.053 mg/L dissolved Al, indicating the potential of flushing for metal recovery and to retain long term hydraulic conductivity.

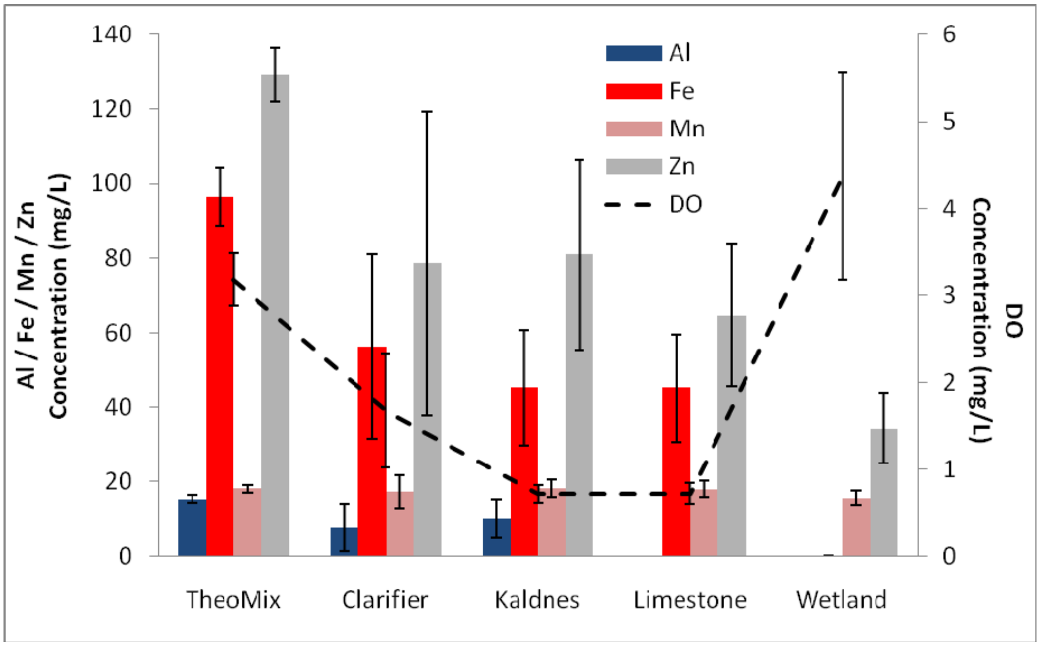

Figure 5.10 Mean of all sampling events for dissolved Al, Fe, Mn and Zn concentrations (bars) with respect to DO (line). Error bars represent one standard deviation above and below the mean.

5.4.3.2 Arsenic

Due to the rapid removal of As to below detection limits (< 0.022 mg/L) in the clarifier (Figure 5.11), the full extent of removal is unknown throughout the remainder of the system. The clarifier likely had a gradient of DO concentrations decreasing from the surface to the organo-metallic sludge at the bottom. This gradient would allow for aerobic (i.e., Fe(II) oxidation) and anaerobic processes (i.e., BSR). It is likely that As sorbed to Fe oxyhydroxides formed in the clarifier. Extensive research has documented the affinity of As for Fe hydroxides. Amorphous Fe oxyhydroxides such as would be formed in the clarifier have the highest adsorption capacity (Mohan and Pittman, 2007). It is also likely that As_2S_3 or AsS formed from BSR (e.g. Luo et al. 2008).

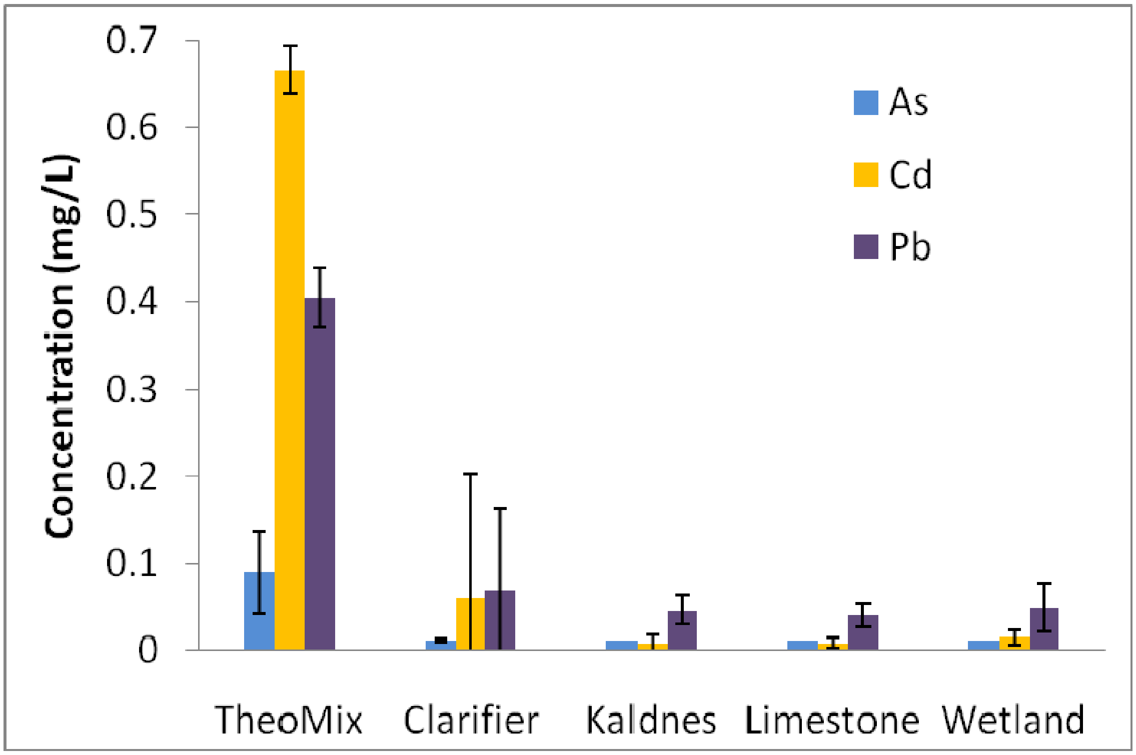

Figure 5.11 Mean of all sampling events for dissolved As, Cd and Pb concentrations. Error bars represent one standard deviation above and below the mean.

5.4.3.3 Cadmium

Cd was removed in the clarifier (Figure 5.11), likely from sulfide formation via BSR and complexation with organic matter. Fristoe and Nelson (1983) demonstrated that between pH 4-5 Cd has a high affinity for organic ligands with which it will complex as well as adsorb to bacterial solids. As in the case of Al, the mean 0.16 mg/L total vs. 0.059 mg/L of dissolved Cd in the clarifier effluent was indicative of inefficient sedimentation. The Cd-enriched floc did not likely pass through the Kaldnes stage as there was no statistical difference between dissolved and total Cd in the Kaldnes effluent. Although Cd can complex with Fe oxyhydroxides (Olivie-Lanquet et al., 2001; Carroll et al., 1998), Cd increased slightly within the wetland, likely due to evapoconcentration and the lack of aerobic removal mechanisms. Zn sorption can suppress Cd sorption to Fe oxyhydroxides (Carroll et al., 1998) and significant Zn sorption likely occurred in the wetlands. Therefore, it is unlikely that sorption to Fe oxyhydroxides was an important Cd removal mechanism in the clarifier as well, where Zn concentrations were approximately 3 times higher than in the wetland.

5.4.3.4 Iron

Fe was removed from solution via multiple means in the clarifier and wetland unit processes (Figure 5.10). Flocculation with phosphate (e.g., Omioke and Vanloon, 1999; Parsons and Smith, 2008) likely removed some Fe from solution in the clarifier. Combination with organic ligands (e.g., Fletcher and Beckett, 1987; Machemer and Wildeman, 1992) and the formation of Fe sulfides (e.g., Kolmert and Johnson, 2001) also likely decreased Fe concentrations in the clarifier. Aqueous Fe is primarily trivalent at the influent AMD pH (2.60) (Kirby and Cravotta, 2005). The increase in pH to 4.47 likely caused some remaining Fe(III) precipitation by oxyhydroxide formation because Fe(III) is rapidly hydrolyzed and precipitated from solution in waters with pH > 4 (Younger et al., 2002). These conditions favored the formation of the oxyhydroxide schwertmannite. Minimal Fe(II) oxidation is likely to have occurred in the clarifiers due to low DO concentrations. The mean 94.7 mg/L total vs. 56.3 mg/L of dissolved Fe in the clarifier effluent was indicative of inefficient sedimentation. Oxidation, hydrolysis and sedimentation were the likely mechanisms of Fe removal in the wetland due to the high DO and pH. The SRB throughout the first three unit processes and the limestone stages produced sufficient alkalinity which buffered pH through the wetlands during Fe oxidation and hydrolysis, a [H^+]-producing process.

Key to the performance of the system, dissolved Fe remained unchanged through the Kaldnes and limestone unit processes and therefore did not armor the limestone. This indicates that the remaining Fe(III) was reduced to Fe(II) in the clarifiers and/or Kaldnes stages, which allowed it to pass through the limestone without forming $Fe(OH)_3$ solids. Fe reducing bacteria (IRB) can use simple sugars (Lovely, 1991) which are present in substantial concentrations in MWW (Metcalf and Eddy, 1991). IRB populations were estimated at 250,000 and 40,000 cells/100mL in the Kaldnes and limestone stages respectively, indicating that they were especially active in the Kaldnes stage where reduction of any remaining Fe(III) is crucial. The lower IRB concentrations in the limestone stage are possibly due to the efficiency of clarifier and Kaldnes IRB at depleting Fe(III) concentrations. Additionally, Stone et al. (2006) demonstrated that Zn concentrations > 8.5 mg/L can severely inhibit some IRB (Stone et al. 2006). The persistence and activity of IRB in the presence of Zn concentrations nearly an order of magnitude greater may indicate that Fe reduction primarily occurred in the relative protection of biofilm communities in the Kaldnes stage (e.g. Stone et al. 2006).

Aerobic heterotroph bacterial activity is the likely mechanism by which DO was driven below the suggested anoxic limestone drain design parameter of < 1 mg/L DO in the Kaldnes stages to limit the oxidation of Fe(II) within the limestone stages. Limestone in the presence of Fe(III) or Fe(II) and DO > 1 mg/L will become coated with $Fe(OH)_3$, dramatically lowering alkalinity production and porosity (Younger et al., 2002) which can dramatically reduce the effective lifetimes of passive treatment systems (Watzlaf et al.

2004; Santomartino and Webb, 2007). The combined activity of heterotrophic and Fe reducing bacterial activity allowed Fe removal to be isolated in the clarifier and wetland stages where precipitate accumulation is preferred while protecting the VFB emulation stages from limestone armoring or clogging.

5.4.3.5 Manganese

Mn remained unchanged throughout the system until the wetland stage, where it decreased by 13.6% (Figure 5.10). The oxidation and hydrolysis of Mn was possible because pH was greater than 6 (6.95) and Fe was driven to such low concentrations (to 0.18 from 45 mg/L) by the outflow of the wetland. It is likely that Mn removal primarily occurred by the wetland outflow where Fe concentrations were lower because Fe (II) will reduce oxidized forms of Mn when present (Watzlaf et al., 2004). In addition, Mn removal would be slightly greater (20.8%) if accounting for wetland evapoconcentration, which was estimated at 8.4%, assuming that Ca, Cl, and Na were conservative ions.

5.4.3.6 Lead

Pb was removed in the clarifier and remained unchanged throughout the subsequent unit processes (Figure 5.11). In the clarifier Pb likely complexed with particulate organic matter (e.g., Fletcher and Beckett, 1987; Aderhold et al. 1996), formed galena (PbS) (e.g., Younger et al. 2002), and/or sorbed to Fe oxyhydroxides (e.g., Carroll et al., 1998; Wilkin, 2008). The mean 0.14 mg/L total vs. 0.069 mg/L of dissolved Pb in the clarifier effluent was indicative of inefficient clarification. Although Pb can sorb to Fe oxyhydroxides at the pH range found within the wetlands and should not be precluded by

Zn sorption (Carroll et al., 1998), no significant difference was noted between dissolved Pb in the limestone and wetland effluent.

5.4.3.7 Zinc

Zn was primarily removed from solution in the clarifier and wetland (Figure 5.10). In the clarifiers, sphalerite (ZnS) formation, and complexation with organic ligands present in the MWW likely served as the primary Zn removal reactions. BSR can remove Zn from solution via sphalerite formation (Younger et al. 2002). Zn can have a relatively high affinity for abiotic and biotic organic ligands (Fletcher and Beckett, 1987; Machemer and Wildeman, 1992; Norton et al., 2004; Gibert et al., 2005). Although significant concentrations of Zn were removed from solution in the clarifiers there was no significant difference between total and dissolved concentrations in the clarifier effluent, which implied efficient clarification of Zn solids and is contrary to the trend noted in Al, Cd, Pb and Fe. This may also indicate that Zn removal was primarily a result of BSR because the majority of Zn removal occurred at the bottom of the clarifiers in the organo-metallic sludge where SRB activity is thought to have been concentrated. In the wetland stage, Zn removal was likely due to sorption to Fe oxyhydroxides. Zn has a high affinity for Fe oxyhydroxides, especially at the pH present in the wetlands (Dzombak and Morel, 1990; Carroll et al. 1998; Mayes et al. 2009).

5.4.3.8 Minor Metal Constituents

Cr, Cu and Ni were present in low concentrations in the AMD and MWW (Figure 5.12). Cr and Cu were concentrated in the clarifier sludge and a significant decrease was noted between the TheoMix and the wetland effluent. Although there was no significant difference between the TheoMix and wetland effluent, Ni was concentrated in the clarifier sludge which indicates marginal removal. Possible removal mechanisms are Cr reduction and subsequent hydrolysis, Cu sulfide formation or sorption to Fe oxyhydroxides, and Ni sulfide formation and sorption to organic solids and/or Fe oxyhydroxides (e.g. Younger et al. 2002; Wilkin, 2008).

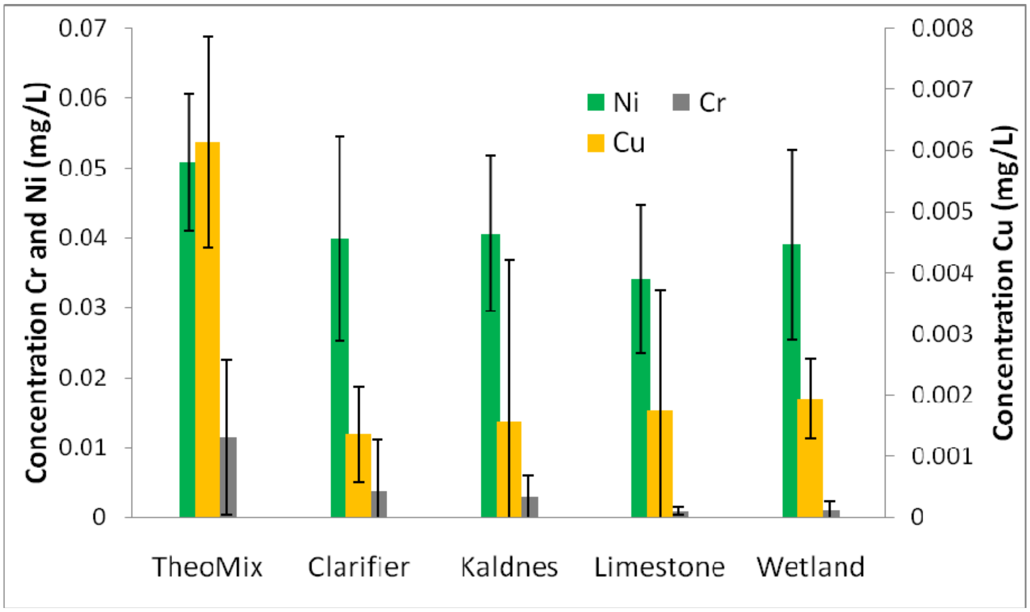

Figure 5.12. Mean of all sampling events for dissolved Ni, Cu and Cr concentrations. Error bars represent one standard deviation above and below the mean.

5.4.3.9 Sulfate

Sulfate concentrations decreased from 680 to 610 mg/L from the TheoMix to the clarifier effluent but did not vary statistically throughout the remainder of the system. BSR was identified as the primary sulfate removal mechanism. However, the formation of schwertmannite, alunite and gypsum are other possible sulfate removal mechanisms. Schwertmannite ($Fe_8O_8(OH)_{8-2x}(SO_4)_x$), where $1 \leq x \leq 1.75$, was likely formed in the clarifiers to some extent and will remove sulfate from solution upon formation (Brady et al. 1986; Bigham et al. 1996). However, if one assumes that all dissolved Fe was removed from solution by schwertmannite formation, which is highly unlikely because of the other aforementioned plausible removal mechanisms, it could only account for a maximum sulfate removal of 15.1 mg/L (21.4% of the total clarifier removal). As previously discussed, alunite formation was likely not a substantial removal mechanism. Sulfate removal was not noted in the limestone stages, which is where gypsum formation could have been favored due to the elevated Ca concentrations. All sulfate removal was assumed to be via BSR despite the marginal error likely inherent in this assumption.

BART tests indicated the presence of SRB in the influent MWW (10^{6-7} cfu/mL), clarifiers (10^{0-2} cfu/mL), clarifier sludge (10^{0-4} cfu/mL), Kaldnes stage (10^{0-3} cfu/mL), limestone stage (10^{1-3} cfu/mL), and wetlands (10^{0-2} cfu/mL). The wide range of values may be explained by the limitations of the BART method, which does not directly measure bacterial concentrations, but the black metal sulfide product of SRB activity. In addition, SRB populations are generally associated with biofilms or biocolloids of which water column samples may not exhibit representative bacterial concentrations (DBI, 2002). The supernatant from clarifier sludge samples was used in the BART tests, while the bulk of SRB were likely bound to the sludge solids. Although the MWW contained high concentrations of organic particulates upon which SRB likely congregated, the very high concentrations of SRB in the MWW and low concentrations in the clarifier water columns suggest die-off of substantial proportions of SRB upon exposure to AMD. The clarifiers may have acted as a species filter or sieve, building a more resistant SRB community throughout the system as non-AMD-resistant SRB perished. Overall, BART tests suggested that SRB were present throughout the system which is evidence of SRB tolerance to low pH (clarifiers, Kaldnes stage), aerobic waters (wetlands) and highly elevated Zn and other metals (entire system).

The substantial concentrations of simple to complex carbohydrates present in MWW (Maki, 1953; Metcalf and Eddy, 1991) likely either directly or indirectly supported SRB populations. SRB require simple sugars or alcohols as substrate, which would have been gleaned from the MWW directly or created by other microbial consortia, such as cellulolytic and fermentative bacteria, breaking down more complex carbohydrates (Pruden et al. 2007).

The mean clarifier sulfate removal rate was 0.56 mol/m^3-d, which is greater than the approximate 0.3 mol/m^3-d found under optimal BSR field conditions (Neculita et al., 2007) yet less than the ~3 mol/m^3-d that Tsukamoto et al. (2004) and Kolmert and Johnson (2001) found under laboratory conditions at similar pH. However, Tsukamoto et al. constructed their reactors with sand and horse manure substrate, and fed those reactors with high quality refined electron donors (methanol and ethanol) and synthetic AMD only containing Fe (100 mg/L) and sulfate (900 mg/L). Kolmert and Johnson (2001) fed ethanol, lactic acid, and glycerol to bioreactors treating nutrient-augmented AMD with acidity and metals concentrations orders of magnitude less than the AMD used in the co-treatment study. The co-treatment clarifier more rapidly reduced sulfate than the various mixes of bark, post peel and compost tested by McCauley et al. (2009) (0.28-0.38 mol/m^3-d) and the pilot-scale bioreactors of spent mushroom compost of Dvorak et al. (1992) (0.214 and 0.333 mol/m^3-d), of which both studies employed AMD with lower concentrations of acidity and most metals of interest.

The BSR rate is unexpectedly high when considering the toxic Zn concentrations, elevated overall metal concentrations and suboptimal pH affecting the SRB. The concentration of Zn present in the clarifiers (78.6 mg/L), Kaldnes (80.9 mg/L), and limestone (64.7 mg/L) unit processes likely limited BSR rates. Zn concentrations ranging from 13 to 65 mg/L have been found to be toxic and severely inhibitive to SRB (Kaksonen and Puhakka, 2007; Neculita et al., 2007). In addition, studies have suggested additive individual metal toxicity (Neculita et al., 2007), indicating that the other toxic metals within the system could have had an additive inhibitory effect on SRB. BSR rates also begin to decline at pH < 5 (Neculita et al., 2007; Koschorreck, 2008), which likely slowed BSR in the clarifier and Kaldnes stages where effluent pH was 4.47 ± 0.61 and 4.42 ± 0.17, respectively. It is assumed that BSR could have been greater if the AMD Zn concentrations were less and/or the ratio of MWW to AMD was increased to raise pH. Because much of the organic solids provided by the MWW settled within the clarifier, BSR in the Kaldnes and limestone stages was possibly also limited by lack of suitable electron donors.

Despite the factors limiting SRB activity, BSR rates were relatively high, suggesting that a rich and diverse SRB community was supported by the electron donors supplied by the MWW. It is likely that microenvironments with elevated pH in the organic and metal-rich sludge at the bottom of the clarifiers supported increased BSR rates (e.g., Fortin et al. 1996; Stockdale et al. 2009). Koschorreck (2008) disputes the feasibility of microenvironments to support BSR at low pH due to the very high sulfate reduction rates that may be required to produce the necessary proton gradient. However, Koschorreck (2008) does not account for diffusion retardation from biofilms and associated metal sulfide precipitates. The capacity of particle enclosures, biofilms, and associated metal sulfide precipitates to allow for suitable SRB microenvironment formation is unknown, yet inferences can be made from the literature. Studies have documented the protective capacity of sheltered attachment surfaces and biofilms, demonstrating that biofilms bind aqueous heavy metals, accumulate metal sulfides, and retard diffusion of hydrogen ions and other toxic species (Keweloh et al. 1989; Vroom et al. 1999; Labrenz et al. 2000; White and Gadd, 2000; Beyenal and Lewandowski, 2001; Wolf et al. 2002; Ito et al. 2002; Teitzel and Parsek, 2003; Curtin and Cormican, 2003; Stone et al. 2006; Kaksonen and Puhukka, 2007). Vroom et al. (1999) documented pH gradients of > 3 units in fewer than 10 μm in heterotrophic biofilms. Teitzel and Parsek (2003) noted that bacteria embedded in biofilms were up to 600 times more resistant to heavy metal stress than planktonic cells. Stone et al. (2006) attributed increased Zn tolerance of the SRB *Shewanella putrefaciens* to the protective capacity of biofilms and related attachment surfaces. Also, researchers have solid evidence of metal sulfide precipitation within and overlying SRB biofilms (White and Gadd, 2000; Labrenz et al. 2000). The activity of acid and metal tolerant SRB, many species of which have been recently isolated (Kolmert and Johnson, 2001; Koschorreck, 2008; Muyzer and Stams, 2008; Winch et al. 2009) and less metal tolerant or neutrophilic SRB embedded in suitable biofilm microenvironments, is likely responsible for the relatively high BSR rates observed.

5.4.3.10 Treatment Rates

Treatment rates are presented in Table 5.7. Johnson and Younger (2006) reported 40-80% Fe removal in a single-stage constructed co-treatment wetland with a mean residence time of 14 hr receiving AMD with ~3 mg/L Fe. The residence time of the multi-stage co-treatment system was approximately 10x that of the Johnson and Younger (2006) system. However, the multi-stage system demonstrated much higher % Fe removal while handling AMD with a Fe concentration approximately 100x greater. The multi-stage co-treatment system performed well with respect to conventional AMD passive treatment systems (Younger et al. 2002; Watzlaf et al. 2004), despite treating AMD that was generally more acidic and metals-rich than that for which passive treatment systems are generally applied. Wetland evapoconcentration likely decreased overall removal performance. It should also be noted that the multi-stage co-treatment system was not optimized for maximum sustainable loading rates and its full potential is unknown. Although 99.6% of Fe was removed from solution in the wetland stage, there was not sufficient Fe loading (0.34 g/m^2-d) to reach typical (10-20 g/m^2-d) aerobic wetland design removal rates (Younger et al. 2002). Overall, the high % removal of key ecotoxic elements indicates that greater removal rates are likely achievable in an optimized system. Additionally, the removal of metals initially at relatively low concentrations (Cr, Cu and Ni) indicates that co-treatment may also be an effective polishing tool for AMD with lower metals concentrations and acidity.

Table 5.7. Removal performance for key dissolved species. Unit process removal is the percent difference between unit process inflow and outflow.

	AMD	TheoMix	Wetland	Overall Removal	Primary Removal Unit Process	Unit Process Removal		Unit Process Treatment Rate			
	mg/L	mg/L	mg/L	%		%	%	g/m^2-d		g/m^3-d	
Al	45.8	15.2	0.034	99.8	C / L	49.5	99.5	0.71	2.5	5.7	6.6
As	0.25	0.090	<0.022	87.8	C	87.8		0.007		0.060	
Cd	2.02	0.67	0.015	97.7	C / K	91.1	85.0	0.06	0.01	0.46	0.02
Cr	0.027	0.011	0.0011	90.4	C	66.2		0.0007		0.006	
Cu	0.005	0.006	0.0019	68.3	C	77.8		0.0004		0.004	
Fe	292	96.4	0.18	99.8	C / W	41.6	99.6	3.8	0.34	30	16
Mn	54.6	18.0	15.5	13.9	W	13.6		0.02		0.88	
Ni	0.145	0.051	0.039	23.2	C	21.4		0.001		0.008	
Pb	1.21	0.40	0.049	87.9	C / K	83.0	32.2	0.03	0.01	0.25	0.01
Zn	391	129	34.3	73.4	C / K	39.1	47.0	4.8	0.23	38	11
SO_4	1920	680	650	4.5	C	10.4		6.6		54	

5.4.3.11 Temporal Variability

Treatment efficiency was relatively stable throughout the duration of the standard operational run of the experiment. The relatively quick realization of peak performance and stabilization exhibited by each unit process and the whole system are exemplified by pH and Zn behavior (Figures 5.13 and 5.14). Metals, FIB, BOD_5 and nutrient processing as well as alkalinity generation performance generally displayed a similar lack of substantial temporal variability. The aforementioned N processing variability was the only constituent that deviated from this pattern.

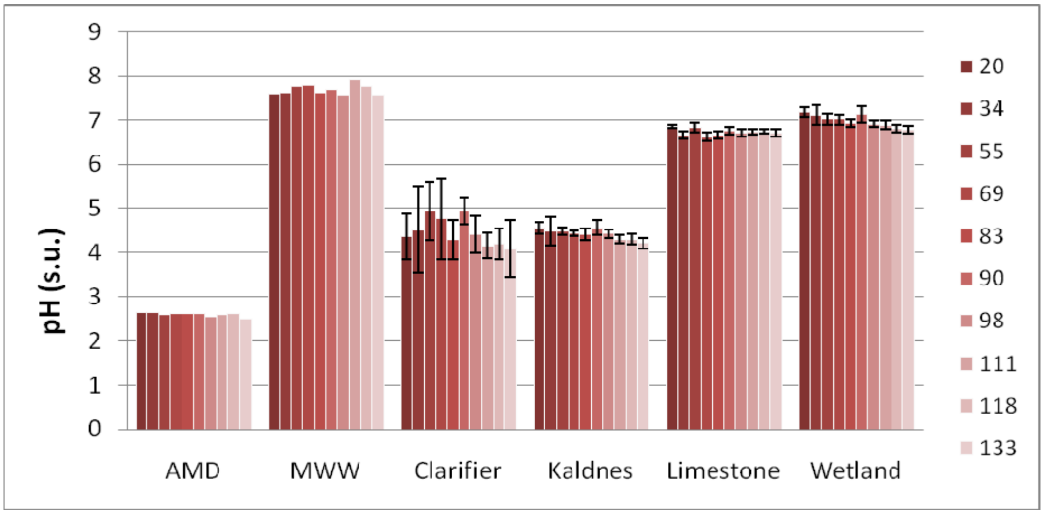

Figure 5.13 Mean pH for each sampling event (n=4). Bars are shaded according to the days elapsed from experiment initiation to the sampling event. Error bars represent one standard deviation above and below the mean. There are no error bars for AMD and MWW because each bar represents a single number.

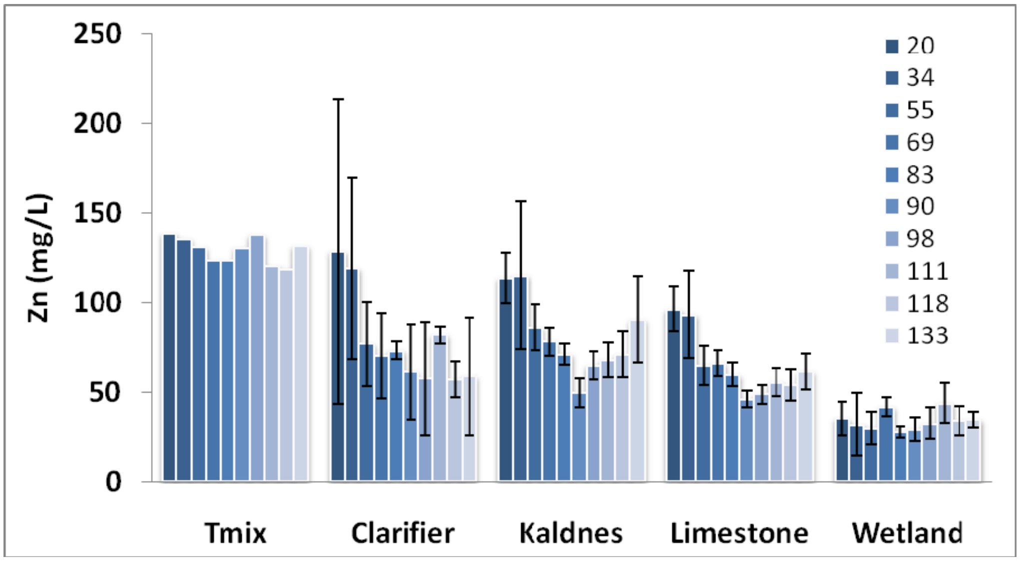

Figure 5.14 Mean dissolved Zn concentrations for each sampling event (n=4). Bars are shaded according to the days elapsed from experiment initiation to the sampling event. Error bars represent one standard deviation above and below the mean. There are no error bars for AMD and MWW because each bar represents a single number.

5.4.3.12 Sludge Generation

Approximately 0.69% of the total flow entering the clarifiers was wasted throughout the experiment. This wasting rate led to sustainable metals removal that did not compromise clarifier residence time. Sludge total Al, As, Cd, Cu, Mn, Ni, Pb and Zn concentrations were negatively correlated with wasting rate (Figure 5.15). The sludge contains concentrations of total Al, As, Cd, Cr, Cu, Fe, Pb and Zn at least an order of magnitude greater than the dissolved TheoMix. Sludge concentrations of total Mn and Ni were also greater than the dissolved TheoMix, indicating that some degree of Mn and Ni removal was occurring in the clarifiers which could not be concluded from solely analyzing the TheoMix and clarifier outflow data. Mn and Ni removal was likely due to sorption to organic matter (e.g. Machemer and Wildeman, 1992; Aderhold et al. 1996)

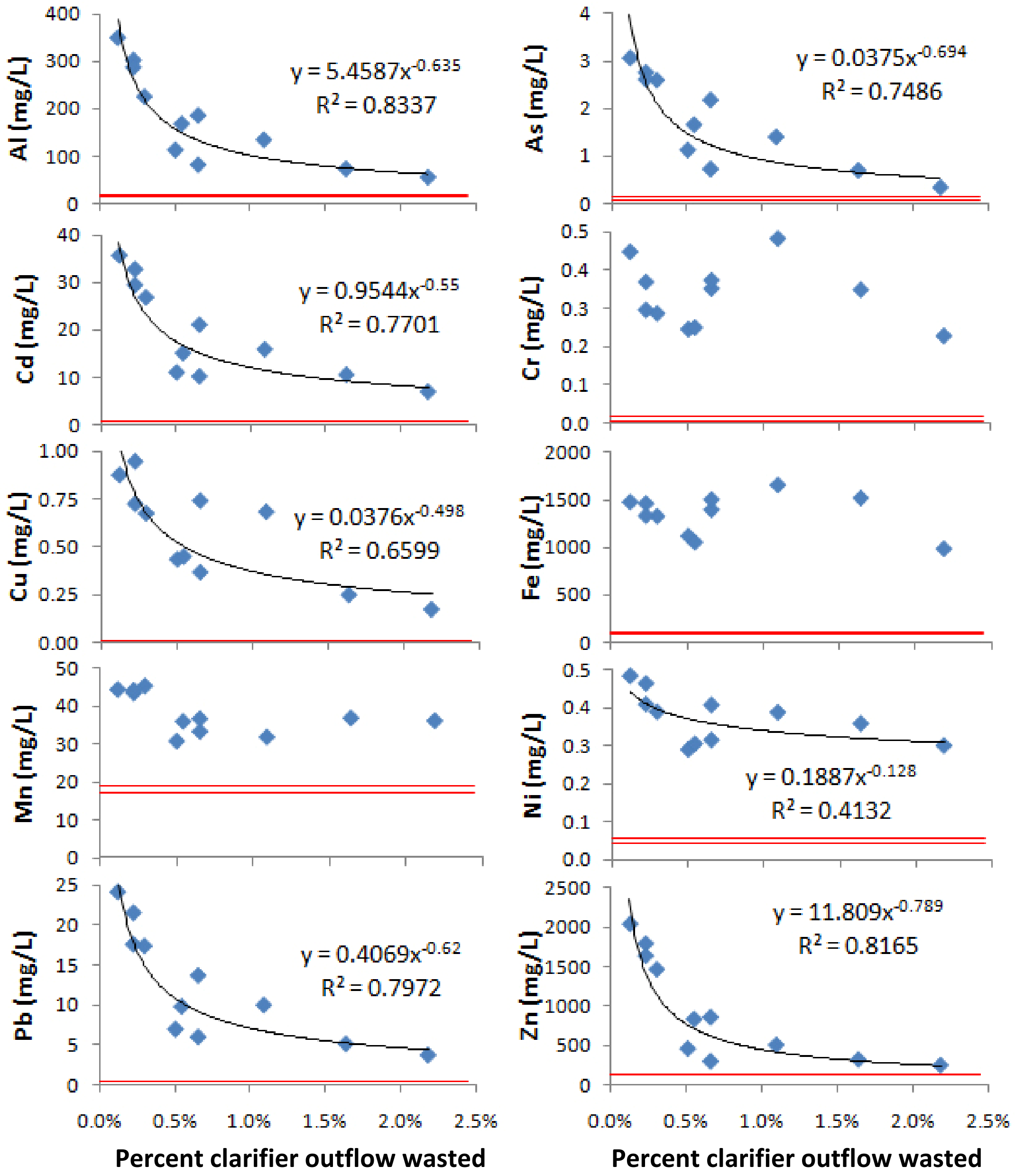

Figure 5.15 Total metals concentrations in sludge with respect to % clarifier outflow wasted. Red bars frame the 95% confidence interval constructed with the *t* statistic of the dissolved metals in the TheoMix.

The sludge data indicate that the "more conservative" metals did not all behave conservatively within the clarifiers (Figure 5.16). K was the only metal tracked for this study that behaved conservatively in the clarifier. The data indicate that Na appears to be dissociating from the sludge for the aqueous phase. Conversely, Mg and Ca appear to be complexing with the sludge.

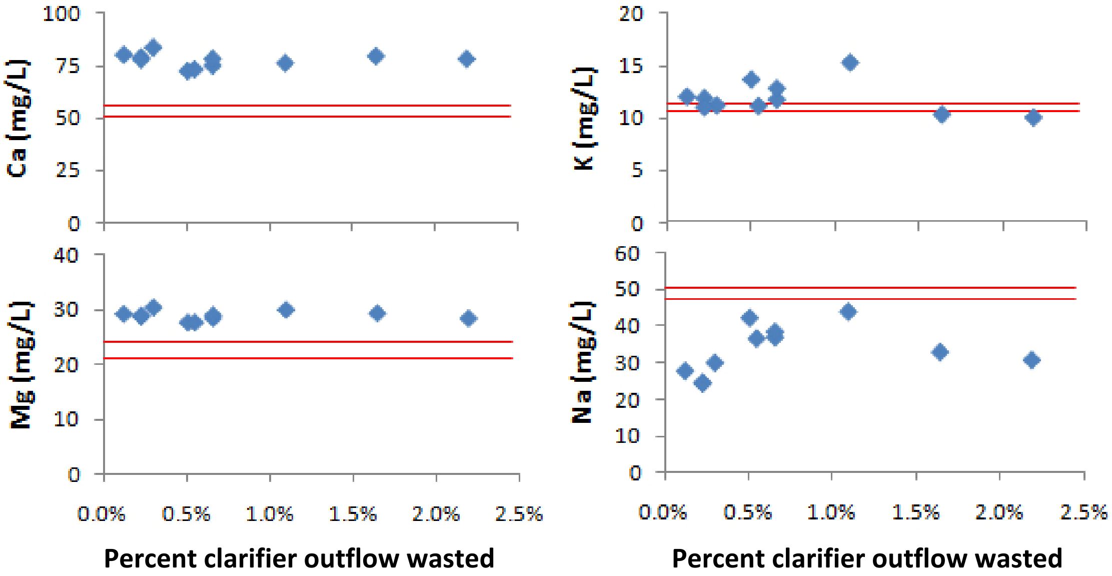

Figure 5.16 Total metals concentrations of possible conservative ions in sludge with respect to % clarifier outflow wasted. Red bars frame the 95% confidence interval constructed with the *t* statistic of the dissolved metals in the TheoMix.

The high concentrations of multiple ecotoxic metals of interest in the sludge present a possible reclamation opportunity or, if mishandled, an environmental hazard. Fe has been economically recovered from oxidation ponds of standard passive treatment systems for years (Hedin, 2003). Mn has been recovered from horizontal flow limestone beds (Denholm et al. 2008). It is important to note that the sludge was not dewatered prior to sampling. It may be possible to drive concentrations of many reclaimable metals from parts per million to per thousand by dewatering. Subsequent smelting or sequential extraction by acidification may be a sensible reclamation approach. Reclaimed metals could be a continual source of revenue for future large-scale passive co-treatment systems.

5.4.3.13 Extended Column Residence Time

Extended column residence time resulted in a pH increase from 6.73 to 7.02 and 31.7% more alkalinity in the limestone stage. There was little evidence of further BSR. Following the limestone exposure results of Watzlaf and Hedin (1993) and Cravotta (2003) the 266 mg/L as $CaCO_3$ was likely near the maximum alkalinity possible with the combination of limestone and mixed effluent present. Limestone dissolved Ca concentrations rose to 319 from 183 mg/L, indicating that the increased alkalinity could be entirely abiotic via limestone dissolution. In the Kaldnes stage, alkalinity rose to 49.1 mg/L as $CaCO_3$ and pH increased to 6.09, either the result of diffusion from the underlying limestone layer, or BSR. However, Kaldnes sulfate concentrations were not significantly different and dissolved Ca concentrations increased to 98.7 from 57.4 mg/L, indicating that substantial diffusion occurred between the stages because there was no Ca source in the Kaldnes.

Unexpectedly, sulfate concentrations in the limestone stage were greater after the extended residence time. This may indicate that some sulfate was released to solution by schwertmannite washed to the Kaldnes via biodegradation or transformation to goethite. Under most conditions, schwertmannite will transform to goethite over time, releasing sulfate and consuming alkalinity (Jönsson et al. 2005). The rate of schwertmannite transformation increases with increased pH (Bigham et al. 1996; Jönsson et al. 2005;

Knorr and Blodau, 2007). Schwertmannite's affinity for sulfate generally decreases with increasing pH as well (Jönsson et al. 2005). The increased pH in the Kaldnes likely accelerated sulfate release which diffused throughout the columns. Depending upon the schwertmannite chemical composition, the transformation of 3.6-5.9 g of schwertmannite would account for the observed sulfate increase. Due to the amount of floc observed in the Kaldnes stage, this is thought to be possible. Cutting et al. (2009) demonstrated that IRB can reduce the Fe in various oxyhydroxides, including schwertmannite, at circumneutral pH in the presence of suitable electron acceptors, which could also add sulfate to solution. It is possible that the decreased sulfate concentrations that would be indicative of further BSR in the Kaldnes or limestone stages were masked by sulfate reintroduction via the aforementioned means.

Removal performance of a few key metals (Al, Mn and Zn) was enhanced with extended residence time. However, dissolved Fe dramatically increased in the Kaldnes stage from 45.3 to 147 mg/L, which may be attributed to IRB activity. Aside from possibly catalyzing schwertmannite and other Fe oxyhydroxide dissolution, IRB and their supporting communities may have utilized Fe(III) sorbed to organic solids protected in biofilms from elevated Zn concentrations (e.g., Stone et al. 2006) where their preferred electron donor is in close proximity to their preferred terminal electron acceptor. IRB activity is the likely reason for increased dissolved Fe because the increased pH boosted the stability of Fe oxyhydroxides and Fe sorbed to organic solids. Mn concentrations were unchanged in the Kaldnes stage, yet dissolved Mn decreased from 18.0 to 14.3 mg/L in the limestone. Mn carbonate formation is a possible removal mechanism (e.g., Waybrant et al. 1998; Bamforth et al. 2006). The extended residence time resulted in more thorough Al removal in the Kaldnes stage. Kaldnes dissolved Al decreased from 10.1 to 0.21 mg/L due to the pH increase which rendered it insoluble. Dissolved Al was unchanged in the limestone and 41.8 mg/L of total Al was flushed from the limestone during sampling, indicating the stability of the Al solid that had been previously formed. Dramatically enhanced Zn removal was noted as dissolved Zn decreased from 80.9 to 5.2 mg/L in the Kaldnes and 64.7 to 17.3 mg/L in the limestone. The increased residence time may have allowed for further Zn sorption to the Fe oxyhydroxide floc. Although evidence of Zn removal in the limestone during standard operation was not previously confirmed by statistical comparisons (i.e., the 84.6 total and 64.7 mg/L dissolved Zn concentrations in the limestone effluent were not statistically different), the extended residence time revealed that a Zn solid was forming within and could be flushed from the limestone in concentrations of 69.2 mg/L. A carbonate mineral, such as smithsonite ($ZnCO_3$), was likely formed (e.g., Nuttall and Younger, 2000). Extended residence time total and dissolved As, Cd, Cr, Ni and Pb concentrations were neither statistically nor substantially different than standard operational concentrations. Although metals sorbed to Fe oxyhydroxides (i.e., As, Pb, Zn, etc.) may be released to solution via IRB activity (Wilkin, 2008), results indicate that metals sorbed to Fe solids washed to the columns were stable or that they had sufficient alternative re-sorption sites available. Overall, the data indicate that Al, Mn and Zn processing and alkalinity generation may be enhanced by increasing residence time. Accumulated Fe can be remobilized and flushed to the subsequent aerobic unit process, which could serve as a unit process regeneration technique to decrease the buildup of solids and avoid clogging.

5.4.4 Plant Community

The wetlands were initially dominated by *Nasturtium officinale* (60-85% at day 29) which quickly became stressed, exhibiting chlorosis, and died off by day 75 to remain <4% coverage throughout the remainder of the experiment. *Hydrocotyle verticillata*, which displayed no stress throughout the experiment, replaced the *Nasturtium officinale* to increase from 10-35% at day 29 to 72-95% by day 133. The second order polynomial model created for estimated total plant coverage of each wetland from days 75-133 had R^2 values of 0.98, 0.89, 0.91 and 0.95, for wetlands 1-4, respectively. Few wetland plant species are tolerant of highly elevated metals concentrations (Younger et al. 2002), and it appears *Nasturtium officinale* was not. However, *Hydrocotyle verticillata* was tolerant of the highly elevated Zn (64.7 to 34.3 mg/L) and Fe (45.1 to 0.18 mg/L) concentrations present from the wetland inflow to outflow.

5.5 Conclusions and Recommendations

The efficient AMD and MWW constituent removal indicate that multi-stage passive co-treatment is capable of effective synergistic performance. In the passive co-treatment system described above, the stage was set for a self-designed microbial ecosystem to develop and take advantage of the available energy flows for preferential biochemical reactions (e.g., sulfate and Fe reduction, FIB disinfection) as well as create suitable conditions (e.g., anaerobic zones) for important abiotic geochemical reactions (e.g., calcite dissolution, phosphate/organic flocculation, metals sorption). Results indicate that high-strength AMD previously suitable only for energy-intensive active treatment can be passively co-treated with MWW.

Due to the promise of passive co-treatment demonstrated in the previous pages, multiple research avenues are recommended. The effect of varied loading rates, improved clarification, inclusion of further unit processes, varied AMD to MWW mixing ratios, and the extent to which BSR can proceed in the presence of more commonly encountered Zn concentrations should be investigated to develop design guidance for full scale co-treatment systems. The relatively quick treatment stabilization to peak performance indicates that full-scale multi-stage co-treatment systems may reach maturity weeks after construction. The lack of denitrification may be addressed by a return loop to increase denitrification such is applied in conventional MWW treatment for enhanced denitrification (e.g. Metcalf and Eddy, 1991). Because the true nitrification potential was not yet observed, longer-term experiments to determine the maximum steady-state nitrification rate may be necessary. There is also the possibility of combining aspects of co-treatment with conventional MWW treatment, such as the use of AMD as a flocculant-source for PO_4^{-3} removal, to create more efficient hybrid facilities. As high-strength AMD and MWW passive co-treatment has proven a viable approach backed by theory and experimental results, field pilot studies are a logical next step. The sludge produced can have concentrations of ecotoxic elements orders of magnitude greater than is present in the mixed influent, which presents a likely reclamation opportunity that should be investigated.

Because co-treatment systems could be used to treat waste streams in communities in developing countries, it would be useful to track specific pathogens through these systems. These pathogens could be tested for expression of metal tolerance and antibiotic resistance genes. The link between antibiotic resistance and metal tolerance has been shown in several studies (Timoney et al., 1978; Foster, 1983; Baker-Austin et al., 2006; Wright et al., 2006; Dib et al., 2008). Although it is unknown whether these systems have the potential to increase the transfer of resistance genes to antibiotics and heavy metals, they may provide a means to consolidate genetic material in order to remove them before they enter the environment. This phenomenon may be tested using bio-assays exposing MWW to AMD in varying proportions to determine the extent of AMD's ability to fully destroy FIB. Additionally, alternative microbiological laboratory techniques, such as direct viability counts, may better estimate the viability of FIB and pathogens in co-treatment systems like the one presented in this study.

Co-treatment augments the affordability and applicability of passive treatment approaches. The Kaldnes biomedia could be substituted with any high surface area naturally occurring material, such the nonreactive river rock used in conventional MWW treatment trickling filter systems. Without the use of plastic in the Kaldnes stage, field-scale co-treatment systems with the same unit processes could be constructed with unrefined low embodied energy (emergy) materials. Aside from low emergy construction, these systems could be operated as gravity flow without ongoing energy inputs. A field-scale passive co-treatment system could use commonly engineered structures, such as ponds, aerobic wetlands, clarifiers, and vertical flow cells, which could decrease engineering costs. Significant cost savings would result from eliminating the need to purchase and transport organic substrate, which can be a major cost of passive AMD treatment. In addition, the use of MWW as a substrate consumes an item with negative societal value in the place of valuable organic substrate, such as compost. Raw MWW and high-strength AMD are often not addressed with passive methods because of the limitations of conventional passive treatment technologies and/or the lack of locally available suitable substrate. The efficiency and rate of which both waste streams can be treated within the same system will determine the extent of savings from reduced system footprint.

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CHAPTER VI

Effects on the Underlying Water Column by Ecologically Engineered Floating Vegetation Mats

Portions of this chapter are included in:

Strosnider, W.H., Nairn, R.W. *In Review*. Effects on the underlying water column by ecologically engineered floating vegetation mats. Proceedings of the American Society of Mining and Reclamation National Conference.

6.1 Abstract

Engineered floating vegetation mats are an emergent application of ecological engineering that have promising water quality improvement and habitat creation applications. However, relatively little research has been published regarding their construction or effects on the underlying water column. The objectives of this study were to determine appropriate design characteristics and the effect of ecologically engineered floating vegetation mats (EFVM) on the underlying water column. Four EFVM designs were constructed of drainpipe, burlap, mulch, utility netting, and reused polyethylene bottles then planted with *Typha* spp. and *Juncus effusus*. The water column beneath EFVM in two test ponds was compared to that in an open water control pond. Dissolved oxygen concentrations and pH were lower, diurnal temperature range was dampened, and sulfate/nitrate reduction was greater under the EFVM with respect to the control. Alkalinity was also greater under EFVM. Results reinforced previous findings indicating that *Typha* spp. is a suitable species for EFVM creation. However, a more robust

planting matrix is necessary to encourage faster growth and protect against wind and wave action damage. Although plant propagation was limited, results suggest that EFVM may be applied to encourage reducing, thermally insulated conditions for passive treatment of acid mine drainage a wide range of other pollutants. Specifically, they may be employed to improve immediate and long-term performance of vertical flow bioreactors for acid mine drainage treatment by lowering dissolved oxygen concentrations in the water column and providing a continual source of organic carbon to the underlying substrate.

6.2 Introduction

Naturally occurring floating vegetation mats (NFVM) are relatively common in various climates and settings (Hunt, 1943; Mitsch and Gosselink, 2000; Mallison et al., 2001; Scheffer et al., 2003; Somodi and Botta-Dukat, 2004; van Duzer, 2004; Kadlec and Wallace, 2009). NFVM often develop as the rhizomes of wetland plants colonize horizontally from the shoreline, from masses of vegetation delaminated from the underlying substrate or via colonization of floating organic substrates (Somodi and Botta-Dukat, 2004; Kadlec and Wallace, 2009). Examples of NFVM range from free-floating *Typha* spp. tussocks in Florida (Mallison et al., 2001), floating sedge fens in Alaska (Racine and Walters, 1994), *Panicum hemitomon*-dominated floating marshes of the Mississippi River Delta Plain (Sasser et al., 1996), floating *Cyperus papyrus* L. marshes of equatorial Africa (Gaudet, 1977; Boar et al., 1999), to boreal quaking bog vegetation (Mitsch and Gosselink, 2000). *Typha* spp. can form stable, buoyant and productive NFVM in a wide variety of climates and hydrogeochemical settings (Hunt, 1943; Racine and Walters, 1994; Sasser et al., 1996; Mallison et al., 2001). *Typha* spp. NFVM are resilient and can recover quickly from drying and even burning regimes (Krusi and Wein, 1988).

NFVM have demonstrated effects on water quality that may be conducive to treating certain types of effluent. NFVM can create anoxic conditions in the water column below them (Mallison et al., 2001; Scheffer et al., 2003). Vascular plants with photosynthetic tissue above the water's surface often deplete dissolved oxygen (DO) in the underlying water column (Kadlec and Wallace, 2009; Janse and Van Puijenbroek, 1998; Caraco et al., 2006). Dense growth of floating vegetation reduces the water surface available for oxygen diffusion as well as lowers turbulence in the neighboring uncovered water surface (Scheffer et al., 2003). NFVM can also decrease DO levels in the water column by shading submersed vegetation (Janse and Van Puijenbroek, 1998; Mallison et al., 2001). Decaying plant material forms loose layers or organic sludge beneath NFVM (Swarzenski et al., 1991) and Alam et al. (1996) found water below NFVM to have depressed DO levels and higher organic matter concentrations when compared to nearby open water. Generally, previous findings indicate that NFVM can help create anaerobic, organic-rich systems in the underlying water column. Similar to NFVM, floating aquatic plant systems of *Eichhornea crassipes* and *Lemna, Spirodela* and *Wolffiella* spp. have been successfully established for municipal wastewater treatment (Kadlec and Wallace, 2009). Like NFVM, these systems of free-floating aquatic plants are generally anaerobic because photosynthesis occurs above the water's surface while oxygen diffusion is limited by vegetative cover (Kadlec and Wallace, 2009).

EFVM (Ecologically-engineered floating vegetation mats) take advantage of the properties of NFVM to provide a desired water quality effect and/or habitat improvement. EFVM are generally constructed of a framework promoting the growth of emergent macrophytes, such as *Typha* spp., suspended in the water column. Although tests and/or full-scale applications have been few, EFVM have been applied to treat meat processing effluent (Van Oostrom, 1995), improve lake water quality (Boutwell, 2001), and treat dilute de-icers from airport runoff (Revitt et al., 2001; Richter et al., 2003).

However, EFVM have been applied in multiple instances to treat AMD by providing a continual carbon source to bacteria in systems dubbed ARUM (Acid Reduction Using Microbiology) (Smith and Kalin, 2000; Kalin and Caetano Chaves, 2003; Kalin, 2004).

These systems have been primarily applied to either flooded pits associated with mining disturbances or in treatment wetlands. Canada hosts EFVM wetlands treating zinc/lead rich contaminated open pits in Buchans, Newfoundland, nickel/copper tailings runoff in Sudbury, Ontario, nickel/arsenic waste rock runoff in Northern Saskatchewan, and aqueous aluminum oxide and coke particles in Kitimat, British Columbia (Smith and Kalin, 2000). Anaerobic conditions beneath the EFVM are reported to enable sulfate and iron reduction reactions that remove acidity and metals from solution while increasing alkalinity (Kalin et al., 2006).

Nevertheless, comprehensive EFVM performance data and design specifications are lacking in the refereed literature, information that is necessary to determine their suitability to treat AMD as well as other effluents. Beyond the exploration of the structural stability and maturation of four EFVM designs, this experiment allowed the comparison of water quality parameters between control and test ponds to provide indications of how passive treatment cells with EFVM may perform.

6.3 Methods

6.3.1 Experimental Design

EFVM trials occurred in newly HDPE-lined ponds cleared of any debris and filled with well water at the University of Oklahoma Aquatic Research Facility. Three approximately 200 m^2 University of Oklahoma Aquatic Research Facility ponds, one control and two experimental, were used (Figure 6.1). The open water control pond had the same general dimensions and volume, approximately 140 m^3, as the test ponds. The ponds were each a closed controlled hydrologic system, receiving very little surface runoff. All ponds were periodically refilled with approximately equal volumes of well water to offset losses from evapotranspiration not compensated by precipitation.

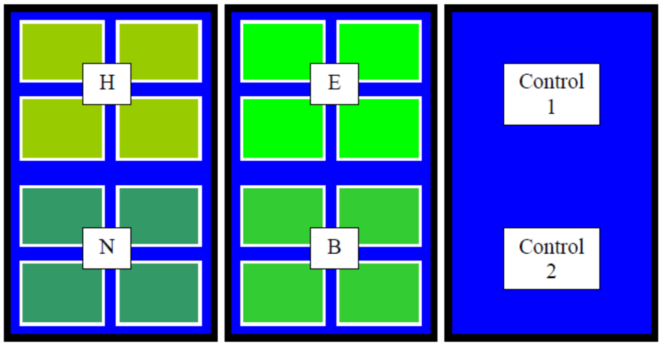

Figure 6.1 Experimental pond layout

Four EFVM designs were installed in the test ponds (Figure 6.1, Table 6.1), each with four replicates. Designs were designated "E", "H", "B" and "N". EFVM "H" had the most intensive planting medium, with cornstover hydromulch sandwiched by burlap accompanying the mulched *Typha* spp. that all designs shared. The "B" design had a medium-intensive planting media. The "E" was a minimalist low embodied energy design defined by a frameless bed supporting minimal planting media overlain by burlap. "N" had the least intensive planting media, with no supportive burlap. Each EFVM had one water sampling unit aligned in its center with fixed sampling ports located 10 cm below the water surface, 40 cm below the water surface and 10 cm above the bottom of the pond.

Table 6.1 EFVM design descriptions

Design Feature	**Design**			
	H	N	E	B
15 x 17 ft ABS drainpipe frame	X	X		X
15 x 17 ft 3/4 in utility net	X	X	X	X
7oz untreated burlap - over rhizomes	X		X	X
7oz untreated burlap - under rhizomes	X			
45 kg cornstover hydromulch	X			
1.5 x 6 ft access slot centered and perpendicular to long axis	X	X	X	X
7 kg of long cut Typha spp. shoots	X	X	X	X
4 kg of mulched Typha spp. shoots and seed heads	X	X	X	X
2 sealed 16oz float bottles placed at each corner	X	X	X	X
4 sealed 16oz float bottles placed around central access slot	X	X	X	X
1 sealed 16oz float bottle placed at each edge midpoint			X	
Tension applied at corners directed away from the center			X	

On June 13-16, 2006 the EFVM were constructed and initially planted with 52 *Typha* spp. rhizomes (~7.6 g dry weight each) at regularly spaced intervals and four *Juncus effusus* culms, one at each corner. Locally harvested rhizomal plantings were chosen following the observations of Mitsch and Gosselink (2000) that this is a viable planting method and Kadlec and Knight (1996) that locally harvested plants may have a more fitting genotype for the applied setting. All EFVM were designed to support the plantings at water depths between 5 and 30 cm following the findings of Grace (1989) that *Typha* spp. have higher productivity in this range. High winds and multiple storm events blew over and killed some of the established vegetation. Therefore, following the findings of Van Oostrom (1995) that interlocked emergent macrophytes patches can be separated from terrestrial substrate and successfully set upon floating frames, seven (10 x 10 cm, 54 g dry weight each) sections and one large (~60 x 60 cm, 1500 g dry weight each) section of rhizomally interlocked *Typha* spp. were added to each mat on May 20-21,

2007. The EFVM were qualitatively and quantitatively monitored until deconstruction on October 10, 2008.

6.3.2 Data Collection

Vegetation growth and any structural degradation were monitored monthly by elevated photography using a 3.6-m stepladder and digital camera. Diurnal DO, pH, Eh and temperature (T) were continuously logged for an average of three days in duration at two or five minute intervals using YSI 600QS multiparameter sondes and YSI 650MDS displays. Diurnal data were gathered by hanging the sondes at the middle of a randomly determined EFVM with sensors located approximately 35 cm below the water's surface. Control diurnal data was gathered by hanging sondes in the same fashion at the center of either half of the control pond.

Waters were sampled from the fixed sampling ports for 13 monthly sampling events. The same sondes and displays were coupled with a peristaltic pump and flow-through cell for the monthly sampling events. Sampling generally occurred from 11:00 to 17:00 over consecutive days. One level of all stations (all EFVM replicates and both control) was sampled a day, over the three day sampling event. The order of sampling was randomized to avoid systemic error due to the diurnal range of various parameters. Alkalinity titrations were conducted with samples from the fixed sampling ports following standard methods (APHA, 1998) and Hach Method 8203 (Hach, 2006). Following Sharp et al. (1995), water samples for dissolved organic carbon (DOC) and dissolved total nitrogen (DTN) were gathered from the fixed sampling ports, immediately filtered through 0.45-μm nylon filters, then stored at <-4°C in 40-mL amber glass EPA vials with polypropylene caps and Teflon septa until quantification with an Analytik Jena multi N/C 2100 Analyzer. DOC and DTN samples were processed from five events that were evenly spread among the 13 overall sampling events.

Grab samples for anion concentrations were taken from the well heads and the center of each pond at irregular temporal intervals throughout the experiment using 250-mL HDPE containers. These samples were stored at 4°C until filtered through Dionex OnGuard® II H cartridges and 0.2 μm nylon filters. A MetrOhm® 761 compact ion chromatograph unit was used to quantify anion concentrations following EPA method 300.

6.3.3 Data Analysis

Due to the non-normality yet relatively similar distribution and equal variances of the diurnal data sets, the Mann-Whitney test was applied for statistical comparisons of diurnal DO, pH and T. A one-tailed homoscedastic Student's *t*-test was applied to compare the diurnal T range differences between the control pond and under the EFVM. To do so, the absolute value between each sequential daily maximum and minimum T during diurnal sampling periods were compiled. Prior to statistical analysis, each monthly sampling event's alkalinity, pH, DO, Eh and T of the control and each EFVM type were averaged, pooling each level and replicate. As the reduced data sets were normal with similar variances, each EFVM was tested against the control using two-tailed homoscedastic Student's *t*-tests. Due to the small size of the DOC and DTN data sets from the monthly sampling events, they were not reduced and medians of the full non-normal data sets were compared with the Mann-Whitney test. One-tailed homoscedastic Student's *t*-tests were applied to means test anion data. All statistical tests employed an alpha of 0.05.

6.4 Results

6.4.1 Structural Performance

Various design lessons were learned from qualitative observation of each EFVM (Figures 6.2 and 6.3). From observation of EFVM "H", it was deduced that a stronger, more buoyant frame was necessary to support the mass of more intensive planting medium. From observation of the other EFVM, more durable finer mesh netting is recommended to allow vegetation to firmly establish. Plant survival and propagation was limited. However, interlocked continual rhizomes were more productive, and are thus preferable over singular separated *Typha* spp. rhizomes. *Juncus effusus* displayed survivability yet remained isolated at the corners of the mats and did not propagate. The depth of planting should be kept at a minimum to avoid any additional unnecessary vegetative stressors. All designs suffered damage from high winds, and consequential wave action, that frequent central Oklahoma. Boutwell (2001) observed the same difficulty with EFVM in Nevada. More robust planting matrices to firmly root plantings are suggested for applications in regions commonly experiencing high winds.

Figure 6.2 The central pond with EFVM set “E” in the foreground and “B” in the background

Figure 6.3 EFVM “E” approximately one year after construction and installation

6.4.2 Anions

Well water (influent) NO_3 concentrations averaged 11.5 ± 1.0 mg/L, yet NO_3 was consistently below detection limits (<0.5 mg/L) in the EFVM ponds. In the control pond, NO_3 was greater than detection limits (0.65 and 0.67 mg/L) for two out of the six anion sampling events. Well water SO_4 averaged 59 ± 5.5 mg/L and was significantly less in the EFVM and control ponds. However, control pond SO_4 (28 ± 2.1 mg/L) was found to be significantly greater than the ponds with EFVM “E” and “B” (24 ± 2.0 mg/L) and “N” and "H" (20 ± 2.8 mg/L). Phosphate was consistently below detection limits (<0.75 mg/L) in the well water and all ponds.

Denitrification and SO_4 reduction are the likely mechanisms for the observed decrease of NO_3 and SO_4 concentrations within the test and control ponds. Dilution is unlikely because the only possible pond outflow was evapotranspiration, which would serve to concentrate dissolved constituents. Denitrification and SO_4 reduction require labile organic electron donors and reducing conditions to proceed. Van Oostrom (1995) observed that his EFVM successfully encouraged denitrification as well. The likely occurrence of these processes to a greater extent in the EFVM ponds indicates that EFVM can help create more reduced organic-rich conditions beneath them.

6.4.3 Diurnal Results

Median diurnal T was significantly greater in the control than the EFVM ponds, except for the diurnal sampling period during the coldest period of the study (Table 6.2). DO was significantly less under the EFVM for the majority of diurnal logging periods (Table 6.3). The pH was also significantly lower under the EFVM for most periods (Table 6.4). Eh was consistently significantly less under each EFVM than the control (Table 6.5). Although the differences are not large in magnitude, the DO, pH and Eh data combine to indicate the presence of less oxidizing conditions with lower oxidation reduction potential under the EFVM than in the control pond. It can be expected that greater differences would be noted for mature EFVM with more intensive planting media, established root mass, and productive emergent vegetation.

Table 6.2 Median T (°C) for each diurnal sampling period. EFVM values are bolded or underlined where statistically less or greater than the control, respectively. Mean air T data are from the Oklahoma Climatological Survey (2010). The "-" denotes that no data were taken at this station.

Logging Period	Station					
	Air	Control	"N"	"B"	"E"	"H"
04/25/08 - 04/27/08	18.83	20.36	18.94	19.50	-	-
03/20/08 - 03/22/08	21.28	15.07	-	-	13.99	14.18
12/28/07 - 12/30/07	0.89	4.17	-	-	-	**4.44**
10/26/07 - 10/29/07	11.47	15.42	-	-	14.27	14.43
10/18/07 - 10/21/07	18.47	19.64	-	-	18.61	-
09/28/07 - 10/01/07	17.04	25.10	-	23.42	-	-
09/18/07 - 09/21/07	25.13	27.06	-	-	-	25.19
08/21/07 - 08/24/07	27.88	31.41	-	-	29.06	29.22
08/10/07 - 08/13/07	29.76	33.83	31.17	30.88	-	-
07/21/07 - 07/23/07	26.39	33.07	-	30.49	30.42	-
07/08/07 - 07/11/07	25.63	31.10	-	27.88	27.90	-
06/30/07 - 07/01/07	23.58	26.98	-	-	-	25.53

Table 6.3 Median percent saturation of DO for each diurnal sampling period. EFVM values are bolded or underlined where statistically less or greater than the control, respectively. The "-" denotes that no data were taken at this station.

Logging Period	Station				
	Control	"N"	"B"	"E"	"H"
04/25/08 - 04/27/08	102.2	77.3	**103.5**	-	-
03/20/08 - 03/22/08	99.0	-	-	**101.3**	96.2
12/28/07 - 12/30/07	91.0	-	-	-	96.4
10/26/07 - 10/29/07	85.6	-	-	**93.1**	**100.2**
10/18/07 - 10/21/07	102.0	-	-	88.2	-
09/28/07 - 10/01/07	124.6	-	94.4	-	-
09/18/07 - 09/21/07	134.1	-	-	-	83.3
08/21/07 - 08/24/07	119.6	-	-	99.9	66.0
08/10/07 - 08/13/07	125.5	52.2	98.0	-	-
07/21/07 - 07/23/07	102.4	-	94.2	96.1	-
07/08/07 - 07/11/07	94.1	-	74.7	74.1	-

Table 6.4 Median pH for each diurnal sampling period. EFVM values are bolded or underlined where statistically less or greater than the control, respectively. The "-" denotes that no data were taken at this station.

Logging Period	Station				
	Control	"N"	"B"	"E"	"H"
04/25/08 - 04/27/08	8.64	7.95	**9.11**	-	-
03/20/08 - 03/22/08	8.51	-	-	8.46	8.46
12/28/07 - 12/30/07	8.13	-	-	-	**8.45**
10/26/07 - 10/29/07	8.71	-	-	8.47	8.10
10/18/07 - 10/21/07	8.99	-	-	8.35	-
09/28/07 - 10/01/07	8.77	-	8.23	-	-
09/18/07 - 09/21/07	8.12	-	-	-	7.63
08/21/07 - 08/24/07	9.22	-	-	8.81	8.05
08/10/07 - 08/13/07	9.22	7.73	8.72	-	-
07/21/07 - 07/23/07	8.94	-	8.59	8.60	-
07/08/07 - 07/11/07	8.93	-	8.28	8.34	-
06/30/07 - 07/01/07	9.17	-	-	-	7.66

Table 6.5 Median Eh (mV) for each diurnal sampling period. EFVM values are bolded or underlined where statistically less or greater than the control, respectively. The "-" denotes that no data were taken at this station.

Logging Period	Location				
	Control	"N"	"B"	"E"	"H"
04/25/08 - 04/27/08	334	242	-	-	-
03/20/08 - 03/22/08	332	-	-	267	-
12/28/07 - 12/30/07	367	-	-	-	309
10/26/07 - 10/29/07	352	-	-	-	251
10/18/07 - 10/21/07	369	-	-	257	-
09/28/07 - 10/01/07	348	-	247	-	-
09/18/07 - 09/21/07	440	-	-	-	242
08/21/07 - 08/24/07	373	-	-	205	-
08/10/07 - 08/13/07	311	-	196	-	-
07/21/07 - 07/23/07	262	-	213	-	-
07/08/07 - 07/11/07	305	-	227	-	-
06/30/07 - 07/01/07	280	229	-	-	-

6.4.4 Diurnal Temperature Range

Temperature profiles were dampened beneath the EFVM through all seasons with respect to the control. Figure 6.4 presents a representative logging period. EFVM designs “B” and “H” demonstrated statistically greater diurnal T range for all five and four of their diurnal logging periods, respectively. EFVM “N” only displayed statistically significant dampening for one of three diurnal logging periods. However, its T ranges were numerically greater, and a longer logging period would likely reveal statistical significance. EFVM “E” demonstrated significantly greater T ranges for five of six logging periods. Overall, it can be expected that greater differences would be noted for mature EFVM with more intensive planting media, established root mass, and productive emergent vegetation.

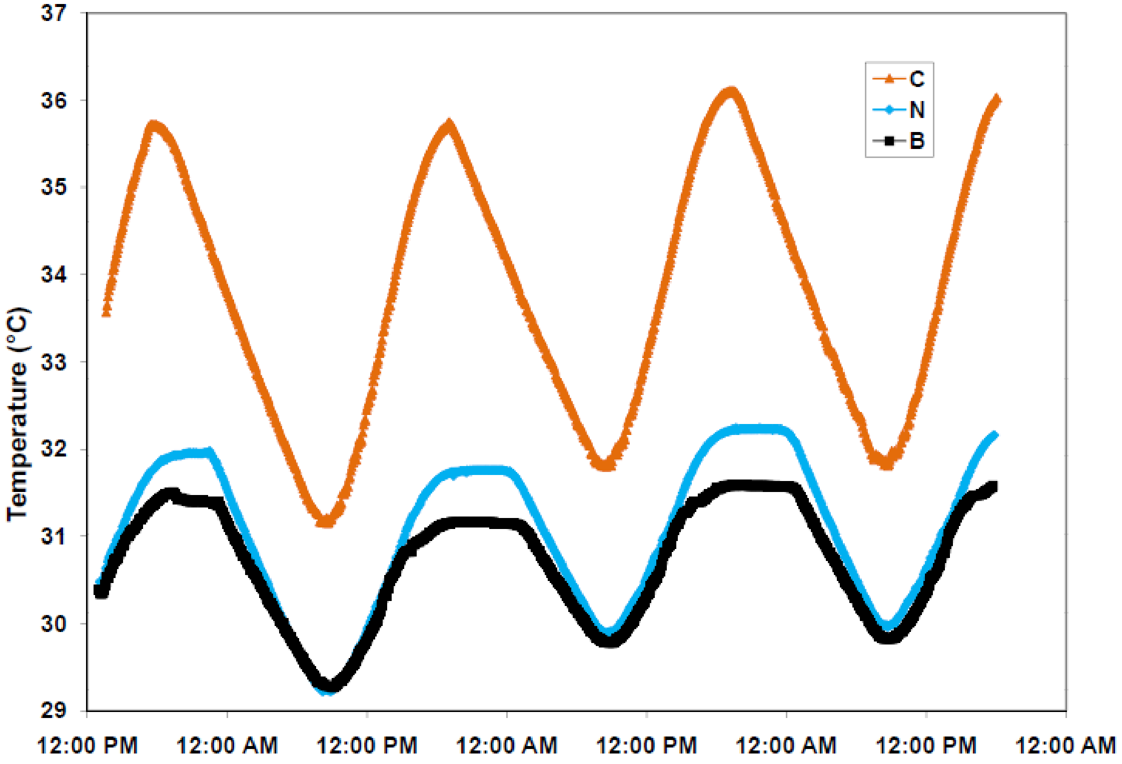

Figure 6.4 Diurnal T variation August 8-13, 2007. “C” is representative of the control.

6.4.5 Monthly Sampling Results

There was no statistical difference in mean T between any of the EFVM and the control (Table 6.6). Mean T was not significantly different between EFVM and the control possibly due to the small size of the data set (n = 13). “N” and “H” had mean DO less than the control (Table 6.7). However, “E” and “B” mean DO was not significantly different than the control. “N” and “H” had mean alkalinity less than the control (Table 6.8). However, “E” and “B” mean alkalinity was not significantly different than the control. Increased alkalinity under “N” and “H” was likely due to the increased presence of organic ligands in the pond from the hydromulch in the “H” design. The hydromulch also likely helped further deplete DO in the water column. The other EFVM may have shown statistically greater alkalinity and lower DO if the size of the data set was expanded. All EFVM had mean pH less than the control (Table 6.9), generally reflecting the diurnal pH results. Median DOC was not significantly different between EFVM and the control (Table 6.10). It may be that the bulk of organic matter released by the mats was in particulate form. Median DTN was slightly, yet significantly less under “N” and “H”.

Table 6.6 Mean T for each monthly sampling period. Mean air T data are from the Oklahoma Climatological Survey (2010). Note that air T is a daily mean, while water T was recorded only during the daytime/evening sampling windows.

Sampling Period	Air	Control	B	N	E	H
		Temperature (° C)				
10/7/2006-10/9/2006	18.4	23.2	21.4	20.7	21.5	20.6
1/9/2007-1/11/2007	9.8	9.9	8.3	9.3	9.3	9.5
2/10/2007-2/12/2007	3.6	5.4	4.5	5.2	5.0	5.3
6/9/2007-6/11/2007	25.5	31.1	28.6	29.2	28.8	29.4
7/21/2007-7/23/2007	26.4	33.5	30.6	30.6	30.7	30.4
8/21/2007-8/23/2007	27.8	32.7	30.8	30.9	31.4	31.1
9/29/2007-10/1/2007	23.2	27.1	25.6	25.9	25.9	25.8
10/26/2007-10/28/2007	10.5	16.2	15.7	15.4	16.2	15.7
11/29/2007-12/4/2007	6.3	10.6	9.9	9.5	9.8	9.9
12/28/2007-12/30/2007	0.9	5.5	5.0	5.0	5.4	4.8
1/26/2008-1/28/2008	9.0	8.0	7.1	6.5	8.4	7.0
2/27/2008-3/1/2008	10.7	11.7	11.2	11.7	11.0	11.8
3/20/2008-3/22/2008	14.1	17.0	16.5	16.0	16.5	16.5
Mean	**14.3**	**17.8**	**16.6**	**16.6**	**16.9**	**16.7**
Standard Deviation	**9.1**	**10.5**	**9.8**	**9.8**	**9.7**	**9.8**

Table 6.7 Mean DO for each monthly sampling period.

Sampling Period	Control	B	N	E	H
	DO (%)				
10/7/2006-10/9/2006	142	151	101	153	92
1/9/2007-1/11/2007	117	108	120	119	124
2/10/2007-2/12/2007	95	90	90	93	84
6/9/2007-6/11/2007	146	98	79	99	77
7/21/2007-7/23/2007	120	96	68	98	46
8/21/2007-8/23/2007	126	114	99	127	97
9/29/2007-10/1/2007	142	113	108	112	105
10/26/2007-10/28/2007	103	102	97	105	97
11/29/2007-12/4/2007	96	93	93	92	95
12/28/2007-12/30/2007	97	93	97	95	97
1/26/2008-1/28/2008	100	98	103	100	104
2/27/2008-3/1/2008	100	104	108	103	108
3/20/2008-3/22/2008	103	115	107	113	105
Mean	**114**	**106**	**98**	**108**	**95**
Standard Deviation	**19**	**16**	**13**	**17**	**19**

Table 6.8 Mean alkalinity for each monthly sampling period

Sampling Period	Control	B	N	E	H
	Alkalinity (mg/L as $CaCO_3$ eq.)				
1/9/2007-1/11/2007	179	214	218	214	220
2/10/2007-2/12/2007	177	208	213	212	218
6/9/2007-6/11/2007	136	167	176	166	179
7/21/2007-7/23/2007	91	108	127	106	125
8/21/2007-8/23/2007	73	88	109	87	109
9/29/2007-10/1/2007	125	145	154	144	156
10/26/2007-10/28/2007	114	137	167	141	168
11/29/2007-12/4/2007	161	181	200	179	197
12/28/2007-12/30/2007	154	169	179	169	179
1/26/2008-1/28/2008	161	177	179	177	176
2/27/2008-3/1/2008	171	181	171	180	169
3/20/2008-3/22/2008	170	161	158	161	157
4/25/2008-4/27/2008	161	116	173	115	173
Mean	**144**	**158**	**171**	**158**	**171**
Standard Deviation	**34**	**38**	**30**	**38**	**31**

Table 6.9 Mean pH for each monthly sampling period

Sampling Period	Control	B	N	E	H
			pH (s.u.)		
10/7/2006-10/9/2006	8.11	7.91	7.36	7.93	7.32
1/9/2007-1/11/2007	8.54	8.50	8.24	8.49	8.18
2/10/2007-2/12/2007	8.05	8.00	7.99	8.04	7.92
6/9/2007-6/11/2007	8.89	7.83	7.71	7.77	7.60
7/21/2007-7/23/2007	8.72	8.16	7.70	8.27	7.63
8/21/2007-8/23/2007	8.74	8.23	7.97	8.39	7.73
9/29/2007-10/1/2007	8.52	7.70	7.45	7.57	7.51
10/26/2007-10/28/2007	8.17	7.62	7.36	7.66	6.85
11/29/2007-12/4/2007	7.62	7.52	7.54	7.58	7.31
12/28/2007-12/30/2007	8.13	8.19	8.28	8.13	8.23
1/26/2008-1/28/2008	7.98	7.99	8.20	7.90	8.18
2/27/2008-3/1/2008	7.85	7.85	8.07	7.92	7.92
3/20/2008-3/22/2008	8.16	8.18	8.17	8.19	8.13
Mean	**8.27**	**7.98**	**7.85**	**7.99**	**7.73**
Standard Deviation	**0.38**	**0.28**	**0.34**	**0.30**	**0.42**

Table 6.10 Median DOC and DTN from monthly sampling

	Control	B	N	E	H
			mg/L		
DOC	15.4	14.1	15.6	14.3	15.5
DTN	1.05	0.91	1.11	0.96	0.93

6.5 Discussion

EFVM have promising passive AMD treatment applications. For example, organic carbon availability and temperature influence the effectiveness of vertical flow bioreactors (VFB), which are also known as reducing and alkalinity producing systems (RAPS) or successive alkalinity producing systems (SAPS). VFB generally consist of a layer of limestone with drainage piping overlain by organic material with a ponded depth of approximately 1 m of overlying water (Watzlaf et al., 2004). Influent flows vertically down through the water column, organic matter, and limestone sequentially. The organic material in VFB serves to deplete oxygen and fuel sulfate reduction, which generates alkalinity and removes metals from solution. The limestone generates yet further alkalinity. VFB are typically followed by oxidation ponds where Fe or Mn can be optimally removed from the buffered solution.

Several studies have shown a sulfate reduction rate decline over time in treatment cells that rely on organic matter oxidation (Drury, 2000; Chang et al., 2000; Gibert et al., 2003; Eger and Wagner, 2003). VFB treatment efficiency decreases with age before organic carbon sources are fully depleted as the supply of short chain organics decreases and supplemental carbon is currently introduced to address this issue (Eger and Wagner, 2003; Kalin et al., 2006). *Typha* spp. and *Juncus effusus* contribute labile dissolved organic carbon to their surroundings during senescence or via root exudates which can be readily used by sulfate reducing bacteria and fermenters, key consortia of VFB (Mann and Wetzel, 1996; Johnson and Hallberg, 2002).

Emergent macrophytes are among the most productive plant communities worldwide (Mitsch and Gosselink, 2000) and Eger and Wagner (2003) suggest that decaying wetland plants could provide a renewing carbon source to VFB. Batty and Younger (2007) showed that wetland plant litter in AMD treatment systems can be a key source of organic and inorganic nutrients to bacterial populations, even under conditions of depressed pH (6.5-3.0). *Typha latifolia* productivity ranged from 0.76 to 2.7 kg dry wt m^{-2} yr^{-1} in data compiled by Cronk & Fennessy (2001). Average *Juncus effusus* productivity is similar (Mitsch and Gosselink, 2000). However, *Typha* spp. productivity is reported to be roughly halved in wetlands receiving mine drainage (Mitsch & Jorgensen, 2004). Neculita et al. (2007) note optimal VFB field conditions at 0.3 mol

SO_4/m^3-d and this comports well with data from Dvorak et al. (1992) and McCauley et al. (2009). In sulfate reduction, for each mol of SO_4 reduced, two moles of C are required (Neculita et al., 2007). From samples of *Typha* spp. at an AMD passive treatment system, it was determined that 90% of dry wt. is organic matter (Nairn, unpublished data). By halving the productivity of the range noted by Cronk and Fennessy (2001), applying the approximation from Mitsch and Gosselink (2000) that approximately 50% of the dry wt. of organic matter is C with the percent organic matter from Nairn (unpublished data) it was calculated that EFVM in AMD could provide 0.04-0.14 mol C/m^2-d of the 0.6 C/m^2-d that an optimally functioning VFB of 1 m substrate depth would require. EFVM as productive as *Typha* spp. in optimal growth settings could, in theory, fix half the C required by VFB.

Aside from the organic matter contributions, EFVM ability to increase alkalinity, encouragement of reducing conditions, and temperature insulating effects could be a welcome addition to VFB. Passive AMD treatment systems, such as VFB, require strategies to limit the impact of low ambient temperatures (Heal and Salt, 1999; Johnson and Hallberg, 2002; Watzlaf et al., 2004; Champagne et al., 2005; Gusek, 2005), especially in extreme latitudes or higher elevations. The results suggest that in a colder climate, EFVM would insulate the underlying water column and substrate from the cooler temperatures that often limit the preferential biogeochemical reactions central to VFB performance. VFB sizing is often based on the conservative expected cold-weather removal/processing rates. The insulative effects of EFVM could inhibit bacterial activity during warmer periods when activity may otherwise be unnecessarily elevated, consuming more substrate than is needed and producing excess hydrogen sulfide gas, which can be a nuisance odor and environmental toxin. The reduction of the water column by EFVM would allow more of the organic substrate's vertical profile to be allocated to sulfate reduction, other than oxygen stripping, which could increase sulfate reduction and therefore alkalinity production and metal removal rates. Continual additions of organic matter and nutrients from the EFVM to the VFB would help renew essential labile substrate. Increased sulfate reduction and hence alkalinity production before VFB waters contact the underlying limestone could decrease the reliance on limestone for alkalinity production, enabling cost-savings. All these factors would combine to increase the lifetime, efficiency, and overall sustainability of VFB while decreasing necessary land footprint and seasonal odors with an aesthetically pleasing addition of floating emergent wetland vegetation.

However, there is the possibility of creating a wildlife attraction that may increase exposure to whatever ecotoxic constituent is present. Floating islands are an attractive habitat for many species (van Duzer, 2004). For example, in Minnesota, floating *Typha* spp. mats are prime nesting substrate for red-necked grebes (*Podiceps grisegena*) because of their isolation from predators (Nuechterlein et al., 2003). However, overall the selection of *Typha* spp. should decrease the chance of biomagnification because it is of limited value to wildlife when compared to many other macrophyte species (Mitsch & Jorgensen, 2004).

There are a myriad of other possible EFVM applications. EFVM could be incorporated into existing ecological engineering applications that require anaerobic and/or organic-rich conditions, like those described by Guterstam (1996) for aquaculture wastewater, Schaafsma et al., (2000), Pattanaik et al., (2007) and Travieso et al., (2006) for distillery waste, Lansing and Martin (2006) for dairy effluent, and Kadlec and Wallace (2009) and Hamersley and Howes (2002) for municipal wastewater denitrification. The dechlorination of chlorinated compounds, an application of ecological engineering proven viable by Kassenga et al., (2003) and Amon et al., (2007), could be enhanced by EFVM. Also, EFVM could help create the reducing conditions noted to encourage the biodegradation of diesel fuel (e.g., Boopathy, 2003), ethanol (e.g., Zhang et al., 2006), other petroleum hydrocarbons (e.g., Hunkeler et al., 1998) and nitro-aromatic compounds (e.g., Kulkarni and Chaudhari, 2007).

6.6 Conclusions

Results indicate that EFVM are a suitable ecological engineering tool for influencing water quality and temperature that may have a wide variety of applications. EFVM can encourage more reducing conditions beneath them with greater alkalinity as well as provide insulation from extreme temperatures. Better performing EFVM can be expected by employing more intensive planting media, joined rhizomal plantings, and stronger more buoyant frames than those presented in this study. Future studies should investigate incorporation of EFVM into VFB for AMD treatment and other applications where organic-rich, thermally insulated, and reduced conditions are advantageous. Pilot studies to investigate the possibilities of passively treating the constituents listed in the previous paragraph are also suggested. Testing EFVM over flow-through systems is necessary to determine the rate at which EFVM can alter the underlying water column.

6.7 Acknowledgements

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