Long Term Operation of Engineered Anaerobic Bioreactors and Wetland Cells Treating Zinc, Arsenic and Cadmium in Seepage – Results, Longevity, Cost and Design Issues

by

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M.Sc., University of Idaho, 1984
G.D.B.A., Simon Fraser University, 2004

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in the Department of Earth and Ocean Sciences

Supervisory Committee

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Supervisor

Dr. Fariborz Goodarzi (Department of Earth and Ocean Sciences)
Co-Supervisor

Dr. Kevin Telmer (Department of Earth and Ocean Sciences)
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Abstract

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At the Trail Smelter, contaminated seepage water is collected and a portion is diverted for treatment to a large pilot-scale wetland system. The design, construction (in stages from 1997 to 2002) and long term sampling (1998-2007) of the wetland system treating high concentrations of zinc, arsenic and cadmium is presented. The final system configuration has been operating year-round since 2002 treating approximately 15,000 L/d. The system is comprised of two vertical upflow anaerobic (compost) bioreactors followed by three horizontal subsurface flow vegetated wetland cells, a slow sand filter and a final holding cell. Operational sampling was done for water quality (metals and various anions), bacterial communities (MPN, PFLA and DGGE) and vegetation (metals content). After several years of operation one of the anaerobic cells was taken apart and rebuilt in 2002. Extensive solid substrate sampling during deconstruction was analyzed for mineralization (SEM/EDS), metals and carbon content (Rock-Eval pyrolysis) to estimate the potential cell life.

The system treats seepage with zinc up to 3800 mg/L (average ~ 260 mg/L), arsenic to 3600 mg/L (average ~ 150 mg/L) and Cd to 83 mg/L (average ~ 4.7 mg/L) which are reduced to <0.5 mg/L (<0.02 mg/L for Cd). Vegetation sampling showed variable uptake into exposed plants at much higher levels than control plants. Plant toxicity was experienced in the system. Evapotranspiration and rhizofiltration are the preferred use of plants as opposed to metal hyper-accumulating plants. Bacterial sampling indicated the presence of sulphate reducing bacteria and a diverse anaerobic microbial community throughout the system despite the high metals entering the system. The predicted life of

the anaerobic cell by Rock Eval 6 was 18 years with a range from 17 to 21 years, while based on biomass calculations could range from 14 to 34 years. Where wetlands systems can be successfully used, their cost and environmental and social sustainability is very favourable when compared to chemical treatment systems (e.g. lime-dosing systems). Based on author's experience at the Trail and other sites, the design issues faced by full scale wetland systems are presented and recommendations made to ensure a successful system.

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List of Abbreviations and Nomenclature

APB	Acid producing bacteria
DGGE	Denaturing gradient gel electrophoresis
DO	Dissolved oxygen
DOC	Dissolve organic carbon
EPS	Extracellular polymeric substances
GHG	Greenhouse gasses
HI	Hydrogen Index (as mg HC/g TOC as determined by Rock-Eval 6)
HRT	Hydraulic retention time
IRB	Iron reducing bacteria
MPN	Most probable number
OI	Oxygen Index (as mg HC/g TOC as determined by Rock-Eval 6)
%PC	Percent pyrolysable organic carbon (wt% as determined by Rock-Eval 6)
PCR	Polymerase chain reaction
PLFA	Phospholipid fatty acids
PRB	Permeable reactive barrier
%RC	Percent residual organic carbon (wt% as determined by Rock-Eval 6)
Rock-Eval 6	Rock-Eval pyrolysis method
SEM/EDS	Scanning electron microscopy/energy dispersed spectroscopy
SSU rRNA	Small subunit ribosomal RNA
SRB	Sulphate-reducing bacteria
SR	Sulphate reduction
SRR	Sulphate reduction rate
TML	Teck Metals Ltd.
TOC	Total organic carbon (wt% as determined by Rock-Eval 6)
TSS	Total suspended solids

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Finally, thanks to Al Mattes and Jim Hall of Nature Works Remediation Corporation for their ongoing dedication to this project. Al has been there from the start when idea became the reality and we continually scientifically challenge each other as we try to understand the system. Jim has been the construction manager that brought our ideas to the physical reality and got the wetlands built. Doug Gould (NRCAN – Ottawa) provided advice on microbial processes and MPN analyses.

Dedication

I dedicate this dissertation to Dr. Fariborz Goodarzi for launching me on this journey and for his unwavering support over the years. He continues to challenge me and make me a better scientist. I thank him for that.

As well, I dedicate this dissertation to my family, my wife – Karen and my daughters – Mauriah, Kimberly, Gabrielle and Kendra. Their support and understanding of this journey has made it possible.

Introduction

At the time of initial wetland conception and construction at Trail, BC (1996-97), there were few other systems operating anywhere in North America that attempted to treat metal-contaminated water with the high concentrations of metals (particularly Zn and As) as those found from the seepage emanating from the historic landfill sites on either side of the Stoney Creek basin, Trail, British Columbia. In the US there were some investigations of such treatment systems for metal removal, specifically for acid rock drainage situations where water was contaminated by various metals – often with very high concentrations of Fe – and very low pH readings. In many areas of the US, particularly in the coal belt areas, wetlands treatment systems were constructed in the 1980s to both treat the low pH and remove and sequester metals (Ziemkiewicz et al 2003). In 1993, Wildeman et al prepared a manual entitled "Wetland Design for Mining Operations" that brought together much of the US experience with acid mine drainage based on a pilot treatment system (0.61 m deep by 3.05 m wide by 18.3 m long) and experience from eastern coal mines. Dunbain and Bownmer (1992) suggested that based on metal chemistry in natural wetlands, constructed wetlands could be designed to enhance the various removal mechanisms likely in a cost-effective manner but concluded that more information was required "on the long-term performance of larger-scale systems applied to a range of industrial effluent qualities". As with all scientific endeavours, numerous other researchers were also developing and piloting large scale wetland pilot field projects to treat other types of mine and industrial drainage in the mid-1990s.

These include several large scale pilot projects such as the Wheal Jane System in Cornwall, UK treating tin mine drainage with low pH and high iron (Johnson and Hallberg 2005) and the Fankou Wetland System treating lead-zinc mine drainage with near neutral pH and high TSS, lead and zinc (Yu et al 2005; Yang et al 2006). The Wheal Jane System has many parallels to the Trail system and will be reviewed more thoroughly in a subsequent section.

Over a period of years from 1997 to 2002, a series of engineered anaerobic bioreactors and wetland cells have been designed and built near the Teck Metals Ltd. (TML) Smelter at Trail, BC. This system has successfully treated metals seepage from a smelter landfill via sulphate reduction with metal sulphide formation, filtration (e.g., Kottigite, metal sulphides), coprecipitation and adsorption. In a large scale system consisting of two vertical upflow anaerobic solid substrate reactors designed to promote sulphate reduction were followed by three wetland cells, high metals removal rates were obtained with a corresponding but smaller decrease in sulphate concentrations.

Seasonal trends in influent concentrations and treatment efficiencies have been experienced from 1998 to present. Highest treatment efficiencies were generally in the summer and during long, stable periods of operation. During these periods, treatment efficiencies often reached 99.9% reduction for all metals. During periods of start-up or excessive loads (i.e., over the design capacity or large increases in influent), re-release or breakthrough of metals was observed especially for arsenic and zinc. Addition of acetate or liquid invert sugar as additional electron donors during these periods generally restored functioning of the system and increased metal removal reductions. All these events and system modifications provide insight into the operation of large scale systems and are examined in this dissertation.

During the removal and subsequent reconstruction of the second anaerobic cell, core samples of the solid substrate were taken to determine the utilizable carbon source remaining and to determine the possible longevity of the anaerobic cells. Analysis indicated the presence of metals as sulphides and that lower TOC values were correlated with higher metal concentrations suggesting carbon mineralization within the substrate. Rock Eval 6 analysis was used to determine the potential life span of these cells confirming the usefulness of Rock Eval 6 as a cost-effective method of determining readily available and residual carbon in an engineered wetlands system.

The overall sustainability of wetlands systems are examined using a variety of economic, environmental and social measures and contrasted to typical chemically-based neutralization systems for treating metals in waste streams. Finally, the lessons learned in the operation of a large scale pilot wetlands system are presented to aid others in designing and constructing such systems.

The system in Trail is unique in many ways – using waste by-products (composted biosolids from aerobic treatment ponds) of the pulp and paper industry as a carbon source for bacterially-mediated metal removal in a large scale passive treatment system; treating high concentrations of metals (Zn, Cd, and As) year-round (where outside ambient winter temperatures can reach minus 20° C) thus providing insight to the seasonal fluctuations experienced by biological systems; detailed substrate sampling of a de-constructed anaerobic cell that had been operating for 5 years using statistical analysis of metals versus TOC (measured using of Rock Eval 6) as a technique to assess possible life span and carbon dynamics; and the long 5 year period of year-round operation of the system.

The seepage collection and wetlands system were conceived by the author and funded largely by Teck Metals Ltd. The design of the bioreactors and wetlands was a joint effort of the author and Al Mattes of Nature Works Remediation Corporation (Nature Works). Some initial anaerobic column work on the use of Zellstoff Celgar pulp mill biosolids was completed by Dr. Mark Edwards (Teck Research, Trail, B.C.) which also assisted in the design of the first anaerobic bioreactor. Nature Works also secured additional government funding for the project in the initial years. Given the size and complexity of the system, many people were involved in the sampling and analytical work. Under the author's direction and supervision the system was built and ran by Nature Works. Sampling was carried out by the author, Nature Works personnel or Teck Environment technicians. Analytical work was carried out by Teck Analytical Services (metals, anions), Natural Resources Canada – Ottawa (MPN) and Microbial Insights Inc.-Rockford, Tennessee (bacterial work) and data provided to the author for interpretation and statistical analysis. Rock Eval 6 and SEM/EDX sample preparation and analyses

were carried out by the author at the Geological Survey of Canada – Calgary research facility with the help of the GSC technicians and researchers.

Given the unique nature of this system and its extended period of operation, many other researchers have been involved in collaborative or parallel research associated with the wetlands system at Trail, B.C. including:

- Nature Works Remediation Corp – Al Mattes (consultant to Teck Metals), PhD candidate looking at microbial processing and sequestration of arsenic in the system (University of Guelph - Mattes et al 2002; Mattes et al 2004);
- NRCAN, CANMET, Ottawa – Dr. W.D. Gould, J.D.E. Kawaja, K. Morin, M. Smeu and L. Morin, performed column and laboratory mesocosm studies based on the Trail System, (Kawaja et al 2005; Kawaja et al 2006);
- University Missouri-Rolla - Drs. Joel Burken and Mark Fitch with graduate students Cem Selman looking at metal bioavailability in wetland plants (MSc Dissertation 2006) and Chang Ye looking at the efficiency of constructed wetlands in the removal of lead and zinc (PhD dissertation 2006);
- Royal Military College of Canada, Environmental Sciences Group – Drs. I. Koch and K. J. Reimer – Arsenic speciation in lab-scale anaerobic bioreactors used to treat arsenic-contaminated water based on Trail System (Koch et al 2003); and
- University of British Columbia – Dr. Sue Baldwin is developing genomic tools for monitoring microbial communities and metabolic processes in passive treatment system. Her student, Jana Schmidtova (PhD Dissertation 2010) worked on tools for assessing microbial processes and carbon utilization SRB in high sulphate situations (e.g. Trail site).

Chapter 1 - Wetland Treatment – Natural and Engineered

Wetlands can remove metals through various physical, chemical and biological processes. The processes are similar in natural and engineered wetlands. In engineering wetlands or bioreactors, ways of enhancing and promoting these processes are explored.

Some of the main metal removal processes in wetlands which were summarized in a review by Sobolewski (1999) include 1) adsorption onto organic matter; 2) filtration of solid, precipitates or colloids; 3) precipitation of carbonates; 4) coprecipitation with iron or manganese oxides; 5) metal hydrolysis by bacteria under acidic conditions; 6) reduction to non-mobile forms by bacteria; 7) precipitation of metal sulphides; and 8) the biological methylation and subsequent volatilization to the atmosphere. Additional processes related to the vegetation in surface wetlands include: 1) uptake by vegetation; 2) formation of root plaque; and 3) evapotranspiration (producing pure water vapour). While all these processes may occur for various metals or metalloids, the dominant processes will vary with the wetland's design. The promotion of various pH and redox conditions by system design, the presence or absence of vegetation in the system, physical substrate composition (organic, sand, slag or gravel that may or may not promote filtration or adsorption) and the input chemical concentrations (e.g., high metal concentrations may be toxic to vegetation), sulphate (required for sulphide formation), and nutrients will together determine the dominant metal removal processes occurring in that particular wetland. The relative importance of these various processes will vary under the anaerobic conditions experienced in the vertical upflow anaerobic bioreactors as compared to aerobic conditions present the horizontal subsurface flow vegetated cells or the final holding (an open water wetlands system) where interactions with plants and algae can occur.

Physical Processes

Adsorption of metals by organic matter can be attributed to the various functional groups present. Peat moss, for example, contains lignin and cellulose which have polar

functional groups such as alcohols, aldehydes, ketones, acids, phenolic hydroxides and ethers which are deemed to be the active species involved in the adsorption process (Bailey et al 1999). However, adsorption to peat is pH controlled with higher pH of 4 to 6 required depending on the metal ion. At a pH of 4.7, metals were adsorbed to humic acid from 100% to 10% of the amount of metal ion in solution in the following order: Hg = Fe = Pb = Cu = Al = Cr> Cd > Ni = Zn > Co > Mn (Kerndorf and Schnitzer 1980). When adsorption of a metal ion to humic materials occurs the subsequent desorption of another metal or hydrogen ion results (i.e., an easily reversible reaction) and there is an ultimate limit to metal adsorption to humic materials (Wildeman et al 1993). So while the complex organic matter fuelling the anaerobic bioreactors has a high absorption capacity for heavy metals it is at best a temporary pool to retain metals that need to be transformed to more stable forms in the bioreactor for permanent sequestration (Sobolewski 1999). As well, breakdown of the complex organic matter as "fuel" will release any adsorped metals.

Filtration of colloids and solids by wetlands is an important process to remove the fine metal precipitates formed by other processes in the wetlands or entering the system as TSS. In a surface flow system, the Fankou wetland treating Pb/Zn mine drainage (pH of ~8.2 and TSS of ~ 2500 mg/L which was composed mostly of tailings), a mass balance determined that settling and increased storage (wetland was expanded over time) was the largest removal mechanism (Yang et al 2006). Filtration or settling will be dependent on hydraulic resident time and the characteristics of substrate. A recent paper by Kuypers *et al* (2009) that compared the cost of reed-bed versus slow sand filtration for a potable water supply in Australia found that a reed bed could provide higher quality water at about half the cost. The use of a polishing wetland, reed-bed or holding pond after an anaerobic bioreactor can greatly enhance the overall system performance.

Chemical Processes

Wetlands are the early stages of organic rock formations and with diagenesis form sedimentary products such as bog deposits, coal, lignite and black shale. So by examining the mineral forms in these types of sediments, one can find the most thermodynamically stable phases. In sediments formed by precipitation the stable iron

minerals include hematite (Fe_2O_3), pyrite (FeS_2) or siderite ($FeCO_3$); for manganese they include pyrolusite (MnO_2) and rhondochrosite ($MnCO_3$); and for trace elements (Co, Ni, Cu, Zn, Ag, Cd, Au, Hg, and U) which can occur as sulphides, oxides and carbonates (Wildeman et al 1993). Most metals are retained in the organic fraction of organic-rich reducing sediments in the inorganic forms. This implies that processes in constructed wetlands should focus on the conditions promoting precipitation of these inorganic forms and that the organic material is primarily there to develop the conditions for this to occur (Wildeman et al 1993).

These microbial mediated precipitation reactions can be described as follows from Wildeman *et al* 1993 (where CH_2O represents organic material in the substrate):

1. Sulphate reduction with the precipitation of metal sulphides with alkalinity generation (anaerobic; generally *Desulfovibrio* genus)

   $SO_4^{2-} + 2\ CH_2O \rightarrow H_2S + 2\ HCO_3^-$

   $H_2S + M^{2+} \rightarrow MS\downarrow + 2H^+$

   where M is a cationic metal such as Cd, Zn, Fe, Cu or Ni.

2. Precipitation of ferric and manganese hydroxides (aerobic; most important species - *Thiobacillus ferrooxidans*)

   $4\ Fe^{2+} + O_2 + 10\ H_2O \rightarrow 4\ Fe(OH)_3\downarrow + 8\ H^+$

3. Adsorption of metals by the ferric (or Al or Mn) hydroxides (aerobic)

   $Fe^{3+} + 3\ OH^- \leftrightarrow Fe(OH)_3$ (amorphous)

   $Fe(OH)_3 \leftrightarrow FeOOH + H_2O$ (goethite)

   $2\ Fe(OH)_3 \leftrightarrow Fe_2O_3 + 3\ H_2O$ (hematite)

   The amorphous hydroxide precipitates first and ages to hematite in dry conditions or goethite in moist situations. The hydroxides create a negative charge as pH increases (>3 to 7 for Mn, >6.5 to 8.5 for Fe and >5 to 9 for Al) which attract positive metal ions. The polymeric properties of these hydroxides can help coagulate suspended material in the water removing

adsorbed metal contaminants to the sediments. This process can be assisted by algae (Whitehead et al 2005).

4. Neutralization and precipitation by NH_3 and HCO_3^- by bacterial decay of organic matter (anaerobic)

   $3\ CH_2O + 2\ N_2 + 3\ H_2O \rightarrow 4\ NH_3 + 3\ CO_2$

   This mechanism was speculated by Wildeman *et al* (1993) where the microbial breakdown of protein could produce ammonia which would hydrolyse to NH_4OH and increase the pH. Most wetlands work to date stresses the sulphate reduction process being a dominant process in providing alkalinity and metals precipitation as sulphides (Neculita et al 2007).

Biological Processes

Plant uptake of metals

The uptake by plants in stems and leaves is not considered the main removal process accounting for only 1 to 5% of metal accumulation in wetland systems (Wildeman et al 1993). However, metal removal by roots and rhizomes may be somewhat more significant, not in the root *per se*, but the microenvironments created at the plant roots (including the iron plaque formed by iron oxidation by plants transmitting oxygen to their roots).

Vymazal *et al* (2009) found that concentrations of trace elements (Al, Fe, Mn, Ba, Zn, Hg, U, Co and Cd) in *Phragmites australis* decreased from roots > rhizhomes > leaves > stems with root/leaf ratio averaging 70:1 for the various elements and up to 392:1 for Co. This result is favourable by reducing the possible environmental risk of metals transfer to animals feeding on aboveground vegetation. Peverly et al (1995) similarly found that metals (Fe, Cu, Pb and Cd) were not translocated to or accumulated by shoots or rhizomes in *Phragmites australis* but exhibited elevated levels in the roots. With the roots providing an effective filter to metals entering the plant. Only Zn accumulated in

the plants approaching that of the leachate entering the constructed reed beds. Using SEM with X-ray microanalysis of root cross-sections by the authors indicated Fe accumulation at the root surfaces, with Fe and other metals at lower concentrations inside the root tissue leading the authors to conclude that the rhizosphere provides a locally oxidized environment for metal precipitation and absorption outside of the actual root.

Yang et al (2006) also found greater accumulations of metals in the roots and litter in the belowground tissue of four different wetland plant species (*Typha latifolia, Phragmites australis, Cyperus malaccensis and Cyperus dactylon*) compared to aboveground tissues. They concluded that in their wetland system metal removal by plants was negligible. As well, Nyquist and Greger (2009) found in a small scale field study that in surface flow wetlands (with and without plants) treating mine tailings water, the metal uptake in plants accounted for only 0.002-2.9% of the total metal removal by the wetland system. Similar to Vymazal et al (2009), they found root:shoot ratios in *Phragmites australis, Carex rostarta* and *Epilobium angustifolium* to be always greater than 1:1 and as high as 81:1 for Cd, Cu and Fe with Zn ranging from 0.2:1 to 19:1. However, they did note that wetland plants increased the pH, decreased the redox potential and increased metal concentrations in the sediments. This suggests that plants promote metal sedimentation and adsorption.

Emergent plants also increase the evapotranspiration rates which decrease the volume requiring treatment during the active growing periods and any water release to the atmosphere directly can be considered 100% clean for most metals (excluding Se and Hg). In the Trail system summer flow rates have to be increased to ensure adequate flow rates are available for the grass and *Typha* cells. The volatilization of Se and Hg to the atmosphere by macrophytes can be used to remove these elements from contaminated waters. The volatilization of Se as dimethylselenide (which is 500-700 times less toxic than the oxyanions) has been proposed. Although it has been recognized that the Se will be re-deposited in other areas, this may not be an issue in areas deficient in Se such as California (LeDuc and Terry 2005).

Algal-assisted removal of metals

Algae by being photosynthesizing organisms can produce alkalinity through the alkalinity production through the assimilation of nitrate assuming no ammonium is present and the biomass is removed by being buried in the sediments (Kanti Das et al 2009) or by consuming a weak base (bicarbonate) and producing a strong base (hydroxyl ions) (Johnson and Hallberg 2005). This leads to metal hydroxide precipitation. Removal of Mn by algal ponds has been demonstrated to be somewhat effective at the Wheal Jane Project (Whitehead et al 2005).

Metal adsorption to extracellular polysaccharides by algae, subsequent death and settlement of the algal cells to the sediments can sequester metal from the water column into the sediments. The breakdown of dead algal cells can serve as a nutrient source for SRB. In fact, this process can be facilitated in pit lakes through nutrient addition with algal cells generating alkalinity at the surface, and the bacterial breakdown of dead algal cells can create anoxic conditions in the sediments suitable for SRB activity (Kanti Das et al 2009). As well, inactivated algal biomass can absorb metals into their cell walls and be used as biosorbents (Kanti Das et al 2009).

Possible role of fungi in wetland systems

The potential role of fungi is explored conceptually by Kanti Das et al (2009). There is evidence that suggests fungi absorb or assimilate heavy metals. They may also play a pivotal role in the degradation of complex organic carbon sources (like compost or wood chips) used as "fuel" sources in compost or anaerobic bioreactors. Degradation of complex organic carbon (e.g., cellulose) by fungi to simpler organic carbon (e.g., fatty acids) may increase the available organic carbon pool for SRB. Fungi can be directly involved in the reduction of ferric iron and sulphur and may contribute to biological alkalinity generation if appropriate solid products are formed (Kanti Das et al 2009).

Bacterial precipitation and immobilization of metals

As discussed under chemical precipitation, many of the chemical reactions are microbially-mediated under both anaerobic and aerobic conditions. A significant reaction with respect to the anaerobic bioreactors and anoxic zones of the plant cells is sulphate reduction to produce sulphides and the subsequent precipitation of metals. As

such a detailed review of sulphate reduction and sulphate reducing bacteria is warranted and follows in subsequent sections. Indeed, Faulwetter *et al* (2009) conclude that given the importance of the sulphur cycle and its potential influence on transformations within treatment wetlands that "more research emphasis on the microbial populations driving sulphur transformations seem warranted". They go on to state that the complexity of the microbiological processes has lead to the "black box" approach to design and to operate constructed wetlands. However, newer molecular and genetic microbial techniques will lead to a greater understanding which will allow us to fully optimize these systems.

Other precipitation reactions that may be significant in other situations include reductive precipitation and phosphate precipitation. Reductive precipitation is useful where the reduction of a metal to a lower redox state reduces the mobility and/or the toxicity of the metal. The bacterial reduction of Cr(VI) to Cr(III) reduces its toxicity, while the reduction of U(VI) to U(IV) by *Geobacter metallireducens* reduces the solubility of U assisting in its removal (Pal and Paul 2008). The reduction of Se(VI) to insoluble Se(0) has been employed in the bioremediation of contaminated waters. Although the reduction of the oxyanions of As and Se can occur by many different mechanisms, dissimilatory reduction appears to be the most environmentally significant process. The oxyanions of As and Se can be used as terminal electron acceptors by microbial anaerobic respiration providing energy for growth. Their reduction can be coupled to the oxidation of a variety of organic substrates (and these As and Se respiring bacteria belong to various genera and are ubiquitous in many different habitats (Stoltz and Oremland 1999).

Phosphate precipitation, where biologically-produced phosphates precipitate metals as phosphates, can treat a range of metals and radionuclides. The process is a mixture of accumulative and chemisorptive mechanisms (Pal and Paul 2008).

Other bacterial mechanisms for addressing metals include:

- Biosorption of metals by dead and sometimes modified bacterial biomass has been explored under a wide range of reactor formats and a variety of physical

and chemical conditions (Gadd 2000). The success of this technology is largely related to the microbial extracellular polymeric substances (EPS). These extracellular polymeric substances are a complex mixture of biopolymers produced by both prokaryotic and eukaryotic microrganisms. EPS are localized at or outside the bacterial cell surface. They are comprised of high molecular weight substances including polysaccharides, proteins, nucleic acids, phospholipids, humic substances and other nonpolymeric constituents of low molecular weight (Pal and Paul 2008). These biogenic polymers mediate the bacterial contact with and exchange with the surrounding biotic and abiotic environment. The EPS are important in cell to cell adhesion leading to the formation of biofilms, flocculus's, sludges and biogranules which help to protect the cells from hostile environments. EPS plays a role in the degradation of particulate substances and sorption of dissolved substances including many metals (Pal and Paul 2008).

Exposure to toxic substances (including metals) stimulates the production of EPS. The nature of the EPS changes with environmental conditions, for example, when *Rhodopseudomonas acidophila* was grown in the presence of Cu, Cd and Cr, it had a higher protein content suggesting the protein was the major metal binding component that increased along with the removal of metal ions (Sheng et al 2005). The biofilms EPS protects the cells from metal ions by binding them and retarding their diffusion within the biofilms. Precipitated metal sulphide within the biofilms of SRB can play a major role in reducing the mobility of metal ions in constructed wetland systems (Webb et al 1998; White and Gadd 1998 – Cd accumulation; White and Gadd 2000 – Cu accumulation).

- Metal-binding molecules, such as eukaryotic metallothioneins and other metal-binding peptides, have been found in cell membranes to play a role in metal detoxification within the cell (Pal and Paul 2008).

Sulphur Cycling and SRB Processes

While focusing primarily on microbial sulphate reduction as it relates metals treatment, the complete sulphur cycling in aquatic systems (primarily in the sediments) involves both reductive and oxidative processes (Jørgensen 1988). Sulphate is reduced to hydrogen sulphide which can be oxidized to organic sulphur under anoxic conditions or form pyrite. If it diffuses upward to the oxic zone it can be oxidized to sulphate. These processes have been somewhat neglected in freshwater sediments given the lower concentrations of sulphate compared to marine sediments (Holmer and Storkholm 2001). The measurement in freshwater has been constrained by the lack of sensitive methods of sulphate analysis prior to the introduction of ion exchange methods in the 1980s (Bak et al 1991). One critical factor controlling sulphate reduction rates (SRR) in freshwater is sulphate concentration, which is often the rate limiting factor for sulphate reducing bacteria (SRB) (Lamers et al 2002). Increased anthropogenic inputs of sulphur (via agricultural runoff, atmospheric deposition, groundwater, or effluent) may stimulate sulphate reduction and greatly alter carbon, nitrogen, phosphorous and iron cycling in lakes and other freshwater systems (Holmer and Storkholm 2001).

Lamers *et al* 2002 warns that increased sulphur loads threaten the biogeochemical functioning and biodiversity of freshwater wetlands citing that values in wetlands have increased from less than 200 μmol L^{-1} to 500 μmol L^{-1} and higher (over 3000 μmol L^{-1}). Impacts include possible phytotoxic effects (in wetlands or littoral areas) and reduced benthic fauna colonization due to accumulation of dissolved sulphide (HS^-), and possibly increased eutrophication by disturbance of the iron-phosphate bond as the formation and precipitation of insoluble iron sulphide compounds reducing the binding of phosphate to iron oxides and potentially releasing phosphate to the water column (Lamers et al 2002; Holmer and Storkholm 2001). Sulphate additions may suppress fermenting- and methanogenic bacteria as SRBs out-compete them due to their high affinity for common substrates in sediments (e.g., H_2, acetate) thus potentially altering carbon cycling.

For example, higher sulphate concentrations in a mire in Italy was related to air pollution via precipitation (Bragazza et al 2003). Such peatlands have been known to

lose species richness and develop into species-poor systems dominated by fast-growing and HS^--tolerant species (Lamers et al 2002). Fertilizer use and irrigation with sulphate-laden waters of fields has increased sulphate concentrations in agricultural runoff (Lamers et al 2002). Mining activities can result in acid mine drainage (AMD characterized by low pH, high in metals and sulphate) via the oxidation of pyrite and other sulphide minerals (Castro et al 1999).

The use of various bioremediation techniques control or modify SRRs either *in situ* or in separate processes have been proposed to reduce pollution impacts. Bacterial sulphate reduction generates alkalinity that can partially counteract the acidity of AMD (Herlihy et al 1988). Controlling *in situ* microbial sulphate reduction has been proposed to reduce impacts related to AMD pollution in pit lakes (Castro et al 1999). Engineered wetlands and anaerobic reactors have also been proposed to treat AMD (Drury 1999; O'Sullivan et al 1999; Greben et al 2002). Gypsum additions to oil sand tailings used to consolidate the tailings, inhibit methane production in a very large tailings settling pond, thereby reducing greenhouse gas emissions (Fedorak et al 2002). The success or application of these techniques will be explored in further detail in this review.

Sulphate Reducing Bacteria – Culturing and Identification

From when the first SRB were discovered in 1895 (Beijerinck 1895) to the present, our views of these microrganisms have changed radically. The focus is now on problems related to phylogenetic and evolutionary relationships, cell physiology and microbial ecology (Madigan et al 2003). A wide variety of methods are now available to identify and enumerate SRB. However, as with many anaerobes, SRB are difficult to culture and isolate for detailed physiological studies.

This inability to culture ecologically relevant bacteria is a pressing problem for current microbial ecology as discussed in a review paper by Overmann and van Germerden (2000). Since 50% or more bacterial cells in natural samples appear to be metabolically active, they should be culturable. However, the fraction of culturable cells is lower and often less than 1% (Overmann and van Germerden 2000). Inherent in the cultivation techniques is that the specific growth conditions only allow very few metabolic types of

bacteria to grow. As well, microbial interactions cannot be reproduced adequately as many bacteria in natural habitats are patchy; forming microcolonies, aggregates or biofilms on solid substrates (Overmann and van Germerden 2000). Given the small diffusion distance within a microbial consortium or patch, essentially all geochemical transformations will be mediated biologically. Many ecologically significant compounds exchange between bacterial cells including signalling compounds (e.g. quorum sensing), growth factors and compounds directly involved in energy metabolism (e.g. exchange of electron donors/acceptors). An example showing exchange of electron donors/acceptors is the close association of the filamentous, colourless sulphide-oxidizing bacteria, *Thioplaca* being covered by the filamentous SRB of the genus *Desulfonema,* indicating a rapid recycling of sulphur compounds occur between the two species. These two organisms have not yet been cultured separately. In addition to the possible organic carbon supply by *Thioplaca*, *Desulfonema* may be able to use reduced sulphur intermediates as opposed to sulphate (Overmann and van Germerden 2000).

Identification and enumeration of SRB has been done using anaerobic plate counts with specific electron donors (e.g. lactate, propionate, acetate) or using most probable numbers (MPN) techniques (Madigan et al 2003). While improvements of the enrichment media have resulted in better approximation of SRB *in situ,* these numbers may still be under estimates (Vester and Ingvorsen 1998; Brandt et al 2001). Using a new tracer MPN-technique, Brandt *et al* (2001) found SRB numbers to be 1-4 orders of magnitude higher than conventional MPN technique. Phospholipids fatty acids (PLFA) analysis using marker fatty acids for SRB and other anaerobic bacteria has been used to profile SRB communities along depth profiles in sediment samples (Llobet-Brossa et al 2002).

Over the last decade, molecular techniques have come to the forefront, including recovering of genes directly from environmental samples and determining the microbial phylogeny based on gene sequencing (Madigan et al 2003; Llobet-Brossa et al 2002). By using probes (based on sequence data, i.e. 16S rRNA), one can identify and classify microbial communities. Probes can be general (for all bacteria) to specialized probes for specific genera, e.g. *Desulfobacterium, Desulfobulbus, Desulfobactus* (Ramsing et al

1996). Probes can be used directly on nucleic acids extracted for natural samples or by *in situ* hybridization (FISH). These techniques have increased the evidence for many SRB that have to date not been culturable. While they have been primarily used for SRB identification, they are now being used to study ecological questions, such as, studying the depth distribution of different SRB in sediments and the *in situ* activity of different SRB in the environment (Llobet-Brossa et al 2002).

These various techniques have shown that there are often two distinct density peaks in the vertical distribution of SRB in sediments, one at the transition zone of oxic to anoxic conditions and one deeper in the reduced sediment layers (Marschall et al 1993). Molecular techniques indicate the peaks represent different populations along the vertical sediment profile as high genetic diversity among isolates from different sediment depths is noted (Llobet-Brossa et al 2002). This likely indicates the bacteria are using differing substrates or have differing environmental tolerances.

The gram-negative SRB are the most common type in freshwater sediments (Holmer and Storkholm 2001). While sulphate is the terminal electron acceptor, most SRB can utilize a wide variety of others (e.g. thiosulphate, sulphite, elemental sulphur and nitrate). Indeed if sulphite and thiosulphate are added to SRB cultures, cell yield may actually increase as the energy-consuming intercellular activation of sulphate by ATP sulphurylase is not necessary for these electron acceptors (Postgate 1984; Holmer and Storkholm 2001). In freshwater lake sediments the concentration of other electron acceptors is usually very low and sulphate is usually the most important (Bak et al 1991; Hadas and Pinkas 1995). High rates of sulphate reductions even with low sulphate concentrations indicate that freshwater SRB have acquired high-affinity uptake systems for sulphate (Ingvorsen and Jorgensen 1984). Alternatively, in cases where sulphate is limited, then anaerobic electron flow can be shifted to methanogenesis (Fedorak et al 2002).

The SRB are considered to be obligate anaerobes whose ability to reduce sulphate is inhibited by traces of oxygen. It has been proposed that oxygen inactivates or inhibits

enzymes and proteins that are utilized in the reduction process (Holmer and Storkholm 2001). However, SRB can survive under oxic conditions and have been shown to consume nitrate and O_2 by respiration (Marschall et al 1993). Numbers of SRB can be as high or higher in the oxic surface layers as compared to the anoxic zones. Further, the strains isolated from oxic sediments had high oxygen tolerance and capacity for oxygen respiration than strains from anoxic sediment layers (Holmer and Storkholm 2001).

The electron donors of SRB are primarily low-molecular-weight compounds that are fermentation products of bacterial degradation of carbohydrates, proteins and other organic detritus. The most important electron donors are H_2, acetate, lactate and propionate (Postgate 1984). Which donor is most important varies among different freshwater systems, and it is very difficult to identify and quantify specific electron donors because of low concentrations in pore water and the rapid turnover by bacteria (Holmer and Storkholm 2001).

Sulphate reduction rates (SRR) are measured using radiotracer techniques, or the measurement of the diffusive flux across the sediment-water interface or diagenetic modelling (Holmer and Storkholm 2001). The radiotracer techniques use radiolabelled $^{35}S\text{-}SO_4^{2-}$ injected directly in the sediment core and incubated over a known period. Incubation is terminated by rapid freezing in liquid nitrogen or addition of zinc acetate. The labelled sulphide is extracted as acid volatile sulphides (H_2S, HS^-, S^{2-} and FeS) and chromium reducible sulphur (FeS_2, S^0 and some organic sulphur). The acid volatile sulphides and chromium reducible sulphur rates are determined by a two-step distillation, whereas total SSR can be obtained by a single step distillation. Diffusive flux measurements obtain SRRs by using Fick's first law of diffusion, using tangents fitted to the sulphate concentration gradient at the sediment-water interface. However, to be accurate other sources and sinks within the sediments must be negligible (Holmer and Storkholm 2001). As well, the effects of bioturbation and resuspension events have to be considered when using diffusive fluxes or diagenetic models.

Learning from SRBs in Natural Aquatic Systems

Understanding of microbial sulphate reduction in natural aquatic systems is critical to designing and developing large scale engineered treatment systems. As well, natural environments will likely provide the SRB culture material for engineered systems. Natural aquatic systems run the gamut of oligotrophic to eutrophic, from high acidity to high alkalinity, from low dissolved to high dissolved solids, oxic to anoxic, littoral to profundal, high to low iron concentrations and so on. By examining systems along these various gradients we can hope to determine the controlling mechanism(s) in each case.

As sulphate concentrations in freshwaters are generally low, sulphate only penetrates to less than 10 cm in freshwater sediments and this is the zone of most active sulphate reduction. Sulphate is one of the most important processes for anaerobic decomposition of organic matter (Hadas and Pinkas 1995). An important difference between lakes depends on the amount of organic matter present and the availability of sulphate. In oligotrophic lakes, having low deposition of organic matter, the majority of the organic matter is aerobically oxidized at the sediment-water interface with little matter available for sulphate reduction in the lower anoxic layers. However, of the remaining carbon, sulphate reduction accounts for the major part (up to 81%) of the anaerobic carbon oxidization (Holmer and Storkholm 2001). Eutrophic lakes usually have high SRR in the surface layers, but sulphate is rapidly depleted and methanogenesis will become the most important carbon mineralization process under anoxic conditions. As well, seasonal changes in shallow eutrophic lakes (e.g. Lake Loosdrecht, Netherlands) will alter the balance between the roles of sulphate reduction (SR) and methanogenesis (Sinke et al 1992). The seasonal change in Lake Loosedrecht was controlled by changes in primary production and sedimentation rate as well as changes in temperature and sulphate availability (was sufficient to support SR). Methanogenesis was the most important process during high periods of mineralization in the summer and the autumn, whereas SR dominated in the winter and spring when mineralization was low. In Lake Kinneret (Israel), high SRRs are experienced year round with the peak during collapse of *Peridinium* blooms that sink and provide abundant organic substrates to the sediments (Hadas and Pinkas 1995). In spring and summer, SRR is stimulated by increasing

temperature and sedimentation of the spring bloom. In Lake Kinneret, sulphate does not become limiting thus allowing the SRB to out compete fermenting and methanogenic bacteria (Hadas and Pinkas 1995).

Bacterial SRRs were measured over a salinity gradient in Great Salt Lake (Brandt et al 2001). They found high SRRs in moderately saline areas but lower rates in the hypersaline area. Bacterial SR activity was greatest in sediments contain more organic material and showed an optimum activity at 5-6% NaCl in the moderately saline areas to around 12% NaCl in the hypersaline area. The authors believe that most SRB active in extremely hypersaline conditions are incomplete oxidizers producing acetate as the end product and the huge energetic cost of coping with salt stress makes SRB very susceptible to energetic constraints.

Sulphate reduction in acidic lakes is generally low, which is consistent with low microbial activity and low sulphate concentrations. The rate of degradation of fresh organic matter is reduced compared to non-acidic lakes due to the low microbial activity. Even with added sulphate, SRRs remain low but can be stimulated with addition of acetate, indicating rate was more impacted by lack of organic matter rather than sulphate (Blodau et al 1998). However, higher SRRs, in neutral mesotrophic lakes, were found in a naturally acidic lake (Lake Cavihue, Argentina) whose acidity was due to volcanism (Koschorreck et al 2003). These rates are high when compared to mine-impacted lakes where the lack of suitable organic substrate and competition with iron-reducing bacteria reduce SRRs in AMD situations. In Lake Cavihue sediments contain high concentrations of DOC and available acetate and low concentrations of reactive ferric iron. They confirmed that microbial SR is possible at a pH $\leq$ 3 in permanently acidic sediments in a natural system. SRB cultures from this lake maybe valuable resource for engineered systems.

The SRR responds strongly with temperature and generally scales with a Q_{10} value (the rate of change for biological activity for a 10 ^{0}C increase in temperature) of between 2.3 and 3.0 (Holmer and Storkholm 2001). An optimum temperature of 35-40^{o}C has been

found for SRB in culture, much higher than most *in situ* temperatures, but a similar pattern is found for mesophilic SRB in sediments (Isaksen and Jorgesen 1996).

Rooted macrophytes can suppress SRR when the density of plants is high, whereas higher SR rates can be found with lower densities. The differing effects of macrophytes on SR are likely due to their impact on sediment redox conditions. At high densities the plants can aerate the sediments in the rooted zone and oxidative respiratory pathways predominate, but at low densities they are not able to fully oxidize the sediment. Under these conditions release of labile microbial substrates from roots or plant detritus may stimulate SRR (Holmer and Storkholm 2001).

Tidal marshes, where high underground biomass production occurs and sediments have high organic matter content, can have high SRRs (Miley and Kiene 2004). Areal SRRs in tidal marshes are strongly correlated with temperature and vary seasonally from a low in January to a high in August. Net oxidation of sediment sulphides occurred during March through May, following a period of infrequent tidal flooding and during a period of high plant production when plants were able to aerate the sediments.

Low elevation salt marshes are characterized by high metal sulphide concentrations compared to higher elevation salt marshes due to the longer periods of tidal flooding in lower salt marshes thereby creating anoxic conditions in the surface layers (Otero and Macias 2002). While root exudates favour the activity of the SRB, they also facilitate partial oxidation of the sulphides generated by the SRB, which forms polysulphides that allow the formation of pyrite. Many different types of SRB are associated with root exudates, e.g., a non-described SRB which degrades sucrose may be specifically associated with root exudates of *Zostera nolti* (Cifuentes et al 2003). The oxic sediment conditions created by plant metabolism at the roots can cause two opposite effects: on one side, the oxidation of sulphides and release of metal sulphides, while on the other, the oxidation of soluble Fe(II) to insoluble Fe(III) oxides with concomitant trace metal adsorption and coprecipitation (Ugo et al 1999). As well, porewater biogeochemistry can

change spatially within a site and from site to site or temporally as a result of high tides or rain events.

Sulphate Reduction in Modified Natural Aquatic Systems

The response of SRR in natural aquatic systems subjected to AMD or other sulphate stressors can be used to elucidate biotechnology strategies to eliminate or ameliorate pollution impacts. Bacterial SR is an alkalinity-generating reaction that can partially counteract the acidification of lakes receiving acidic pollution (Herlihy et al 1988). However, the reverse is also true with the oxidation of reduced sulphur compounds consuming alkalinity. So in order for the alkalinity to be permanent, the sulphide formed by SR must be held in the reduced form or removed from the system (e.g. evolution of H_2S gas). As some sulphides are more mobile and some more readily oxidized by bacteria, the way in which reduced S is stored in the sediments plays a role in how permanent a solution the alkalinity generation from SR in aquatic systems receiving anthropogenic S inputs.

The major inorganic forms of reduced S are amorphous metal sulphides, crystalline disulfides (greigite – $Fe^{2+}Fe_2^{3+}S_4$, mackinawite – $(Fe, Ni)_9S_8$ and pyrite – FeS_2) and elemental sulphur. Sulphur is also found in organic forms as ester sulphates or various carbon-bonded species (e.g. amino acids – cysteine and methionine) (Herlihy et al 1988).

Herlihy et al (1988) showed that because of SR, the sediments of Lake Anna, Virginia, act as a major sink for incoming AMD pollutants (Fe, SO_4^{2-}, H^+). Acid volatile sulphide, elemental S and pyrite in the sediments were significantly greater in a polluted arm of the lake compared to unpolluted sections of the lake (containing primarily organic sulphur) and made up 60 to 100% of the total sediment S concentration in the polluted section. They suggest that AMD inputs can greatly skew the distribution of SR end products from the natural system. The high concentrations of ferrous Fe resulted in the rapid precipitation of FeS and very little dissolved sulphide in the porewater. They conclude that the burial of these reduced S products indicates that the alkalinity generated in the sediments represents a permanent neutralization of the acid pollution in these sediments with high TOC content of up to 15% (ranging from 3 to 15%).

Accumulation of porewater sulphides is usually found in eutrophic lakes with intense sulphate reduction. However, iron availability will strongly affect the dynamics of dissolved sulphides in porewater (Holmer and Storkholm 2001). The high SRRs in the eutrophic Lake Kinneret (Israel) were accompanied by disappearance of dissolved iron and significant accumulations of dissolved sulphides in the porewater, suggesting that the availability of iron-controlled sulphide dynamics (Hadas and Pinkas 1995).

In an experiment to elucidate the Fe(III) reduction in mining lake sediments (Mining Lake 111, near Lauchhammer, Germany), large enclosures (10 m diameter) were amended with an organic carbon and lime waste byproduct of the sugar industry (Wendt-Potthoff et al 2002). The authors showed that both SR and direct microbial Fe(III) reduction occurred simultaneously in the top 10 cm of the sediments and both processes contributed to alkalinity generation. However, the initial process was Fe(III) reduction with rates at least 3.5 fold higher than the SRRs. This indicates that suitable anions are required (such as sulphides or carbonate) to precipitate Fe(II) to prevent loss of alkalinity by upward Fe(II) diffusion and subsequent reoxidation. The effect of their additions was short-term (5 months) as the substrate was used up and autumn turnover accelerated Fe(II) oxidation and likely caused aerobic decomposition of the remaining organic matter. To be successful, they suggest that Fe(II) be immobilized as a solid (sulphide or carbonate precipitates). As well, the provision of excess organic carbon and limiting the mixing of sediments with the water column are needed to ensure long-term anoxia at the sediment surface.

In a smaller laboratory-scale study, where organic wastes were added to a mine-impacted pit lake waters (Summer Camp Pit, Humboldt County, Nevada) to create anoxic sediment conditions, it was found that in selected microcosms SRB increased with time and sulphide was generated by SR (Castro et al 1999). Correspondingly, sulfate, iron and arsenic concentrations (1200, 100 and 5 mg/L respectively) approached zero and pH approached neutrality (~6). The added organics having two effects: firstly, reducing oxygen and other highly oxidizing species (NO_x, Fe(III), etc.) to establish the redox

conditions for dissimilatory SR; and second, providing directly or indirectly, the low-molecular weight organic compounds required by SRB. Two readily available cheap organic wastes examined were waste from a potato-processing plant and composted cattle manure. The added amendments caused nitrate concentrations to drop from 90 mg N/L to zero over 31 days, while over the same period nitrite increased from 0 to 30 mg N/L then decreasing to 0 after 60 days. A pattern that is consistent with dissimilatory nitrate reduction by bacteria. However, ammonia concentrations varied over time in a manner dependent upon the amount and type of organics added. Dissolved Fe(II) was initially oxidized by nitrate to Fe(III) precipitating as oxyhydroxides at the prevailing pH. As nitrates and nitrites were depleted, Fe(III) was reduced to Fe(II). As sulphides appeared Fe(II) precipitated again and dissolved iron concentration dropped again. Arsenic concentrations fell with the onset of sulphide generation consistent with the precipitation of As_2S_3 but as sulphide concentrations increased further, re-dissolution of As_2S_3 to form thioarsenite complexes occurred. The potato waste amendment showed the best results and based on lake volume it was calculated that 1000 ton (approximately 50 dump truck loads) was required to treat the lake. While not mentioned in the article, the logistics of placing that amount of material evenly over the lake would be daunting. Additional material may have to be added if oxidizing waters continue to be discharged from the underground mine. The establishment of eutrophic conditions may produce enough organic matter to obviate the need for further waste additions.

Engineered Treatment Systems

Engineered wetlands and anaerobic reactors have also been proposed by many researchers to treat AMD (e.g. O'Sullivan et al 1999; Drury 1999; Greben et al 2002). The Wetland Ecology Research Group at University of Dublin has examined "volunteer" wetlands that have treated mine drainage for over a century (O'Sullivan et al 1999). By examining the metal concentrations porewater and sediments along a gradient from mine source to wetland's outlet, they determined that the marsh has been effectively retaining metals for at least a century and with its' remaining metal-binding capacity could continue to do so for another two centuries. They also examined sulphate retention in a constructed wetland in a series of 6 cells with a total surface area of 96 m^2 in a field situation at the Outukumpu Zinc-Tara Mines Ltd, Co. Meath, Australia. The biological

system was expected to be more cost-effective than the current chemical treatment processes employed. To date they have had some qualified success but the system was only run for one year at the time of publication.

Drury (1999) ran mesocosm-sized (66 L) anaerobic solid-substrate reactors containing a solid substrate of 2:1 (weight) cow manure to sawdust. To one series of mesocosms, he added an additional electron donor source in the form of cheese whey. He found this additional electron donor source helped maintain near neutral pH and increased removal efficiencies of sulphate and the dissolved forms of iron, manganese, zinc, copper, cadmium, and arsenic as compared to the un-amended system. As well, a 63-day period of excessive loading lead to permanently decreased treatment efficiency in systems without whey addition. He concluded that when designing systems without continual addition of carbon and energy source, one needs to account for the decrease in SRR over time as only the more refractory carbon substrates remain. Replenishment of the carbon and energy source, in reactors without continual electron donor additions, may eventually be necessary for reactors requiring high SSRs to treat their waste streams.

In smaller scale 2-L stirred microcosms, the effects of biomass concentration, temperature and concentration of the energy source (e.g. sugar, ethanol) on sulphate removal rates was examined (Greben et al 2002). They found that adequate biomass was required to effectively remove sulphate and when biomass was less than 1g/L sulphate removal was insufficient. As temperature was decreased from 20 to 5 ^{o}C the sulphate removal rate decreased by ~8 fold and the lowest critical operational reactor temperature was 15 ^{o}C. They found that while ethanol addition provided energy for the sulphate reduction reaction, cell yield was minimal and that the addition of sugar provided energy for cell maintenance and growth, thereby, increasing cell yields and overall sulphate removal rates of the system.

In a fortuitous manner, the experimental gypsum additions to oil sand tailings to help consolidate the tailings in the Mildred Lake Settling Basin (Athabasca Basin, Alberta, Canada) also inhibited methane production (Fedorak et al 2002). The addition of gypsum

consolidates tailings in hours as compared up to up to 150 years without. This is desirable when focusing on a "dry landscape" approach to reclamation which will allow revegetation. Methanogens have become very active in the basin and methane production is thought to be detrimental reclamation of the tailings. Production of methane is detrimental because (*i*) bubbles of released methane in the mature fine tailings (MFT) may prevent solidification of fine tailings; (*ii*) gas released from the MFT in water-capped fine tailings may resuspend fines and destabilize the fine tailings interface; (*iii*) bubbles rising through the MFT and water column may strip dissolved organic compounds causing fugitive emissions of low molecular weight hydrocarbons and organosulphur compounds; (*iv*) methane released to overlying waters may decrease dissolved oxygen levels leading to anoxic conditions; and (*v*) methane is a greenhouse gas and steps to reduce it are being sought. As SRB are known to out-compete methanogens if sulphate is sufficiently abundant in the habitat, the addition of gypsum ($CaSO_4 \cdot 2H_2O$) should favour SRB. In serum bottle microcosms showed that sulphate in the consolidated tailings stopped methane production. However, if the microcosms were amended with readily utilizable electron donors, the sulphate was consumed and when sulphate concentrations reached 20 mg/L, methane production began. It was concluded that both MFT and consolidated tailings have the potential to become methanogenic but that added sulphate would inhibit methanogenesis for a very long time. Further, methane evolution was not detected in Mildred Lake Settling Basin for the first decade of its operation. But methanogenesis did start after the porewater sulphate concentrations were depleted. So addition of sulphate in the form of gypsum to the basin may reverse this trend and help further consolidate *in situ* MFT.

The Wheal Jane Passive Bioremediation System

The Wheal Jane wetlands project came about in response to an "environmental disaster" that occurred January 13th 1992 when an uncontrolled release of up to 50,000 m^3 acidic, metal laden mine water (> 3,500 mg/L of dissolved metals) entered the Carnon River in southeast Cornwall, England (Younger et al 2005). Zinc and cadmium exceeded the Environmental Water Quality Standards for the Carnon River by 900 and 600 times, respectively. This prompted many scientific investigations into the development of a remediation plan. One possible option considered was a passive treatment system. This

was initially thought possible (even given the large water flows) as the metal concentrations dropped quickly the mine flush event when continual mine pumping was instituted at 300 L/s. A large scale pilot "wetlands" system was built in 1994 and sized to treat up to 2% of the pumped flow. The system was based on the prevailing knowledge at the time and relied heavily on the experience with coal mines in the eastern United States. Parts of the system were rebuilt in 2000 to address operational issues and many research papers have been written on the system including a 14 paper issue of the journal Science of the Total Environment (Volume 338; Whitehead and Neal 2005).

However, early in the program it was determined that passive remediation would not be able to address the high volumes that required treating from the Wheal Jane and surrounding mines. However, the setup did provide a unique research opportunity as a large-scale pilot could be built within the confines of the mine to treat "real" mine waters under field conditions (varying flows, temperature, metal concentrations and pH). With final treated effluent being sent to the tailings pond to be treated by the active treatment system and any problems with the experimental wetlands system would not create an environmental impact (Whitehead and Prior 2005).

For the active treatment system, a conventional aeration and lime-based precipitation High Density Sludge System (HDS) was commissioned in fall 2000 to treat up to 350 L/s of mine water (Younger et al 2005). Trail's active system (Effluent Treatment Plant (ETP)) is also a HDS without the aeration (as Trail smelter's iron bleed is via high iron slag) that treats large volumes effluent. While passive bioremediation systems have relatively low maintenance costs and the solid phase products are retained within the system, they can be expensive to install, require large land areas, their performance is less predictable than traditional chemical treatment systems (hence the need for research), and the long-term fate and stability of the solid phase products is still uncertain (Johnson and Hallberg 2005). However, the active treatment system is no panacea for environmental sustainability. To operate the system requires mining limestone and roasting it to produce lime, which is then ground and transported several hundred miles to the mine site with all these processes requiring large amounts of energy and producing CO_2 along

the way (Younger et al 2005). Then there is the energy required to run the HDS plant and disposal of the copious volumes of iron hydroxide sludge into the existing tailings pond. Once that space is filled land-filling on another part of the mine site will likely be needed. While the traditional approach wins out at present, further research and long-term experience with passive bioremediation systems may tip the balance in favour of these systems over a wider range of applications then are currently being considered.

Comparison of the Wheal Jane and Trail Passive Bioremediation Systems

Many of the lessons learned at Wheal Jane are similar to the Trail system as are the goals. The main areas of interest were to better understand the physical, chemical and biological processes of passive remediation and to explore the potential for passive technology for long-term treatment of Wheal Jane minewater (Whitehead and Prior 2005). Specific research issues and objectives included:

1. Examination of constraints of using anoxic limestone drains (ALD) in treating high concentrations of dissolved oxygen, ferric iron and aluminium;
2. Develop criteria for sizing reed beds to promote iron hydroxide precipitation;
3. To evaluate the role of reeds and algae in promoting iron hydroxide precipitation;
4. To assess seasonal performance of passive systems;
5. Examine the time required for passive treatments to reach "maturity" and shorten this time;
6. Test "robustness" of passive systems to varying minewater quality and flows;
7. Examine the chemical composition and environmental stability of the retained solid-phase products; and
8. Develop models to optimize system performance and aid in the design of new passive systems.

With respect to the stability of the solid-phase products, several important questions need to be answered to promote the use of passive bioremediation systems (Whitehead and Prior 2005). These include:

1. How suitable are the sediments for the long-term disposal of solid-phase metal products?

2. Are the metals retained in an environmentally inert form?
3. Will the sediments require active management for the foreseeable future? and
4. Is the recovery and recycling of precipitated metals practical and if so, under what conditions or constraints?

The Wheal Jane wetland system of 2000 consisted of three parallel treatment trains with only the pre-treatment stage varying among the parallel trains. The mine drainage enters the pre-treatment step for pH control (lime dosing, anoxic limestone drain or no treatment (control)); followed by 5 aerobic reed beds in series for precipitation of iron hydroxide and arsenic removal; then into a compost bioreactor for alkalinity production, sulphate reduction and precipitation of zinc, copper, cadmium and remaining iron as sulphides; then into an aerated rock filter to promote algal growth, reduce total organic carbon and precipitate manganese; and finally released to the tailings pond.

The compost or anaerobic bioreactor followed by the aerobic rock filter (a polishing phase of rock pools to promote algal growth) is somewhat similar to the final holding pond at Trail. The low pH and high dissolved iron and aluminium at Wheal Jane presented additional challenges which required additional treatment when compared to Trail. The anaerobic bioreactors were designed for a 30 year lifespan before substrate replenishment but in reality required fresh organic matter and SRB inoculum to enhance performance (Whitehead et al 2005). The bioreactors were sized based on similar "rule-of-thumb" of a volume 0.3 mol $[Me^{+2}]m^3$/day based on total divalent metal concentration and a surface area of 20 m^2/L/min at pH<5 based on flow rate as compared to the Trail system. The bioreactors were large (87.5 m long x 8.75 m wide and 1 m deep). The substrate (95% softwood sawdust, 5% hay and a small amount of cow manure as an inoculum) was placed in a HPDE lined trench and capped with 400 mm layer of gravel and soil. The system did experience numerous operational issues, e.g., plugging, low performance and design flaws. In hindsight, one critical design flaw was the "back-to-front" issue where acid-generating aerobic processes were upstream of the alkalinity-generating anaerobic processes (Younger et al 2005). The anaerobic bioreactor had low pH and high dissolved oxygen inputs which are detrimental to the function of such

systems and unnecessarily processed rainwater collected by the open cells. The use of appropriate inoculum suitable for the system (as opposed to cow manure) is critical to the success of such systems. In fact, it was the 10 month shutdown of one of the compost bioreactors that provided the most insight into the functioning of an SRB system as it allowed the system to "mature". These issues will be explored more in subsequent sections as similar problems are discussed with respect to the Trail system. While the performance of the 3 compost bioreactors was highly variable, if operated properly they can be highly efficient at generating alkalinity and removing metals from even extremely acidic minewaters (Younger et al 2005).

A key finding with respect to modelling how these systems work was that while adapted Pourbaix diagrams could be used to depict the most thermodynamically stable form of the elements as a function of pH and Eh and allowed determination of which chemical equilibrium equations could be included in the model, they did not adequately describe the system. It was found that most of the transition between metal speciation forms was microbial mediated and using equilibrium equations was not the best approach (Whitehead et al 2005). In field and laboratory experiments to determine reaction rate controls (Hall et al 2005; Hall and Puhlmann 2005), they found that most processes are first order microbiologically-controlled reactions with measurable reaction rates and this can be used to model bioremediation processes.

Summary and Discussion

The use of wetland technology has expanded greatly over the last 30 years, first being applied to sewage treatment and stormwater runoff; it has expanded to the treatment of a wide variety of industrial wastes including organic compounds and metals. This thesis focuses on an industrial application to treat very high concentrations of metals (in particular Zn and Cd) and metalloid (As) in large scale pilot system in a field situation. For simplicity, in this thesis "metals" will be used as a generic term that includes the metalloids (e.g., As). The high metal concentrations and the long-term operation of this system make it unique in the world. While the main focus of this thesis is on the processes occurring in the anaerobic bioreactors (sulphate reduction and carbon cycling), it presents the results of the whole process train.

As seen in many of the examples described, the S cycle is complex and linked to other biogeochemical cycles, such as carbon and iron. While our knowledge of biological sulphate reduction has increased greatly over the last century since SRB were first discovered in 1895, there is still much to learn. Multi-method approaches using advanced chemical and molecular methods (e.g. Llobet-Brossa et al 2002) will greatly improve our knowledge of microbial SR in natural aquatic systems.

Studies of natural aquatic systems can elucidate many of the mechanisms of microbial SR but are often hampered by many confounding variables. Spatial and temporal variations in sampling make it difficult to extrapolate SRR results from the areas sampled to large scales across a marsh or a lake. Examining impacted natural systems allows us to examine the field response of SR to the pollution inputs and may suggest ways in which these systems can be restored. Small microcosm systems may help us work out relationships of SR to temperature, substrate availability, competing processes (e.g. iron reduction, nitrate reduction) and others but microcosms do not adequately represent the complexity of natural systems. Mesocosm studies help us to bridge the gap from laboratory to the field but still lack some of the complexity found in larger systems. While pilot-scale or large scale field trials are necessary to put our knowledge in practice under the vagaries of varying influent concentrations, seasonal temperature changes, trying to make uniform solid-substrate on the large scale, bacterial consortiums versus single species tests, and to test stability and longevity of systems in the real world situation.

It is through these large scale tests coupled with detailed field sampling that we will gain the knowledge that will help us build cost-effective, reliable biological SR treatment systems or bioengineer SR processes in impacted natural or constructed aquatic systems. With sufficient knowledge, it may be possible to convert mine tailings ponds into long-term anaerobic bioreactors negating the need for use of conventional chemical processes, such as, lime precipitation with their inherent problems.

While much remains to be worked out with bacterial SR and the S cycle, tremendous progress has been made over the last 20 years with advances in chemical and molecular methods. This new knowledge will help us to better understand the S cycle and reverse some of the problems created by anthropogenic S emissions.

Chapter 2 - Wetlands System Evolution and Results to Spring 2002

Research Funding – The Practical Side

The wetland system was not originally conceived as a research project but an applied project in an industrial setting. Originally, the wetlands were presented to Teck Metals Ltd. (TML) by the author as a "black box" approach that should work based on the author's review of many bench-scale laboratory studies, mesocosm studies and small scale pilot systems (several square meters in area and several cubic meters in volume) in the literature that seemed to have "proven" the technology. TML agreed to build one and see if it works for the Trail Smelter Facility with its unique issues. Initial funding was very limited and some additional monies were obtained through the Industrial Research Assistance Program. Most funds received were directed to the construction and modifications of the system until it reached its final configuration by summer 2002 and was capable of year-round operation. Less funding emphasis was placed on the sampling and monitoring. As such this chapter is a history and a synopsis of the work during this period. Many practical lessons were learned and while the science conducted, i.e. detailed sampling and monitoring, was not as rigorous as generally required by academic standards is still useful and is presented here as a record of the history of the bioremediation operation.

This chapter (with related Appendix A) and the rest of the thesis bring together the all relevant information up to 2007 into one package for my employer (TML) and as such brings together information from various memos, annual reports, spreadsheets and file notes are incorporated here into a coherent package. With successful year round operation and only minor maintenance issues by 2002, funds provided by TML during the subsequent period were more fully directed to wetlands monitoring and also, with success came additional annual funding. The "black box" was now opened but funding was still limited especially in regards to this researcher's ability to fund analytical work beyond the normal Teck Analytical Services was limited even during the 2002 to 2007 period. Much of the key data (Chapter 4) in this thesis came about by the author's ability

to access the laboratory and other resources of Geological Survey of Canada-Calgary through the gratuitousness of Dr. Fari Goodarzi.

Summary of Design and Construction History

From 1997 to 2002 the system evolved from a summer-only plant-based wetland to the current year-round treatment system capable of treating full strength seepage for all contaminants present. The wetlands were originally conceived as plant-based horizontal flow wetlands with the idea of increasing the percentage of seepage flow to clean water until plant toxicity was noted. However, construction problems delayed the planting of the wetlands until late in the summer of 1997 and only clean water was fed into the system while the plants were being established. Subsequent greenhouse experiments using synthetic seepage indicated that plant toxicity would occur as low as 25% seepage strength. So the idea of an anaerobic bioreactor prior to the "wetlands" cell was conceived and built in 1998.

Initially, the system was only operated during the summer with very high metal reductions noted. The question quickly became how well will it operate in the winter and how long such a system would last. The system was winterized in 2000 but many issues arose (e.g., frozen supply lines and pump failures) and successful winterization was not completed until 2002. An additional anaerobic bioreactor was added in 2000 for improved treatment of zinc. In 2002 the original anaerobic bioreactor was taken apart, sampled and reconstructed with improved flow characteristics. A detailed system construction history is provided (Appendix A), outlining the many issues that were faced and overcome during the "wetlands" evolution. These issues will be examined and their solutions or "lessons learned" discussed for the benefit others embarking on such programs.

Although the collected seepage waters contain other metals, the primary focus was on zinc, arsenic and cadmium due to their elevated concentrations and to a lesser extent, on lead due to its relationship to the Pb-Zn smelter (Table 1). The horizontal subsurface flow plant cells were designed and built using typical constructed wetland techniques

(Kadlec and Knight 1996). The original pilot scale project was designed to treat 10 – 15,000 L of seepage water per day (approximately 13 –20% of total volume).

Table 1. Total volume of water and mean metal concentrations in Stoney Creek Seepage (Trail, B.C.) used for initial design considerations.

Total Flow (L/day)	Zinc (mg/L)	Arsenic (mg/L)	Cadmium (mg/L)	Lead (mg/L)
~77,000	434	6.0	4.6	0.056

Given that these concentrations were determined to be toxic to plants, a vertical upflow anaerobic compost bioreactor was constructed upstream of the wetland cells in 1998. The composition of the material used in the bioreactor was composed of Zelstoff Celgar pulp mill biosolids (60% by volume), sand (35% by volume) and cow manure (5% by volume). The biosolids were added as the carbon source while the manure acted as a bacterial inoculum while also providing additional available carbon. Sand was added to assist hydraulic conductivity and prove a surface for biofilm attachment. Total cell volume was approximately 1000 m^3 with a surface area of 450 m^2 (see Appendix A for detailed design calculations). A cell schematic (Figure 1 - circa 1998) outlines the basic design of the bioreactor (note standing water on top of bioreactor).

The total treatment system at the end of summer 1998 consisted of one anaerobic bioreactor, three plant cells and final holding pond (Figure 2; Figure 3 – photograph circa 1999). In August 1998, an eight week trial (55 days) of treating seepage commenced. The system was shutdown in early October prior to freeze up.

Before and after various repairs the system ran for 18 weeks (124 days) during the summer of 1999 prior to shutting down for the winter (see Appendix A for details). To test system robustness, flow rates were increased to 50% higher than designed rates during July and August to test the limits of the system's capacity and to determine if the system could be recovered upon failure. During July and August, input flow rates were as high as 20,000 L/day with total system flow rates up to 40,000 L/day due to rain

events. As well, average metal concentrations in the inputs had increased by 140% higher for As, 76% higher for Cd, and 109% higher for Zn as compared to 1998.

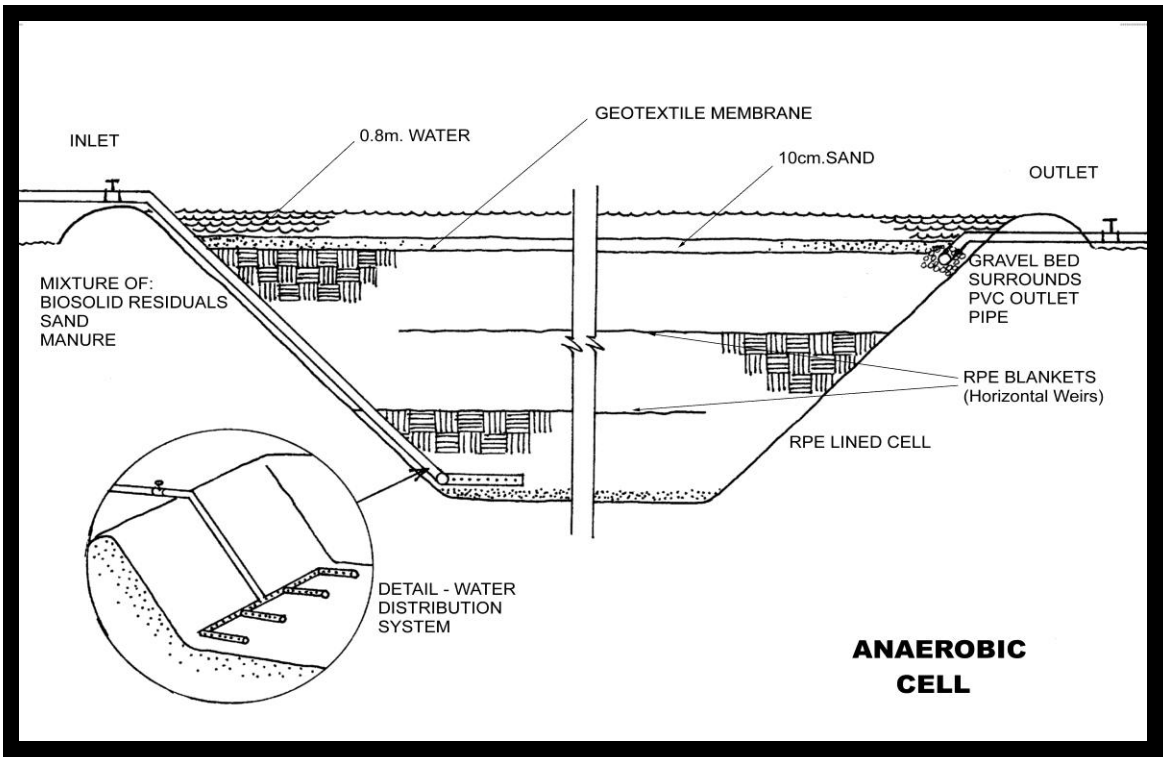


Figure 1. Schematic of anaerobic cell design as conceived showing inlet and outlet structures and horizontal weirs (Duncan et al 2004).

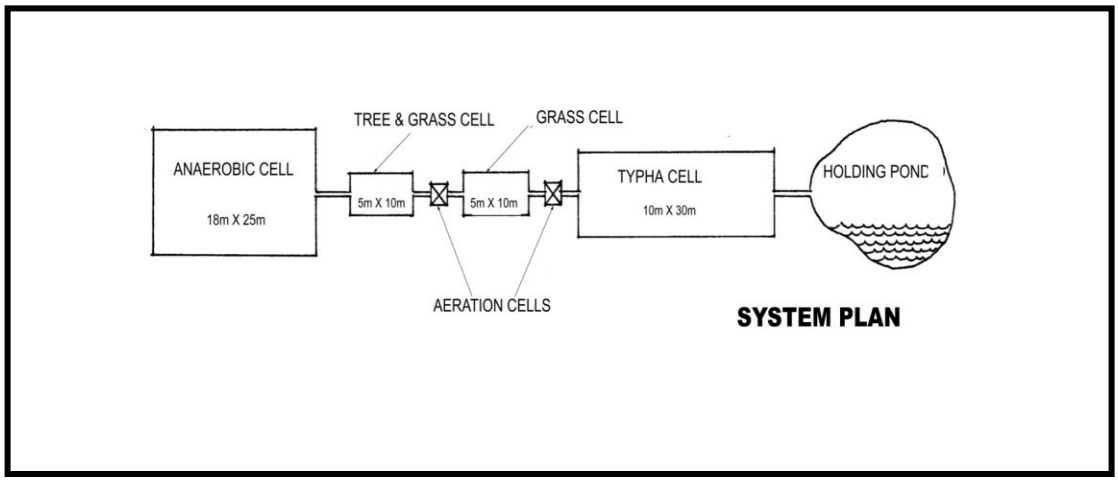


Figure 2. Schematic of the biological treatment system as installed in 1998 (water flows from left to right; from Mattes et al 2002).

Figure 3. Aerial photograph of wetlands in 1999 showing the 5 treatment cells (Note: anaerobic cell pink due to rhodamine dye test for HRT determination).

System failure appeared to be due to a lack of biological activity in the anaerobic cell (no bubbles and no H_2S odour observed at the surface of the bioreactor) which resulted in reduced metal reductions. The system was temporarily shutdown. During the shutdown, additional biological substrate was added to the bioreactor and various hydraulic issues addressed. When the system had stabilized, water from the final holding pond was pumped back into the anaerobic cell. Also, 150 kg of liquid invert sugar was added to the system as it filled to stimulate bacterial growth. The water from the holding pond was then allowed to remain in the anaerobic bioreactor for a few days and then the entire

system was returned to normal operating flow rates. Total downtime for repairs, re-charging and restarting was 10 days.

The addition of liquid invert sugar did induce increased bacterial growth and activity. Extensive bubbling at the surface of the anaerobic bioreactor accompanied by the smell of H_2S gas was noticeable after this treatment. The plants in the downstream cells previously showing signs of phytotoxicity recovered. As well, flow rates were reduced below design treatment rates and the system recovered.

Tests during the summers of 1998 and 1999 demonstrated that the system worked well during the summer. The system could handle the high and variable metal loads. As well, it could be recovered if stressed beyond design capacity. However, to be useful as an industrial treatment option, year-round treatment capability was required. Additionally, the effect of reduced temperatures and evapotranspiration rates on the overall treatment capabilities during the winter months needed to be determined. Therefore, the existing system was retrofitted in 2000 to achieve year-round treatment capability.

Also in 2000, an additional bioreactor with limestone was constructed to optimize Zn removal. Higher Zn removal rates were required as higher concentrations of Zn were found in the collected seepage (as high as 750 mg/L in 1999 compared to the 400 – 500 mg/L designed for in 1997). As well, the existing bioreactor had not been able to raise pH levels to desired optimal range. The addition of limestone in the new bioreactor would help increase the pH and also provide additional treatment capacity for winter operations (see Appendix A for details).

During start up in June 2000, the new bioreactor was filled with water available from a nearby stream. This water was amended with approximately 150 kg of liquid invert sugar (an aqueous solution of glucose and fructose which are monosaccharide sugars) to quickly stimulate the fermentative bacterial communities to produce the anaerobic conditions and low molecular carbon compounds necessary for SRBs. High rates of bacterial activity were obvious as frothing and bubbling occurred over most of the cell

surface. After one week, the water was released to the second anaerobic cell and seepage was added in a controlled manner to ensure adequate residency time. Initially, the outflow into the second bioreactor was limited to 15,000 L/day. The main pipeline froze during the winter (November) and as a result the system was shutdown after a total 164 days of operation in 2000.

A variety of vegetation issues from phytotoxicity to limited metal uptake (lack of hyperaccumulation in aboveground plant biomass noted in tissue sampling) resulted in many changes to the plant cells (see Appendix A for details). From 1997 to 2002, the system evolved from using hyperaccumulating plants for enhanced metal treatment to primarily phytoexcluders relying on evapotranspiration and rhizofiltration as treatment mechanisms. For example, in 2000 the *Typha* were growing unevenly throughout the *Typha* cell with poorer growth in the centre. Therefore it was decided to replant the cell. Given the large differences between total and dissolved metal exiting the *Typha* cell, it was apparent that this cell was not effectively filtering out fine metal sulphides. Therefore, a slow sand filter was constructed at its output in 2001. An aerial photograph documents the system as it looked in 2001 (Figure 4). As well, repairs to input pipeline to prevent freezing were made. After start up on May 1, 2001, the system ran for 342 days until to April 8, 2002 when it was shutdown for additional construction and repair.

During 2002, substantial changes were made when the initial 1998 bioreactor was deconstructed and rebuilt. The rebuilt bioreactor included addition of limestone and other design features (see Appendix A for details). During deconstruction extensive horizontal and vertical sampling of the biological substrate was done to determine both the bacterial populations and the metal and carbon concentrations throughout the cell from top to bottom (See Chapter 4 for details).

Figure 4. Labelled aerial photograph of wetlands system with sampling points indicated.

By summer of 2002 the system had evolved to its current configuration with no standing water on anaerobic bioreactors (Figure 5). The system is capable of year-round treatment and has operated with minimal issues or interruptions from 2002 to 2007 (data cut off for this thesis). It continues to operate to present and has become an acceptable treatment technology for treating the Stoney Creek seepages. The possibility of a larger system in the Stoney Creek valley to treat the total seepage volume is being considered by TML.

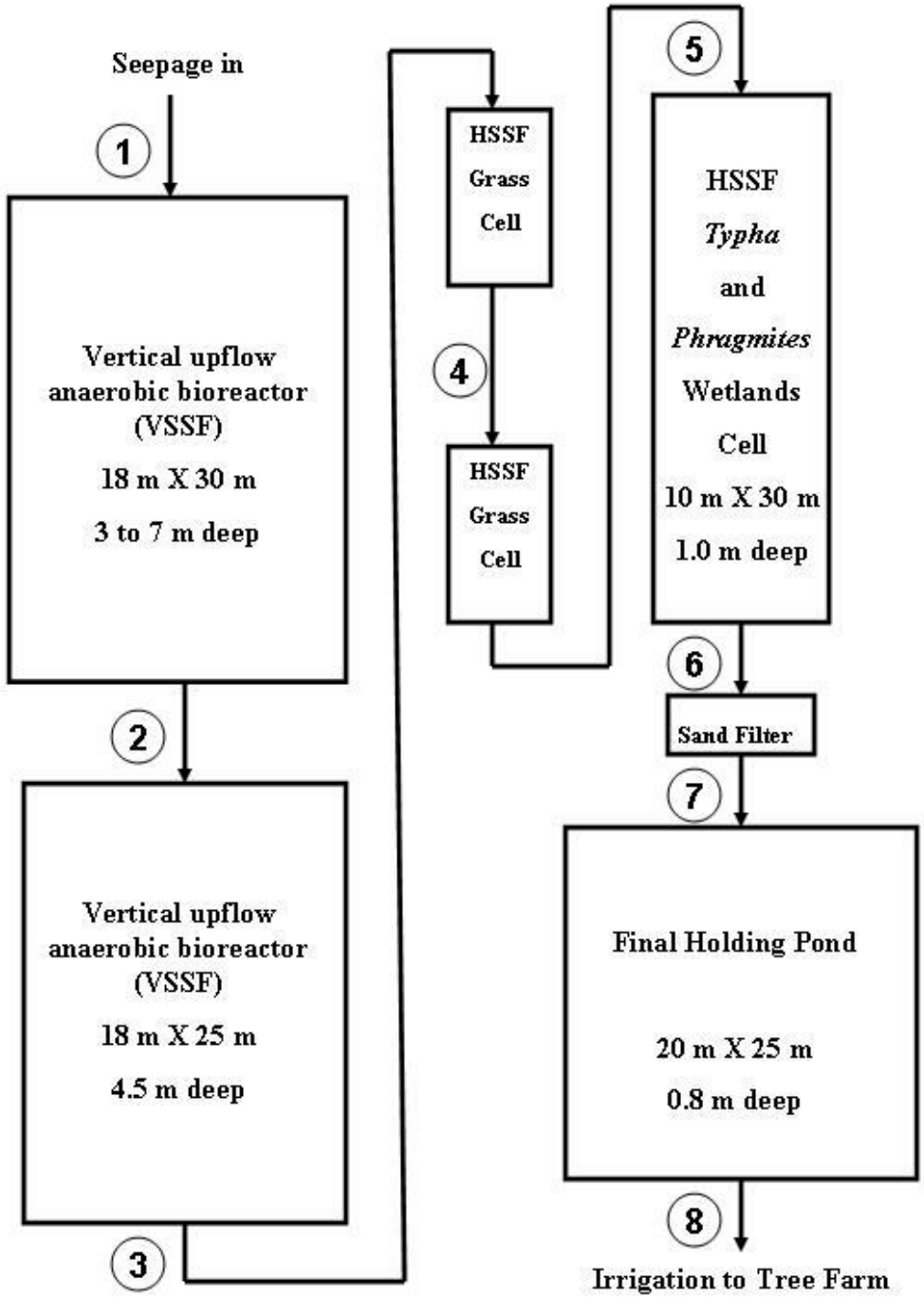


Figure 5. Schematic diagram of the wetlands system (cells are approximately to scale) as of summer 2002 with sampling points (SP) indicated in circles (from Duncan et al 2008).

A series of photographs from 2009 showing the system at various points (Figure 6; Figure 7; Figure 8; Figure 9; Figure 10; Figure 11) as compared to the schematic representation (Figure 5). Similar control structures as shown for the first anaerobic cell are at the inlet and outlet of the second anaerobic cell and allow for bypass of each cell if required (Figure 6). The first wetland or grass cell has an inset of an aeration control chamber which occurs before the second wetland cell and the *Typha* cell (Figure 7). The pipe height in these chambers sets the level of the cells and is buried in the winter which precludes winter sampling at these points. In the *Typha* cell, the *Phragmites australis* have over taken center with the *Typha* at the edges (Figure 8). The Typha cell flows through the buried slow sand filter (Figure 9) into the final holding cell (Figure 10). While originally just a lined pond the final holding cell has been colonized and developed into an open surface wetland. Water from the final holding pond is then used to irrigate the tree farm (Figure 11) or surrounding remediation trials at the site.

Figure 6. First anaerobic cell in 2009 showing control shed with sample points shown.

Figure 7. First wetland cell in summer 2009 showing aeration chamber and sampling point.

Figure 8. *Typha* cell in 2009 with *Phragmites australis* in the foreground.

Figure 9. Buried sand filtration unit in 2009 with sample points shown.

Figure 10. Final holding pond in 2009 with sample point and inset of irrigation pump.

Figure 11. Spruce trees growing in tree farm irrigated by treated water from the wetlands.

Methods - Sampling and Analysis

Water Quality

Seepage was first introduced into the system in early August of 1998. Water samples were taken several days after the initial loading to allow for a first flush (August 9) and then after a short stabilization period on August 24, September 22 and October 22. Grab samples were collected directly into Nalgene® bottles from the input and output of the anaerobic cell and the outlets of the three subsequent plant-based wetland cells. Samples were subsequently filtered, preserved with nitric acid and analyzed by Teck Analytical Services using ICP-AES for Zn, Cd and As.

In 1999, water samples were collected weekly from mid-June until late October from 5 sample points (See Figure 2 for system configuration; SP1, SP3, SP4, SP5 and SP 8 as shown in Figure 5). Grab samples were collected directly into Nalgene® bottles from the input and output of the anaerobic cell and the outlets of the three subsequent plant-based treatment cells. Water samples were split and one half filtered for dissolved metals and the remaining half unfiltered and then preserved with nitric acid for Zn, Cd and As

analysis by ICP-AES. Field measurements of pH, temperature and dissolved oxygen were taken *in situ* using portable field meters at the five sample points.

In 2000, water samples were collected weekly from early June until mid-October (then less frequently until system shutdown) from 7 sample points with the addition of the new anoxic limestone cell in 2000 and sampling of the final holding pond. Grab samples were collected directly into Nalgene® bottles from the input and output of the anaerobic cells and the outlets of the three subsequent plant-based treatment cells. Samples were immediately sent to Teck Analytical Services. Water samples were split and one half filtered for dissolved metals and the remaining half unfiltered and then preserved with nitric acid for Zn, Cd and As analysis by ICP-AES. Field measurements of pH, temperature and dissolved oxygen were taken *in situ* using portable field meters at the seven sample points.

In 2001, water samples were collected three times per week from May 7th until October 30th from 8 sample points (due to the addition of the sand filter in 2001; Figure 5). After October 30th, winter sampling was weekly at four points in the system – the input and output to the first anaerobic cell, the output of the second anaerobic cell and the final holding pond during the winter period. Grab water samples were collected directly into Nalgene® bottles. Water samples were split and one half filtered for dissolved metals and the remaining half unfiltered and then preserved with nitric acid for Zn, Cd and As analysis by Teck Analytical Services by inductively coupled mass spectrometry (ICP-MS). Field measurements of pH, temperature and dissolved oxygen were taken *in situ* using portable field meters at the eight sample points.

Statistical analysis for each year was performed using Statgraphics© Centurion XV (Version 15.2.00) software package. Statistical procedures are described in Results section for each particular year analysed.

Vegetation

In August 1999, plant tissues from various cells were collected: the leaves of hybrid poplar trees, willows and stems of the grass (*Tripsicum dactyloides)* in the first cell; and

stems of the grasses (*Agrostis stolonifera* and *Calamagrostis canadensis)* in the second cell; and leaves from *Typha* in the third cell. A second round of plant tissues (excluding willows) were collected in October 1999. Plant samples were bagged, labelled, and then oven dried for 24 hours at 80 ^{o}C and metals determined by ICP-AES after nitric acid digestion by Teck Analytical Services.

During 2000, selected plants in each cell were tagged for identification and sampled up to 6 times depending on availability of plant material (monthly from May to October). Aboveground tissue of plants was harvested, bagged, labelled, and then oven dried for 24 hours at 80 ^{o}C and metals determined by ICP-AES after nitric acid digestion by Teck Analytical Services.

On four occasions over the summer of 2001, 10 species of plants were harvested, placed in labelled bags then dried in a shed on the property. Plants were digested in nitric acid and analysed using ICP-MS by Teck Analytical Services.

Bacterial MPN

On July 4th, 2001, four samples were taken from the two anaerobic cells by driving 2.5 cm diameter PVC pipes into the cell at depths up to 1.5 m. The pipes were then removed, sectioned into 20 cm lengths and sealed with saran wrap. On the same date four liquid samples were taken from the lower cells (two plant cells, *Typha* cell and final holding pond). As well, four bulk biosolids samples were taken from the surface of the first anaerobic cell and three were taken from the surface of the second anaerobic cell. All samples stored at 4^{o}C until they could be processed. Samples were analysed for MPN (most probable number) by Dr. Doug Gould (NRCAN, Ottawa) as described below.

The sulphate reducing bacteria (SRB) were grown in a modified Postgate (1984) medium C in 20 mL serum bottles. The medium had the following composition in g/L: KH_2PO_4 - 0.5; NH_4Cl – 4.5; $CaCl_2$•$2H_2O$ – 0.04; $MgSO_4$•7H2O – 0.06; Na lactate (60%) – 2.92; Na acetate – 1.28; yeast extract – 1.0; $FeSO_4$•7H2O – 0.004; Na citrate•$2H_2O$ – 0.3; and resazurin – 0.005 (as a redox indicator). Preparation of the medium and dilution of the samples for enumeration is described in Benner et al (2000). The serum bottles

were incubated in the anaerobic chamber for 30 days. Positive growth of SRB was indicated by the precipitation of Fe-sulphides. The iron reducing bacteria (IRB) medium and dilution procedure is described in Mattes et al (2002). After 30 days the serum bottles were injected with a 0.1% ferrozine solution according to Sorensen (1982). A positive result was indicated by a purple complex formed between ferrozine and ferrous iron. The procedure for the enumeration of fermentative bacteria is described in Hulsof et al (2003). The numbers of SRB and IRB were calculated from an MPN table developed by Cochran (1950).

PLFA Analysis - Biomass

Surface substrate samples (from each anaerobic bioreactor as described above) were sent Microbial Insights Laboratories (in Rockford, Tennessee; see Appendix B for data table) to characterized the bacterial populations present by phospholipid fatty acid content (PLFA Analysis) and by profiling a conserved region of the 16S rRNA gene using denaturing gradient gel electrophoresis (DGGE). Additionally, most probable number estimates were determined for sulphate reducing bacteria. For the PLFA analysis the lipids were recovered using a modified Bligh and Dyer method (as described by White et al 1979). Recovered lipids were dissolved in chloroform and fractionated on disposable silicic acid columns into neutral-, glycol- and polar-lipid fractions. The polar lipid fraction was transesterified with mild alkali to recover PLFA as methyl esters in hexane. PLFA were analyzed by gas chromatography with peak confirmation performed by electron impact mass spectrometry (GC/MS).

DGGE Analysis - Bacterial Composition

Nucleic acid extraction was performed using a bead-beating method (Stephen et al 1999) by Microbial Insights Laboratories on sub-samples as described above. Sodium phosphate buffer, chaotropic reagent, glass beads, and the sample were agitated in a microcentrifuge tube using a high-speed bead beater. Chloroform was added, mixed thoroughly, and the tube was recentrifuged. The aqueous supernatant was collected and phenol/chloroform/isoamyl alcohol (24:24:1) extracted. Glycogen was added and the DNA was precipitated from the aqueous phase with an equal volume of isopropanol. DNA was pelletted by centrifugation, washed with 80% ethanol, air-dried, and re-

dissolved in Tris buffer, pH 8.0. The DNA was purified by a glass-milk DNA purification protocol using a Gene Clean™ kit as described by the manufacturer.

PCR amplification of 16S rRNA gene fragments was performed as described in Muyzer et al (1993) with modifications. Thermocycling consisted of 35 cycles of 92°C for 45 sec., 55°C for 30 sec., and 68°C for 45 sec. Using 1.25 units of Expand High Fidelity polymerase and 10 pmole each primer (forward primer contained a 40 bp GC-clamp) in a total volume of 25 μL, thermocycling was performed using a "Robocycler™" PCR block. The primers targeted eubacterial 16S rDNA regions corresponding to *E. coli* positions 341-534. A portion (20%) of each PCR product was analyzed by agarose gel electrophoresis (1.5% agarose, 1x TAE buffer) and ethidium bromide fluorescence. The amount of DNA used for DGGE was standardized to 150 ng by comparison to molecular weight standards using Alpha Imager™ software. DGGE employed a D-Code 16/16 cm gel system maintained at a constant temperature of 60°C in 6L of 0.5 x TAE buffer (20mM Tris acetate, 0.5mM EDTA, pH 8.0). Denaturing gradients were formed between 30 – 65 % denaturant (with 100% denaturant defined as 7 M urea, 40% v/v formamide).

Gels were electrophoresed at 35V for 16 hr. Gels were stained with ethidium bromide (0.5 mg/L) and destained twice in 0.5 x TAE for 15 min. each. Gel images were captured using an Alpha Imager™ system. The central 1mm portion of intensely fluorescing DGGE bands were excised using a razor blade and soaked in 50 μL of purified water overnight. A portion (15 μL) was used as the template in a PCR reaction as described above. The products were purified by electrophoresis through a 1.2% agarose/TAE gel followed by glass-milk extraction (Gene-Clean™ kit). Purified DNA was sequenced with an ABI-Prism automatic sequencer model 377 with dye terminators. Sequence identifications were performed using the BLASTn program of the National Center for Biotechnology Information (http://ncbi.nlm.nih.gov/Blast) and the "Sequence Match" of the Ribosomal Database Project (http://www.cme.msu.edu/RDP/analyses.html).

Mineralogical Sampling and Analysis

Samples of the compost substrate from the second anaerobic cell were collected at several levels by inserting a 5 cm plastic pipe in the bioreactor substrate as it was being deconstructed in 2002. The pipe was then capped to maintain the samples in an anaerobic state. The samples were analyzed at Geological Survey of Canada - Calgary, Alberta by scanning electron microscopy/energy dispersive spectroscopy (SEM/EDS) using a Philips XL 30 SEM instrumented with PGT Prism - EDS having a digital spectrometer with Si detector. The work was completed at 20 kV with a spot size of about 500 nm; the instrument uses a LaB6 emitter.

Results and Discussion

Water Quality - Summer 1998

Seepage was first introduced into the system in early August of 1998 and the system ran for approximately 55 days before the final sample was collected October 22. Mean dissolved Zn, Cd and As concentrations based on 4 sample dates are presented (Table 2).

The system was designed to treat 10-15,000 L a day during the summer months when plants are growing most rapidly and have high rates of evapotranspiration. Due to construction delays, the seepage was not introduced until relatively late in the growing season when some plants had ceased their active growing phase and plants were beginning to senesce. Therefore, treatment flow rates in August were initially kept at less than 50% of the potential treatment volume (approximately 5,000-6,000 L/day) to ensure the plants could handle the metal concentrations entering the plant cells. Even with low flow rates the metal sulphides formed had plugged the wrapped outlet pipes within two weeks. As a consequence the cells flooded and surface flow drained directly to the final holding pond via overflow drains.

The outlet systems were fixed allowing proper water flow through the cells. While the additional aeration in the plant cells removed the brackish colour and strong odour associated with the anaerobic conditions previously experienced. With these changes made, the designed treatment flows (12,000 to 15,000 L/day) could be attained. Overall treatment rates were high at 99.1%, 98.6% and 98.5% for Zn, Cd and As respectively

Table 2. Mean dissolved Zn, Cd and As (mg/L; *n*=4), ranges and percentage reduction for each stage in 1998 (55 days).

Sampling Location	Zinc (mg/L)	Zinc (% reduced)	Cadmium (mg/L)	Cadmium (% reduced)	Arsenic (mg/L)	Arsenic (% reduced)
Seepage Input *(mean)*	138		2.58		31.8	
Seepage Input *(min-max)*	*75-205*		*1.0-3.6*		*17-45*	
Anaerobic Output *(mean)*	79	**42.6**	0.31	**87.9**	8.45	**73.4**
Anaerobic Output *(min-max)*	*72-88*		*0.16-0.45*		*8.0-9.5*	
Tree Cell Output *(mean)*	40.8	**48.5**	0.28	**27.2**	5.18	**38.8**
Tree Cell Output *(min-max)*	*7.9-74*		*0.11-0.36*		*1.5-8.4*	
Grass Cell Output *(mean)*	7.7	**81.1**	0.13	**41.8**	1.59	**69.4**
Grass Cell Output *(min-max)*	*0.56-24*		*0.02-0.29*		*0.62-3.4*	
Typha Cell Output *(mean)*	1.2	**83.9**	0.035	**73.6**	0.47	**70.4**
Typha Cell Output *(min-max)*	*0.12-3.9*		*0.01-0.1*		*0.05-0.79*	
Total % Removed		**99.1**		**98.6**		**98.5**

(Duncan and Mattes 1999; Table 2). The anaerobic cell was responsible for removing a large percentage of the metals.

Water Quality - Summer 1999

Based on weekly sampling from mid-June until late October, the field pH of the system in 1999 was generally ranging from 5.45 to 7.07 (Figure 12). The lowest pH is found in the seepage inputting to the anaerobic cell (SP1) with a median pH (pH range in brackets) of 5.88 (5.45-6.87). The alkalinity generated by the anaerobic cell (SP3) increases the outlet median pH to 6.51 (6.05-6.99). After the tree cell (SP4) the median pH was 6.48 (5.96-7.07), after the grass cell (SP5) was 6.54 (6.19-6.46) and at the *Typha* cell outlet to

final holding pond (SP8) was 6.29 (5.50-6.79). Water temperature was similar throughout the system for any given date but ranged from 17 to 20 °C in June, rising in July and August to 22 to 25 °C and then declining through September (11 to 18 °C) to lows of 9 to 10 °C in October (Figure 12).

Daily evapotranspiration rates as a percentage were determined for the total system by dividing flow at system output (SP6) by the input flow (SP1) for the same day. Rates were highly variable depending on temperature, cloud cover and precipitation. Daily evapotranspiration rates ranged from less than 0% during precipitation events to 55% on a clear, hot summer day. Maximum daily evapotranspiration rates increased through June (up to 37%); to the highest rates (up to 55%) in July and August; and then decreasing through September (21%) and October (8%).

The field dissolved oxygen (DO) of the system in 1999 was quite variable through the system and within the individual cells and ranged from a low of 1.13 mg/L from the tree cell during June 7 to 12.4 mg/L in October from the anaerobic cell (Figure 12). The highest mean DO was found in the seepage inputting to the anaerobic cell at 6.87 mg/L. The mean DO was decreased by the anaerobic cell (5.68 mg/L) with further decreases in the tree cell (2.57 mg/L). This was followed by slight increases in mean DO from the grass cell (3.46 mg/L) and subsequently the *Typha* cell (4.19 mg/L).

A wide range in the seepage input concentrations of total and dissolved Zn, As and Cd to the system was noted (Table 3; Table 4). This likely due to precipitation effects on the seepage sources and subsequent discussion will focus primarily on mean metal concentrations treated. During the 1999 season, As and Cd percent reduction was > 99% through all four stages even with the higher mean total input concentrations experienced in 1999 (Table 3). Mean seepage input concentrations of total Zn, As, Cd in 1999 were 76.4, 4.56 and 289 mg/L as compared to 31.8, 2.58 and 138 mg/l in 1998 respectively.

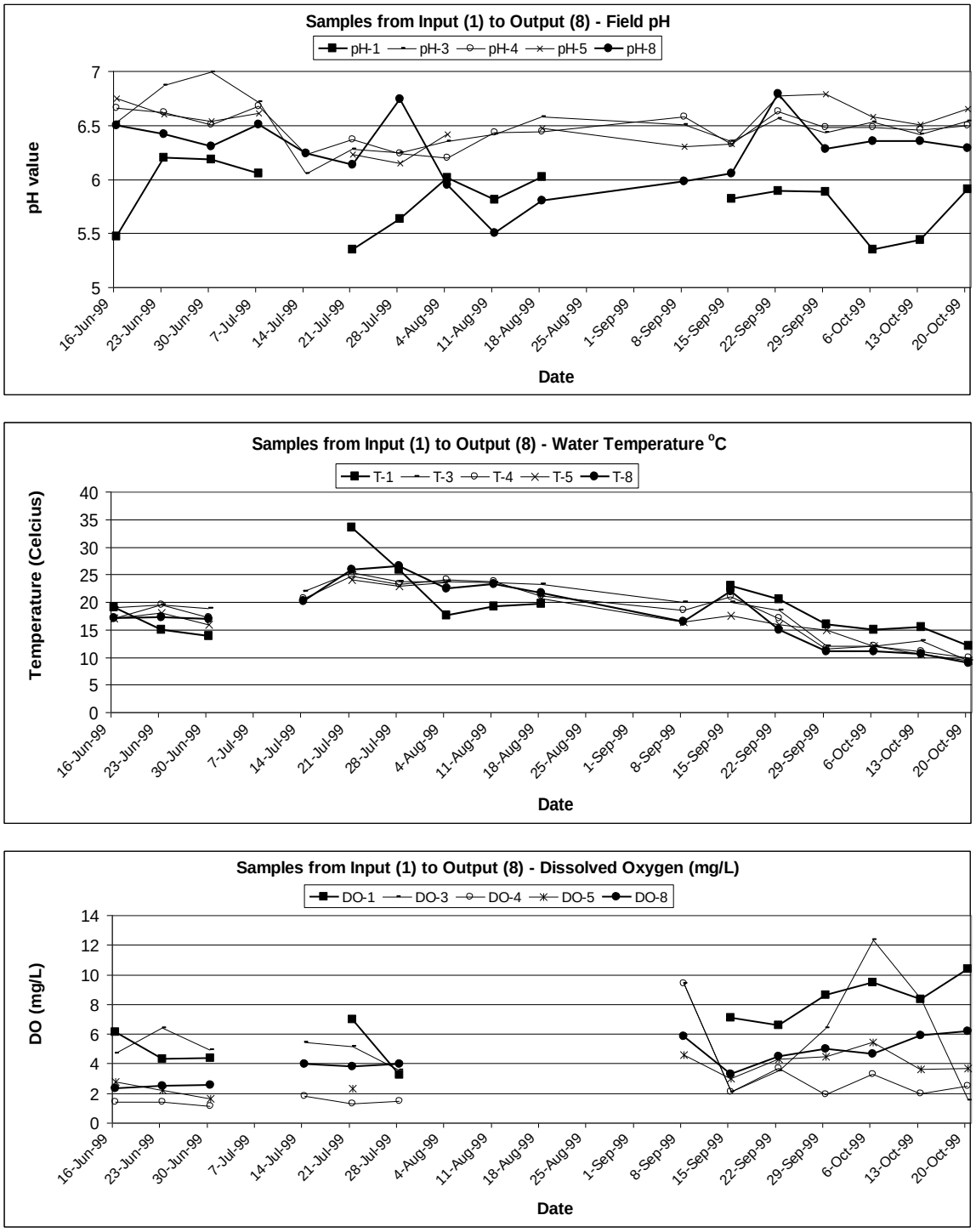


Figure 12. Field pH, water temperature (°C) and dissolved oxygen (mg/L) in 1999 at five sample points (See Figure 2 - system configuration; Figure 5 - sample points).

However, percent Zn reductions were lower at 75% in 1999 compared to > 97% reduction experienced in 1998. The reduced Zn reductions led to the temporary shutdown in August after high Zn concentrations were noted following the high flow rates (up to 32,000 L/day; Figure 13) experienced in July. The minimal decreases in DO in the anaerobic cell during the high flow rates may indicate that minimal carbon utilization with possibly flushing of low molecular weight carbon compounds produced was occurring (Figure 12). This is somewhat supported by the much lower mean DO

Table 3. Mean total Zn, Cd and As concentrations (mg/L; *n*=17), ranges and percentage reductions for each stage in 1999 (124 days).

Sampling Location	Zinc (mg/L)	Zinc (% reduced)	Cadmium (mg/L)	Cadmium (% reduced)	Arsenic (mg/L)	Arsenic (% reduced)
Seepage Input *(mean; SP1)*	289		4.56		76.4	
Seepage Input *(min-max)*	*146-748*		*2.1-12.0*		*27-288*	
Anaerobic Output *(mean; SP3)*	194	**33**	1.69	**63**	14.4	**81**
Anaerobic Output *(min-max)*	*122-273*		*0.4-3.5*		*5.6-16*	
Tree Cell Output *(mean; SP4)*	164	**15**	0.89	**47**	2.80	**81**
Tree Cell Output *(min-max)*	*11-262*		*0.06-1.8*		*1.7-6.8*	
Grass Cell Output *(mean; SP5)*	117	**29**	0.11	**88**	1.28	**54**
Grass Cell Output *(min-max)*	*11-188*		*0.023-0.20*		*0.56-3.7*	
Typha Cell Output *(mean; SP6)*	72.8	**38**	0.024	**82**	0.29	**77**
Typha Cell Output *(min-max)*	*0.60-155*		*0.002-0.092*		*0.07-0.96*	
Total % Removed By All Cells		**74.8**		**99.6**		**99.6**

found in tree cell immediately downstream. A possible decrease in bacterial activity could also explain the low alkalinity generation as measured by pH decrease from 6.99 to 6.05 (Figure 12).

Table 4. Mean dissolved Zn, Cd and As concentrations (mg/L; *n*=17), ranges and percentage reductions for each stage in 1999 (124 days).

Sampling Location	Zinc (mg/L)	Zinc (% reduced)	Cadmium (mg/L)	Cadmium (% reduced)	Arsenic (mg/L)	Arsenic (% reduced)
Seepage Input *(mean; SP1)*	231		3.40		23.3	
Seepage Input *(min-max)*	*140-515*		*1.6-9.3*		*12-37*	
Anaerobic Output *(mean; SP3)*	173	**25**	1.33	**61**	3.3	**86**
Anaerobic Output *(min-max)*	*90-236*		*0.29-2.3*		*0.2-25*	
Tree Cell Output *(mean; SP4)*	158	**9**	0.81	**39**	1.30	**61**
Tree Cell Output *(min-max)*	*7.6-228*		*0.01-1.5*		*0.4-5.0*	
Grass Cell Output *(mean; SP5)*	111	**30**	0.08	**90**	0.65	**50**
Grass Cell Output *(min-max)*	*10-185*		*0.001-0.16*		*0.23-2.8*	
Typha Cell Output *(mean; SP6)*	70.6	**36**	0.019	**75**	0.11	**83**
Typha Cell Output *(min-max)*	*0.36-155*		*0.004-0.080*		*0.05-0.92*	
Total % Removed By All Cells		**69.4**		**99.4**		**99.5**

Flow rates were reduced to approximately 15,000 L/day from July 23rd until August 24th when the system was temporarily shutdown (Figure 13), while pH out of the anaerobic cell gradually increased through the period of lower flow (Figure 12). The increased pH out of the anaerobic cell appears to be related to the increased hydraulic

retention time (HRT) as a result of the lower flows. Similar to the initial start-up, Zn and Cd concentrations are lower after a shutdown period of no flow where HRT was dramatically increased for a short period likely making a larger temporary pool of available low molecular weight carbon compounds.

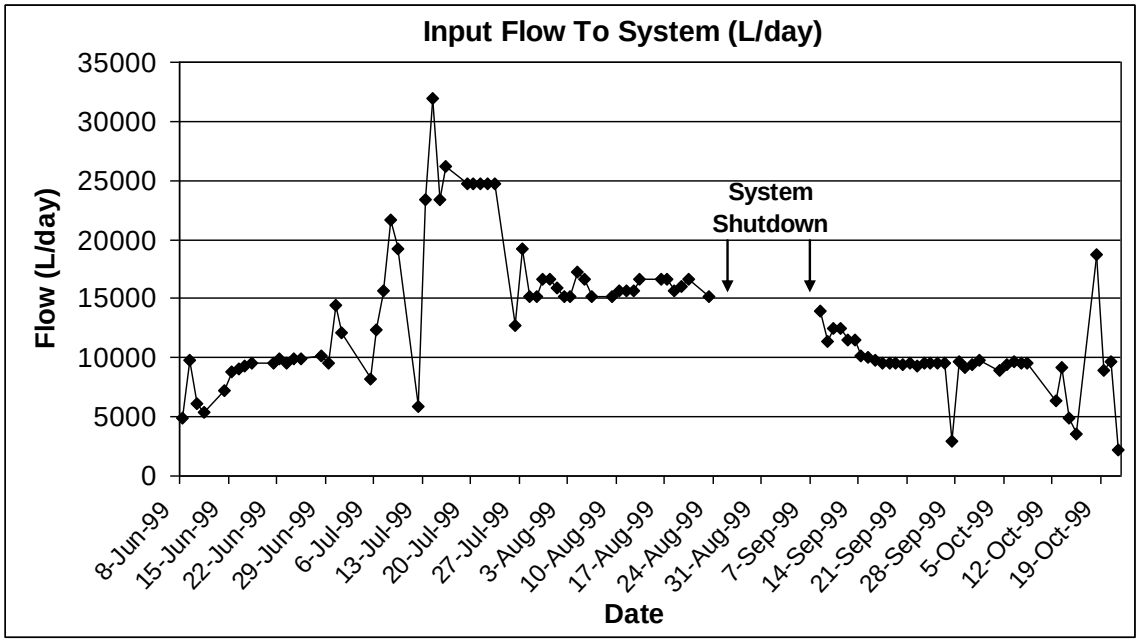


Figure 13. Daily inputted flow rates to system (SP1) from June 16 to October 20, 1999 with shutdown period noted.

During the shutdown approximately 150 kg of liquid invert sugar was added to the anaerobic cell to stimulate bacterial growth and ensure anaerobic conditions in the cell. Once re-started on September 8th at lower flow rates of approximately 10,000 L/day (Figure 13), the system returned to higher percent Zn reductions and handled a concentration spike in October of 748, 288 and 12 mg/L for total Zn, As and Cd respectively (Figure 14; Figure 15; and Figure 16) which were 224%, 277% and 163% higher than the mean seasonal concentrations for Zn, As and Cd respectively. Following the spike, the total percent Zn reduction in the anaerobic cell increased to 48% from no percent reduction in August (Figure 14). This is likely due to the reduced HRT from the lower flow rates and readily available carbon source due to the addition of the liquid invert sugar after the spike event.

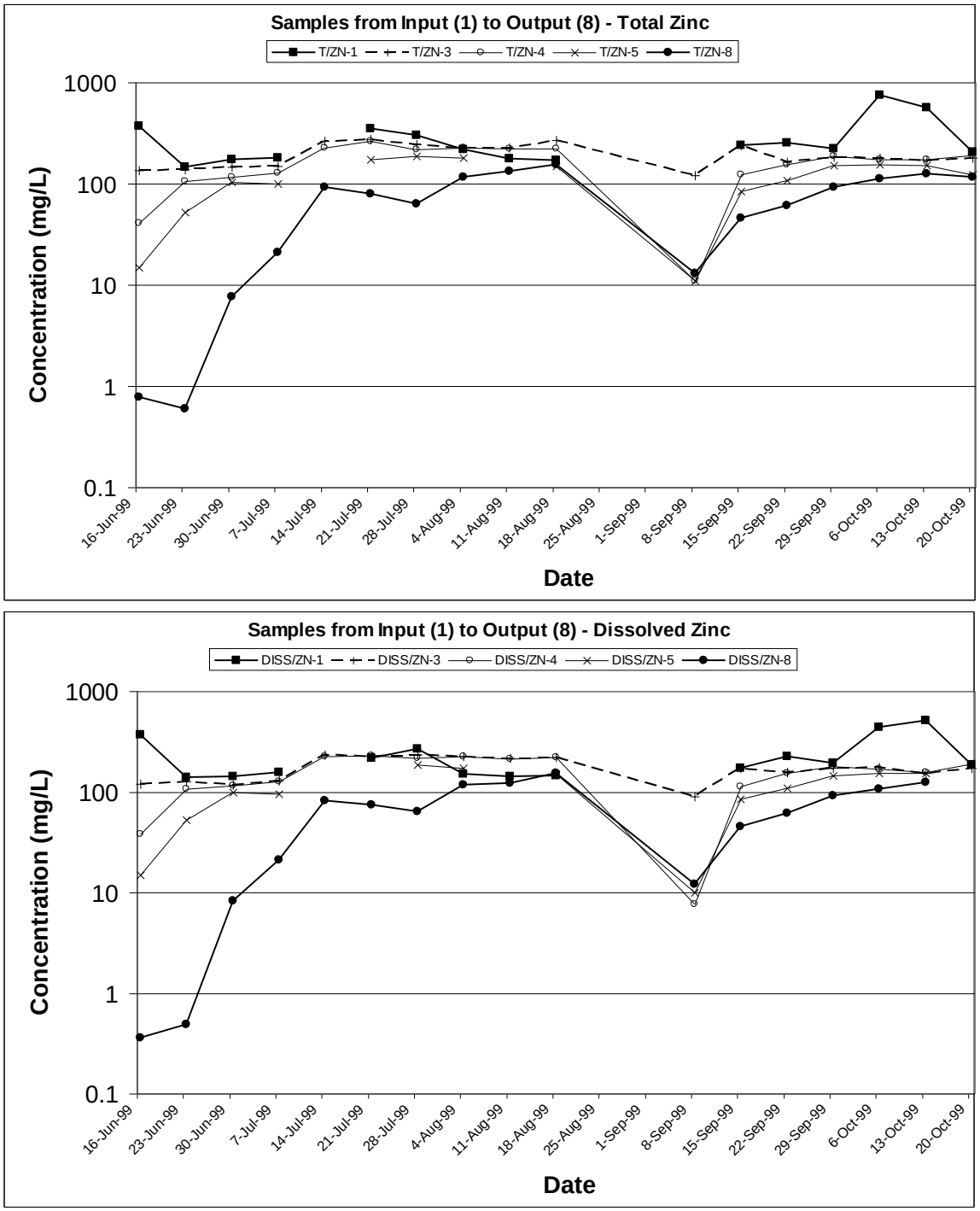
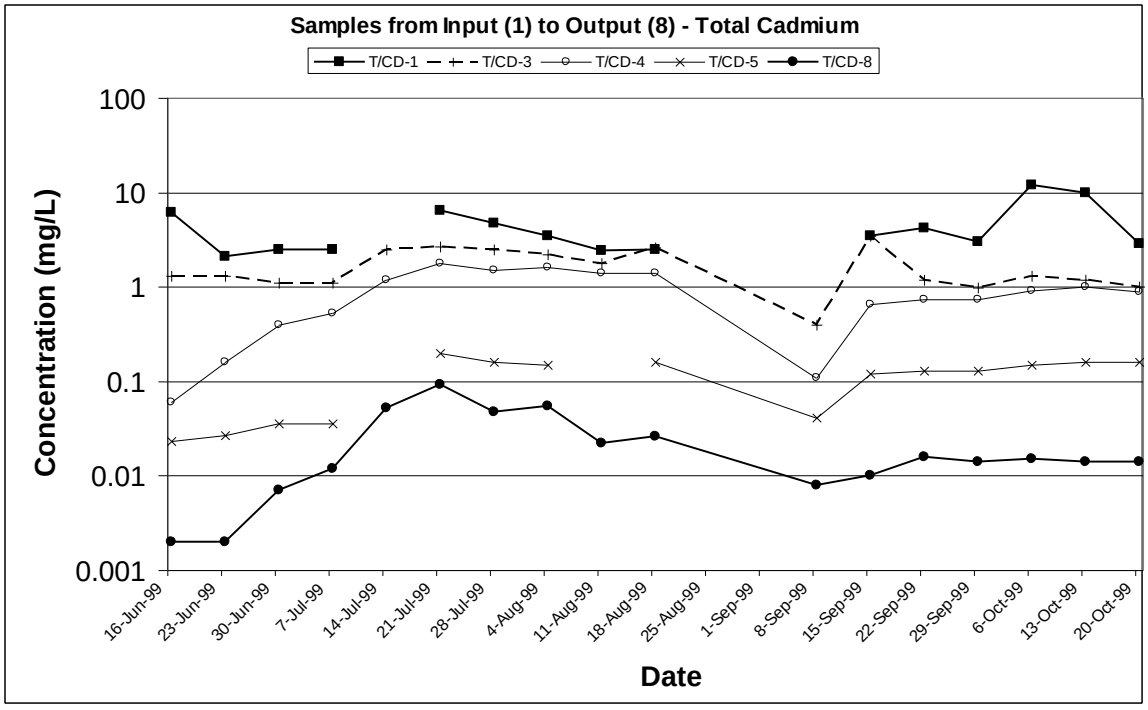


Figure 14. Total and dissolved Zn concentrations (mg/L) at sample points 1, 3, 4, 5 and 8 (refer to Figure 3) from June 16 to October 20, 1999.



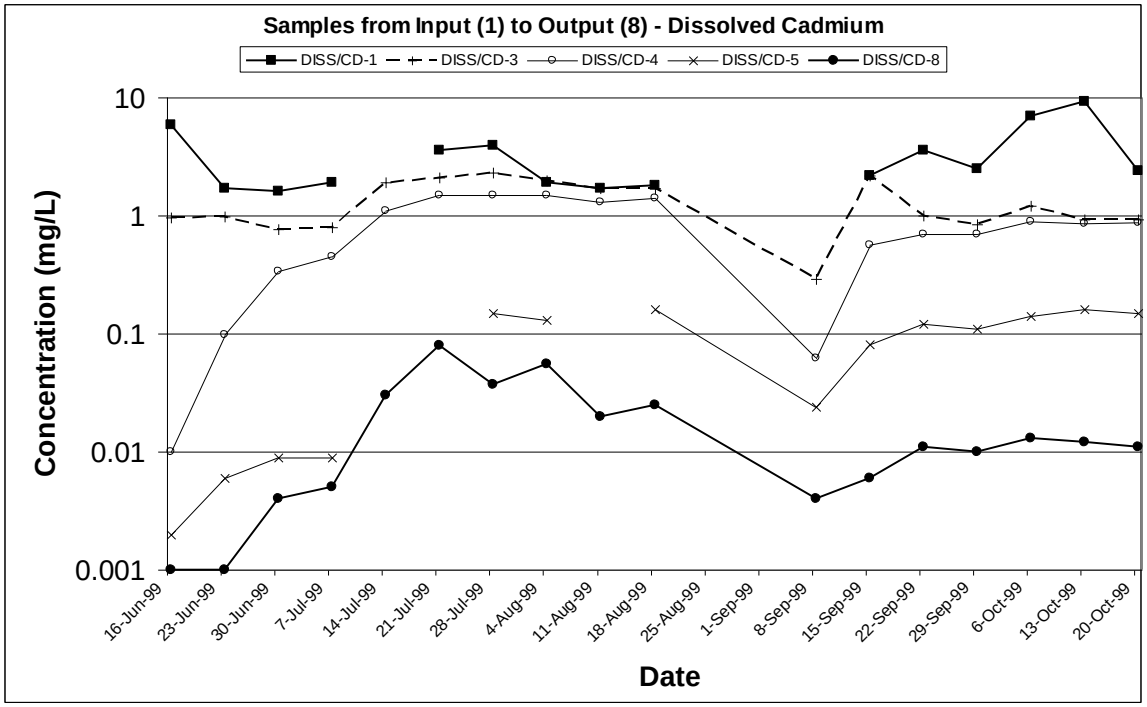


Figure 15. Total and dissolved Cd concentrations (mg/L) at sample points 1, 3, 4, 5 and 8 (refer to Figure 3) from June 16 to October 20, 1999.

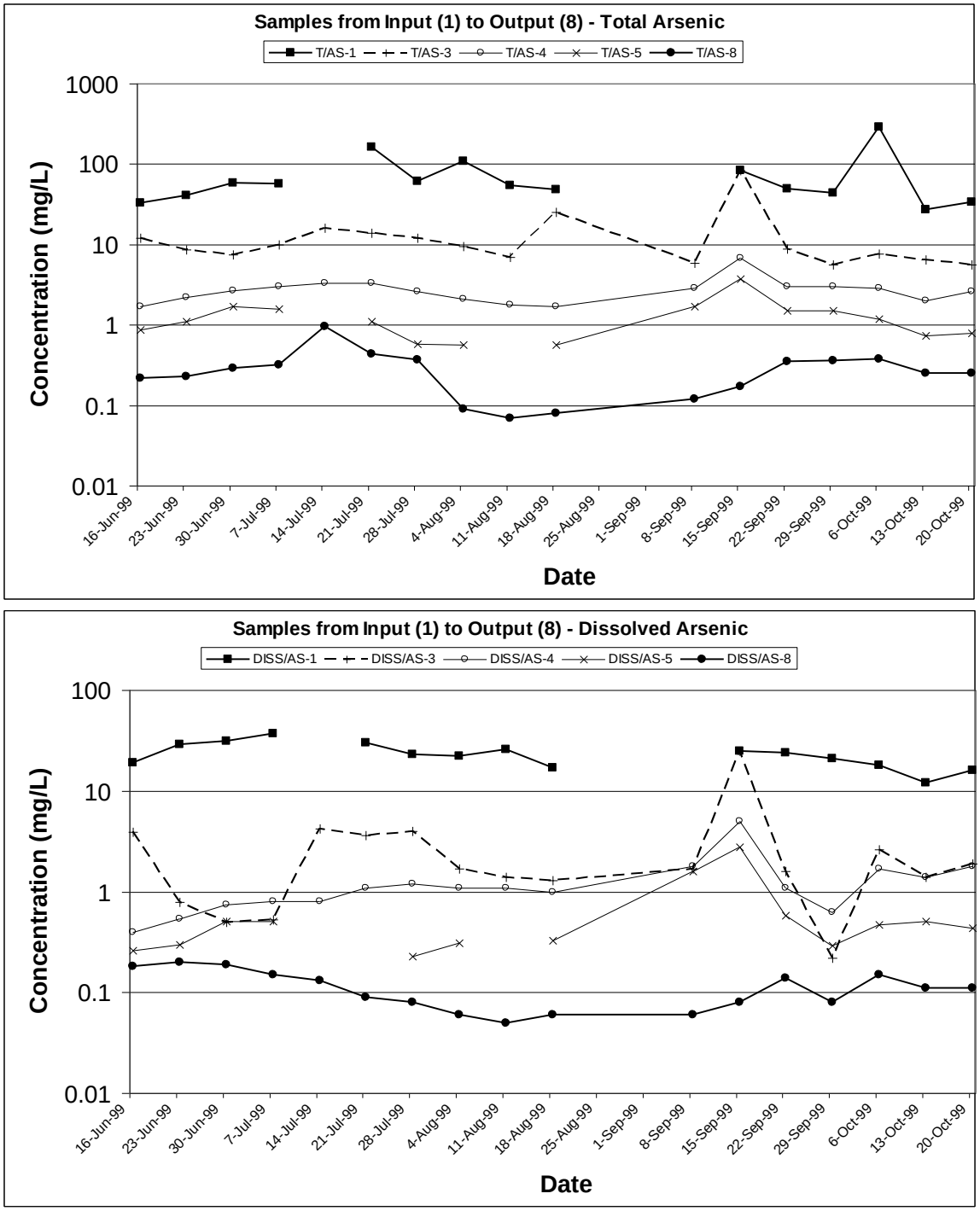


Figure 16. Total and dissolved As concentrations (mg/L) at sample points 1, 3, 4, 5 and 8 (refer to Figure 3) from June 16 to October 20, 1999.

The treatment of total suspended solids (TSS) and subsequent decrease in metal concentrations by filtration appears to be an important aspect of the anaerobic cell and the system in general (Figure 17). Indeed during the high July flows system filtration as measured by TSS removal was compromised. The overall mean TSS at the input (233 mg/L) was reduced by 61% by the anaerobic cell to a mean of 86 mg/L. The 81% reduction of total As concentrations (Table 1; Table 3) compared to the lower reduction of total Zn and Cd concentrations of 33% and 63% respectively is likely due to a large part the form of the metals entering the system. Only 30% of the As is dissolved compared to 80% and 75% for Zn and Cd respectively. The mean molar ratio of Zn:As of the inputted solids is 1.2 which is similar to 1.5 indicative of zinc arsenate (Kottigite – $Zn_3(AsO_4)_2 \bullet 8(H_2O)$) known to be found in the input (Duncan et al 2008).

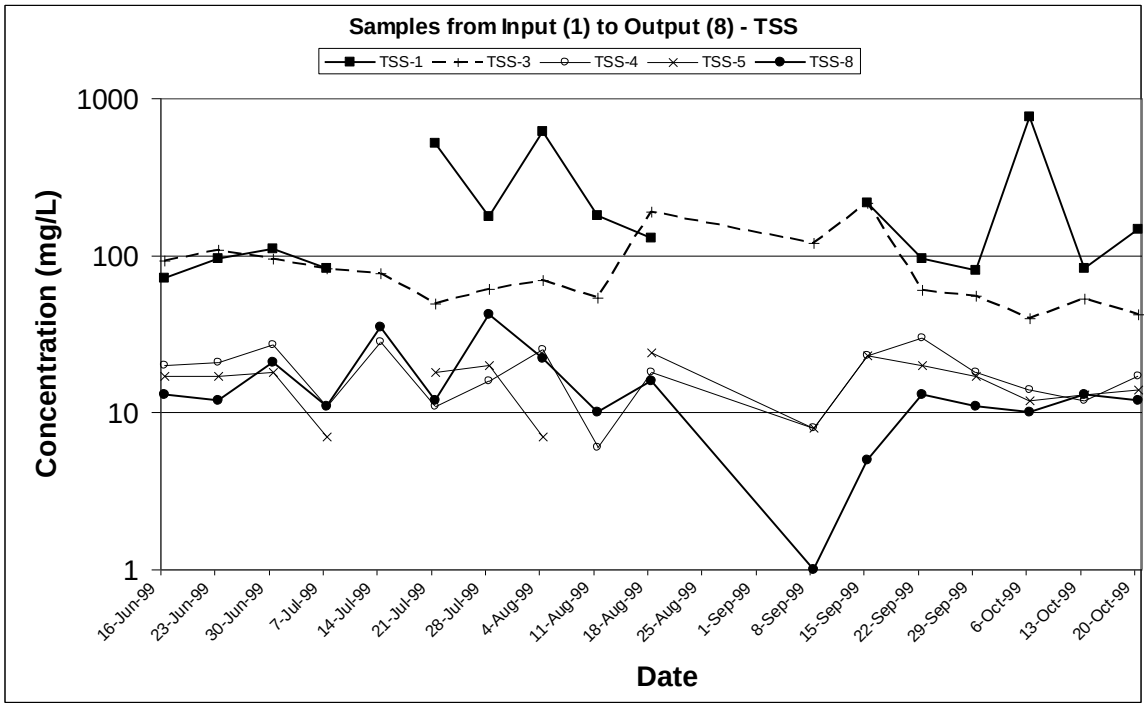


Figure 17. Total suspended solids (TSS - mg/L) at sample points 1, 3, 4, 5 and 8 (refer to Figure 3) from June 16 to October 20, 1999.

Pearson Product-Moment Correlation analysis of the measured water quality parameters (maximum n=17) was done for each sample point and only statistically significant results ($p<0.05$ or lower) and a Pearson correlation coefficient (r) > 0.5 are

discussed. At the input, total As concentrations were strongly positively correlated with TSS (r=0.9; $p<0.001$). Total Zn and Cd concentrations were also correlated with TSS but to a lesser degree (r=0.5; $p<0.05$). These findings further support that most of the As is entering as solids (likely predominantly Kottigite – $Zn_3(AsO_4)_2 \bullet 8(H_2O)$), while Zn and Cd are less associated with the inputted solids. Total and dissolved Zn and Cd concentrations were strongly negatively correlated with pH (r=-0.8; $p<0.001$). These results corroborate with the different sources of the three parameters with the primary source of Zn and Cd from the old landfill (lower pH as well) as compared As primarily coming from an As storage area. Seepage from both source areas are collected with separate collection systems and combined prior to entering the final collection sump which pumps the combined seepage to either the wetlands or the TML effluent treatment plant or both. Comparison of input pH and Zn concentrations (Figure 12; Figure 14) show lower pH tracking with higher Zn concentrations which likely relates to varying seasonal contributions of the two seepage sources to the input to the system.

In water exiting the anaerobic cell, only total As concentrations remained strongly positively correlated with TSS (r=0.8; $p<0.001$). Total and dissolved Zn concentrations were strongly negatively correlated with pH (r=-0.7; $p<0.005$) and total and dissolved Cd concentrations are negatively correlated with pH but to a lesser degree (r=-0.5 and -0.6 respectively; $p<0.005$).

Water from the tree cell had no correlations with respect to TSS which has been reduced to a mean of 18 mg/L at this point. Total and dissolved Zn concentrations were strongly negatively correlated with pH (r=-0.7; $p<0.005$) and total and dissolved Cd concentrations as well (r=-0.8; $p<0.005$). Comparison of pH to Zn and Cd concentrations at various point through the system confirm the correlations (Figure 12; Figure 14; Figure 15) and at pH lower than 6.4 that lower reductions of Zn and Cd concentrations are noted.

Water Quality – Summer/Fall 2000

Water samples were collected weekly in 2000 from early June until mid-October (then less frequently until system shutdown). In general, the seepage is slightly acidic with a median pH of 5.41 in 2000 (Table 5) which was lower than in 1999 with a median pH of 5.88. While the pH a 7.0 or higher is desired to precipitate ZnS, reasonable Zn treatment rates are observed at pH > 6.4 in the Trail system. During sulphate reduction alkalinity is produced and the pH is raised. To further assist in raising the pH and treat Zn, the construction of the anoxic limestone cell was completed prior to 2000 system start-up. Higher median pH values were noted leaving the first cell and throughout the system as compared to 1999 (Table 5; Figure 12; Figure 18). The median pH in the final holding pond was 7.00. These higher pH values correspond with higher total and dissolved Zn removal rates in 2000 as compared to 1999 especially in the anaerobic cell (Table 3; Table 4; Table 6; Table 7).

Water temperature was similar throughout the system from input to output for any given date (generally within 5 °C) but varying seasonally by up to 25 °C (Figure 18) Water temperature generally ranged from 15 to 23 °C in June, rising in July and August to 20 to 25 °C and then declining through September (12 to 18 °C), October (6 to 12 °C) and November (1 to 6 °C).

Table 5. Median pH and mean DO (mg/L) and range as measured at each treatment stage (n=47; June 2 to November 17, 2000).

Parameter	System Input	Anoxic Cell Out	Anaerobic Cell Out	1st plant Cell Out	2nd Plant Cell	*Typha* Cell Out
pH	5.41	6.70	6.94	6.91	6.96	7.00
Range	*4.29-5.87*	*6.42-7.07*	*6.12-7.30*	*5.97-7.25*	*5.75-7.25*	*6.00-7.22*
DO	6.73	3.20	2.40	2.37	2.24	3.40
Range	*3.3-8.2*	*2.2-5.2*	*0.6-5.3*	*0.5-5.1*	*0.6-7.07*	*1.1-6.3*

Dissolved oxygen concentrations generally decreased as water moved through the system with the biggest reductions seen from the anaerobic cells as expected (Figure 18; Table 5). The reduction in mean DO seen in the system from 6.24 to 3 mg/L (or less in subsequent stages) is an indicator of how well the system is operating. To further reduce DO and increase metal treatment by the anaerobic cells 1 kg of sodium acetate (readily available carbon source) was added to the collection sump each time the pump was started (six times from September 22 to October 15, 2000). The addition resulted in a reduction in the DO concentrations in the input to the system, this reduction did not track through the other treatment cells (Figure 18). The amount added was minimal and was likely used up rapidly by the system. However, dissolved Zn concentrations out of the second anaerobic bioreactor declined dramatically through this period (Figure 19).

In 2000, the mean total Zn concentration in the input was 437 mg/L ranging from 192 to 744 mg/L (Table 6) versus a mean of 289 mg/L in 1999 (Table 3). These high Zn concentrations along with the high variability further confirmed the need for additional capacity to treat Zn and the addition of the anoxic limestone cell greatly enhanced system performance and increased HRT. Total Zn, Cd and As mean concentrations generally decreased through the system while dissolved concentrations for Cd and As did not show reductions across all treatment cells (Table 6; Table 7; Figure 19; Figure 20; Figure 21).

Mean dissolved Cd concentrations out of the tree cell were higher than from the preceding cell (Table 7). Higher total and dissolved As concentrations exited the anaerobic cell than the newly constructed anoxic cell, especially the during the start-up period (Figure 21). This may reflect the very low initial concentrations leaving the anoxic cell as well as release of previously stored As in the anaerobic cell. It does demonstrate the need for stable operation of wetland systems as flushing of metals can occur as changes in redox, pH and the bacterial activity result from a prolonged system shutdown or the addition of new treatment cells.

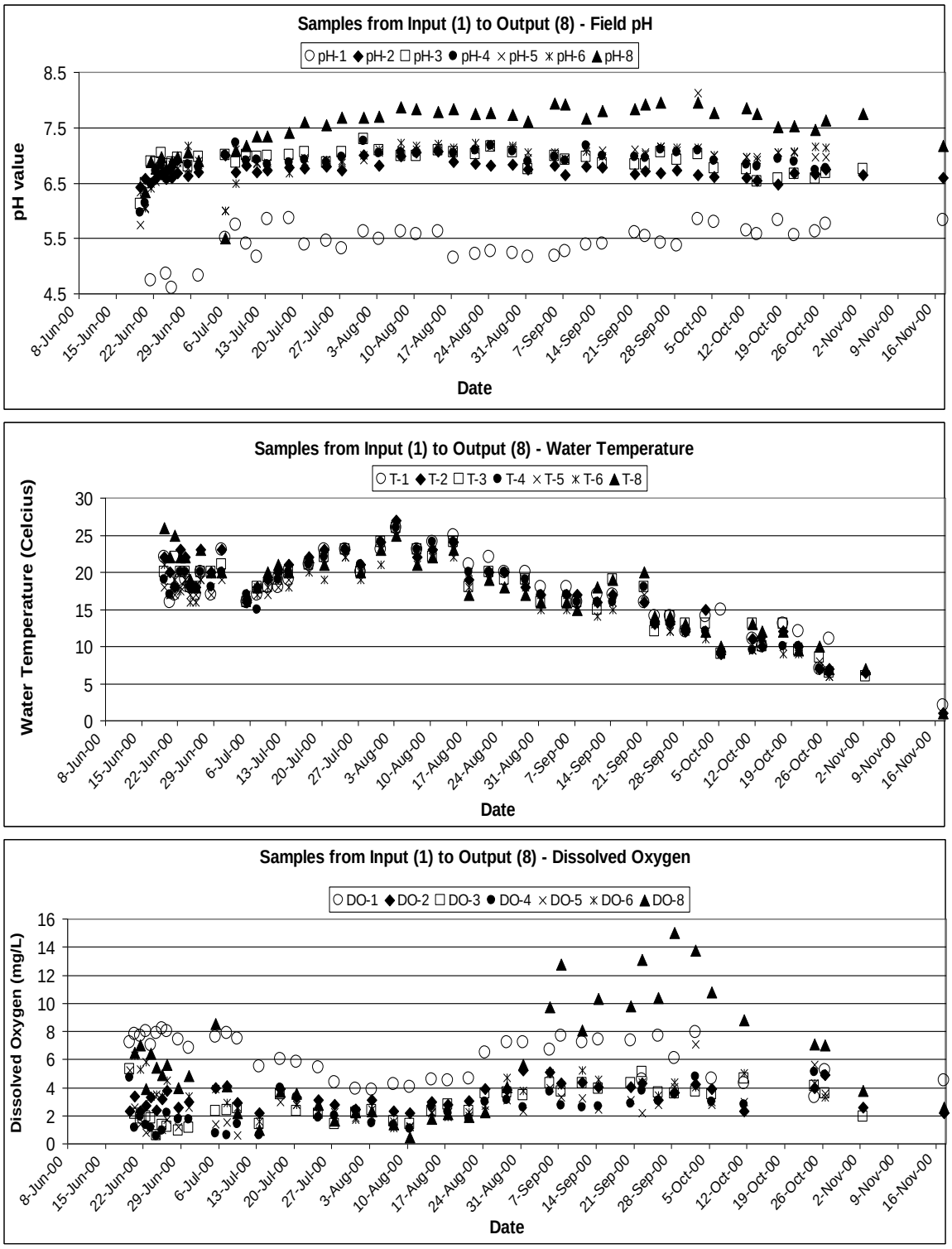


Figure 18. Field pH, water temperature (°C) and dissolved oxygen (mg/L) in 2000 at seven sample points (See Figure 5 for sample labels).

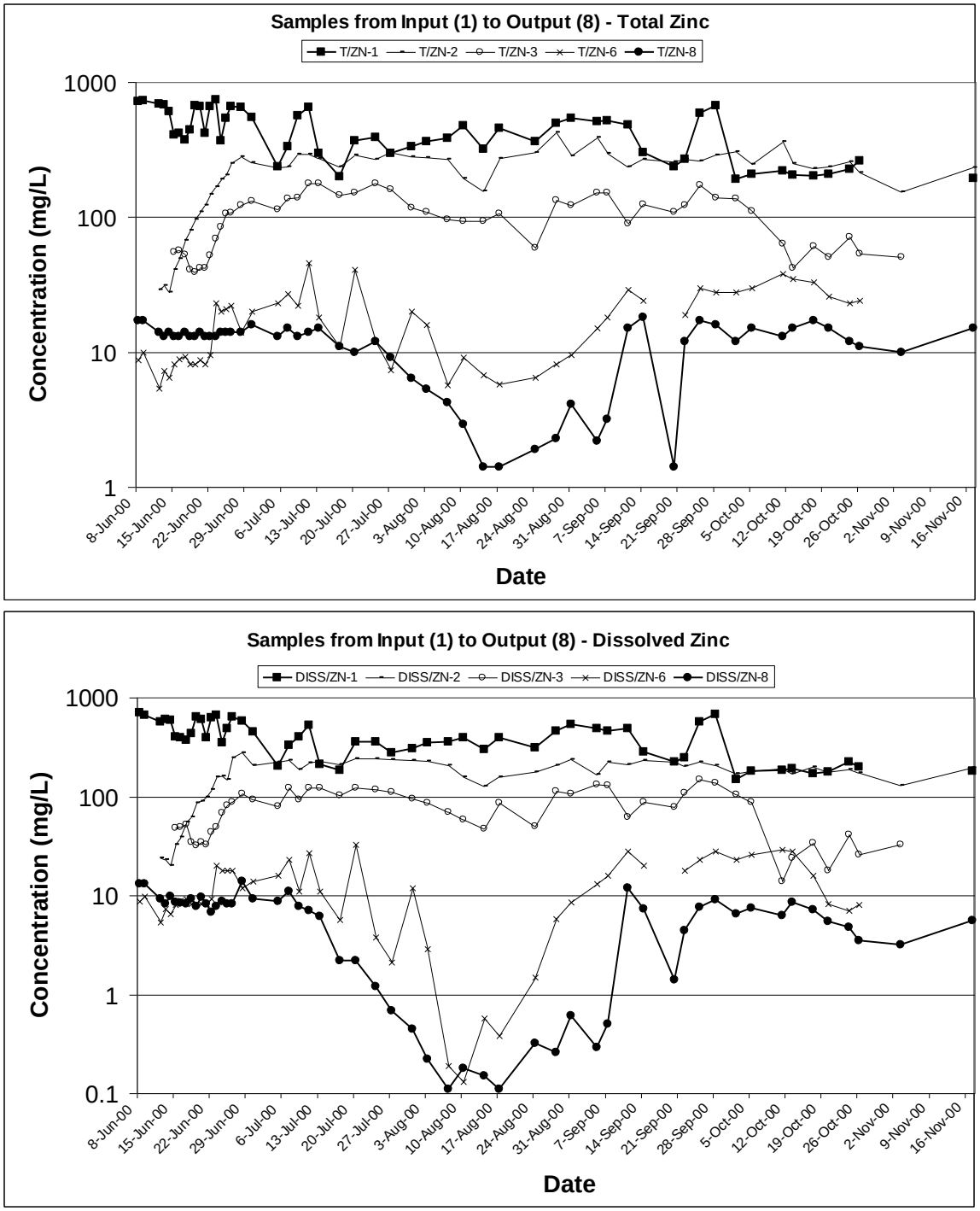


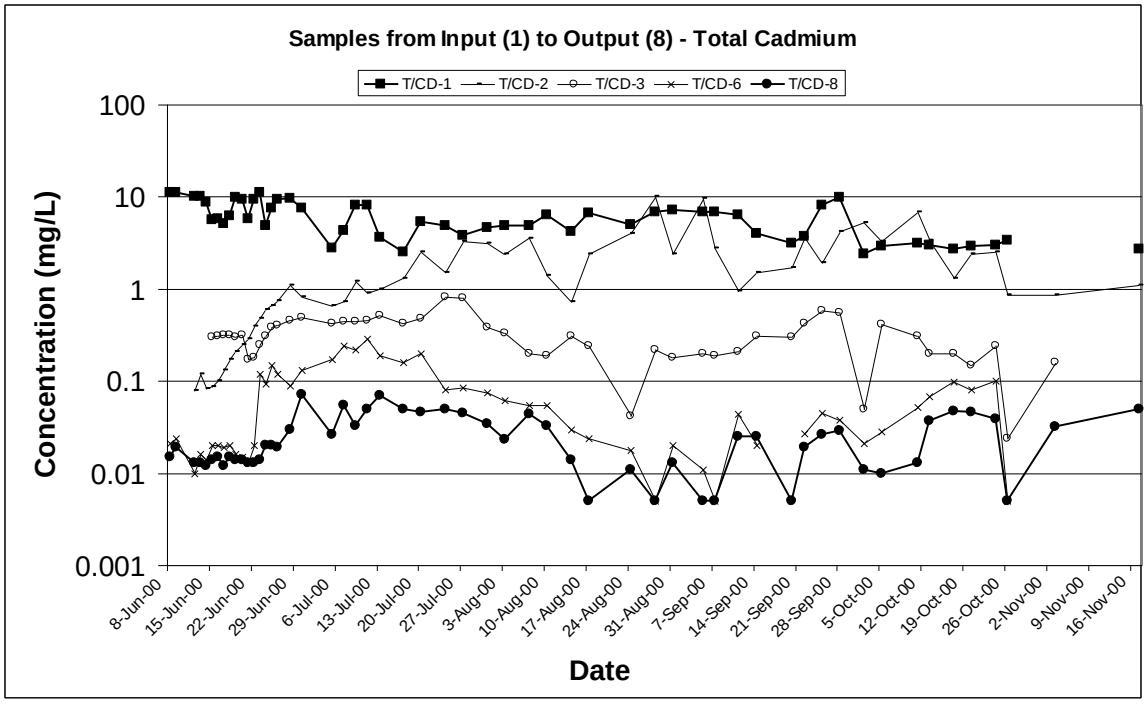
Figure 19. Total and dissolved Zn concentrations (mg/L) at sample points 1, 2, 3, 6 and 8 (refer to Figure 5) from June 8 to November 17, 2000.

Table 6. Mean total Zn, Cd and As concentrations (mg/L), ranges, and percentage reduction for each stage (sampled from June 8 to November 17, 2000; operated 164 days).

Sampling Location	Zinc (mg/L)	Zinc (% reduced)	Cadmium (mg/L)	Cadmium (% reduced)	Arsenic (mg/L)	Arsenic (% reduced)
Seepage Input *(mean; n=54)*	437		6.06		47.6	
Seepage Input *(min-max)*	*(192-744)*		*(2.4-11)*		*(12-106)*	
Anoxic Output *(mean; n=53)*	223	**49**	1.95	**68**	9.30	**80**
Anoxic Output *(min-max)*	*(28-427)*		*(0.079-10.0)*		*(1.1-39)*	
Anaerobic Output *(mean; n=49)*	103	**54**	0.32	**83**	10.5	**-13**
Anaerobic Output *(min-max)*	*(39-179)*		*(0,24-0.81)*		*(1.3-35)*	
Tree Cell Output *(mean; n=53)*	63.8	**38**	0.26	**19**	8.43	**20**
Tree Cell Output *(min-max)*	*(5.2-139)*		*(0.036-1.0)*		*(1.2-41)*	
Grass Cell Output *(mean; n=53)*	53.6	**16**	0.20	**24**	8.32	**1**
Grass Cell Output *(min-max)*	*(2-125)*		*(0.005-0.74)*		*(0.41-40)*	
Typha Cell Output *(mean; n=52)*	17.7	**67**	0.068	**92**	2.97	**64**
Typha Cell Output *(min-max)*	*(5.4-46)*		*(0.005-0.29)*		*(0.13-11)*	
Total % Removed By All Cells		**95.9**		**99.7**		**93.8**
Final Holding Pond with Total % removed *(mean; n=55)*	11.4	**97.4**	0.025	**99.6**	1.64	**96.5**
(min-max)	*(1.4-18)*		*(0.005-0.71)*		*(0.11-5.0)*	

Table 7. Mean dissolved Zn, Cd an As concentrations (mg/L) and their ranges at each stage with the percentage reduction for each stage (sampled from June 6 to November 17, 2000; operated 164 days).

Sampling Location	Zinc (mg/L)	Zinc (% reduced)	Cadmium (mg/L)	Cadmium (% reduced)	Arsenic (mg/L)	Arsenic (% reduced)
Seepage Input *(mean; n=54)*	399		5.52		31.2	
Seepage Input *(min-max)*	*(149-710)*		*(1.8-11)*		*(11-93)*	
Anoxic Output *(mean; n=53)*	172	**57**	0.331	**94**	1.27	**96**
Anoxic Output *(min-max)*	*(20-276)*		*(0.026-1.1)*		*(0.19-7.9)*	
Anaerobic Output *(mean; n=49)*	77.4	**55**	0.064	**81**	5.47	**-331**
Anaerobic Output *(min-max)*	*(14-150)*		*(0.005-0.17)*		*(0.52-24)*	
Tree Cell Output *(mean; n=53)*	63.8	**32**	0.095	**-49**	5.48	**0**
Tree Cell Output *(min-max)*	*(5.2-139)*		*(0.005-0.44)*		*(1.1-28)*	
Grass Cell Output *(mean; n=53)*	45.4	**14**	0.060	**37**	5.64	**-3**
Grass Cell Output *(min-max)*	*(1.5-109)*		*(0.005-0.30)*		*(0.22-37)*	
Typha Cell Output *(mean; n=52)*	12.7	**72**	0.016	**74**	1.29	**77**
Typha Cell Output *(min-max)*	*(0.13-33)*		*(0.005-0.047)*		*(0.11-4.6)*	
Total % Removed By Treatment Cells		**96.8**		**99.7**		**95.8**
Final Holding Pond with Total % removed *(mean; n=55)*	5.94	**98.5**	0.007	**99.9**	0.69	**97.8**
(min-max)	*(0.11-14)*		*(0.005-0.027)*		*(0.09-2.9)*	



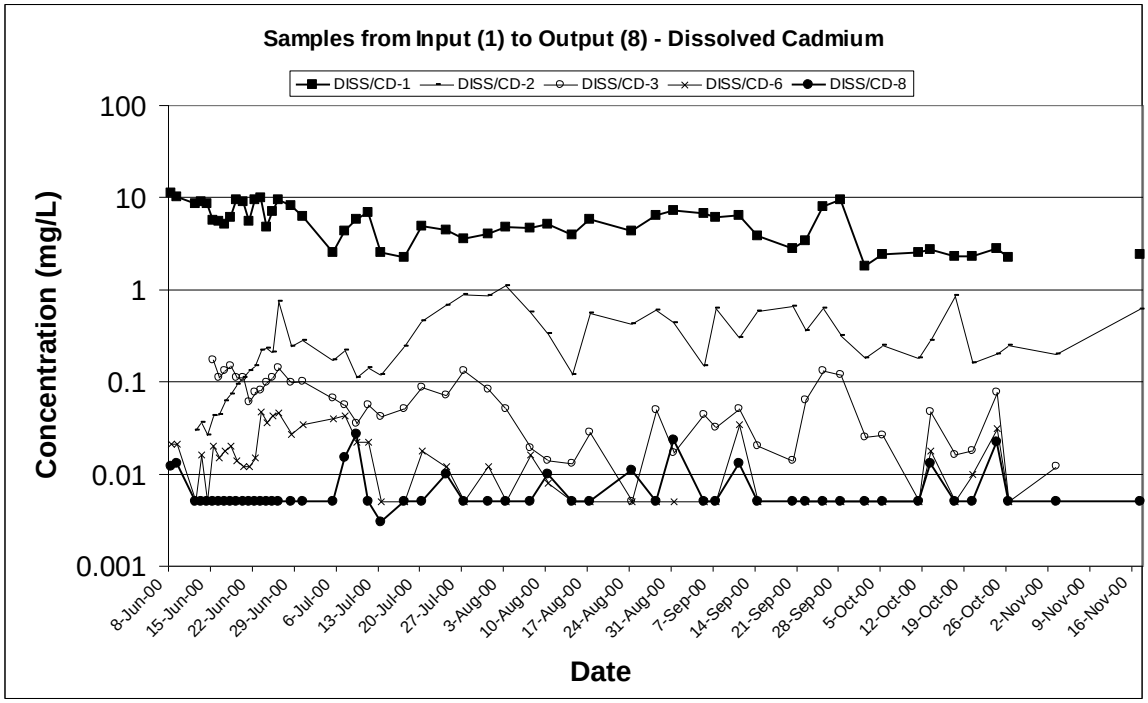


Figure 20. Total and dissolved Cd concentrations (mg/L) at sample points 1, 2, 3, 6 and 8 (refer to Figure 5) from June 8 to November 17, 2000.

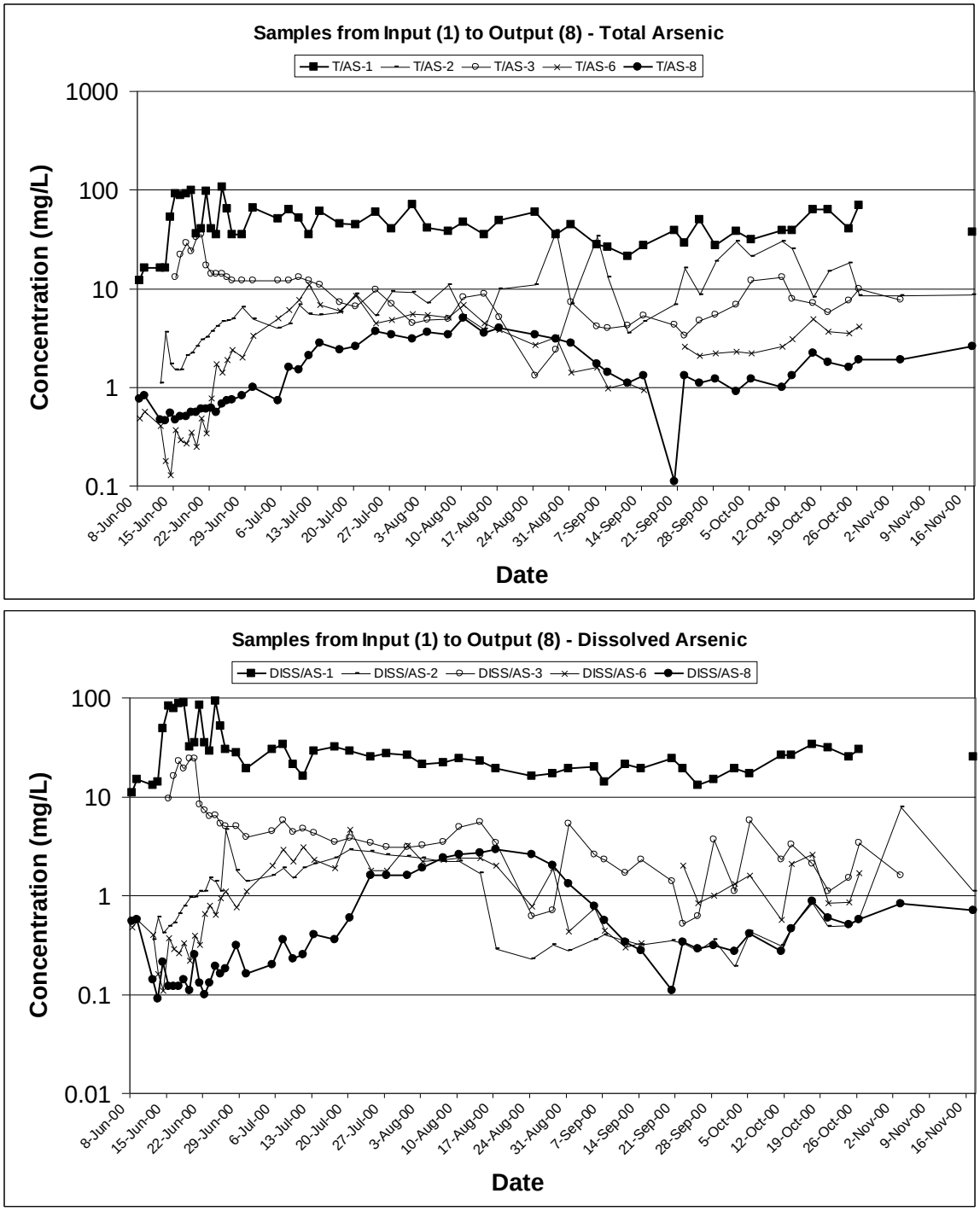


Figure 21. Total and dissolved As concentrations (mg/L) at sample points 1, 2, 3, 6 and 8 (refer to Figure 5) from June 8 to November 17, 2000.

During the first few weeks of start-up, large quantities of Zn, Cd, As and sulphate were retained in the new anoxic cell (Figure 19; Figure 20; Figure 21; Figure 22). This may be due to adsorption to the complex organic materials in the anoxic cell during its initial start-up. The initial concentrations exiting the cell are low increasing in a linear fashion plotted on a log scale until a plateau is reached and other treatment mechanism start to predominate the system. Other researchers have described this phenomenon in the initial phases of wetland start-up. Machemer and Wildeman (1992) note that at start-up of a constructed wetland the adsorption of dissolved metals onto organic sites in the substrate material was an important process but diminished over time with sulphide precipitation becoming the dominant metal removal process. Similarly, Willow and Cohen (2003) found in small column reactors that adsorption is a transitory metal removal mechanism. They also found that Mn and Zn were loosely sorbed, while Cd tended to be strongly sorbed and would displace Zn and Mn when the sorptive capacity of the system was exhausted.

Another interesting phenomenon is the higher TSS concentrations exiting the anoxic cell then the input TSS after the initial start-up which continue through the year (Figure 22). The TSS is higher through the rest of the system as well when compared to 1999 (Figure 17). This is in spite of the lower mean TSS into the system of 70 mg/L in 2000 compared to 223 mg/L in 1999. The mean TSS values in 2000 exiting out of the anoxic cell, anaerobic cell, tree cell, grass cell and *Typha* cell were 239, 123, 53, 44 and 50 mg/L respectively all higher than comparable sample points in 1999 which decreased to15 mg/L after the *Typha* cell. The TSS exiting the anoxic cell could be a release of complex organic carbon compounds in a particulate form that is not readily amenable to filtration. This flush of organic material could complex with and release previously sequestered metals in the downstream cells. This was most noticeable for As which experienced significant release from the anaerobic cell and overall lower treatment rates in 2000 compared to 1999 (Table 3; Table 4; Table 6; Table 7).

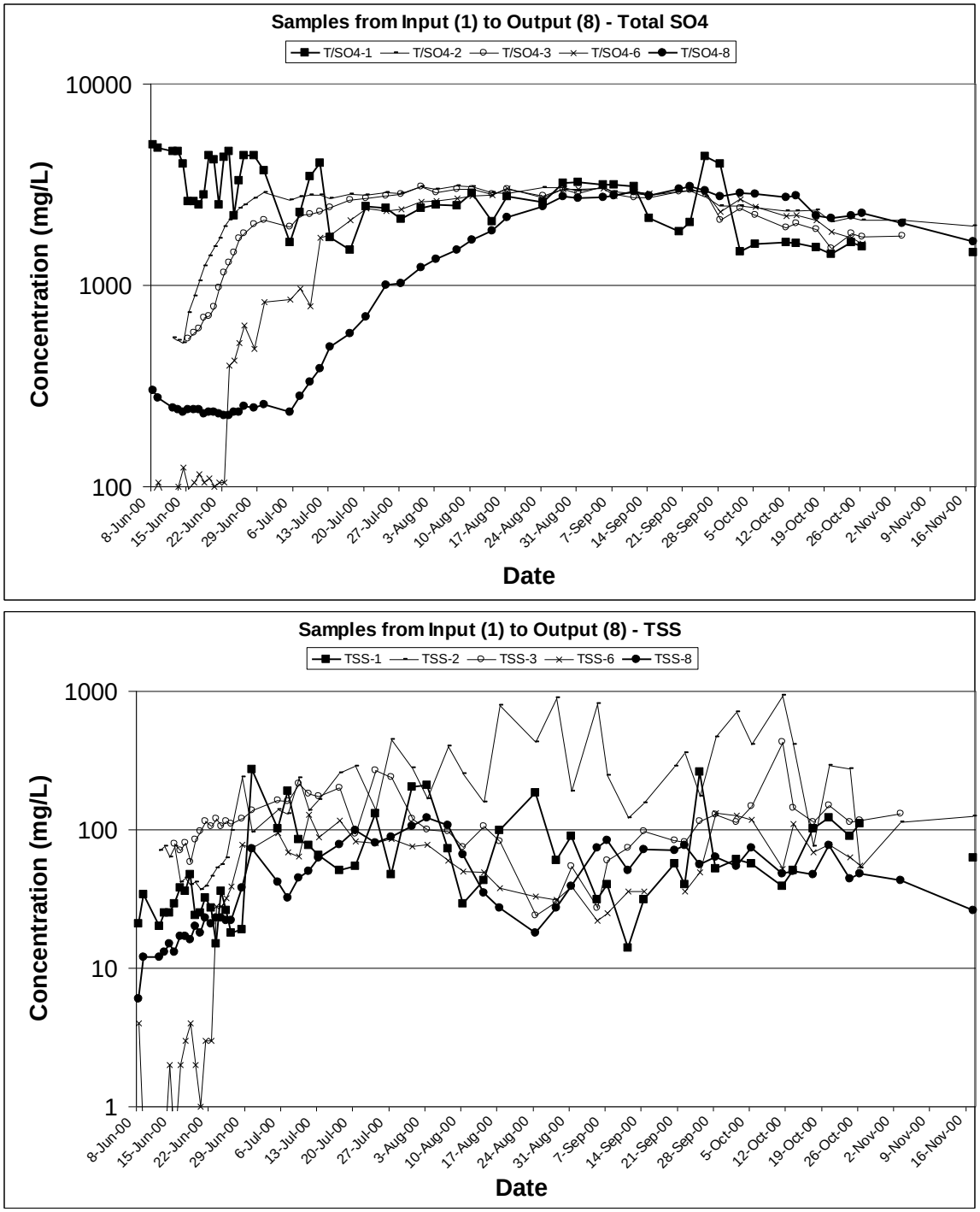


Figure 22. Total sulphate and total suspended solids concentrations (mg/L) at sample points 1, 2, 3, 6 and 8 (refer to Figure 5) from June 8 to November 17, 2000.

The mean percent dissolved concentrations in the seepage input as compared to total concentrations were 91%, 91% and 65% for Zn, Cd and As respectively (Table 6; Table 7). As the seepage was treated through the system, the mean percent dissolved concentrations decreased compared to the seepage input to 78%, 75% and 48% for Zn, Cd and As respectively out of the *Typha* cell. If the water exiting was filtered to remove the particulate portion (e.g., by slow sand filter) then better overall metal treatment rates could likely be achieved. The final holding cell does act as a settling basin, as well, its large holding capacity averaging out the peak concentrations exiting the *Typha* cell. Based on the observed difference between total and dissolved metals, a slow sand filter was added to the system in 2001.

Pearson Product-Moment Correlation analysis of the measured water quality parameters was done for each sample point (maximum n=55) and only statistically significant results ($p<0.05$ or lower) and a Pearson correlation coefficient (r) of at least 0.5 are discussed. At the input, total As, Zn and Cd concentrations were not correlated with TSS which would be expected as were mostly in the dissolved phase. But total As, Zn and Cd concentrations were strongly correlated TSS exiting the anoxic cell (r=0.9, 0.9 and 0.7 respectively; $p<0.001$). Only total Cd concentrations were correlated out of the anaerobic cell (r=0.5; $p<0.001$). Total As, Zn and Cd concentrations were correlated with TSS out of the tree cell (r=0.8, 0.5 and 0.6 respectively; $p<0.001$), the grass cell (r=0.5, 0.6 and 0.7 respectively; $p<0.001$) and for total As only from the *Typha* cell (r=0.5; $p<0.001$).

Input total and dissolved Zn and Cd concentrations were strongly negatively correlated with pH (r=-0.8, -0.8, -0.9 and -0.8 respectively; $p<0.001$) and total sulphate (r=1.0; $p<0.001$). No strong correlations were found for As but mean total As concentrations in the input decreased from 76.4 to 47.6 mg/L while mean total Zn increased from 289 to 437 mg/L comparing 1999 to 2000. Similar to 1999, these results corroborate with the

different sources of the three parameters with the primary source of Zn, Cd and sulphate from the old landfill (lower pH as well) as compared As primarily coming from an As storage area. Both areas are collected and combined prior to the final collection sump that provides the input to the wetlands. Comparison of input pH and Zn concentrations (Figure 18; Figure 19) show lower pH tracking with higher Zn concentrations which likely relates to varying seasonal contributions of the two sources to the input to the system. The lower median pH (5.41) and higher total mean Zn (437 mg/L) indicate a greater contribution from the landfill side in 2000.

Total and dissolved Zn and Cd concentrations were not correlated with pH exiting any of the cells. This may be due to the fact that pH was quite high through the system with median pH ranging 6.70 to 7.00 which is well above the pH of 6.4 that is associated with lower Zn and Cd percent reductions in the Trail system.

Water Quality - May 2001 to April 2002

After thaw, the pipeline break was repaired, input to the system was restored May 1st and sampling began May 7th. Throughout the course of the summer flow rates were gradually increased until the system was operating at designed maximum flow rate of 15,000 to 20,000 L/day by August 10th. This rate was continued until September 15th then it was lowered to 10,000 to 12,000 L/day as reduced Zn removal efficiencies were noted. After October 30th, the system was further slowed down to between 8,000 and 10,000 L/day for winter operation until April 8th, 2002 when system was shutdown for 2002 construction activities.

Water samples were collected three times per week in 2001 from May 7th until October 30th at eight points then winter sampling was weekly at four points in the system – the input and output to the first anaerobic cell, the output of the second anaerobic cell and the final holding pond. The median summer pH of leachate into the system in 2001 was 5.52 slightly higher than 5.41 in 2001 (Table 5; Table 8). Whereas the median summer pH exiting the first anaerobic cell was 6.83 in 2001 versus 6.70 in 2000, while during the winter period 2001 the median pH is only 6.04 (Table 8). A similar winter pH decrease occurred exiting the second anaerobic cell where median summer pH was 7.18 while the

median winter pH was 6.26. During the winter no samples were taken from plant cells. However, as the median winter pH in the final holding pond was 6.33 versus 7.63 during the summer, the median winter pH in these cells was likely lower than summer. These pH reductions are important given that Zn treatment by this system is most effective above a pH of 6.4. The pH from the anaerobic cells decreased steadily from May to August with increasing flow to the system and corresponding lower HRT (Figure 23; Table 8). Additionally, 2001 was a very wet summer (Figure 23) with numerous rain events (acidic rain with a pH 4.5 to 5.0) that which added to the volume treated (reducing HRT) and further reduced HRT. These rain events are likely responsible for large temperature shifts (>10°C) and highly variable DO concentrations observed in the system from May through August (Figure 23). These factors are likely responsible for reduced Zn and Cd treatment observed through this period.

As flow was decreased for winter operation (increasing HRT), the winter pH may be lower because the bacteria in the system are not as active at the lower temperatures (<4°C; Table 8) and the pH adjustment process that is bacterially-based was not operating as effectively. A temperature decrease could decrease biological activity of APB and hamper the biological neutralization by SRB in the cells. The higher mean DO out of the anaerobic cells in the winter likely indicates reduced carbon utilization during this period (Table 8).

During the summer 2001 period the mean total and dissolved Zn seepage input concentrations of 321 and 247 mg/L respectively were lower (Table 9; Table 10) being 437 and 399 mg/L respectively for 2000 (Table 6; Table 7). However, the ranges in Zn concentrations were quite large but comparable to 2000. Mean total and dissolved Cd concentrations like Zn were lower in 2001 compared to 2000. While As had higher total mean concentrations in 2001 with similar dissolved concentrations when compared to 2000. As in 2000, the anaerobic cells are removing most of the Zn and Cd and in 2001 most of the As. Overall summer system percent metals' reductions were fairly similar between the years but somewhat lower for Zn in 2001.

Table 8. Median pH, mean dissolved oxygen (DO; mg/L) and mean temperature (^{o}C) measurements at each treatment stage.

Median or Mean	*Input*	*Anoxic Out*	*Anaerobic Out*	*1st Plant cell out*	*2nd Plant cell out*	*Typha Cell out*	*Holding Pond*
Summer (May 7 to October 31, 2001; 3 times weekly)							
Median pH	5.52	6.83	7.18	7.13	7.26	7.34	7.62
DO(mg/L)	3.79	2.66	3.31	1.71	2.04	3.60	4.74
Temp(^{o}C)	21.7	21.4	20.5	20.1	19.6	19.3	19.8
Winter (November 1, 2001 to April 8, 2002; weekly)							
Median pH	5.53	6.04	6.26				6.26
DO(mg/L)	5.29	3.45	3.42				6.46
Temp(^{o}C)	3.6	2.8	1.8				2.7

However, during the winter 2001-2 percent reductions for Zn and Cd out of the anaerobic cells were reduced compared to the summer period (Table 11). Overall system Zn percent reduction was reduced for total Zn from 97.8% to 38.3% for total Zn and from 97.5% to 42.0% for dissolved Zn. This is consistent with previous observations where decreased Zn percent reductions were noted when pH is less than 6.4. Percent reductions for As were similar between the summer and winter periods (Table 9; Table 10; Table 11) which may be indicative of a differing sequestration mechanism as compared to Zn and Cd.

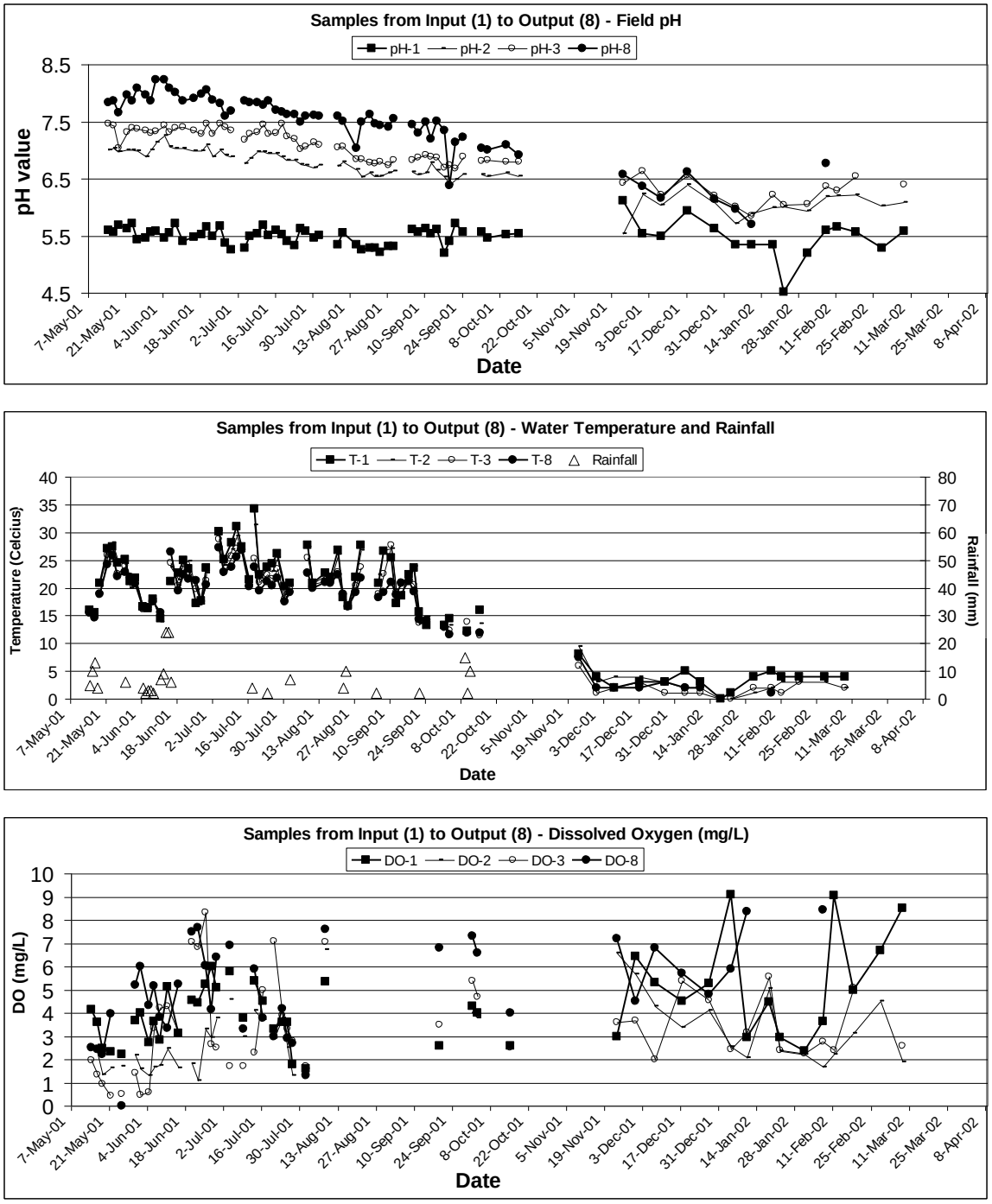


Figure 23. Field pH, water temperature (°C), rainfall (mm) and dissolved oxygen (mg/L) at four sample points from May 14, 2001 to April 8, 2002 (See Figure 5 for sample labels).

Table 9. Mean total Zn, Cd and As concentrations (mg/L) and their ranges at each treatment stage with percentage reduction for each stage (May 7 to October 30, 2001).

Sampling Location	Zinc (mg/L)	Zinc (% reduced)	Cadmium (mg/L)	Cadmium (% reduced)	Arsenic (mg/L)	Arsenic (% reduced)
Seepage Input *(mean; n=66)*	321		4.97		99.6	
Seepage Input *(min-max)*	*(175-651)*		*(2.3-11.0)*		*(24-285)*	
Anoxic Output *(mean; n=66)*	194	**40**	2.03	**59**	28.2	**72**
Anoxic Output *(min-max)*	*(78-386)*		*(0.39-6.5)*		*(1.9-115)*	
Anaerobic Output *(mean; n=66)*	92	**53**	0.373	**82**	5.67	**80**
Anaerobic Output *(min-max)*	*(8.0-404)*		*(0.018-1.50)*		*(1.3-65)*	
Tree Cell Output *(mean; n=65)*	45.5	**50**	0.038	**90**	0.96	**83**
Tree Cell Output *(min-max)*	*(0.23-1555)*		*(0.005-0.170)*		*(0.22-4)*	
Grass Cell Output *(mean; n=63)*	38.2	**16**	0.042	**-9**	1.11	**-15**
Grass Cell Output *(min-max)*	*(0.81-161)*		*(0.005-0.075)*		*(0.20-7)*	
Typha Cell Output *(mean; n=62)*	23.6	**38**	0.026	**37**	1.04	**6**
Typha Cell Output *(min-max)*	*(0.39-137)*		*(0.005-0.011)*		*(0.13-3.9)*	
Sand Filter Output *(mean; n=48)*	20.6	**13**	0.007	**74**	0.31	**71**
Sand Filter Output *(min-max)*	*(0.18-88)*		*(0.001-0.025)*		*(0.06-0.85)*	
Total % Removed By Treatment Cells		**93.6**		**99.9**		**99.7**
Final Holding Pond with Total % removed *(mean; n=82)*	7.10	**97.8**	0.013	**99.7**	0.063	**99.4**
(min-max)	*(0.03-82)*		*(0.001-0.062)*		*(0.14-2.8)*	

Table 10. Mean dissolved Zn, Cd and As concentrations (mg/L) and their ranges at each treatment stage with percentage reduction for each stage (May 7 to October 30, 2001).

Sampling Location	Zinc (mg/L)	Zinc (% reduced)	Cadmium (mg/L)	Cadmium (% reduced)	Arsenic (mg/L)	Arsenic (% reduced)
Seepage Input *(mean; n=66)*	247		3.63		31.7	
Seepage Input *(min-max)*	*(140-473)*		*(2.1-7.0)*		*(16-95)*	
Anoxic Output *(mean; n=66)*	124	**50**	0.494	**86**	1.96	**94**
Anoxic Output *(min-max)*	*(27-258)*		*(0.005-1.60)*		*(0.27-13.0)*	
Anaerobic Output *(mean; n=66)*	53.2	**57**	0.063	**87**	0.63	**68**
Anaerobic Output *(min-max)*	*(8.0-404)*		*(0.005-0.37)*		*(0.08-3.0)*	
Tree Cell Output *(mean; n=65)*	41.1	**23**	0.014	**78**	0.57	**10**
Tree Cell Output *(min-max)*	*(0.01-142)*		*(0.005-0.170)*		*(0.07-2.3)*	
Grass Cell Output *(mean; n=63)*	33.4	**19**	0.015	**-7**	0.68	**-20**
Grass Cell Output *(min-max)*	*(0.32-139)*		*(0.005-0.075)*		*(0.08-3.3)*	
Typha Cell Output *(mean; n=62)*	20.4	**39**	0.011	**27**	0.47	**30**
Typha Cell Output *(min-max)*	*(0.21-122)*		*(0.005-0.052)*		*(0.020-1.70)*	
Sand Filter Output *(mean; n=48)*	19.8	**3**	0.004	**65**	0.25	**47**
Sand Filter Output *(min-max)*	*(0.13-82)*		*(0.001-0.016)*		*(0.05-0.80)*	
Total % Removed By Treatment Cells		**92.0**		**99.9**		**99.2**
Final Holding Pond with Total % removed *(mean; n=80)*	6.07	**97.5**	0.006	**99.8**	0.38	**98.8**
(min-max)	*(0.014-44)*		*(0.001-0.062)*		*(0.03-1.69)*	

Table 11. Mean total and dissolved Zn, Cd and As concentrations (mg/L) and their ranges with percentage reduction for three stages (winter - November 1, 2001 to April 8, 2002).

Sampling Location	Zinc (mg/L)	Zinc (% reduced)	Cadmium (mg/L)	Cadmium (% reduced)	Arsenic (mg/L)	Arsenic (% reduced)
Total Metal Concentrations						
Seepage Input *(mean; n=22)*	267		3.35		96.0	
Anoxic Output *(mean; n=21)*	176	**34**	1.46	**56**	21.7	**77**
Anaerobic Output *(mean; n=20)*	142	**20**	0.655	**55**	9.69	**55**
Final Holding Pond with Total % removed *(mean; n=13)*	88	**38.3**	0.025	**96.3**	0.31	**96.8**
Dissolved Metal Concentrations						
Seepage Input *(mean; n=22)*	230		2.84		75.1	
Anoxic Output *(mean; n=21)*	152	**34**	1.05	**63**	14.3	**81**
Anaerobic Output *(mean; n=20)*	129	**15**	0.446	**57**	5.79	**60**
Final Holding Pond with Total % removed *(mean; n=13)*	75	**42.0**	0.010	**98.0**	0.05	**99.2**

The variability in the system is very evident in plots of the individual sample results total and dissolved metal concentrations at four points (input, after anoxic cell, after anaerobic cell and final holding pond) from May 7th, 2001 to April 8th, 2002 (Figure 24, Figure 25, and Figure 26 for Zn, Cd, and As respectively). Total and dissolved Zn concentrations started to increase out of the anaerobic cells in mid to late July and in late August in the final holding cell until the end of October when the flow rates to the system were reduced to winter rates. By March total and dissolved Zn concentrations started to decrease out of the anaerobic cells with increasing pH and decreasing DO (Figure 23). Total and dissolved Cd concentrations behaved in similar manner as Zn with a more

dramatic decrease after March. This may indicate that the system had adapted to the colder temperatures over time. However, total and dissolved As concentrations while quite variable started to increase out of the anaerobic cells in mid to late October (most apparent for dissolved As concentrations) but continued to decrease in the final holding cell through the sample period.

The suspected adsorption phenomena observed in the first anaerobic cell in 2000 during initial cell start-up was not evident during start-up in 2001. This provides additional evidence that this may indeed have been an adsorption process that was observed in 2000. This also supports that while adsorption can be an important factor it is limited temporal until all adsorptions sites are used. Given the high metal and sulphate concentrations in the input, available adsorption sites would likely be quickly used up in this system.

The buffering effect of a large holding cell is readily apparent when comparing the several month delayed response of dissolved Zn concentrations (Figure 24) between the last anaerobic cell (sample point 3) and the final holding cell (sample point 8). A similar delay but shorter can be seen for dissolved Zn and Cd between the two anaerobic cells reflecting their 1 to 2 week hydraulic residency times.

Considerable variability with numerous peaks in the total Zn, Cd, As and TSS concentrations (Figure 27) were noted exiting the anaerobic cells for period May through August. These peaks appear to correspond to the significant rain events that occurred during this period (Figure 23). The flushes of these solids may be in response to the sharp decreases in HRT resulting from the rain events temporarily reducing the filtration capacity of the anaerobic cells. At this point of the system evolution both anaerobic cells had standing water covering the cells. Based on the results of 2001, the decision to “cap” the anaerobic cells with additional biosolids was made to reduce the impacts of precipitation events on the standing open water surface of these cells.

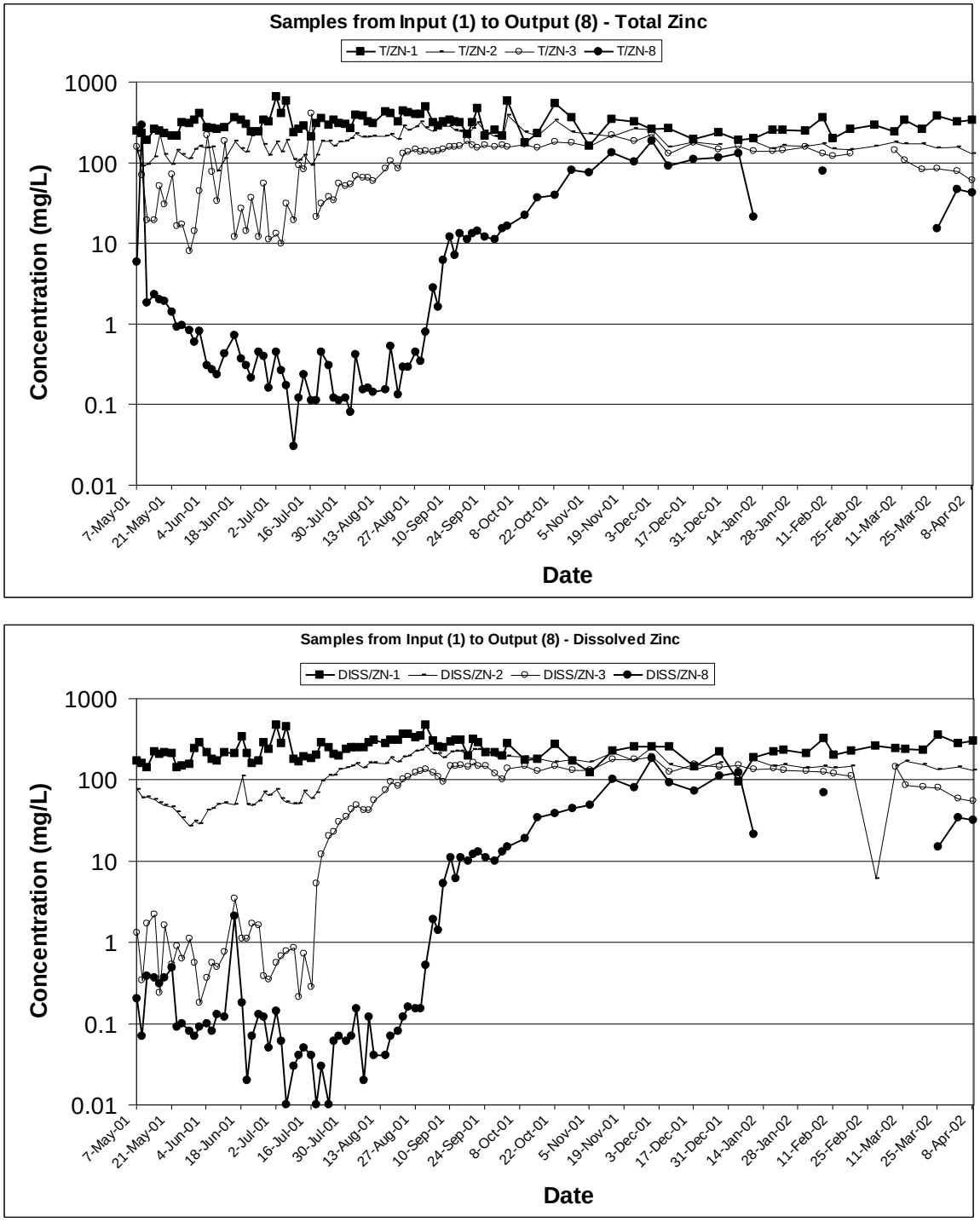


Figure 24. Total and dissolved Zn concentrations (mg/L) at sample points 1, 2, 3 and 8 (refer to Figure 5) from May 7, 2001 to April 8, 2002.

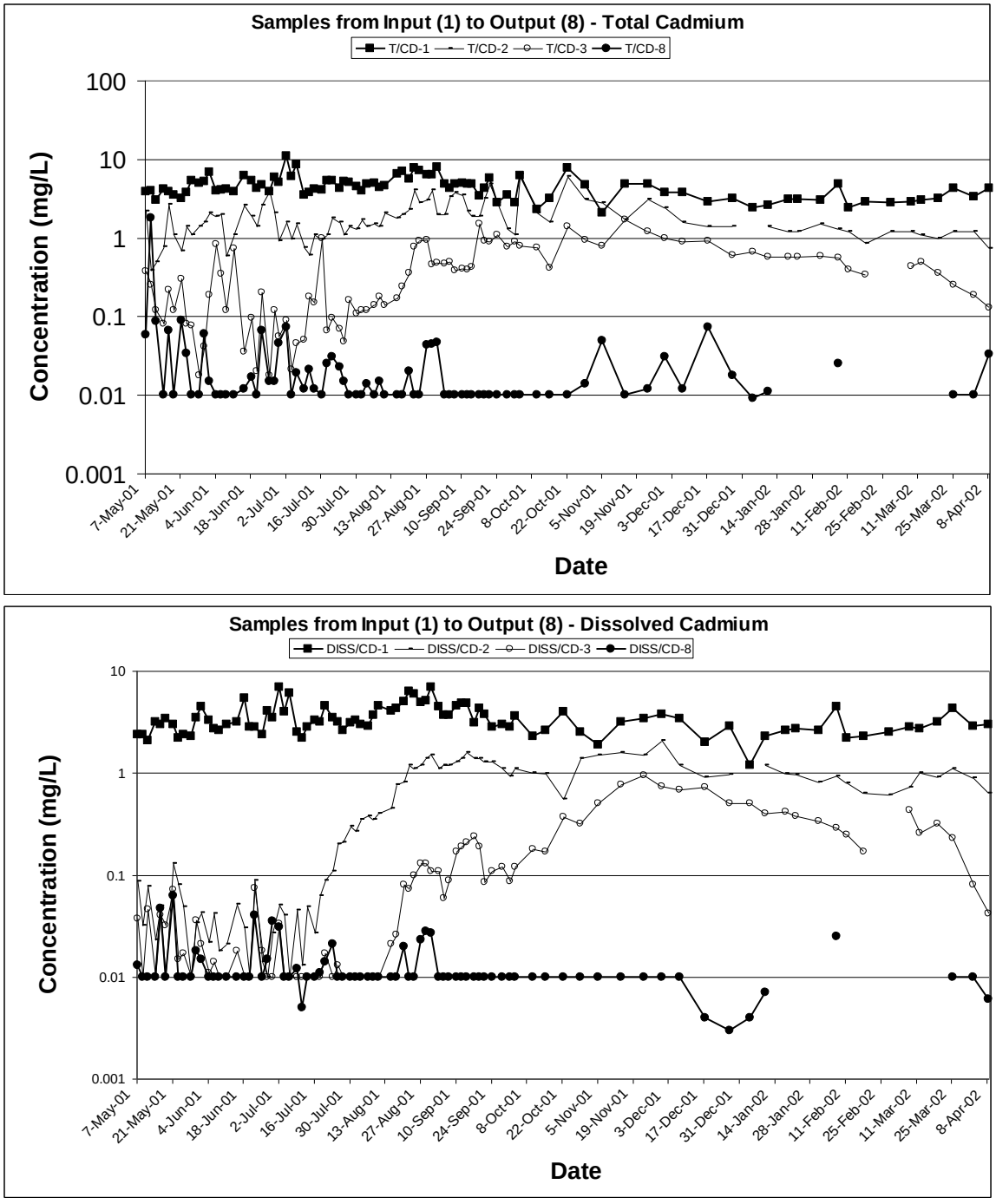


Figure 25. Total and dissolved Cd concentrations (mg/L) at sample points 1, 2, 3 and 8 (refer to Figure 5) from May 7, 2001 to April 8, 2002.

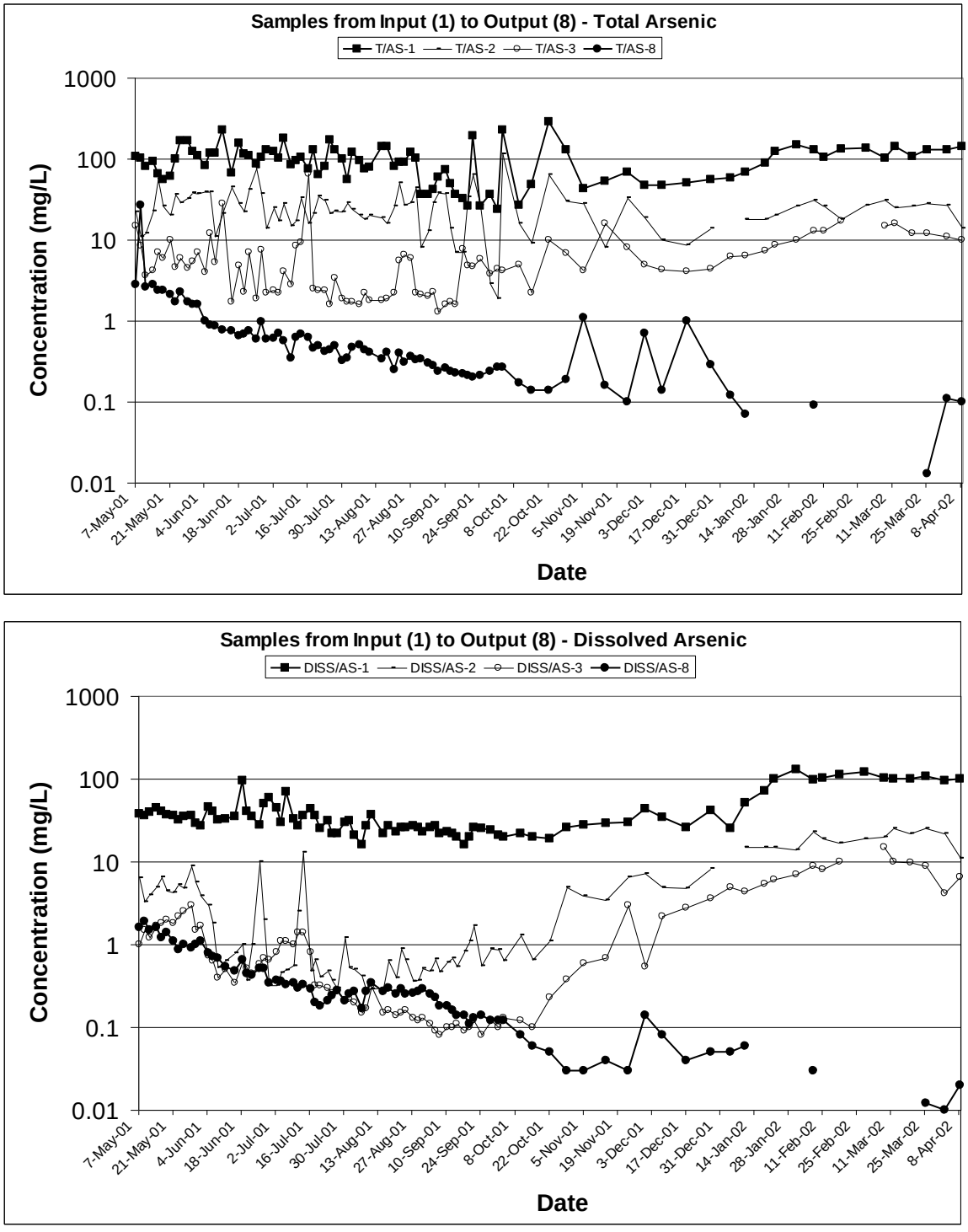


Figure 26. Total and dissolved As concentrations (mg/L) at sample points 1, 2, 3 and 8 (refer to Figure 5) from May 7, 2001 to April 8, 2002.

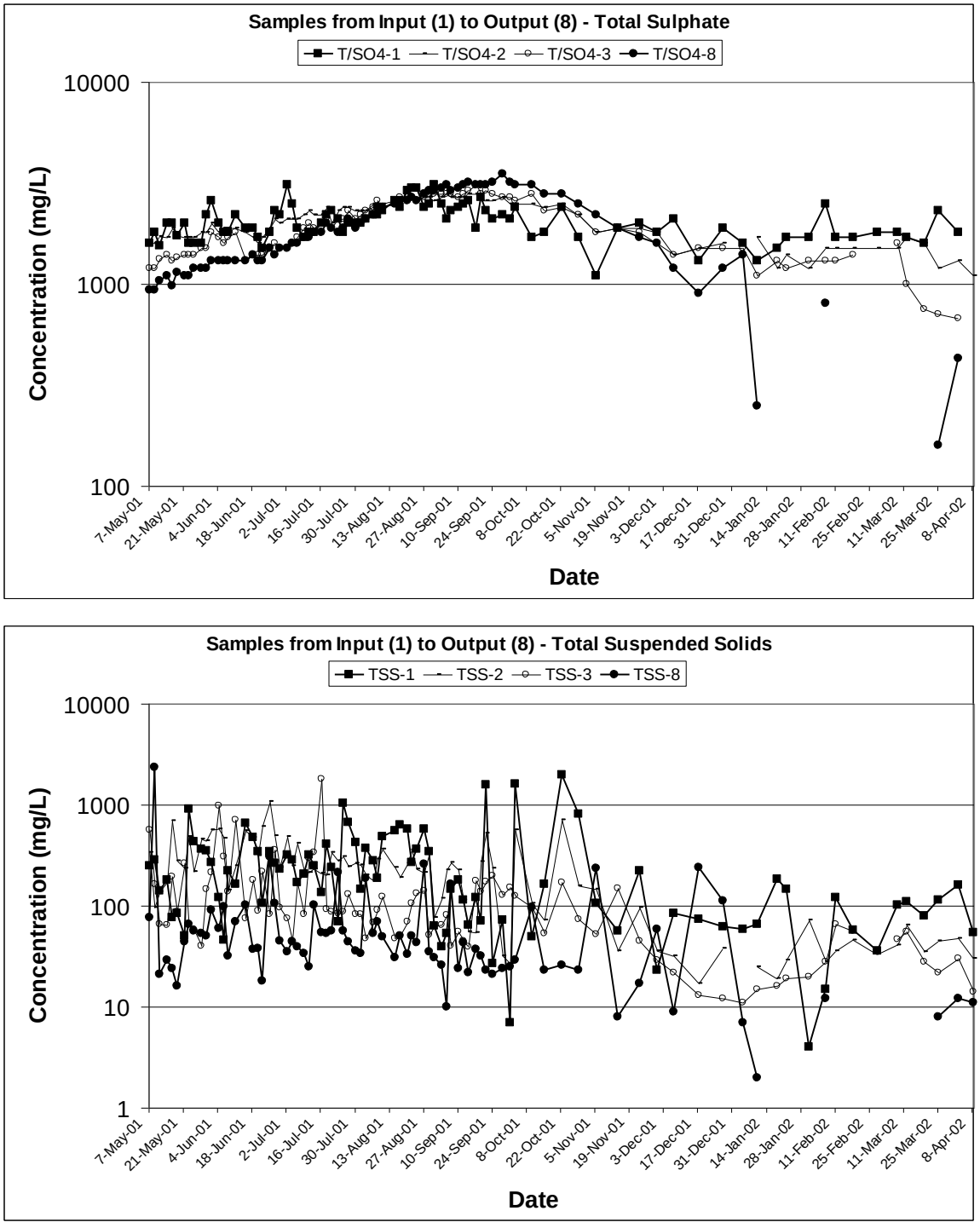


Figure 27. Total sulphate and total suspended solids concentrations (mg/L) at sample points 1, 2, 3 and 8 (refer to Figure 5) from May 7, 2001 to April 8, 2002.

The higher treatment rates of Zn and Cd from May to mid-July are likely due to the higher HRT of this period. Willow and Cohen (2003) found in column reactors that with higher flows and lower HRT that metal loading rates could exceed sulphate reduction rates and reduced metals treatment was noted (particularly for Zn). Additionally, the system was shut from late November 2000 to April 2001 which would have allowed considerable breakdown of complex carbon into simpler carbon compounds usable by SRB. So the system started out with highly available carbon which was utilized and or flushed out mid-July. With the higher flow rates the system appears to reach a state where Zn and Cd are being treated consistently possibly in response to the available carbon that can be produced at the higher flow rates. This further illustrates the need for consistent operation of biological systems and the need to design such systems based on the ability of bacteria to provide a flux of suitable available carbon that is in balance with the metals' load to be treated.

Pearson Product-Moment Correlation analysis of the measured water quality parameters was done for each sample point (maximum n=66) and only statistically significant results ($p<0.05$ or lower) and a Pearson correlation coefficient (r) of at least 0.5 are discussed. In 2001 the input, total As, Zn and Cd concentrations were correlated with TSS (r=0.5, 0.4, and 0.6 respectively; $p<0.001$) reflecting the reduced percentage of the mean dissolved phase of Zn, Cd and As of 76%, 73% and 31% respectively when compared to 2000. Total As and Cd concentrations were correlated TSS exiting the anoxic cell (r=0.7 and 0.5 respectively; $p<0.001$). Only total Zn concentrations were correlated with TSS out of the anaerobic cell (r=0.5; $p<0.001$). Total As, Zn and Cd concentrations were not strongly correlated with TSS out of the tree cell, the grass cell or the *Typha* cell. The reduced correlation with TSS out of the anaerobic treatment cells is consistent with the improved filtration seen in 2001 with mean TSS being reduced from 394 mg/L in the input to 287 mg/l at the anoxic cell to 107 mg/l at the anaerobic cell. While mean TSS was reduced to 22.7 mg/L from the tree cell, to 13.0 mg/L from the grass cell, increasing slightly from the *Typha* cell to 18.8 mg/l and deceasing out of the sand filter to 11.0 mg/L. As well, numerous precipitation events in 2001 also contributed

to TSS variability through the system (Figure 27) which would reduce the number of possible correlations observed.

Input total and dissolved Zn and Cd concentrations in 2001 were not correlated with pH but were with total sulphate (r=0.7, 0.8, 0.7 and 0.8 respectively; $p<0.001$) and dissolved ammonia (r=0.5, 0.6, 0.6 and 0.7 respectively; $p<0.001$). Additionally total sulphate is positively correlated with dissolved ammonia (r=0.8; $p<0.001$). The lack of correlation with pH is likely due to the minimal variation seen in input pH for most of 2001 (Figure 23). No strong correlations were found for As but mean total As concentrations were higher and more variable in the input in 2001. As compared to previous years, these results corroborate with the different sources of the parameters with the primary source of Zn, Cd, sulphate and ammonia from the old landfill with As primarily coming from an As storage area. Both areas are collected and combined prior to the final collection sump that provides the input to the wetlands. The higher median pH (5.52), lower total mean Zn (437 mg/L) and higher total mean As (99.6 mg/L) are indicative of a greater contribution from the As storage side in 2001 compared to 2000.

Dissolved Zn and Cd concentrations were negatively correlated with pH exiting the anoxic cell (r=-0.6 and -0.7 respectively; $p<0.001$). Total and dissolved Zn and Cd concentrations were negatively correlated with pH exiting the anaerobic cell (r=-0.5, -0.8, -0.6 and -0.8 respectively; $p<0.001$). Total and dissolved Zn concentrations were strongly negatively correlated with pH exiting the tree cell (r=-0.8; $p<0.001$), grass cell (r=-0.8; $p<0.001$), *Typha* cell (r=-0.6; $p<0.001$) and in the final holding cell (r=-0.7; $p<0.001$). This is consistent the system pH exhibiting a wide pH range above and below 6.4 that is associated with lower Zn and Cd percent reductions in the Trail system. Willow and Cohen (2003) found that pH was critical to reactor efficiency, more so than DO. As well, metal loading rate capacity can be increased and HRT reduced by modifying the pH of the influent to near neutral. The neutral pH enhanced SRB activity, sulphide production and metal sulphide precipitation.

As well, pH was positively correlated with temperature out the anoxic, anaerobic and Typha cells (r=0.7, 0.8 and 0.6 respectively; $p<0.001$); and in the final holding cell (r=0.8; $p<0.001$). Total and dissolved Zn and Cd concentrations were negatively correlated with temperature exiting the anaerobic cell (cc=-0.5, -0.8, -0.5 and -0.8 respectively; $p<0.001$). Total and dissolved Zn concentrations were negatively correlated with temperature exiting the tree cell (r=-0.5; $p<0.001$), grass cell (r=-0.5; $p<0.001$), and in the final holding cell (r=-0.8; $p<0.001$). These negative correlations of the metals to temperature are consistent the system pH exhibiting a positive response to temperature. While it is believed that reduced pH is the causal factor for the reduced metals removal, the impact of temperature on system operation also appears evident.

Conversely, total and dissolved As concentrations were positively correlated with pH exiting the tree cell (r=0.5 and 0.8 respectively; $p<0.001$), grass cell (r=0.5 and 0.6 respectively; $p<0.001$), *Typha* cell (r=0.5 and 0.6 respectively; $p<0.001$) and in the final holding cell (r=0.5 and 0.6 respectively; $p<0.001$). This hints at an interesting design issue for the vegetated cells where a high pH is beneficial for Zn and Cd retention while a lower pH may be beneficial to As retention.

Some additional correlations noted in 2001 were strong positive correlations of total sulphate with dissolved ammonia through the whole system (ranging from r=0.7 to 0.9; $p<0.001$) which is not surprising the high concentrations of these compounds entering and largely passing through the system. Given the production of ammonia sulphate fertilizer at the TML smelter, the high mean concentrations of ammonia (133 mg/L) and sulphate (2044 mg/L) in the input would be expected. The mean concentrations out of the *Typha* cell of ammonia (108 mg/L) and sulphate (2060 mg/L) indicate minimal treatment of these parameters. Some treatment of ammonia (mean concentration of 65 m/L) does occur in the more oxic conditions of the final holding cell.

Vegetation - Metals Hyperaccumulation and Toxicity in Plants

In August 1999, after a period of high Zn concentrations, the trees in the first plant cell exhibited signs of metal toxicity (e.g., chlorosis) and many were rapidly losing leaves.

Some hybrid willows had died and many hybrid poplars were suffering. Therefore, vegetation samples were collected. In an attempt to alleviate metal toxicity to the trees, the system was flushed for 4 days with clean water in September temporarily reducing Zn concentrations in the plant cells to 11 mg/L. The plant samples were sampled again in October 1999 (when the system was shutdown for winter) to determine their role in metal sequestration and examine issues of metal toxicity to the various plant species

While Zn is a required micronutrient for plants, concentrations experienced by the plant cells proved to be toxic to some plants. In hydroponics solutions for promoting plant growth, Zn is supplied at 0.05 to 0.25 mg/L. The tree cell experienced up to 273 mg/L Zn which caused chlorosis, premature leaf fall and death in some trees. Salisbury and Ross (1992) consider plant tissue concentrations of 20 mg/kg Zn (dry weight basis) as adequate for proper plant growth, where a Zn hyperaccumulator plant would have Zn tissue concentrations of 10,000 mg/kg or 1% dry weight or greater (Baker and Brooks 1989; Baker et al 1991). The mean Zn tissue concentrations were 3413, 3727, 1713, 1815, and 725 mg/kg for willows, hybrid poplars, *Tripsicum dactyloides*, *Calamagrostis canadensis* and *Typha latifolia* respectively were below those of a hyperaccumulator (Table 12). However, these elevated Zn concentrations well over the minimal required for growth and along with the noted chlorosis provide evidence of Zn toxicity in the poplars.

The hybrid poplar trees had over 2 times the Zn in leaves than the popular control (Table 12). The healthy “control” popular was sampled near the plant cells in an area with higher than background soil concentrations of Zn, Pb and Cd due historical air deposition from the nearby lead-zinc smelter. Therefore, higher metal concentrations in the control would be expected than seen in other areas, especially for Cd. The As and Cd concentrations in the hybrid poplars in the cells were lower or comparable to the control poplars. Whereas, the high Zn concentrations in the hybrid poplars compared to controls provide additional evidence for Zn being the primary metal being responsible for the observed toxicity.

Table 12. Zn, Cd, As and Pb (mg/kg dry weight) in above-ground plant tissue samples collected from the three plant cells at the end of August and October 1999.

Plant Species	Cell & Position	Zinc (mg/kg)	Cadmium (mg/kg)	Arsenic (mg/kg)	Lead (mg/kg)
	First Sampling – August 1999				
Coyote Willow	First – Front	2980	32	11	173
Coyote Willow	First – Front	3570	13	8	59
Coyote Willow	First – Middle	3690	10	9	65
Hybrid Poplar	First – Front	2880	15	7	49
Hybrid Poplar	First – Back	4280	16	6	44
Tripsicum dactyloides	First – Front	1980	19	9	79
Tripsicum dactyloides	First – Front	1470	7	11	50
Tripsicum dactyloides	First – Middle	1690	22	10	114
Calamagrostis canaden.	Second – Middle	1750	13	9	79
Typha latifolia	Third – Front	467	3	3	15
Typha latifolia	Third – Middle	638	3	4	24
	Second Sampling – October 1999				
Hybrid Poplar	First- Front	4020	26	9	91
Tripsicum dactyloides	First – Middle	1190	10	6	65
Tripsicum dactyloides	First – Back	890	6	7	54
Calamagrostis canaden.	Second – Front	1430	4	6	30
Calamagrostis canaden.	Second – Middle	1750	9	8	65
Calamagrostis canaden.	Second – Back	2330	12	9	109
Typha latifolia	Third – Front	871	5	8	34
Typha latifolia	Third – Middle	1020	7	6	53
Typha latifolia	Third – Back	629	6	8	43
Poplar (control)	adjacent area	1670	52	7	73

As, Cd and Pb have no known physiological function for plants and are considered to be phytotoxic. Accumulation of these metals is usually expressed as the plant's abilities to accumulate or even hyperaccumulate these elements without suffering irreparable cell damage. A hyperaccumulator plant is defined as dry tissue concentrations of equal or greater than 10,000 mg/kg (1%) for Pb and 100 mg/kg (0.01%) for Cd (Baker and Brooks 1989; Baker et al 1991) and 10,000 mg/kg (1%) for As (McIntyre 1999). By these definitions none of the tested plants could be classified as hyperaccumulators for As, Cd or Pb.

Other elements are essential to plant growth as micronutrients and are accumulated by plants (Table 13). However, for Cr, a metal with no known physiological use in plants, normal plant tissue concentrations of less than 1mg/kg are used to compare to those found in the treatment system (Baker and Brooks 1989). Plants accumulated higher concentrations of essential micronutrients than required by over 5 times for Cu, and over 30 times for Mo and Mn (Table 13). Other essential nutrients (Fe, K, Ca and Mg) are present at up to twice required concentrations. The only essential micronutrient that was low is P. The non-essential metal Cr was present at greater than 40 times the concentration normally found in plants but was comparable to Cr in the control plant.

Table 13. Mean tissue concentrations of metals (mg/kg) found in plant leaf tissue taken from the three plant cells compared plant requirements and a site control.

	Cu (mg/kg)	Mo (mg/kg)	Cr (mg/kg)	Mn (mg/kg)	Ca (%)	Fe (%)	Mg (%)	P (%)	K (%)
Mean	37.1	1.42	41.4	1640	0.74	0.02	0.37	0.12	1.64
(n)	*20*	*12*	*20*	*20*	*20*	*17*	*20*	*20*	*20*
Minimum required	6	0.1	<1.0	50	0.5	0.01	0.2	0.2	1.0
Site control	30.4	2	36	1150	1.85	0.01	0.46	0.18	1.13

A native plant, *Epilobium grandifolia*, had colonized parts of the first two plant cells. Metals analysis of this plant indicate it can accumulate metals with concentrations of greater than 5,000 mg/kg when Zn, As, and Pb are added together. Additional seeding of this plant was done to assess the metals-accumulation potential of this plant.

Metal concentrations in plants were lower in 2000 (Table 14) than in 1999 (Table 12). This is likely due to lower metal concentrations entering the wetland cells after the anaerobic cell where total metal concentrations entering the plants cells decreased from 194, 1.69 and 14.4 mg/L in 1999 to 103, 0.32 and 10.5 mg/L for Zn, Cd and As respectively. In 2000, the perennial grasses (*Tripsicum dactyloides* and *Calamagrostis canadensis)* and willows (*Salix spp*.) had generally increasing metal (Zn, Cd, As and Pb) concentrations throughout the growing season (Table 14). For *Typha latifolia*, Zn

concentrations decreased while other metals increased between July and August. However, for the annual *Epilobium grandifolia*, this trend is reversed with all metal concentrations generally decreasing over the growing period. This annual plant may have been experiencing a dilution effect of the plant's biomass increasing faster than metals uptake over the growing season along with the lower metal inputs. In 2000, *Epilobium grandifolia*, had tissue metal concentrations less than half those in 1999.

The front end of the first plant cell (where metal loading is highest) had been used to test a wide variety of plant species (potential phytoaccumulators) over the initial years of operation. For example in 2001 *Epilobium grandifolia* (fireweed) and *Rheum rhaponticum* (rhubarb) were present in the first cell as experimental plantings. The back half of the first cell is planted with *Tripsicum dactyloides*. The second plant cell is primarily a grass cell with *Calamagrostis canadensis* as the primary grass although there are other plants in the community. The third plant cell is the largest cell and was originally exclusively planted with *Typha latifolia* but has been planted with *Phragmites australis* in areas where die back was noted in the *Typha latifolia* growth. As well, volunteers of other species noticeably several clumps of *Juncaceae* are present. On four occasions over the summer of 2001, 10 species of plants were harvested. The mean metals' concentrations for each species tested for each harvest period was determined (June - Table 15; July - Table 16; August - Table 17; and September - Table 18).

In each sampling *Epilobium grandifolia* is one of the top three accumulators of metals as is *Rheum rhaponticum*. During the first harvest *Spartina pectinata* showed a fairly high concentration of metal accumulation but high values were not seen in subsequent harvests. Generally, the concentrations of all the metals in the cells were higher than those found in the surrounding vegetation, although, as expected, the grasses both in the cells and those used as controls from outside the area of the research site showed minimal metal uptake. This is consistent with the accepted understanding that grasses are not known as accumulators (Morrey 1995; Dahmani-Muller et al 2000). However, *Deschampsia* (a volunteer in the system) accumulated metals up to 10 times more than other grasses (total metal load of 3091 mg/kg in August; Table 17).

Table 14. Metal concentrations (dry weight; mg/kg) in aboveground plant tissue from three plant cells sampled during the 2000 growing season.

Plant Species Sampled	Harvest Month	Zn (mg/kg)	As (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	Total Metals (mg/kg)
Epilobium grandifolia[a]	May	1488	253	21	60	1822
Epilobium grandifolia[a]	June	1215	16	6	39	1275
Epilobium grandifolia[a]	July	1046	8	3	36	1093
Epilobium grandifolia[a]	Sept	1051	11	4	33	1097
Epilobium grandifolia[a]	Aug	1030	9	2	34	1075
Epilobium grandifolia[a]	Oct	987	9	4	49	1048
Epilobium grandifolia[b]	June	1006	28	8	37	1079
Epilobium grandifolia[b]	July	861	9	3	32	904
Mean		**1085**	**43**	**6**	**40**	**1174**
Tripsicum dactyloides	June	313	7	2	8	329
Tripsicum dactyloides	July	332	6	2	8	347
Tripsicum dactyloides	Sept	388	9	2	7	406
Tripsicum dactyloides	Oct	584	8	3	11	605
Mean		**404**	**7**	**2**	**9**	**422**
Salix (streamco)	July	526	6	6	8	545
Salix (streamco)	Aug	879	10	9	11	910
Salix (streamco)	Sept	1164	11	13	15	1202
Salix (streamco)	Oct	928	9	7	22	966
Mean		**874**	**9**	**9**	**14**	**906**
Salix (native)	July	1235	6	7	22	1270
Salix (native)	Aug	1795	7	9	28	1839
Salix (native)	Sept	1875	10	13	39	1936
Salix (native)	Oct	2395	9	12	38	2454
Mean		**1825**	**8**	**10**	**32**	**1874**
Calamagrostis canadensis	June	446	6	1	9	462
Calamagrostis canadensis	July	878	7	2	15	902
Calamagrostis canadensis	Aug	828	7	3	13	851
Calamagrostis canadensis	Sept	1199	9	5	15	1227
Calamagrostis canadensis	Oct	1653	7	7	25	1692
Mean		**1001**	**7**	**4**	**15**	**1027**
Typha latifolia	July	1140	9	1	5	1154
Typha latifolia	Aug	575	11	2	9	596
Mean		**857.5**	**9.5**	**1.3**	**6.8**	**875.0**

[a]*Collected from tree cell* [b]*Collected from grass cell*

Table 15. Mean metal concentration of Zn, As, Cd and Pb (mg/kg dry weight) in above-ground tissues of selected plants (all cells combined) harvested in June 2001.

Plant Species	*n*	Zn	As	Cd	Pb	TOTAL
Vegetation collected from the three plant cells						
Spartina pectinata	1	3090	285	18	29	3422
Rheum rhaponticum	6	2649	245	19	40	2953
Epilobium grandifolia	19	2023	138	10	53	2223
Deschampsia caespitosa	3	1993	36	10	89	2127
Salix (ssp.streamco)	8	1075	12	6	24	1117
Salix (spp. native)	6	1031	18	15	22	1086
Helianthus annuus	1	792	14	9	40	855
Carex marteninsei	1	711	3	2	7	723
Tripsicum dactyloides	3	562	14	2	10	588
Juncaceae	1	323	3	2	5	333
Typha latifolia	12	299	10	1	5	316
Calamagrostis canadensis	3	265	3	2	9	279
Surrounding vegetation near the site for comparison						
Grass (various species)	8	163	5	2	12	182
Epilobium grandifolia	6	172	4	2	9	186
Salix (native)	4	1331	3	26	20	1380
Typha latifolia	3	90	3	1	6	100

Table 16. Mean metal concentration of Zn, As, Cd and Pb (mg/kg dry weight) in above-ground tissues of selected plants (all cells combined) harvested in July 2001.

Plant Species	*n*	Zn	As	Cd	Pb	Total
Vegetation collected from the three plant cells						
Epilobium grandifolia	27	2832	285	20	74	3212
Calamagrostis canadensis	4	523	3	3	919	1448
Rheum rhaponticum	6	1048	15	4	21	1088
Salix (ssp. Streamco)	9	1007	6	5	17	1035
Salix (ssp. Native)	3	743	11	11	21	786
Deschampsia caespitosa	3	647	13	3	13	676
Juncaceae	1	423	3	13	6	445
Typha latifolia	12	400	8	2	8	419
Tripsicum dactyloides	3	359	11	2	6	377
Helianthus annuus	1	274	10	4	21	309
Spartina pectinata	1	182	3	2	11	198
Carex martenensii	1	79	3	1	3	86
Surrounding vegetation near the site for comparison						
Grasses (various species)	8	109	3	1	7	120
Epilobium grandifolia	10	350	3	4	57	415
Salix (ssp. native)	6	896	3	25	10	934
Typha latifolia	12	81	3	1	5	90

Table 17. Mean concentration of Zn, As, Cd and Pb (mg/kg dry weight) in above-ground tissues of selected plants (all cells combined) harvested in August 2001.

Plant Species	*n*	Zn	As	Cd	Pb	Total
Vegetation collected from the three plant cells						
Rheum rhaponticum	7	3147	132	19	38	3335
Deschampsia caespitosa	3	2847	109	19	116	3091
Epilobium grandifolia	17	1892	47	8	96	2043
Salix (native)	7	1078	24	11	19	1133
Salix (sp Streamco	7	980	9	5	15	1009
Helianthus annuus	2	571	19	7	35	631
Juncaceae	3	510	3	12	9	533
Calamagrostis canadensis	7	482	4	3	35	524
Tripsicum dactyloides	8	304	4	1	6	315
Spartina pectinata	7	284	6	2	7	299
Typha latifolia	19	232	8	1	18	259
Carex martenensii	3	207	4	1	6	218
Surrounding vegetation near the site for comparison						
Grasses (various)	8	241	6	1	10	259
Epilobium grandifolia	12	427	4	5	218	654
Salix (native)	6	692	3	25	6	725
Typha latifolia	24	94	3	1	12	110

Table 18. Mean metal concentration of Zn, As, Cd and Pb (mg/kg dry weight) in above-ground tissues of selected plants (all cells combined) harvested in September 2001.

Plant	*n*	Zn	As	Cd	Pb	TOTAL
Vegetation collected from the three plant cells						
Rheum rhaponticum	5	1756	30	7	8	1801
Deschampsia caespitosa	4	1646	15	5	14	1679
Salix (ssp. native)	4	1090	8	8	12	1117
Salix (ssp. streamco)	7	920	5	3	8	936
Juncaceae	2	533	16	1	4	554
Carex marteninsei	3	523	4	1	7	535
Helianthus annuus	4	448	5	4	6	462
Tripsicum dactyloides	3	387	9	3	5	403
Calamagrostis canadensis	6	347	3	2	9	361
Spartina pectinata	6	295	4	1	5	305
Typha latifolia	6	239	4	1	3	246
Surrounding vegetation near the site for comparison						
Grasses (various species)	2	62	3	1	6	72
Typha latifolia	5	347	3	2	12	363
Salix (native)	3	2733	3	66	21	2822

The third cell, originally planted only with *Typha latifolia*, experienced a pronounced lack of growth in the centre section. Plants were stunted and brown at the edges but did not show signs of metal toxicity or necrosis due to high metal loads. Additional nutrients (including Fe and other micronutrients) were added with no result. Dissolved oxygen sampling indicated similar concentrations throughout the cell so did not appear to be the causative factor. Rhizomes examined did not have iron plaque so this too was eliminated as a possible causative agent. No evidence of fungal pathogens on the rhizomes and given the fact that the affected area is localized, the problem is probably not

disease or fungal related. The most plausible explanation is H_2S is being produced in the centre of this cell which is known to be toxic to *Typha latifolia* and other wetland plants (Armstrong et al 1996; Lamers et at 2002). The high subsurface sulphide measurements in the three plant cells in October 2003 ranged from 194 to 3470 mg/L as measured by University of Missouri-Rolla (UMR) personnel (Ye 2006) support this possible explanation. This cell was re-planted in the affected areas with *Phragmites australis* which has adapted well.

Vegetation - Shift to Evapotranspiration and Rhizofiltration

While the original system envisioned hyperaccumulators in the design, the high metals uptake proved toxic to the plants and these plants had several negative aspects. As most hyperaccumulators are annuals they require re-seeding every year thus adding to system maintenance costs. As well, unless harvested annually some of these metals may be subsequently released as the plant ages and dies (Batty and Younger 2004). Additionally, regulator concerns about accumulating high metal concentrations in aboveground tissue that would be accessible by herbivores changed the focus of plants selected for the wetland treatment system. Therefore, the focus shifted to plants that can tolerate and thrive in a high metal environment and that minimize metals uptake in the above ground tissues, have evapotranspiration (high water use) and are suitable for rhizofiltration (abundant root biomass) as opposed to hyperaccumulators.

Many of the perennial grass species (e.g., *Calamgrostis canadensis* and *Tripsicum dactyloides*) and wetland species (e.g., *Typha latifolia*) already established in the system fulfill one or both of these functions very well and are quite tolerant of metals. These three species along with the various volunteer sedges and rushes had the lowest above-ground tissue concentrations for Zn, As, Cd and Pb over the growing season in 2001 (Table 15; Table 16; Table 17; and Table 18). As opposed to the phytoaccumulators their concentrations were generally close to or below the tolerable forage levels for domestic animals of 500-1000, 100, 0.5 and 50 mg/kg air dried forage respectively (NRC 1980). *Phragmites australis* which was planted in the *Typha* cell was determined by Stoltz and

Greger (2002) to be a root accumulator as opposed to a shoot accumulator for most metals and therefore was suitable for phytostabilization of wet-covered mine tailings.

While plants do sequester metal, many researchers have found that accumulation of metals in above-ground plant biomass in common wetland plants account for only a small percentage of the inputted metal loads and the sediment was the main area of metal accumulation. Lesage et al (2007) found that in a *Phragmites australis* HSSF reed bed that only 0.5% and 1.4% of inputted Cu and Zn respectively was retained in the above-ground biomass. Similarly, Nyquist and Greger (2009) found that metal uptake in the plants only accounted for 0.002-2.9% of annual metal loadings to the wetlands.

So beyond metal sequestration in tissue, the reasons for including plants in a wetland system are several. The high summer evapotranspiration rates can treat considerable quantities of water. Peverly et al (1995) found much higher cumulative evapotranspiration (May through September) in planted *Phragmites australis* wetlands (up to 50 cm) versus an unplanted cell (20 cm). These high evapotranspiration rates assisted in rhizofiltration by creating a flow of porewater (leachate) to the root absorptive surfaces creating a build-up of particulate matter consisting of clay minerals, precipitated Fe and microbes (Peverly et al 1995). The release of oxygen from the roots precipitates the Fe to form a plaque. The roots and/or the plaque acts as a filter to metal movement into the rhizomes and above-ground tissues promoting rhizofiltration. Peverly et al (1995) found relative metal concentration decreased in roots moving from the root surface, cortex to the stele (central part of the root containing the vascular tissue) suggesting an internal metal exclusion mechanism in addition to physical/chemical one on the surface. Additionally, plants provide additional carbon to the system and alter the chemistry of the rhizosphere. In surface-flow cattail wetlands, Goulet and Pick (2001) found that vegetated sediments had higher organic content and lower redox potential than non-vegetated sediments. Similarly, Nyquist and Greger (2009) noted that wetland plants increased pH, decreased the redox potential and increased metal concentrations in the sediments suggesting that plants promote metal sedimentation and adsorption. Brix (1997) suggests that macrophytes stabilize the bed surface, provide good conditions for

physical filtration, insulate surface against freezing in winter and provide a huge surface for attached microbial growth but do not increase hydraulic conductivity. He also suggests that macrophytes provide habitat for wildlife and make treatment systems visually appealing.

Additional vegetation sampling of roots and shoots of various species was conducted by UMR personnel in October 2003 in the plant cells (Ye 2006; Table 19). From this sampling they concluded that most of the plants were "excluders" with higher root than shoot concentrations. As well, the plants tested had bioconcentration factors well below 1.0 (0.01 to 0.60) for roots and shoots indicating no potential metal bioaccumulation. Based on the UMR data the root:shoot ratios at Trail were calculated for comparison to other studies (Table 19).

At Trail, the Zn and As root:shoot ratios decreased for both species with generally decreasing Zn and As concentrations in the water through the plant cells and the control site (Table 19). However, the root:shoot ratios for Zn are 10-fold or more lower than found for As even though Zn concentrations in the water are much higher than for As. No similar pattern is observed for Pb but water concentrations of Pb are very low in this system. Considerable variability in root:shoot ratios for Pb, Zn and As is noted for the two species across the other sites is likely reflective of varying metal porewater concentrations and other chemical characteristics (Eh, pH, nutrient, etc.). Deng *et al* (2004) noted that total and DPTA-extractable metal contents, pH, N, P and K in the sediments all affected metal uptake in plants to varying degrees.

Table 19. Calculated or provided mean root:shoot ratios in *Typha latifolia* and *Phragmites australis* for Pb, Zn and As for the Trail wetlands as compared to other sites.

Plant Species	Sample Location	Pb (root:shoot)	Zn (root:shoot)	As (root:shoot)
	Smelter - Trail, BC Canada (Ye 2006)			
Typha latifolia	1st Plant Cell	**8.1**	**5.7**	**267**
Typha latifolia	2nd Plant Cell	**10**	**3.4**	**41**
Typha latifolia	3rd Plant Cell	**3.3**	**1.4**	**15**
Typha latifolia	Control Site	**5.8**	**1.0**	**3.7**
Phragmites australis	1st Plant Cell	**1.5**	**2.4**	**58**
Phragmites australis	3rd Plant Cell	**8.2**	**1.3**	**5.4**
Phragmites australis	Control Site	**4.6**	**1.5**	**1.7**
	Pb/Zn Mine - China (Lan et al 1992)			
Typha latifolia	Pond	**4.9**	**2.9**	*n.a.*
Typha latifolia	Control Site	**2.2**	**2.6**	*n.a*
	Au Mine - NWT, Canada (Dushenko et al 1995)			
Typha latifolia	Downstream Lakes	*n.a*	*n.a*	**13.5**
	Pb/Zn/Cu Mine - Sweden (Nyquist & Greger 2009)			
Phragmites australis	AMD/Tailings	*n.a*	**19**	*n.a*
Phragmites australis	Water/Tailings	*n.a*	**4.3**	*n.a*
Phragmites australis	Control Site	*n.a*	**6.1**	*n.a*
	Boliden Mine - Sweden (Stoltz & Greger 2002)			
Phragmites australis	Tailings Pond	**128**	**19**	**33**
	Landfill Leachate - USA (Peverly et al 1995)			
Phragmites australis	Reed Beds	**136**	**2.8**	*n.a*
Phragmites australis	Control Site	**26**	**3.9**	*n.a*
	Municipal Wastewater – Czech Republic (Vymazal et al 2009)			
Phragmites australis	HSSF Reed Beds	**42**	**3.6**	**29**
	Fankou Pb/Zn Mine - China (Deng et al 2004)			
Phragmites australis	HSSF Reed Beds	**50**	**8.3**	*n.a*
Typha latifolia	HSSF Reed Beds	**25**	**17**	*n.a*

n.a = not available

Bacterial MPN

Most of the metal removal mechanisms in the engineered wetland system are microbially-mediated. Three functional groups of bacteria were sulphate reducing bacteria, fermentative bacteria (or acid producing bacteria) and iron reducing bacteria. SRB are critical to the operation of the anaerobic bioreactor cells as they generate alkalinity and cause metals to precipitate. Fermentative bacteria produce low molecular weight carbon compounds. SRB are obligate anaerobes that use these low molecular weight carbon compounds and hydrogen as electron donors. Most IRB are facultative and can use ferric iron as a terminal electron acceptor in the absence of oxygen.

Appreciable numbers of all three groups of bacteria were found in the surface of the upper cell (Table 20), which indicates that the operation of the cell was satisfactory although considerable variability was noted. High numbers of SRB found in the surface layer also indicate that the entire cell was anaerobic. The second cell had bacterial numbers that were on average an order of magnitude higher than the first cell (Table 20). The higher numbers in the second cell may be that it had been in operation for two years longer than the first cell at the time of sampling. Anaerobic bacteria (SRB and IRB) were also detected in the outlets of the plant cells and the Typha cell, which indicates that these cells also have anaerobic zones (Table 20). It is likely that metal removed in the plant cells may occur by both filtration (removal of suspended solids by the substrate), rhizofiltration (adsorption to and plaque formation on the roots) and by precipitation as metal sulphides in the deeper areas of the cells.

During deconstruction (see Chapter 4 for details), additional samples were similarly taken for SRB analysis. The top cap or surface layer and A (5 to 20 cm) layer were similar previous samples (Table 20). When compared to the new samples taken during deconstruction, bacterial populations were not different in the cap layer or the top biosolids (A) layer. The deeper second (B) layer had 3.5×10^{6} cells/g in one sample

Table 20. Mean microbial populations in the anaerobic bioreactors substrate (cells/g wet weight with ranges) and in plant cell outlets (cells/ml) sampled on July 4, 2001.

Location	Microbial Populations (Mean MPN values)		
	Fermentative Bacteria	**Sulphate Reducing Bacteria**	**Iron Reducing Bacteria**
	Substrate Samples (cells/g)		
First cell: surface	1.3×10^4	1.3×10^6	5.1×10^4
Range (min-max)	$6.8 \times 10^3 - 2.4 \times 10^4$	$3.3 \times 10^5 – 2.4 \times 10^6$	$1.7 \times 10^4 – 6.8 \times 10^4$
First cell: 5-20 cm	2.2×10^6	7.4×10^5	6.3×10^4
Range (min-max)	$6.8 \times 10^5 - 6.2 \times 10^6$	$1.9 \times 10^2 - 2.7 \times 10^6$	$2.4 \times 10^4 – 1.3 \times 10^5$
Second cell: surface	6.4×10^4	8.2×10^6	2.1×10^6
Range (min-max)	$4.9 \times 10^4 - 7.9 \times 10^4$	$3.3 \times 10^6 - 1.3 \times 10^7$	$1.7 \times 10^6 – 2.4 \times 10^6$
Second cell: 5-20 cm	1.1×10^6	1.7×10^7	4.1×10^6
Range (min-max)	$1.4 \times 10^5 - 4.1 \times 10^6$	$2.4 \times 10^6 - 4.9 \times 10^7$	$3.1 \times 10^4 – 7.9 \times 10^6$
	Water Samples (cells/ml)		
Plant cell #1 outlet	n.d.	3.3×10^5	7.9×10^3
Plant cell #2 outlet	n.d.	3.1×10^4	7.9×10^4
Typha cell outlet	n.d.	3.3×10^5	7.9×10^3

n.d. = not done.

whereas in the bottom (C) layer the values ranged from 4.9×10^5 to 1.3×10^7 with a mean MPN of 7.2×10^6 from 4 samples. The wide range of values is indicative of the heterogeneity of the substrate. However, the additional samples were within the previous ranges observed (Table 20).

PLFA - Biomass

Phospholipid fatty acids are found in the membranes of all living cells but decompose quickly upon cell death because cellular enzymes hydrolyze the phosphate group within minutes to hours of cell death. Thus, measuring the total amount of PLFA content provides a quantitative measure of the viable microbial biomass present. Biomass estimates (as determined by the total concentration of PLFA) were relatively high at 2.28×10^8 cells/g dry weight in first anaerobic cell and at 1.02×10^8 cells/g dry weight in

second anaerobic cell (Figure 28). Bacterial biomass is calculated based upon PLFA attributed specifically to bacteria, whereas eukaryotic biomass is based on PLFA associated with higher organisms.

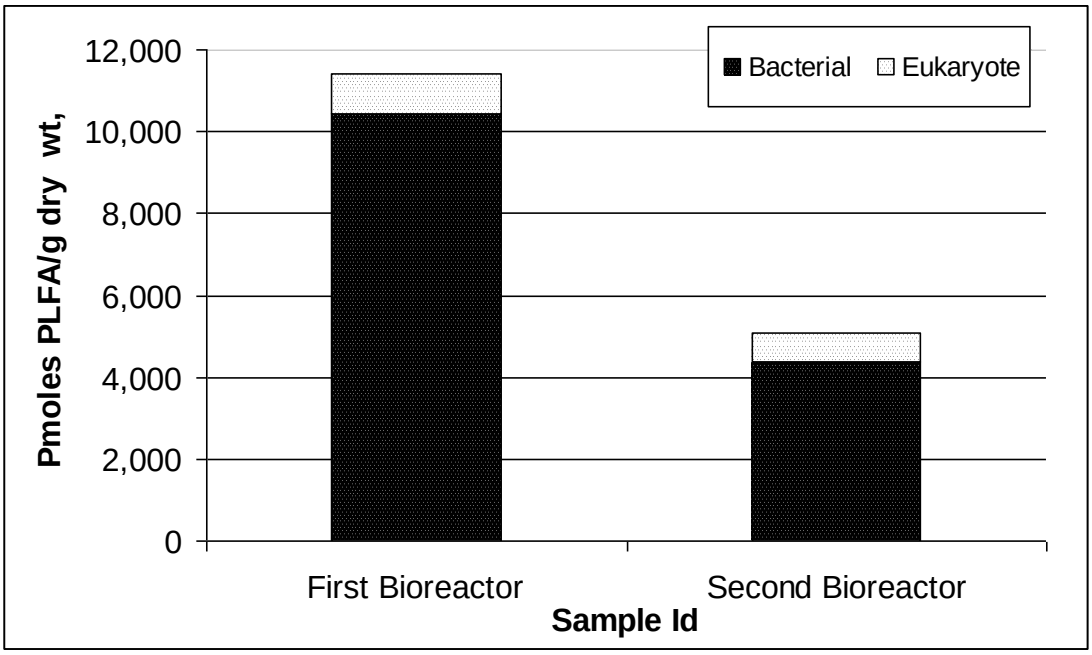


Figure 28. Biomass content is presented as the total amount of phospholipid fatty acids (PLFA) extracted from substrate samples from the two anaerobic bioreactors.

High biomass present can be interpreted as a positive indication of the general health of the bacterial population in the cells. Along with the high bacterial counts (Table 20), these results indicate that the bioreactors are functioning in the high metal concentrations found in these bioreactors. Maximum dissolved Zn, Cd and As concentrations in 2001 entering the first anaerobic cell were 473, 7 and 95 mg/L respectively and entering the second anaerobic cell were 258, 1.6 and 13.0 mg/L respectively (Table 10).

PFLA - Community Structure

The PLFA patterns derived from environmental samples provide a quantitative profile of the microbial population, which accurately mirrors differences in community composition among samples. Specific groups of microbes contain different fatty acid profiles making it possible to distinguish between them. Structural groups are assigned according to PLFA chemical structure, which is related to fatty acid biosynthesis

particular to that group. The PLFA profiles for these two samples revealed relatively diverse bacterial communities, which were dominated by Gram-negative bacteria at 48.8% and at 44.0% for the first and second bioreactors respectively (Figure 29).

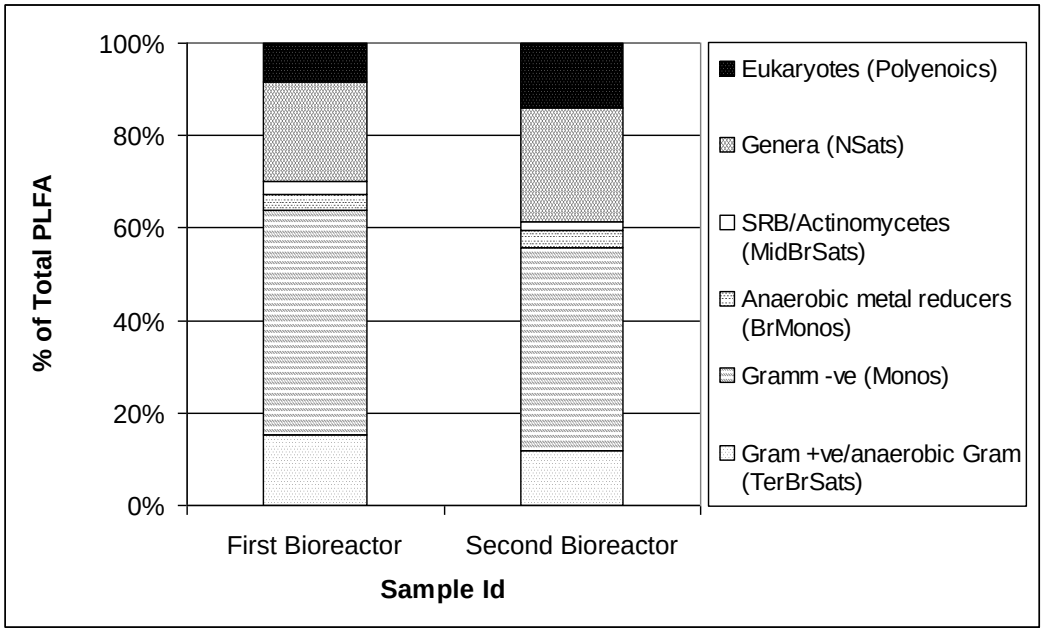


Figure 29. A comparison of the relative percentages of total PLFA structural groups in the substrate samples from the two anaerobic bioreactors.

Terminally branched PLFA were detected in the first and second bioreactors at 15.2% and 11.9% respectively (Figure 29). Terminally branched PLFA are representative of Gram-positive bacteria, but may also be found in the cell membranes of some anaerobic Gram-negative bacteria. Some evidence for anaerobic metal reducing bacteria (branched monoenoic PLFA (3.3-3.6%)) and SRB/Actinomycetes (mid-chain branched PLFA (2.0-2.7%) were present in both samples.

PLFA - Metabolic Activity

Lipid composition of microorganisms is a product of metabolic pathways that reflects phenotypic responses of the organisms to their environment. Knowledge of specific lipid biosynthetic pathways can provide insight into the metabolic activity of the microbial community because certain fatty acids provide indications of turnover rate and physiological responses to environmental conditions. Specifically, Gram-negative

bacteria form cyclopropyl fatty acids (f.a.) (cy17:0 & cy19:0) preferentially over monoenoic f.a. (16:1w7c and 18:1w7c) as the turnover rate decreases. Gram-negative bacteria also generate *trans* fatty acids to minimize the permeability of their cellular membranes as an adaptation to less favourable environments (White and Ringelberg 1995). Ratios of cy/ω7c and ω7t/ω7c can be used as quantitative indicators of how a portion or all of the Gram-negative community is responding to environmental factors (toxicity, starvation, etc.) and/or any engineered treatments or modifications.

Ratios of fatty acid biomarkers that provide indications of activity showed that the Gram-negative community in the second bioreactor had the fastest turnover rate of the two but values were not indicative of fast growth rates (Figure 30). The fatty acid ratios

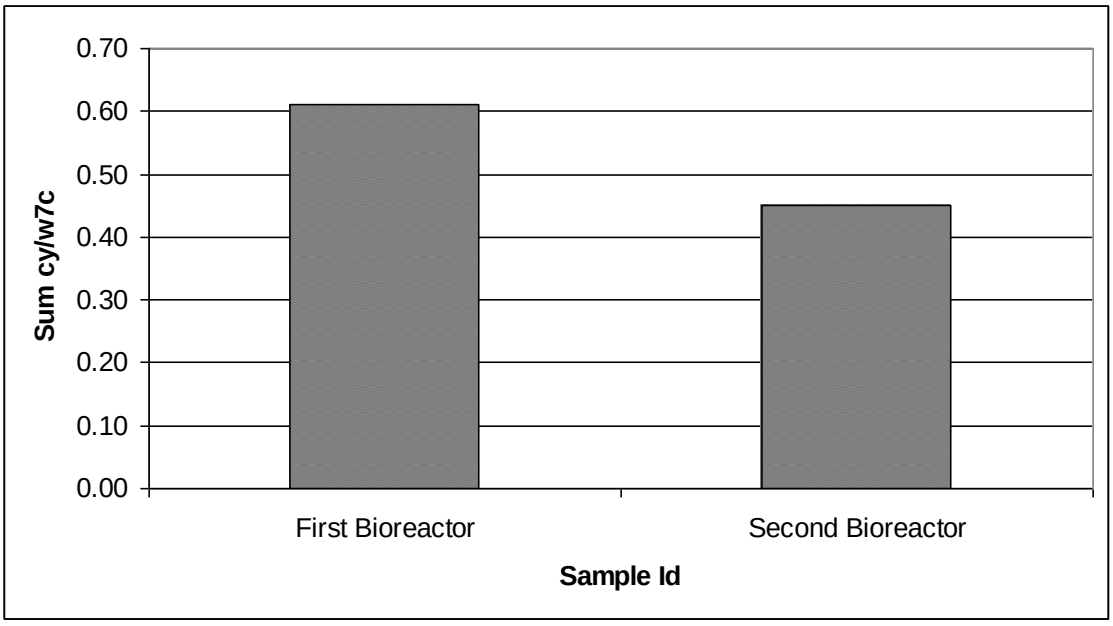


Figure 30. Growth rate of the Gram-negative community as assessed by the ratio of cyclopropyl f. a. to ω7c f. a. from substrate samples from the two anaerobic bioreactors.

are greater than 0.15 which indicates slowed growth rates, whereas ratios less than 0.05 are taken to indicate fast growth rates. Specifically, 16:1ω7c and 18:1ω7c fatty acids are converted to cyclopropyl fatty acids (cy17:0 & cy19:0) as microbial growth slows, therefore, a high ratio indicates decreased cellular turnover rate. Lower ratios would be

anticipated in samples at depth where living conditions to anaerobes would be more suitable. Neither sample contained detectable *trans* fatty acids which suggests that these samples were not forced to respond to conditions of environmental stress (toxicity, starvation, etc.).

SRB MPN

SRB populations were analyzed using a MPN technique using a sulphate reducing medium and incubated for 21 days. MPNs of SRB were estimated at 10^3 and 10^4 cells/g dry weight in the first and second anaerobic cells respectively. These results are consistent with the low proportion of mid-chain branched PLFA (2.0-2.7%) found in the samples. However, the results are lower than found by Dr. Gould (Table 20) which could be due to sample heterogeneity or differing sampling methodology.

DGGE Analysis - Bacterial Composition

Bacterial profiles for these two samples showed that both samples contained relatively diverse bacterial communities (Figure 31). Banding patterns and relative intensities of the recovered bands provide a measure of differences among the communities. The second bioreactor has the most diverse and developed community of the two reactors. This could be due its longer operating time, as well, the lower dissolved oxygen concentrations experienced by this cell promoting a more diverse anaerobic bacterial community.

Labelled bands were excised and sequenced (Table 21). Similarity indices above 0.900 are considered excellent, those between 0.700-0.800 are good, and those between 0.600-0.700 are fair to be considered as unique sequences. Bands A and E appeared to represent one of the more dominant member of the bacterial communities. Sequence analysis of these bands indicated that this organism was associated with cytophaga (clone WCB1-29). Since the remediation of metals is of interest in these samples, discussion of each organism will refer to instances in which these types of bacteria have been used for this type of remediation (particularly arsenic). Honschopp et al (1996) described an arsenic-resistant and arsenic-methylating bacterium within the cytophaga subgroup.

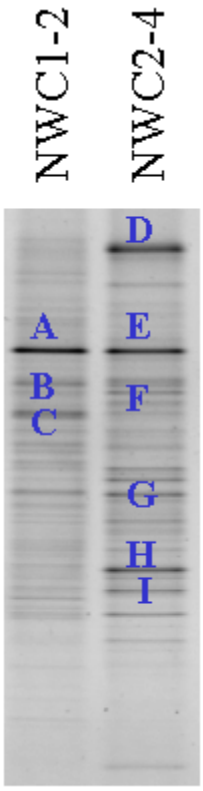


Figure 31. DGGE gel image of amplimers from a conserved region of bacterial 16S rDNA from two substrate samples (nwc1-2 = first bioreactor; 1nwc2-4 = second bioreactor).

Two minor bands (B and C) within the first bioreactor also produced useable sequence information. Band B represented a rather novel bacterium associated with several sulphate-reducing bacteria. There are numerous studies to date that have indicated the ability of sulphate-reducing bacteria to methylate arsenic and even some indicating the methylation of mercury (Loydd and Oremland 2006). Band C appears to represent a rather novel organism that is somewhat related to the genus *Thiomicrospira*. Currently there does not appear to be any published information relating members of this genus with arsenic or chromium reduction. Members of *Thiomicrospira* are nitrate-reducing and sulphide-oxidizing bacteria.

The sample from the second bioreactor contained more bands, which could be excised and sequenced, than in the sample from the first bioreactor. Band D appeared to be a fairly dominant member of this community based upon band intensity. This band

Table 21. Sequence results from bands excised (see Figure 31) with identifications based on information in the Ribosomal Database Project (RDP).

Band	Closest Match	Similarity Index	Phylogenetic Affiliation
	Isolated from the first bioreactor		
A	Clone WCHB1-29	.792	Flexibacter-Cytophaga-Bacteroides
B	Clone WCHB1-67	.628	Delta Proteobacteria
C	Thiomicrospira denitrificans	.654	Epsilon Proteobacteria
	Isolated from the second bioreactor		
D	Prototheca wickerhamii	.606	Unclassified
E	Clone WCHB1-29	.813	Flexibacter-Cytophaga-Bacteroides
F	Failed	-	-
G	Strain 2a/ Unidentified bacterium	.843	Alpha Proteobacteria
H	Nitrobacter sp./ Bradyrhizobium sp./ Afipia sp./ Photorhizobium sp.	.970	Alpha Proteobacteria
I	Bradyrhizobium sp.	.807	Alpha Proteobacteria

represented a rather novel organism that was loosely associated with bacteria found in chloroplasts. Bands G, H, and I were all closely associated with alpha proteobacteria (G; *Sphingomonas* and H and I; *Bradyrhizobium*). Macur et al (2001) reported members of *Sphingomonas* and *Rhizobium* having the ability to reduce arsenic.

Similar molecular analysis of organic substrate from the Lily/Orphan Boy demonstration project after 11 years of operation confirmed the presence of SRBs and a diverse bacterial community (Nordwick 2008). Using BLAST, the returned sequenced

results included two SRB (*Chloroflexi bacterium* and *Flexibacter sp.*) and a sulphur reducing bacteria (*Thermococcales archeon*). Other sequences included three sulphur oxidizers, three compost isolates, four Protobacteria and one Fe/Mn-reducing anaerobe. Nordwick (2008) recommended repeating the analysis into the future so that any changes occurring in the microbial community could be documented. These changes could be used to predict microbial behaviour in other SRB projects and allow for optimization of this technology.

Surface samples displayed a diverse bacterial population, some of which are SRB. However, the SRB MPN that Microbial reported are lower than the numbers reported by Dr. Gould. The combination of spatial heterogeneity separate samples (i.e., not split samples) and differences in transport time to and handling techniques in the laboratories could be responsible for the differences that were seen.

Microbial Insight's findings regarding MPN are internally consistent. They found corroboration for their low SRB populations in the fatty acid assays that were completed where the low presence of branched monoenoic and mid-chain branched phospholipid fatty acids supports their finding that there were few SRB in the sample. Lower SRB numbers result in lower levels of PFLA

Summary of the PLFA, MPN and DGGE analyses revealed the following:

- Biomass estimates provided by the total concentration of PLFA were fairly high in both samples (~10^8 cells/g dry wt.)
- PLFA profiles indicated that both samples contained relatively diverse microbial communities, which were primarily composed of Gram negative bacteria.
- Some evidence for anaerobic sulphate or iron reducing bacteria was found in the PLFA profiles. However, proportions of these fatty acids were fairly low. MPN estimates reiterate this finding with cell estimates for sulphate reducers ranging from 10^3 to 10^4 cells/g dry wt.

- Bacterial profiles provided by the DGGE analysis showed relatively diverse bacterial communities in each sample. Several of these organisms represented rather novel bacteria with only loose affiliations with certain groups.

While these techniques can provide valuable information, the number of samples was very limited. Complete bacterial characterization would require additional research beyond the scope of this thesis. Other researchers are expanding on bacterial community analysis by a variety of techniques (e.g., various genomic tools) at the Trail site.

Mineralogical Results

While one metal removal mechanism for zinc, cadmium and lead is the precipitation of these metals as their sulphides, some removal of these metals may also occur by precipitation as carbonates and adsorption to the pulp mill solids. Arsenic as an oxy-anion will behave differently than the other metals. Some strains of SRB can reduce both sulphate and arsenate to produce the mineral orpiment (As_2S_3) as shown by Newman et al (1997a). The solubility of orpiment is controlled by both the sulphide concentration and the pH. A yellow precipitate found in the input distribution pipes to the second anaerobic bioreactor had the proper stoichiometric ratios of As:S of 2:3. Because the cells also contain calcium carbonate some of the arsenic may also precipitate as calcium arsenate. Preliminary mineralogical work indicates the arsenic is present in the cells as amorphous arsenic sulphides and zinc arsenate. Jong and Parry (2003) described the possibility of arsenic sulphide precipitation or the concomitant removal of arsenic with the zinc and iron sulphides in their bench scale upflow anaerobic packed bed reactors.

Samples of the compost substrate from the second anaerobic cell were examined for their mineral composition using Scanning Electron Microscopy/Energy Dispersive Spectroscopy (SEM/EDS) analysis using carbon coated natural or polished surfaces. Sand grains containing various iron oxides (e.g. magnetite (Fe_3O_4)) are common in the substrate (Figure 32). As iron is liberated from the sand or other sources in the substrate, the iron can be re-precipitated as amorphous FeS found on a calcite mineral (Figure 33 b), incorporated with As, Zn and Si in a crystalline structure (Figure 34 a), form an amorphous FeO associated with organic matter along with other elements Fe(Zn, As, and

Mn) Ca, P, Al, Si, & O (Figure 35 a) or as non-crystalline particles in association with ZnS (Figure 35 b).

ZnS was found as a pure crystalline structure (Figure 33 a), in association with Al, Si, K, Ca, Fe and As (Figure 34 a), or as an amorphous sulphide in association with other elements such as Ca, Al, Si, and Fe (Figure 35). Arsenic was often found in association with Zn and S (Figure 34; Figure 35). ZnAs(Mn) mineralization was found which could be zinc arsenate (Kottigite) with some minor Mn substitution (Figure 34 b). While this preliminary mineralogical work corroborates the formation ZnS, FeS and possibly As_2S_3, it also highlights the complex precipitation and co-precipitation reactions occurring in the anaerobic bioreactor. The stability of these precipitates is crucial to the treatment processes and long-term retention of the various metals within the treatment cells.

Summary

Generally high metal removal rates were found for total and dissolved Zn, Cd and As at greater than 97%, 99% and 98% respectively when the system was operated within designed loads. When overloaded in 1999 the treatment rates for total and dissolved Zn dropped to 74.8% and 69.4% respectively. Lowered treatment rates were also found in winter operation of 2001-2002, especially for Zn (as low as 38.3%). While system had good mean removal rates, considerable day-to-weekly variation is observed likely partially due to varying input concentrations but also likely related to precipitation and temperature events. However, the system did display resiliency to changing loads and can recover from overloading events. The final holding pond was useful in buffering or averaging out the short term variability. Adsorption was observed to be a significant metal removal mechanism but was short term in nature as adsorption sites were quickly used up. The HRT is an important parameter to overall system treatment efficiency.

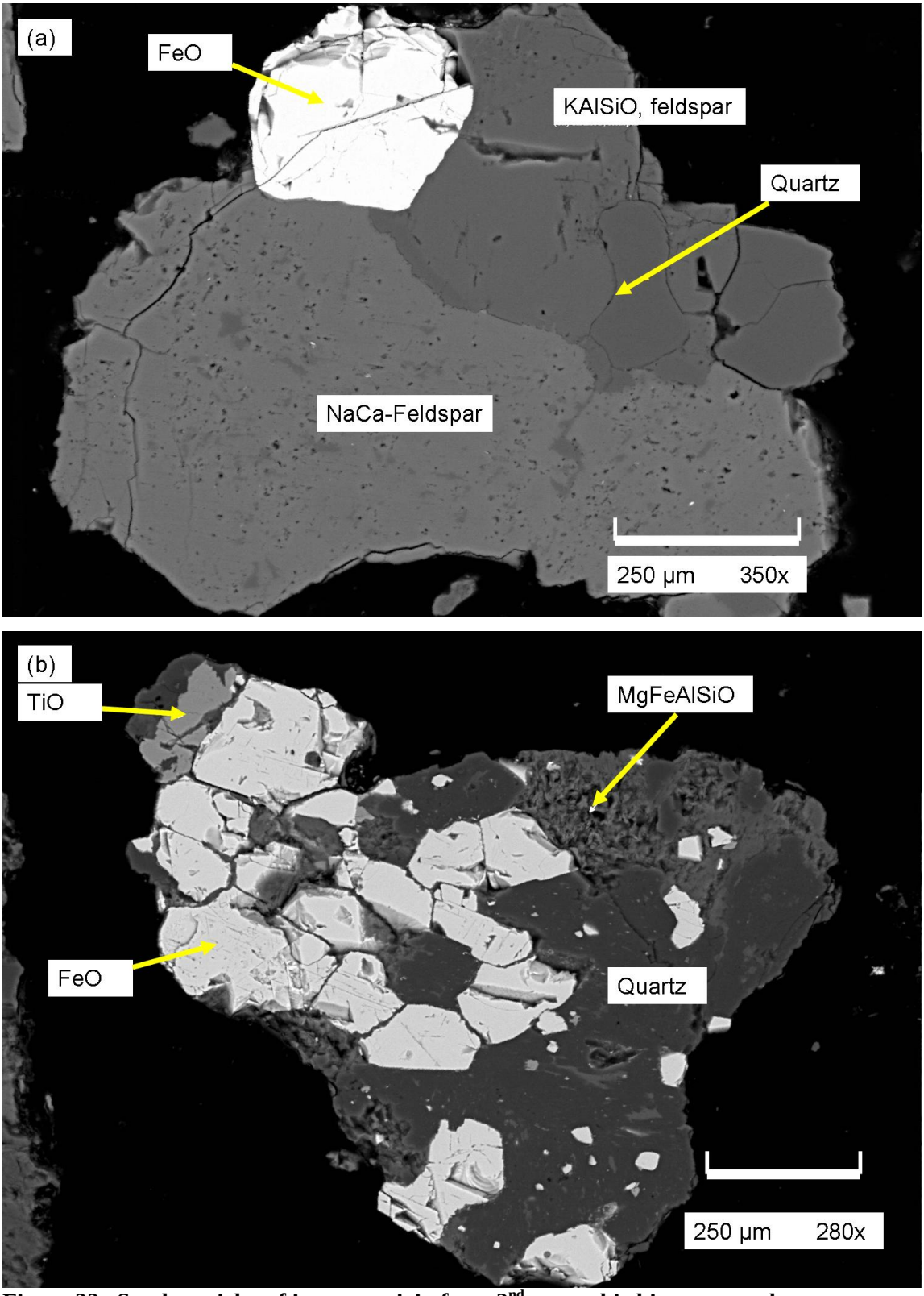


Figure 32. Sand particles of igneous origin from 2nd anaerobic bioreactor substrate consisting of (a) quartz (SiO_2), Na-K-Ca feldspars and magnetite (Fe_3O^4) and (b) Titanite (TiO) - SEM/EDS, carbon coated, polished surface.

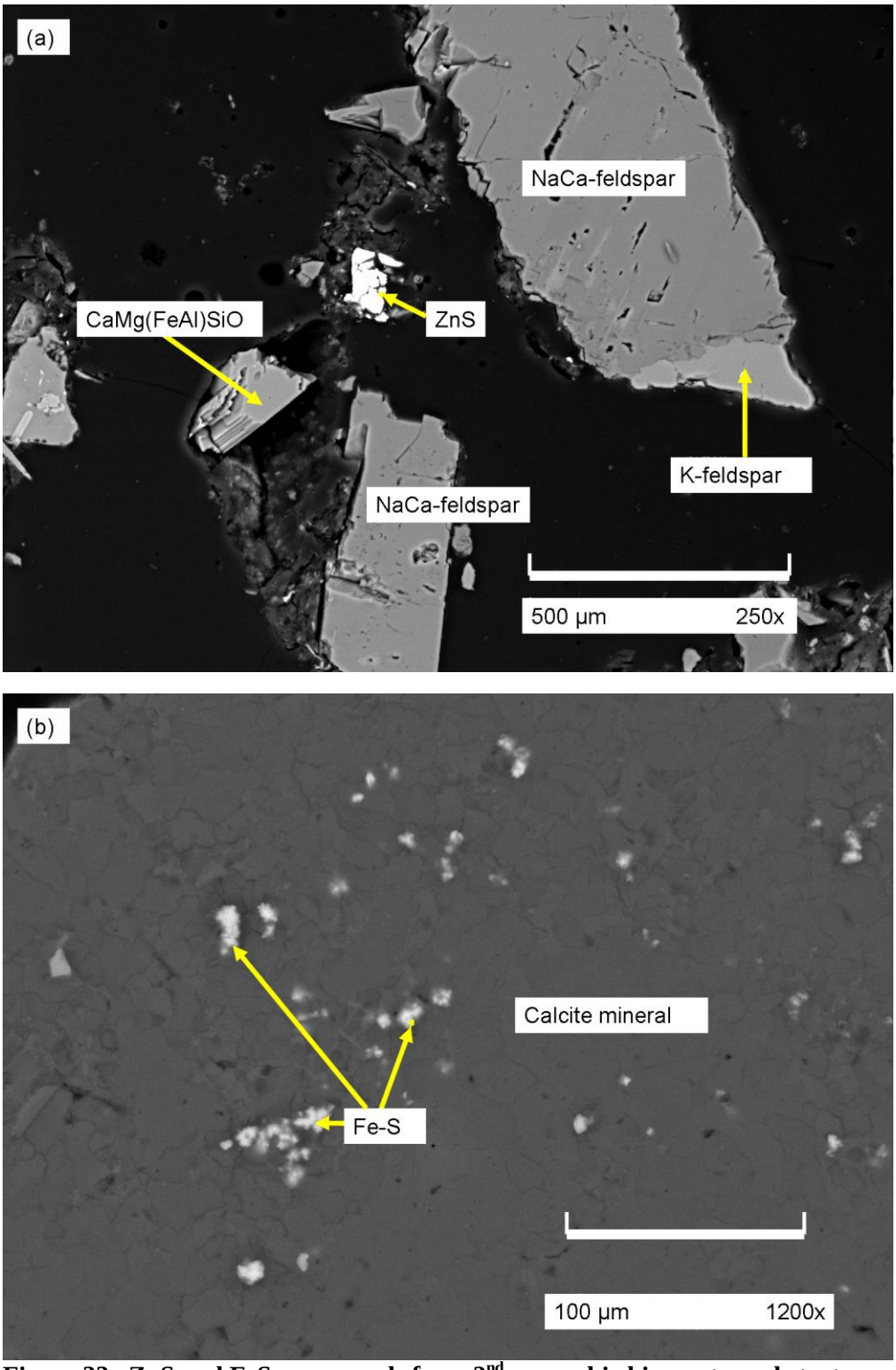


Figure 33. ZnS and FeS compounds from 2nd anaerobic bioreactor substrate consisting of (a) sphalarite (ZnS) and (b) FeS particles in association with carbonate (calcite) - SEM/EDS, carbon coated, polished surface.

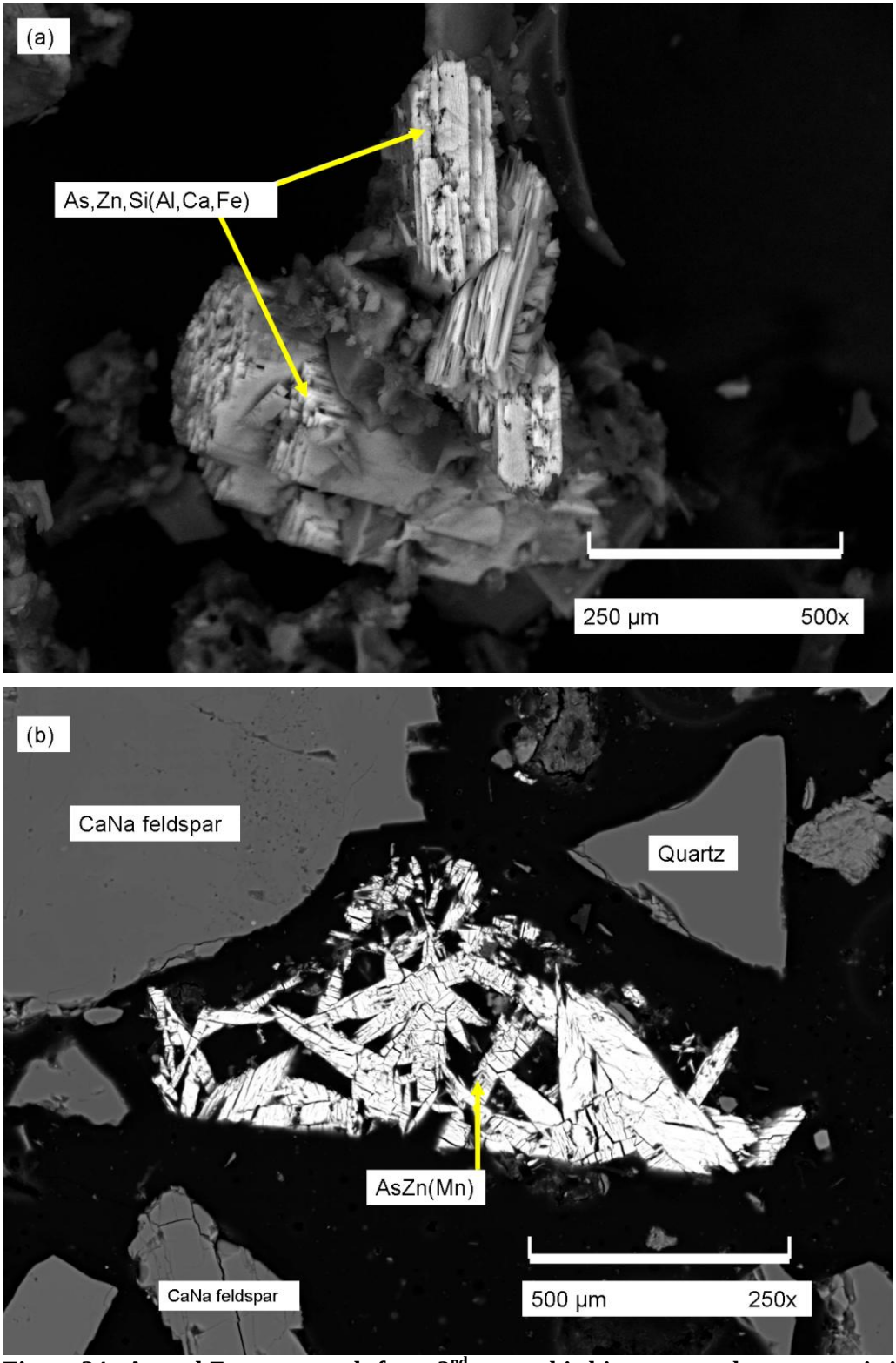


Figure 34. As and Zn compounds from 2nd anaerobic bioreactor substrate consisting of (a) AsZn-Si compound and (b) AsZn(Mn) compound (possibly Kottigite with some Mn substitution) - SEM/EDS, carbon coated natural surface (a) and polished surface (b).

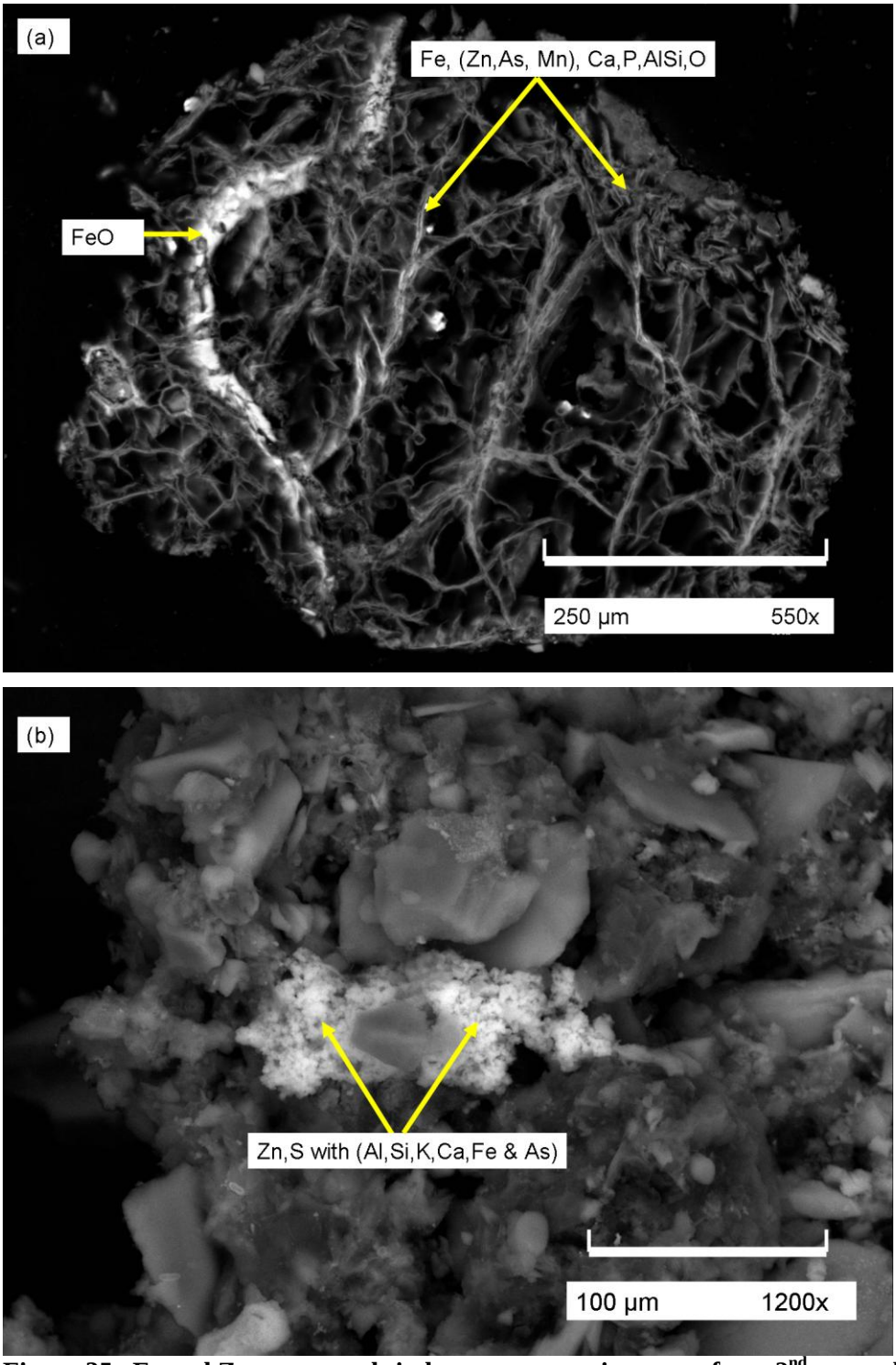


Figure 35. Fe and Zn compounds in lacy type organic matter from 2nd anaerobic bioreactor substrate consisting of (a) iron compounds and (b) finely disseminated amorphous ZnS with Ca, Al, Si and Fe associations - SEM/EDS, carbon coated polished surface (a) and natural surface (b).

The hyper-accumulating plants originally planted in the system reached much higher tissue metals concentrations than control plants; however, they have inherent issues related to their non-perennial nature, small biomass, weedy nature and lack of acceptance by regulators in an open-ecosystem. The wetlands were switched over to more typical wetland species and various high biomass grasses with a focus on evapotranspiration and rhizofiltration and minimal uptake into the above-ground tissues. While trees are very effective at evapotranspiration and can sequester metals into their woody tissue, they could not handle the high Zn concentrations in the system. *Typha latifolia* and *Phragmites australis* both showed preferential metal accumulation at their roots compared to above-ground biomass.

Analysis of microbiological community in the bioreactors (MPN, PLFA, DGGE) showed diverse, well established bacterial populations with cells/g ranging from 10^4 to 10^7 of SRB, IRB and fermentative bacteria even at the high total (to 1 g/L) and dissolved (up to 0.5 g/L) metal concentrations found in the input to the cells. There were minor differences in bacterial populations seen between the anaerobic cells or depth in the cells. SRB activity occurring in the primary and secondary bioreactors is seen as one of the metal removal mechanisms through precipitation of insoluble metal sulphide. Filtration of other insoluble metal precipitates is another potentially important mechanism, for example with the mineral Kottigite ($Zn_3(AsO_4)_2$).

Analysis by SEM/EDS confirmed the presence of sand and its associated iron minerals in the bioreactor (Figure 32). As well, various sulphide mineralization precipitates (particularly Zn and Fe) were observed (Figure 33; Figure 34). The amorphous nature of some sulphides and their association with the organic matter observed (Figure 35) are indicative of recent formation within the cell. Amorphous FeO precipitates were also observed with coprecipitation of other metals (Figure 34 a; Figure 35 b).

Chapter 3 – System Performance from 2002 to 2007

Introduction

The complete system as of the summer of 2002 consisted of five self-contained treatment cells, a slow sand filter and final holding pond that had been successfully winterized for full year-round operation (Figure 5). This chapter will focus on the seasonal treatment rates obtained between 2002 and 2007. The results presented are adapted from (Duncan et al 2008) and further expanded in this chapter. Flow rates are generally 15,000 to 21,000 L/d during the summer (May through September) and 7,000 to 15,000 L/d during the fall-winter (October through April).

Methods - Sampling and Analysis

Regular sampling has taken place since the system became operational year-round. Initially, sampling frequency was three times weekly during summer months and then weekly during spring and summer and bi-weekly during winter operations. By 2005 sampling was reduced to monthly sampling year-round. After flushing the sampling lines by several volumes, grab samples were collected into Nalgene® HDPE plastic bottles at the input and output of each cell in the system corresponding to sampling points one (SP1) through eight (SP8) (Figure 5). The unfiltered non-acidified grab samples were immediately sent to Teck Analytical Services (Trail, B.C) for analysis. Field and travel blanks along with a random replicate sampling were performed and analyzed for each sampling date.

Samples were assayed for both total and dissolved metals using inductively couple plasma mass spectrometry (ICP-MS). For analysis of total metals, a 2.5 mL aliquot of sample is added to a graduated 30 mL polypropylene tube containing 0.5 mL of nitric acid (Environmental Grade, Anachema Science, Lachine, Quebec, Canada) and placed in a hotblock digestion system (Environment Express, Mt. Pleasant, SC, USA) for 1 hour set at 115^{o}C. Samples are then cooled, made up to 30mL volume with de-ionized water and analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS: Agilent

model 7500ce, Santa Clara, CA, USA). Samples for total sulphur are prepared as above and analyzed by Inductively Coupled Plasma Optical Emission Spectrometry (Varian model 735ES, Palo Alto, CA, USA) and reported as total SO_4. For analysis of dissolved metals, a comparable sample aliquot is filtered using a 0.45 micron nylon syringe filter (VWR International, West Chester, PA, USA). The filtered sample is then prepared and analyzed as above. No changes in analytical procedures or instrumentation were experienced over the five year period. Blanks, certified reference standards and controls are employed for quality assurance in the laboratory along with calibration standards and calibration verification procedures. Reference standards and calibration verification must be within 10% of the stated values. While measurement of other water chemistry parameters (including alkalinity, and other cations and anions) would have been useful in assisting in interpretation of results limited funding and lack of availability other than routine procedures through Teck Analytical Services prevented this.

Samples were also tested for suspended solids (gravimetric) and lab pH and conductivity (meter). In the field, samples were tested for pH, dissolved oxygen, and conductivity using portable field meters on a campaign basis. Meters were calibrated prior to each sampling event. Flow rates into the system are maintained by a digital timer that is used to control pump operations and flow rate is monitored by an accumulating digital flow meter installed at the central collection sump. Flow rates between treatment cells were only monitored manually on an occasional basis, where possible. Lack of electricity at the site and the lack of full pipe flow precluded the use of inline flow monitors. Although attempts were made to use passive accumulating meters, these proved unreliable. Therefore, hydraulic retention time can only be inferred which unfortunately limits the ability to fully interpret the water chemistry data. Statistical analysis was performed using Statgraphics© Centurion XV (Version 15.2.00).

With respect to quality assurance and quality assurance for both field and travel blanks, the parameters analyzed were generally below detection for greater than 90% of the samples collected (maximum $n = 131$). Iron and zinc were the elements most often exceeding detection limits in the field and travel blanks, with the detection limits

exceeded more often in the field blanks as compared to travel blanks. The relative percent difference (RPD) between field replicates was generally below 15% for most parameters. However, the median RPD was 39%, 18% and 22% for total suspended solids (TSS), total arsenic and total iron respectively. As well, RPD for total metals was always higher than for the dissolved metals for all metals. This is not surprising given the difficulty in collecting representative water samples high in TSS. When the samples were low in TSS the RPD was generally very good (i.e. well below 15%).

Results and Discussion

While metals, sulphate and ammonia concentrations generally decline through the system as noted in Table 22, there was considerable variability in metal concentrations observed over time in input and final concentrations over the six years examined with a general overall decline (exclusive of the peak) as illustrated by total and dissolved Zn concentrations (Figure 36). While similar graphs for As (Figure 37) and Cd (Figure 38), confirm similar decreases and variably as seen for Zn and, as well, Zn concentrations are highly correlated with As concentrations particularly in the input seepage (Figure 36; Figure 37). Some gaps in the data set are due to pump failures, labour disruptions or frozen sample lines (Figure 36; Figure 37; and Figure 38).

Pearson Product-Moment Correlation analysis of the measured water quality parameters (maximum n=131) was done for each sample point and only statistically significant results ($p<0.05$ or lower) and a Pearson correlation coefficient (r) > 0.5 are discussed. While the large decreases in total and dissolved Zn and total suspended solids (TSS) concentrations can be observed (Table 22) as the seepage (SP1) is treated through the anaerobic bioreactors, the wetland cells, the slow sand filter and ultimately the final holding pond (SP8), the variability seen makes visual interpretation difficult (Figure 36). Large differences between total and dissolved Zn often seen in the input seepage are greatly reduced after complete treatment in the final holding cell, where the total and dissolved Zn concentrations track very closely (Figure 36). The sand filter appears to be functioning well (SP 6 versus SP7) and brings total and dissolved Zn concentrations in

Table 22. Mean values of measured parameters at sample points SP1-SP6 and SP8 (Figure 5) for the 2002 to 2007 sampling period (units as mg/L except pH and conductivity (mS)).

Parameter	SP1	SP2	SP3	SP4	SP5	SP6	SP8
pH	5.7	6.3	6.6	6.6	6.8	6.8	7.5
Conductivity	2.8	3.2	3.2	3.3	3.2	2.9	2.2
DO	4.2	2.1	2.5	2.1	2.5	3.2	5.6
TSS	385	282	82	25	22	78	43
T As	128	23	6.7	10.9	6.5	3.9	0.95
D As	36	8.3	2.7	4.1	2.5	0.79	0.47
T Cd	4.65	0.69	0.15	0.03	0.02	0.02	0.01
D Cd	2.32	0.13	0.05	0.01	0.01	0.01	0.01
T Mn	25	24	21	21	21	22	7.1
D Mn	22	22	20	20	20	20	6.2
T Fe	9.1	27	18.1	7.7	5.7	16.5	1.3
D Fe	0.88	13.1	5.1	3.1	1.7	0.64	0.18
T Sb	0.45	0.11	0.06	0.06	0.05	0.03	0.02
D Sb	0.25	0.09	0.05	0.06	0.05	0.03	0.02
T Zn	289	66.7	35.7	32.3	22.5	9	2.5
D Zn	166	43	30.6	31.6	23.4	7.4	1.2
T SO4	1463	1173	1145	1146	1143	1137	914
D NH3	66	69	59	54	48	28	18

close alignment. Indeed measured total Zn, As and Cd concentrations at SP 7 are highly ($r > 0.9$) and significantly ($p<0.001$) correlated with their respective dissolved metal concentrations. Total Zn and TSS are highly ($r=0.9$) and significantly ($p<0.001$) correlated in the input seepage where TSS is more related to chemical precipitates, whereas, in the final holding cells, the TSS is likely composed largely of organic material and therefore does not correlated with total Zn concentrations (Figure 36). A large peak event is observed in the fall and winter of 2005 and this has been used to separate the performance of the wetlands into three distinct periods as discussed below. The limited samples in the summer period of 2006 are due to pump failures caused by the abrasive nature and high load of chemical precipitates related to the peak event.

Similar large decreases in total and dissolved As and total dissolved solids (TSS) concentrations can be observed (Table 22) as the seepage (SP1) is treated through the anaerobic bioreactors, the wetland cells, the slow sand filter and ultimately the final

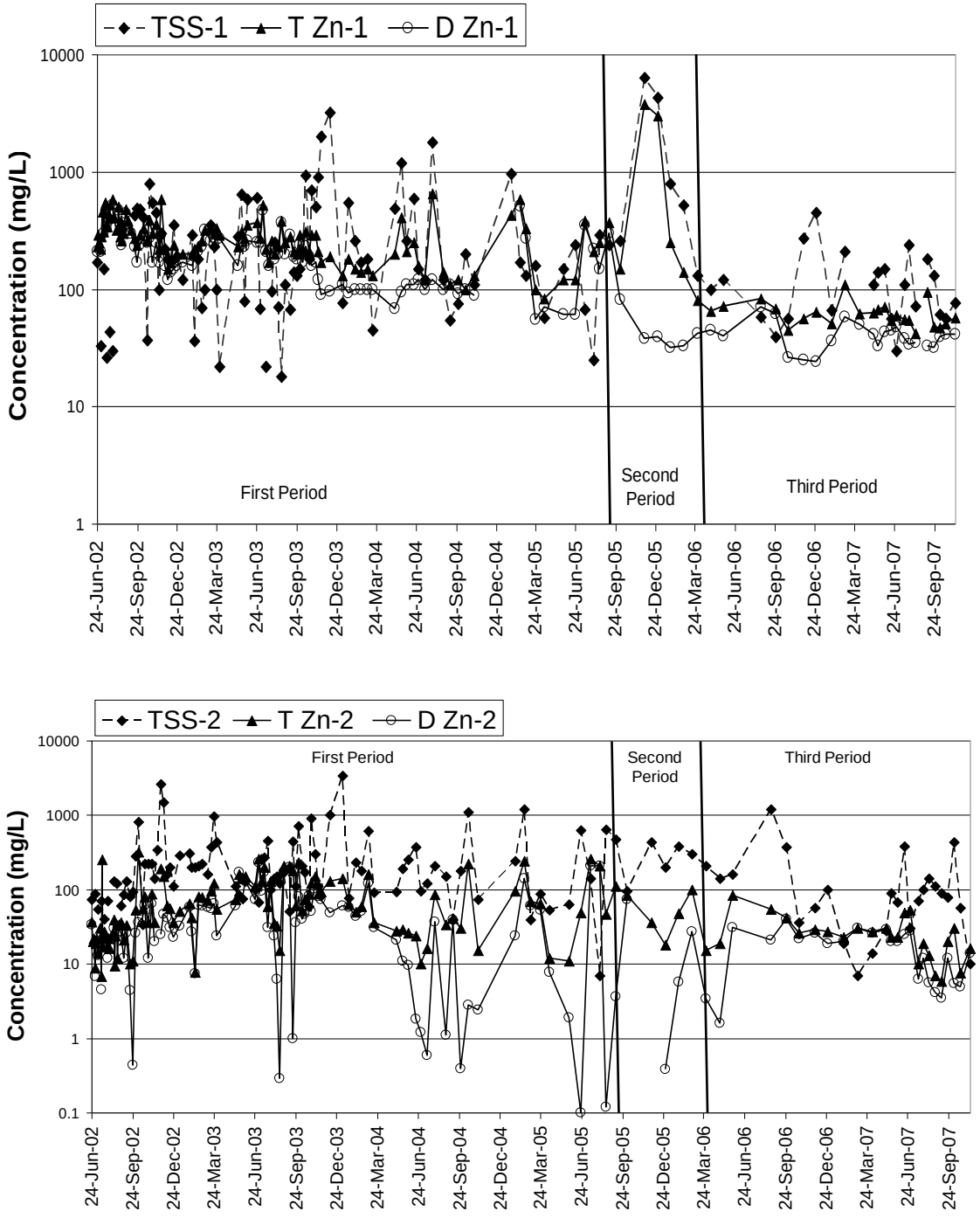


Figure 36. Concentrations of total suspended solids, total and dissolved Zn (mg/L) versus sample dates (June 24, 2002 to November 10, 2007) for sample points 1 through 8 from Figure 5.

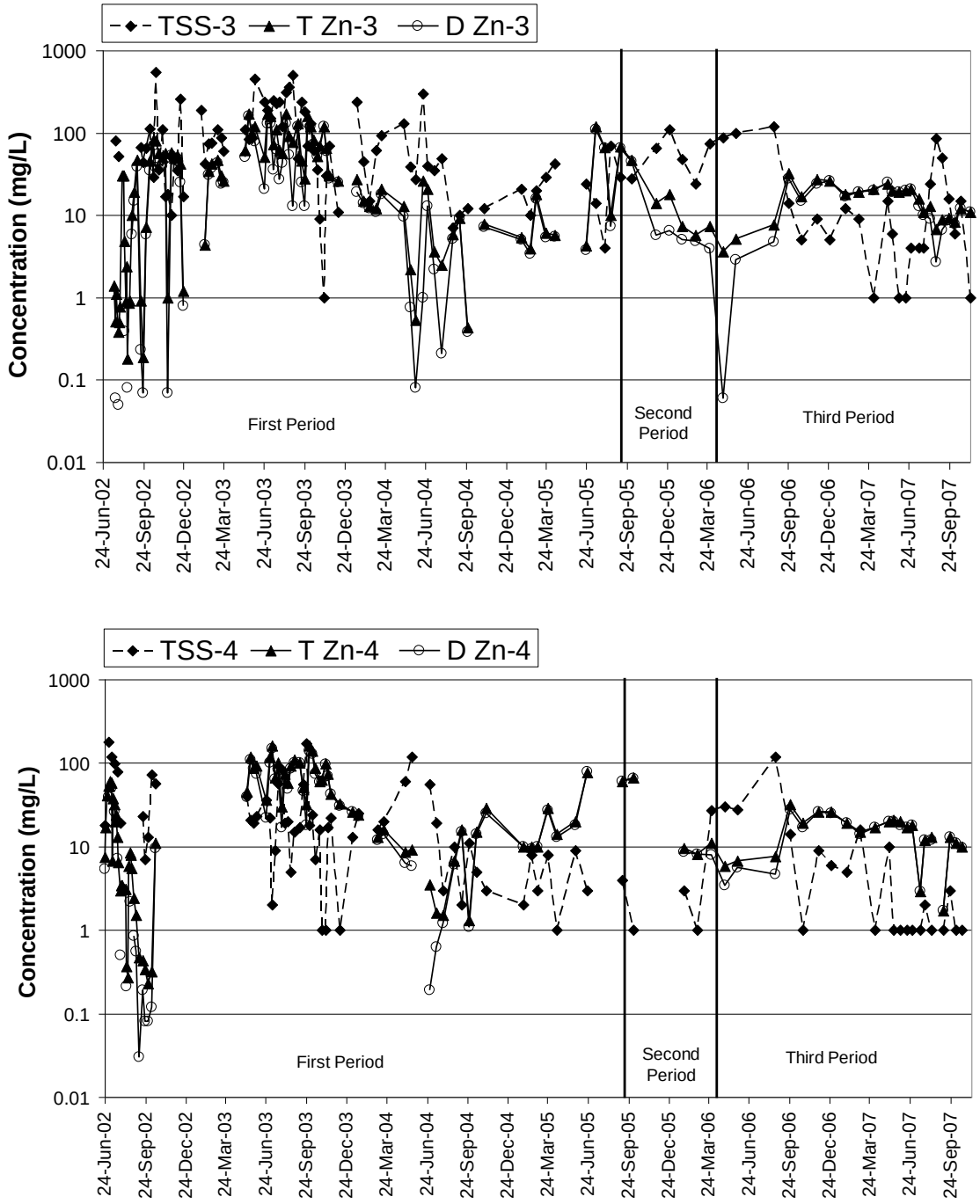


Figure 36 (continued). Concentrations of total suspended solids, total and dissolved Zn (mg/L) versus sample dates (June 24, 2002 to November 10, 2007) for sample points 3 and 4.

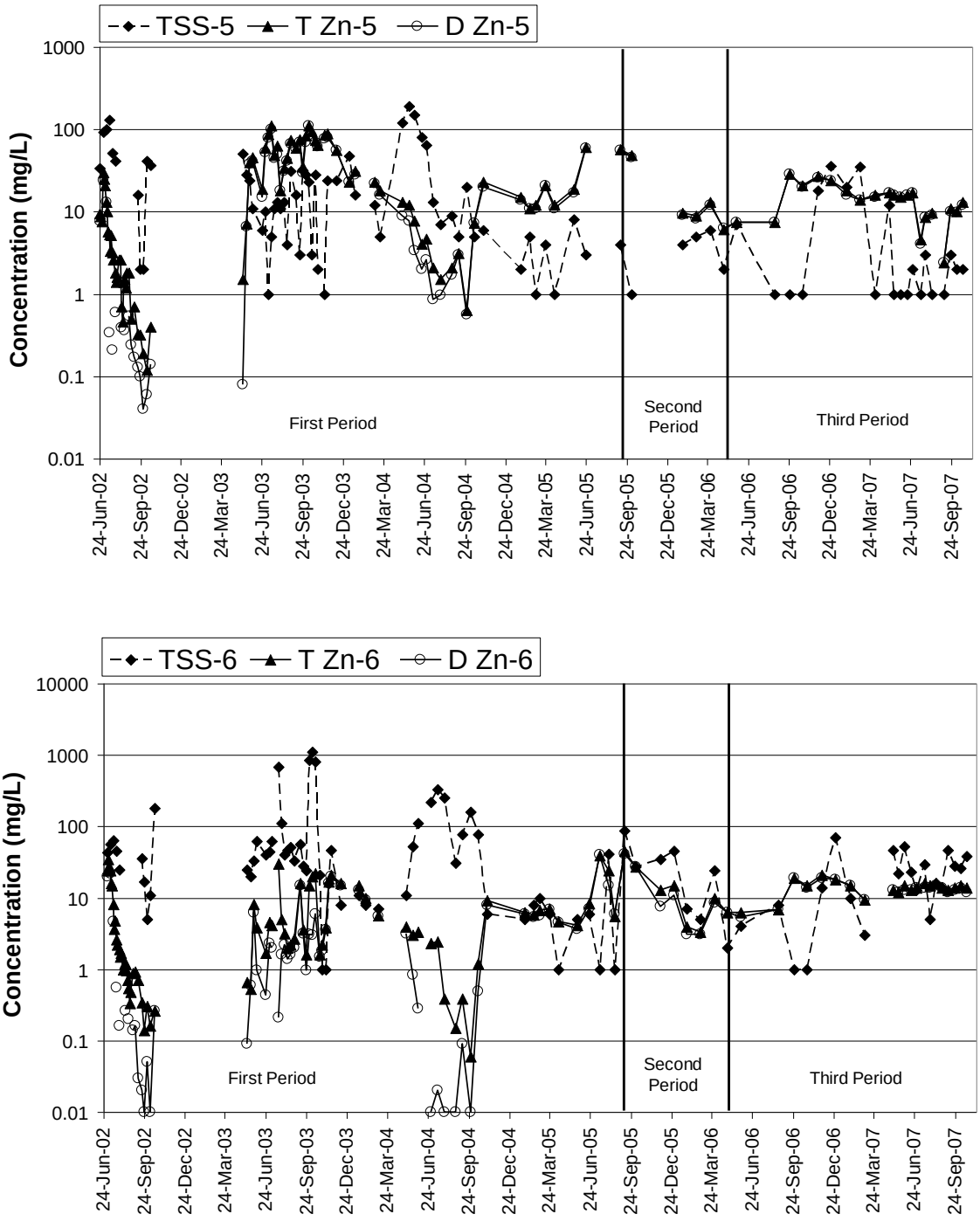


Figure 36 (continued). Concentrations of total suspended solids, total and dissolved Zn (mg/L) versus sample dates (June 24, 2002 to November 10, 2007) for sample points 5 and 6.

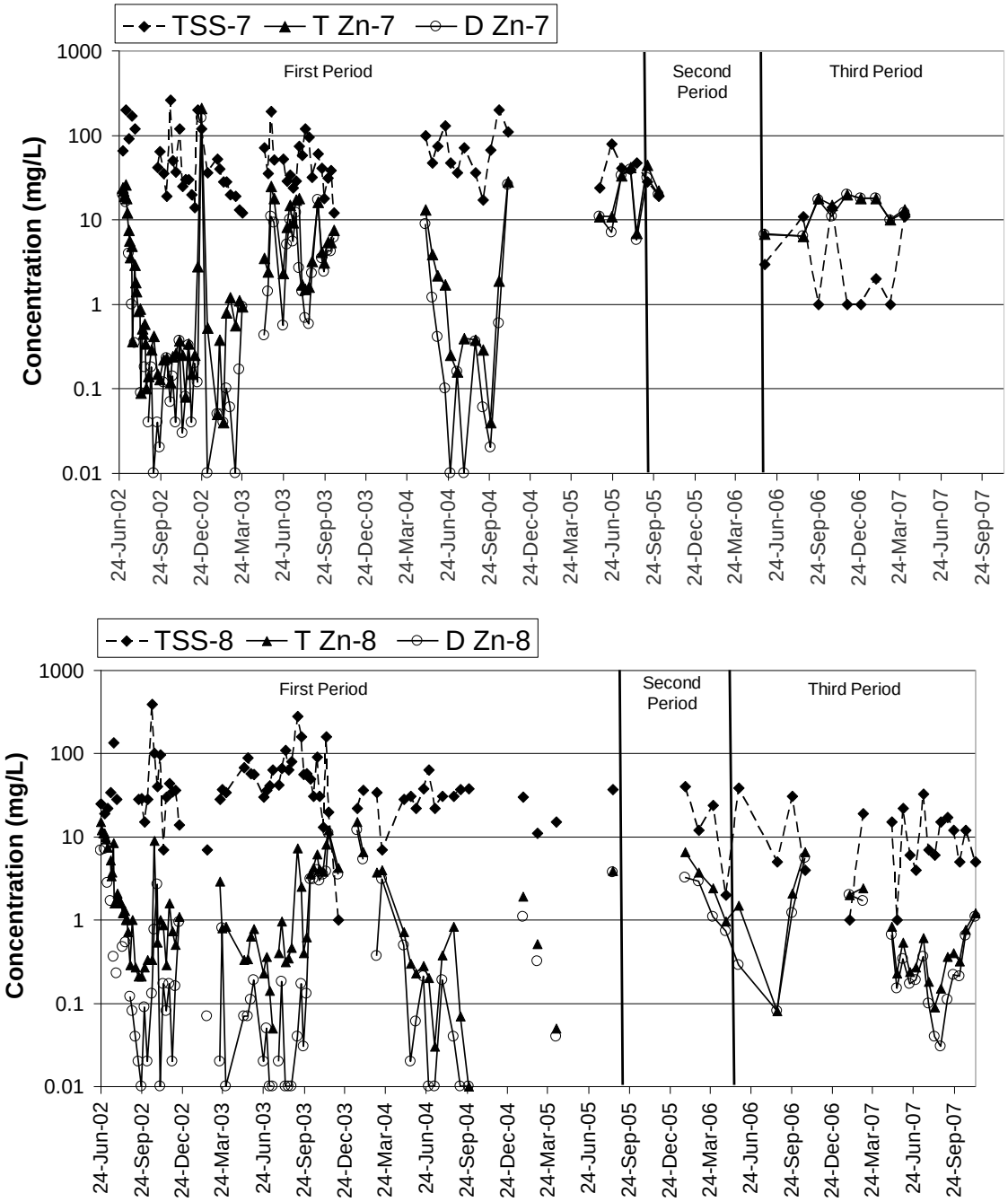


Figure 36 (continued). Concentrations of totals suspended solids, total and dissolved Zn (mg/L) versus sample dates (June 24, 2002 to November 10, 2007) for sample points 7 and 8.

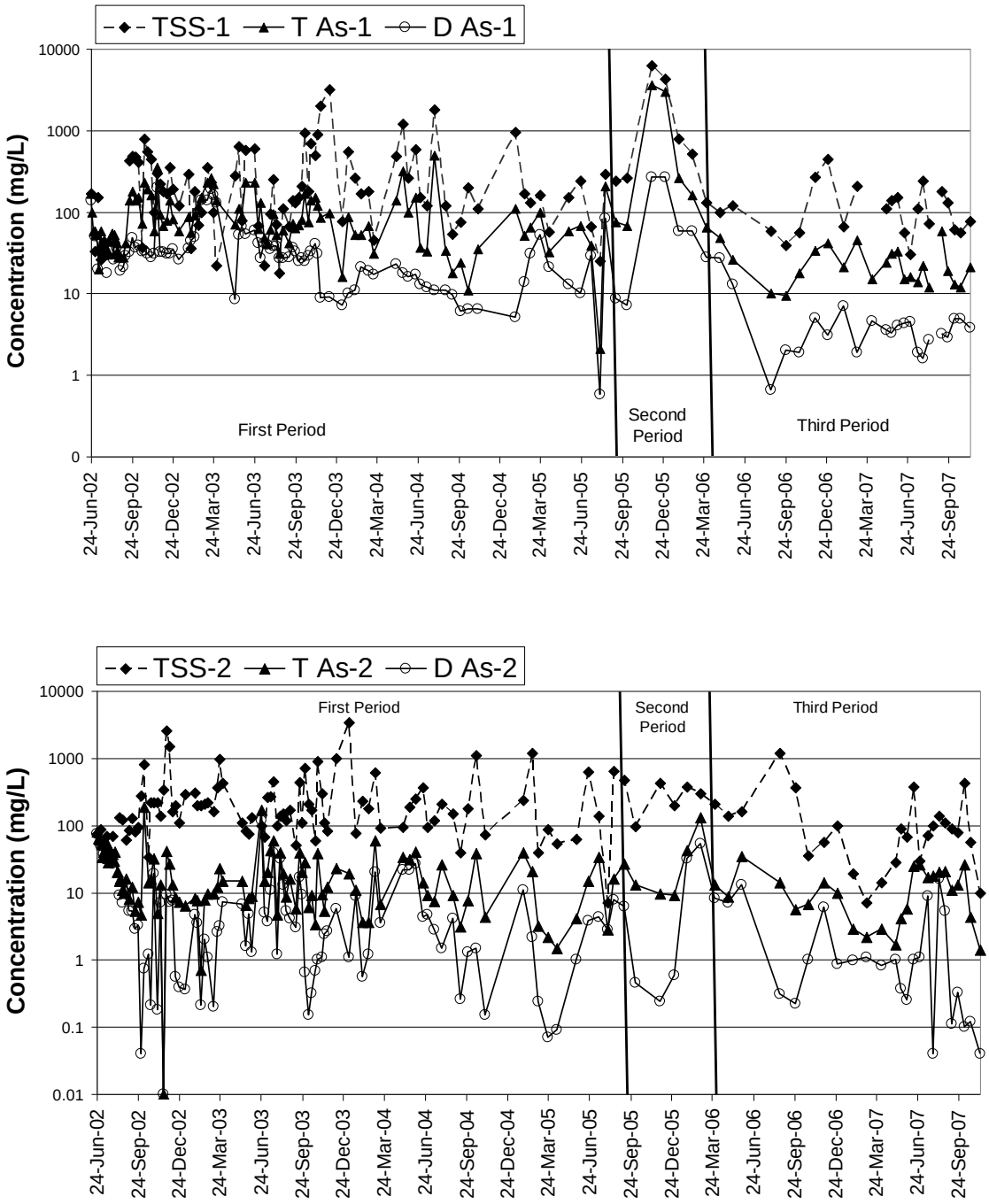


Figure 37. Concentrations of total suspended solids, total and dissolved As (mg/L) versus sample dates (June 24, 2002 to November 10, 2007) for sample points 1 through 8 from Figure 5.

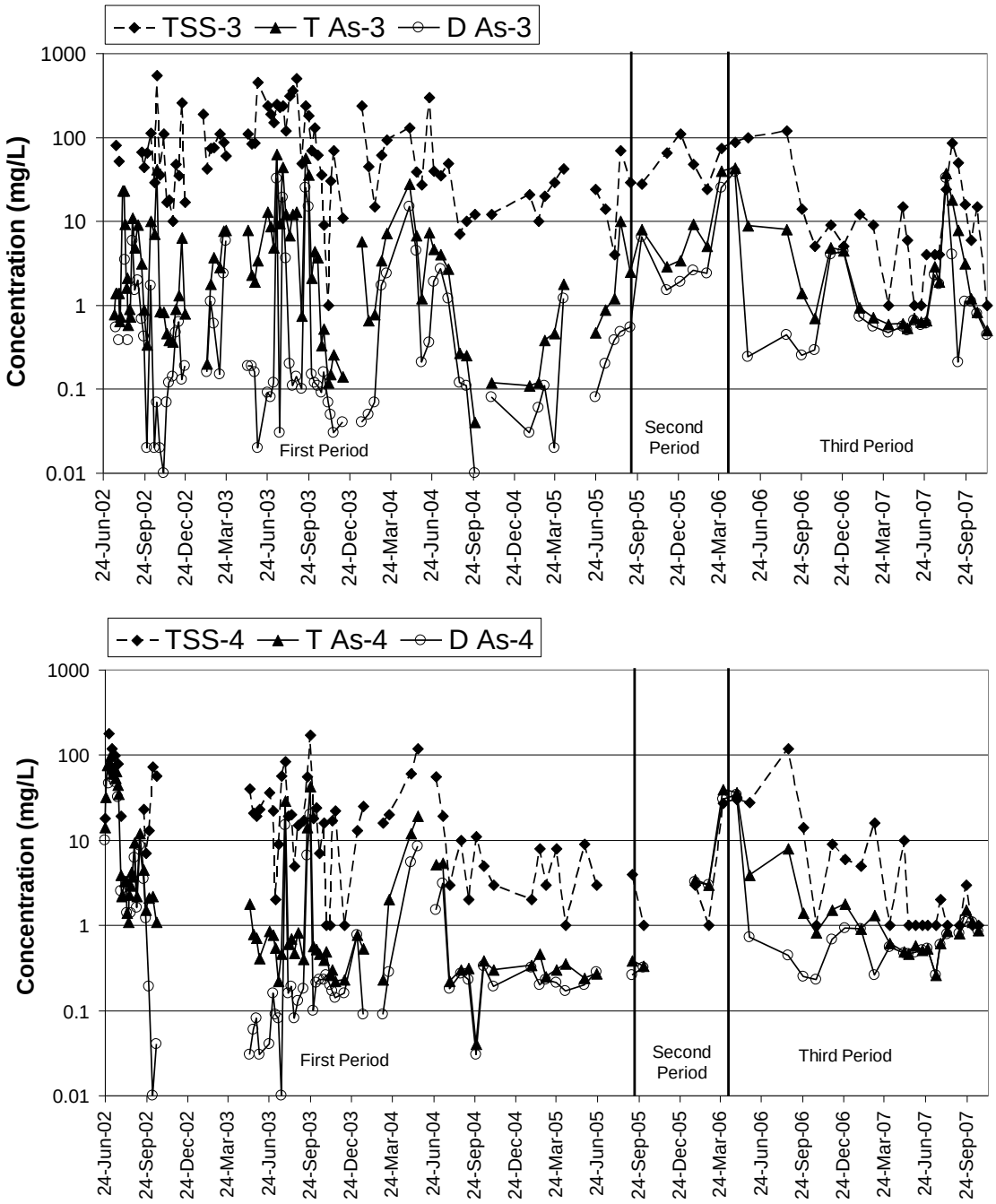


Figure 37 (continued). Concentrations of total suspended solids, total and dissolved As (mg/L) versus sample dates (June 24, 2002 to November 10, 2007) for sample points 3 and 4.

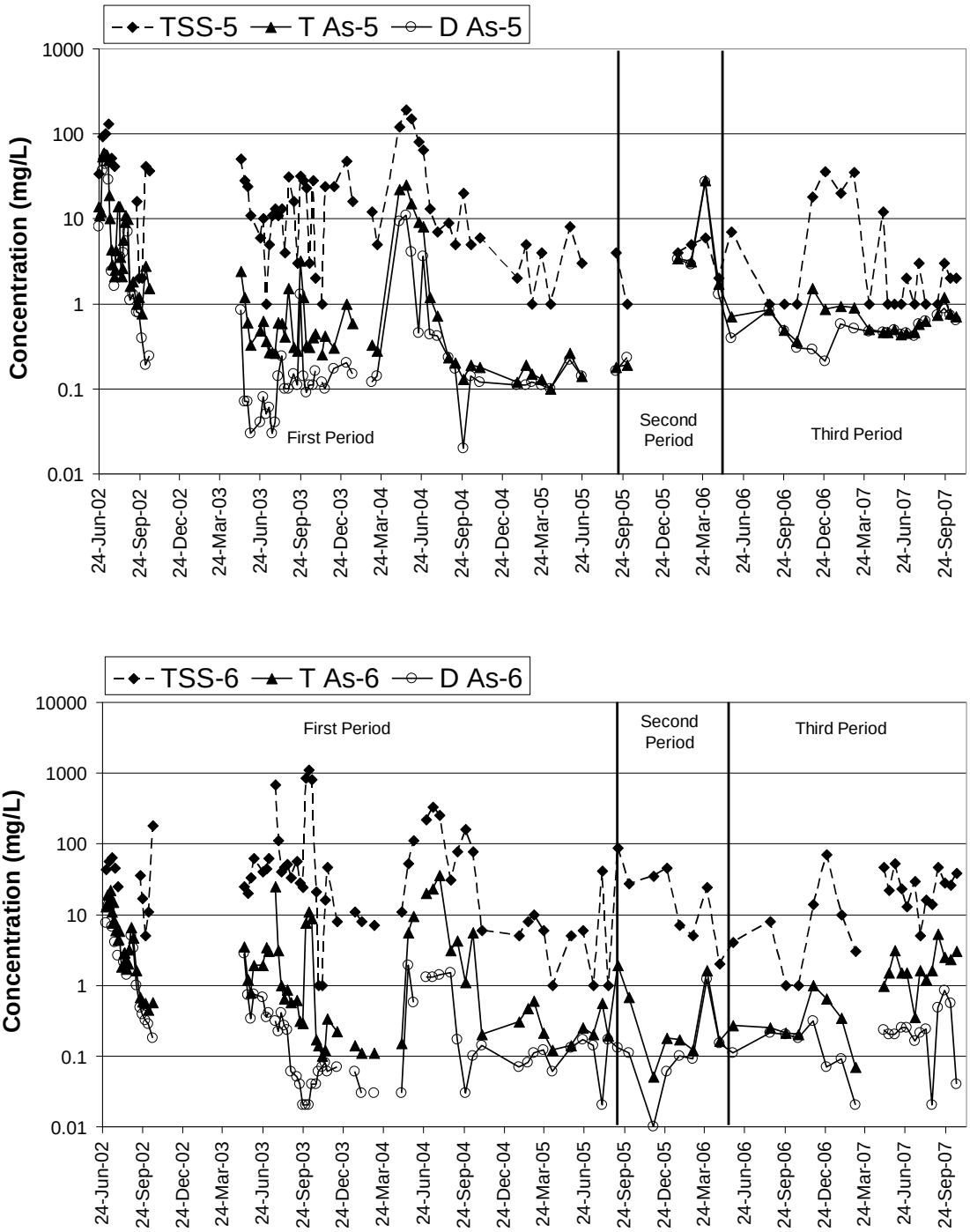


Figure 37 (continued). Concentrations of total suspended solids, total and dissolved As (mg/L) versus sample dates (June 24, 2002 to November 10, 2007) for sample points 5 and 6.

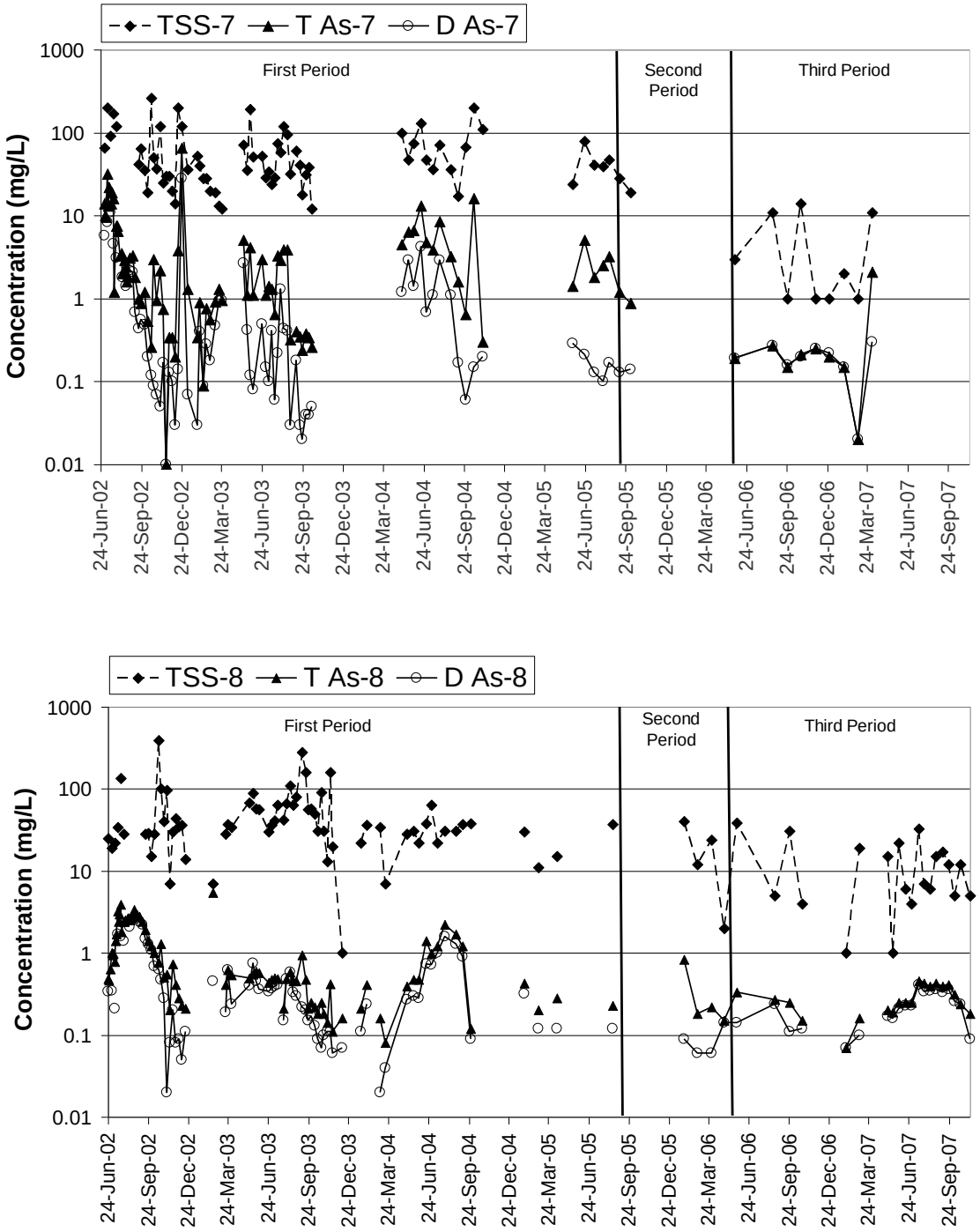


Figure 37 (continued). Concentrations of total suspended solids, total and dissolved As (mg/L) versus sample dates (June 24, 2002 to November 10, 2007) for sample points 7 and 8.

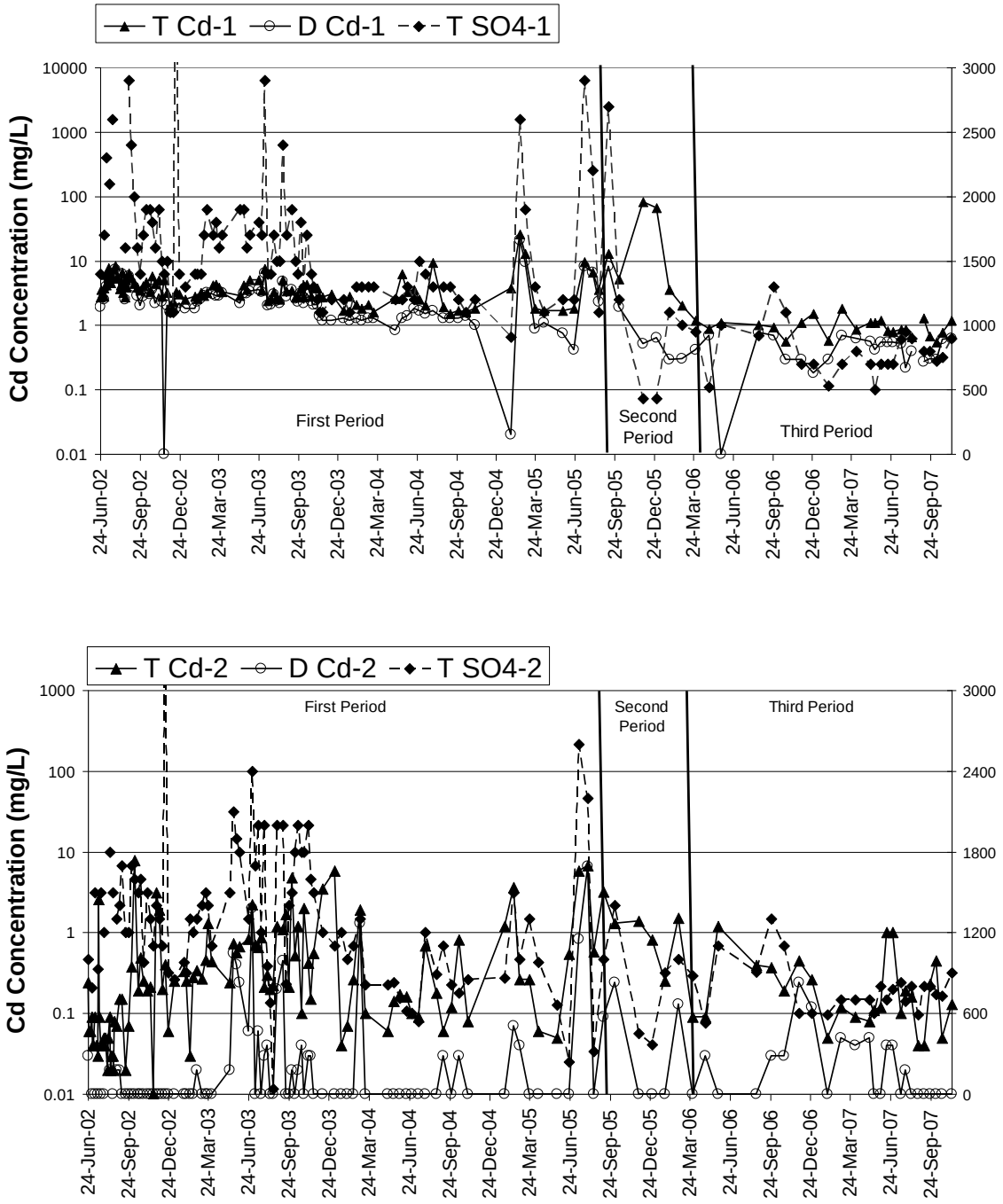


Figure 38. Concentrations of total and dissolved Cd as mg/L and total sulphate (mg/L) (left y-axis) versus sample date (June 24, 2002 to November 10, 2007) for sample points 1 through 8 from Figure 5.

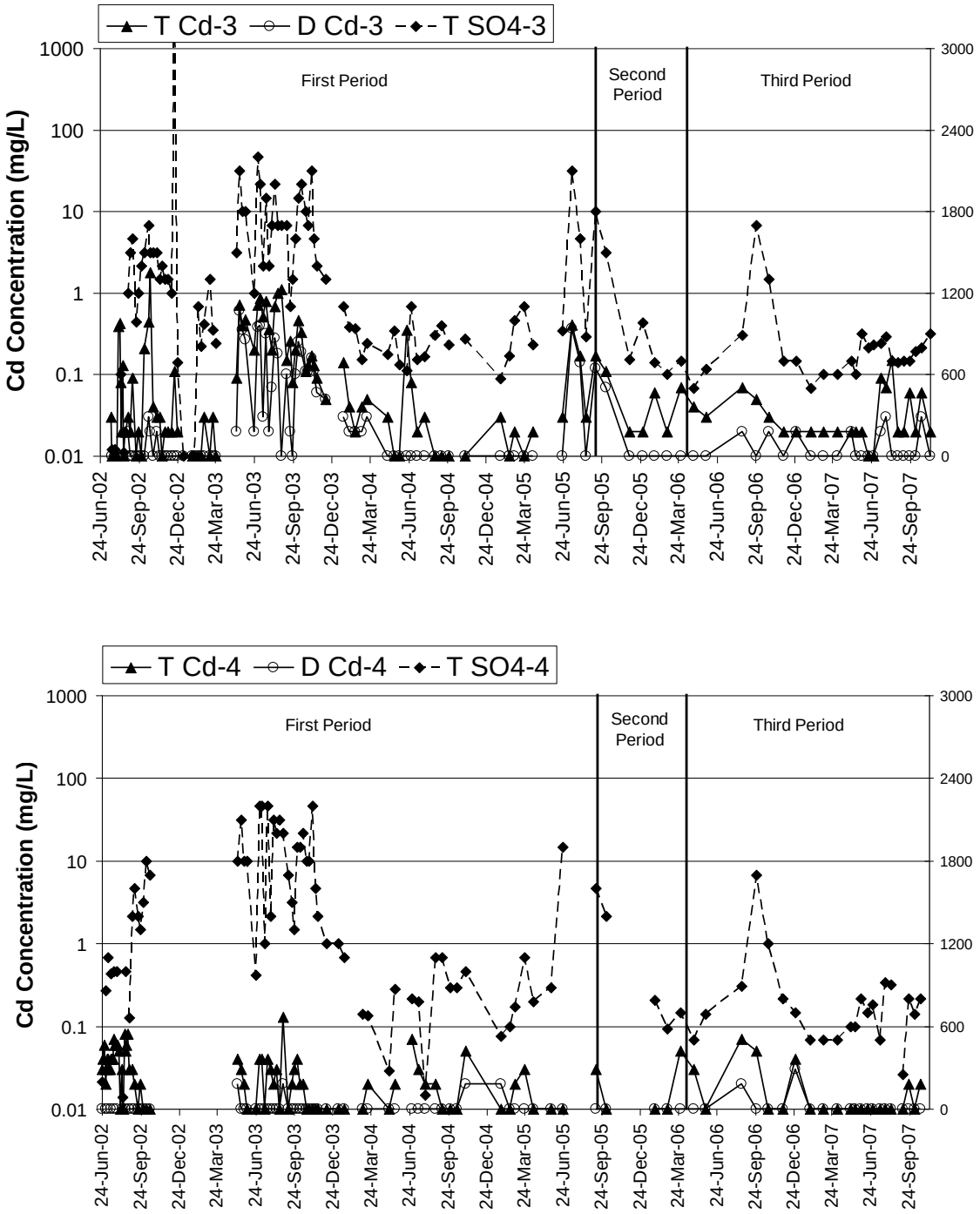


Figure 38 (continued). Concentrations of total and dissolved Cd (mg/L) and total sulphate as mg/L (left y-axis) versus sample dates (June 24, 2002 to November 10, 2007) for sample points 3 and 4.

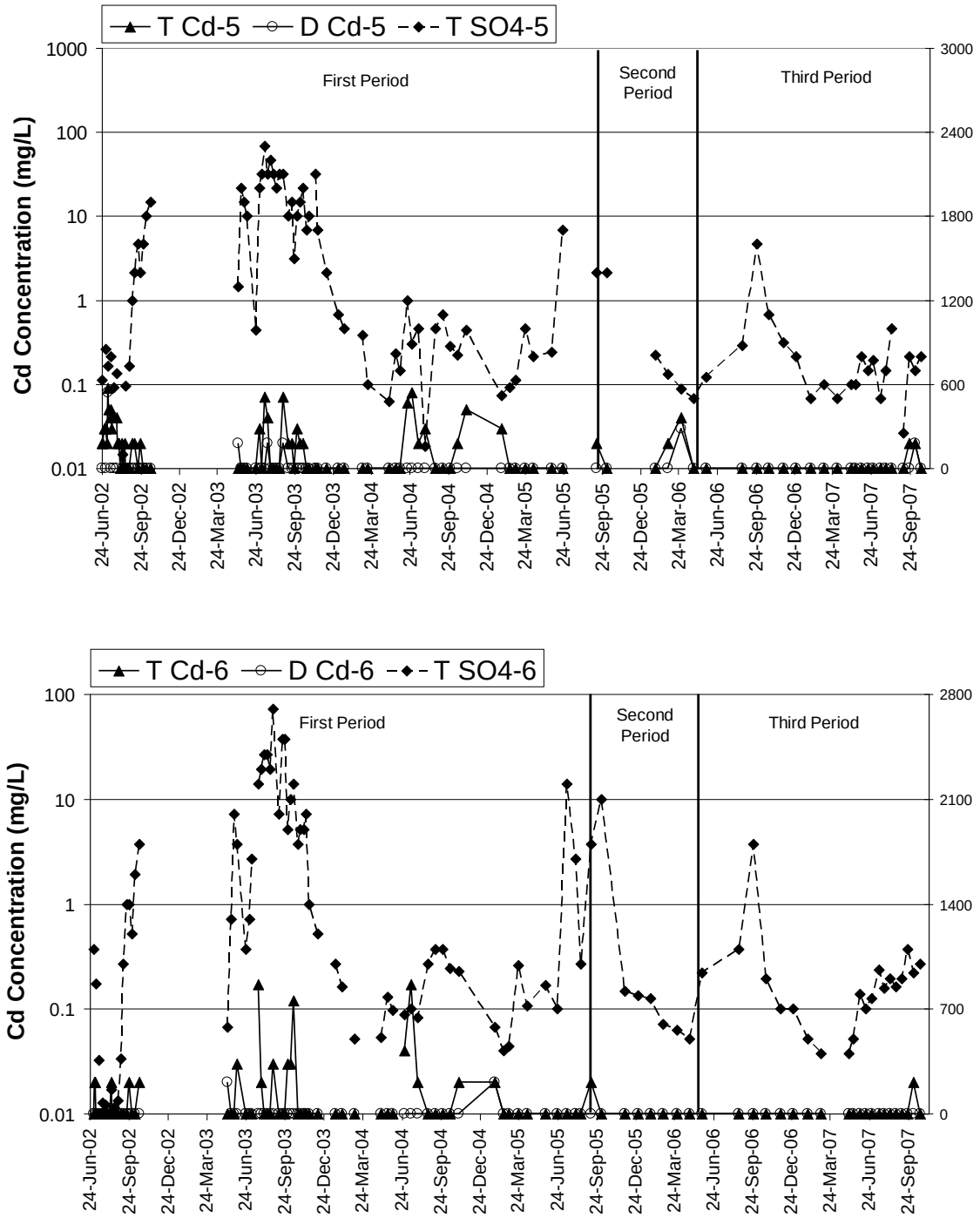


Figure 38 (continued). Concentrations of total and dissolved Cd (mg/L) and total sulphate (mg/L) (left y-axis) versus sample dates (June 24, 2002 to November 10, 2007) for sample points 5 and 6.

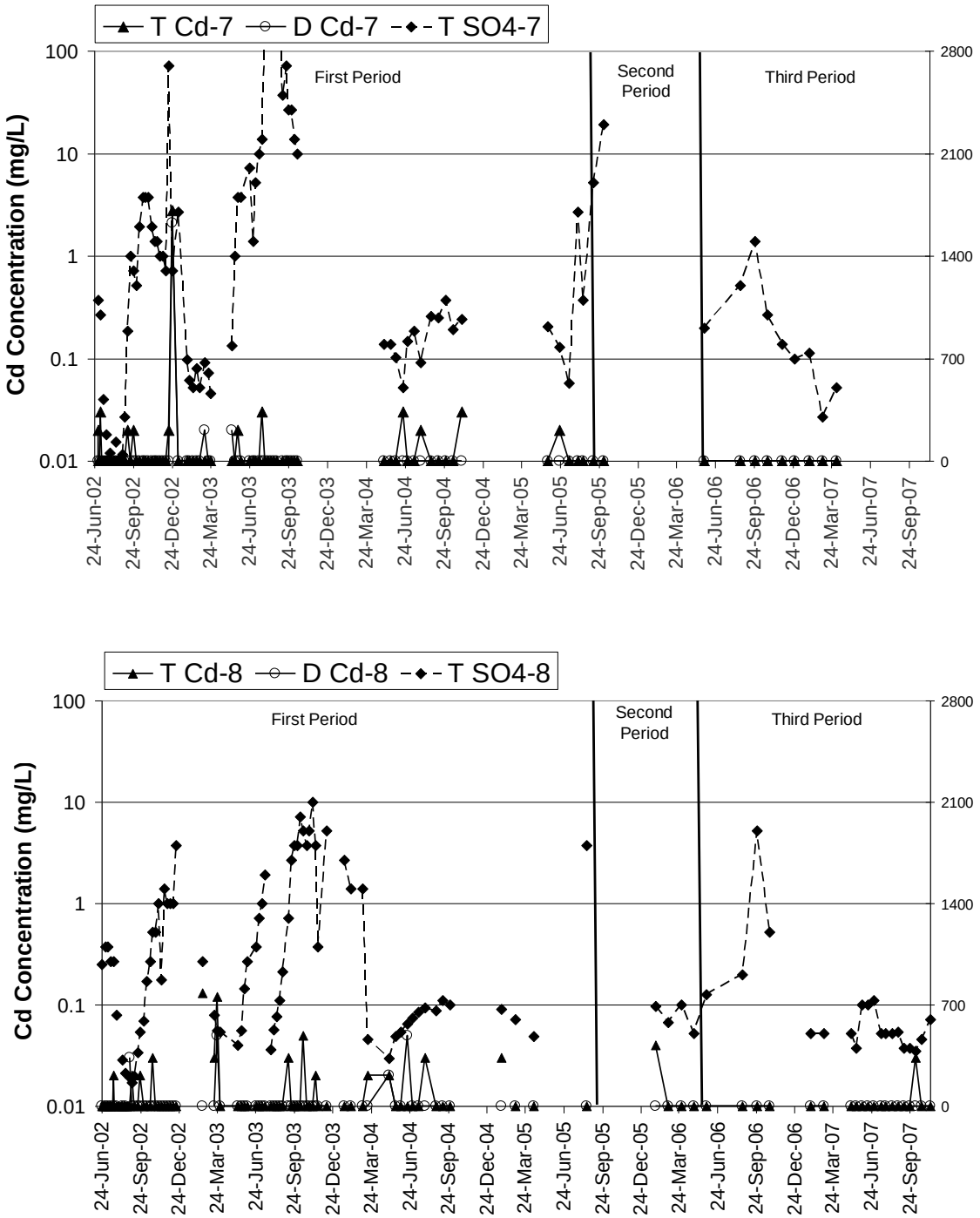


Figure 38 (continued). Concentrations of total and dissolved Cd as mg/L and total sulphate (mg/L) (left y-axis) versus sample dates (June 24, 2002 to November 10, 2007) for sample points 7 and 8.

holding pond (SP8), again considerable variability was noted (Figure 37). Large differences between total and dissolved As often seen in the input seepage are reduced through the anaerobic bioreactor and vegetated cells and are brought in closer alignment by the sand filter and in the final holding cell, the total and dissolved As concentrations are highly and significantly correlated (Figure 37). Total As and TSS are highly ($r>0.9$) and significantly ($p<0.001$) correlated in the input seepage where the TSS is more related to chemical precipitates, whereas, in the final holding cells, the TSS is likely composed largely of organic material and therefore does correlate with total As concentrations (Figure 37). The large As peak event in the fall and winter of 2005 corresponded with the Zn peak.

Starting concentrations of total and dissolved Cd are much lower than compared to Zn and As (Table 22) and frequently drop below detection limit as the seepage (SP1) is treated through the anaerobic bioreactors, the wetland cells, the slow sand filter and ultimately the final holding pond (SP8), variability is less given the lower overall concentrations (Figure 38). Differences between total and dissolved Cd often seen in the input seepage are reduced through the anaerobic bioreactor with both often being below detection limit through the vegetated cells, the sand filter and in the final holding cell (Table 22; Figure 38). The large total Cd peak event in the fall and winter of 2005 corresponded with the Zn and As peak.

Mean total sulphate concentrations decreased in the anaerobic cells remained fairly constant through the vegetated cells; and then decreased in the final holding cell (Table 22). Total sulphate concentrations show considerable variability during the first and second periods that was greatly reduced through the third period (Figure 38). The highest total sulphate concentrations (from 1,200 to > 3,000 mg/L) were found during the first period with a sharp decrease in the second period (from 2,600 to <500 mg/L) when the Zn and As concentrations peaked followed by the third period of generally lower concentrations (500 to 1,200 mg/L) that appear to be deceasing with time. While total sulphate generally decreases through the system, the peaks track through each cell in the

system to in the final holding pond where a smoothing of the peaks occurs due to its dilution capacity (Figure 38).

Some additional parameters assessed for this sampling period included conductivity, Mn and Sb (Table 22). Mean conductivity remains quite constant through the system decreasing slightly in the *Typha* and final holding cells likely due to dilution by precipitation events on these large surface area cells. Mean total and dissolved Mn are not treated by the system at all until the final holding cell where total and dissolved concentrations are reduced approximately 70% in this algal dominated community with high pH and oxic conditions. The final holding pond is acting similar to the trickling rock filters of the Wheal Jane design (Whitehead et al 2005). Not surprisingly total and dissolved Mn concentrations are highly (r=0.9) and significantly ($p<0.001$) correlated through the whole system. Mean input total and dissolved Sb are quite low entering the system and most removal is accomplished in the two anaerobic cells similar to Cd.

Mean dissolved ammonia concentrations decrease slightly through the anaerobic cells and first two vegetated cells then decreasing by 42% in the *Typha* cell and another 36% in the final holding cell as conditions become the most oxic (Table 22). Dissolved ammonia concentrations show considerable variability during the first and second periods that was greatly reduced through the third period (Figure 39). The highest dissolved ammonia concentrations in the input (from <50 to >200 mg/L) were found during the first period with a decrease in the second period (from 25 to >50 mg/L) followed by the third period of generally lower concentrations (from 1 to <40 mg/L) that appear to be deceasing with time (Figure 39).

Dissolved Fe concentrations are generally very low in the input at <0.1 mg/L and showing little variability with occasional spikes up to 10 mg/L (Table 22; Figure 39). Total Fe concentrations however are quite variable (from 0.1 up to 230 mg/L – associated with the Zn, As and Cd peak) and were highly (r>0.9) and significantly ($p<0.001$) correlated with TSS. Interestingly, the total Fe concentrations appear to increase after the fall 2003 and remain somewhat elevated which corresponds to when the historic landfill

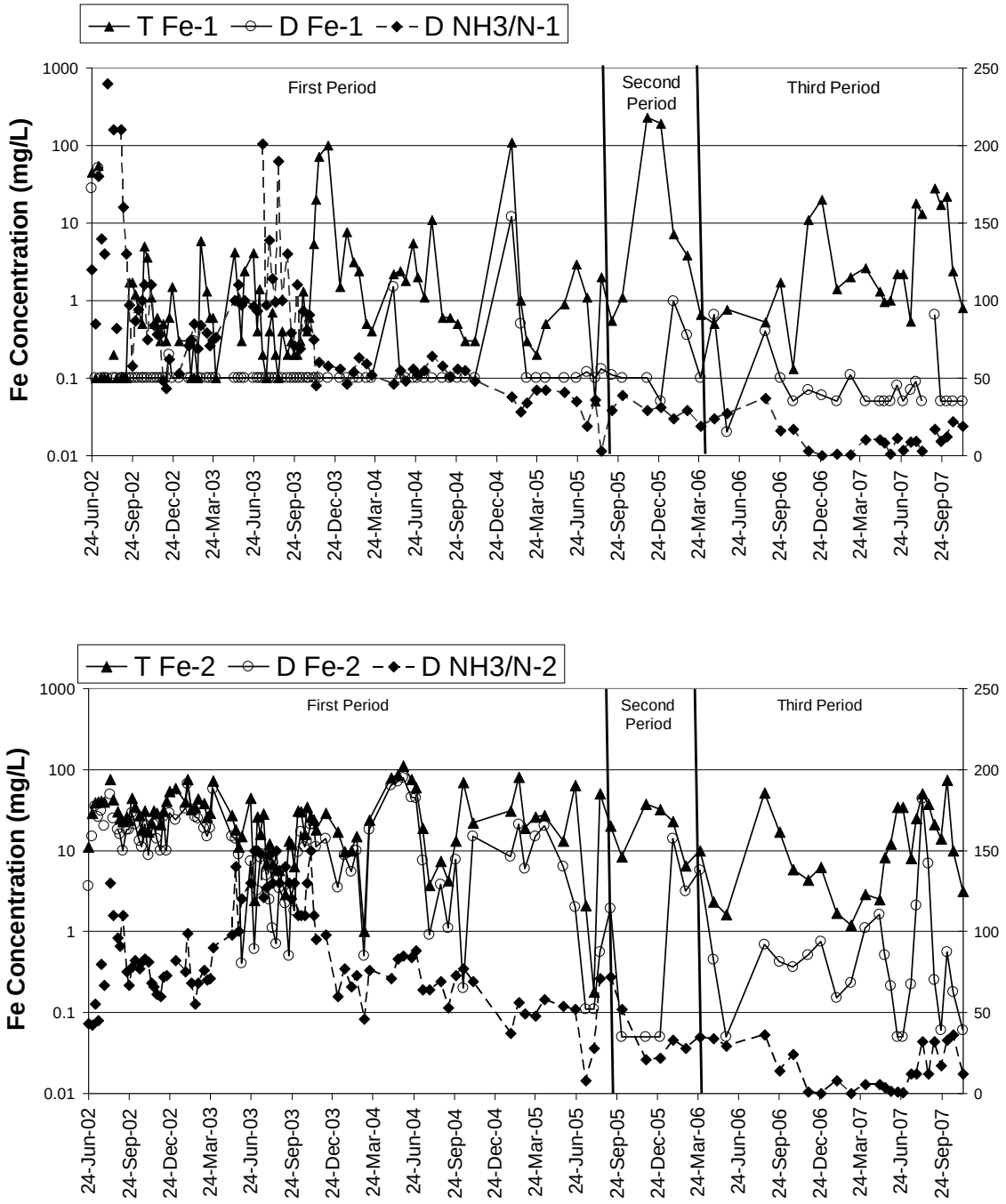


Figure 39. Concentrations of total and dissolved Fe and dissolved ammonia (mg/L) (left y-axis) versus sample dates (June 24, 2002 to November 10, 2007) for sample points 1 through 8 from Figure 5.

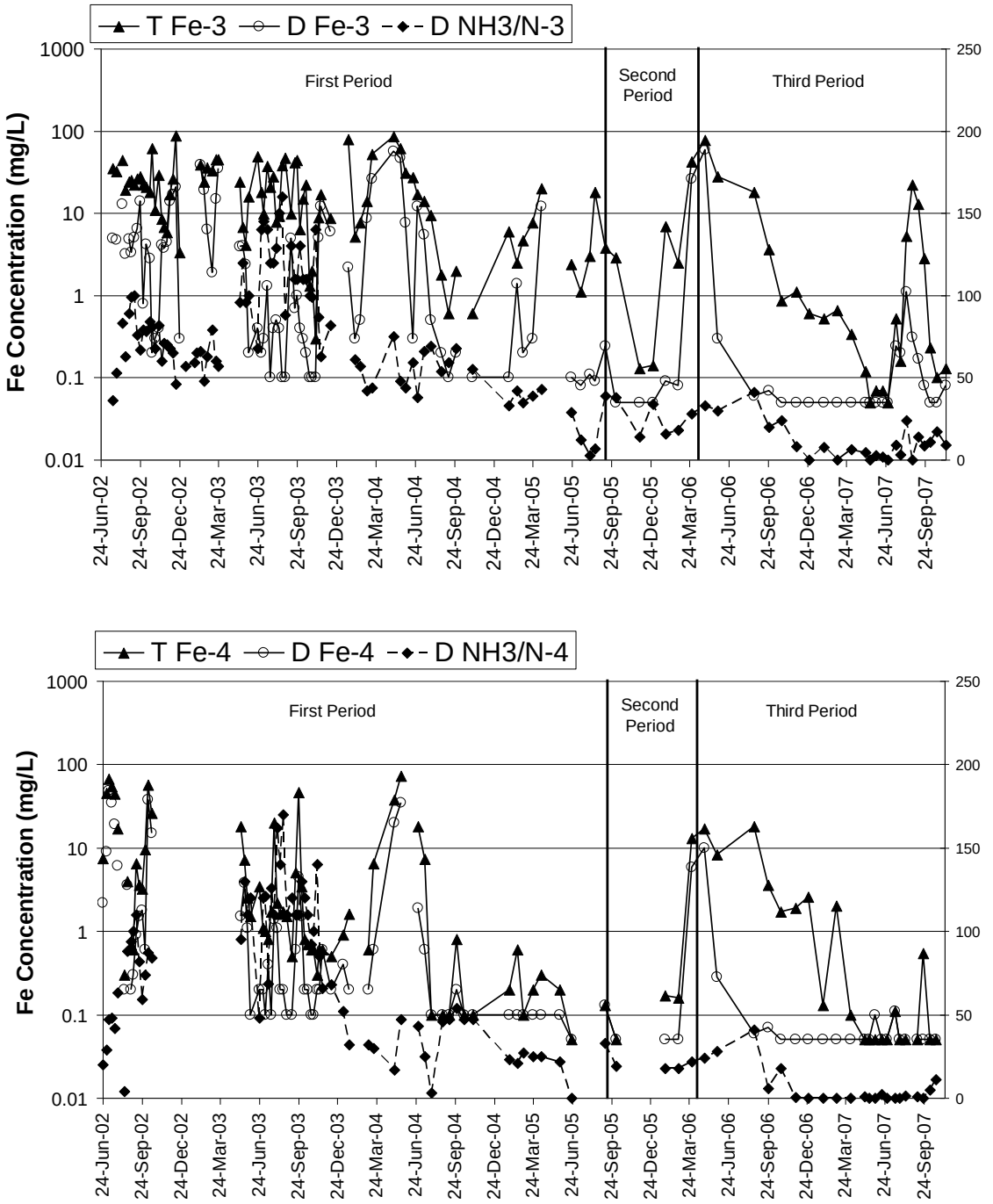


Figure 39 (continued). Concentrations of total and dissolved Fe and dissolved ammonia (mg/L) (left y-axis) versus sample dates (June 24, 2002 to November 10, 2007) for sample points 3 and 4.

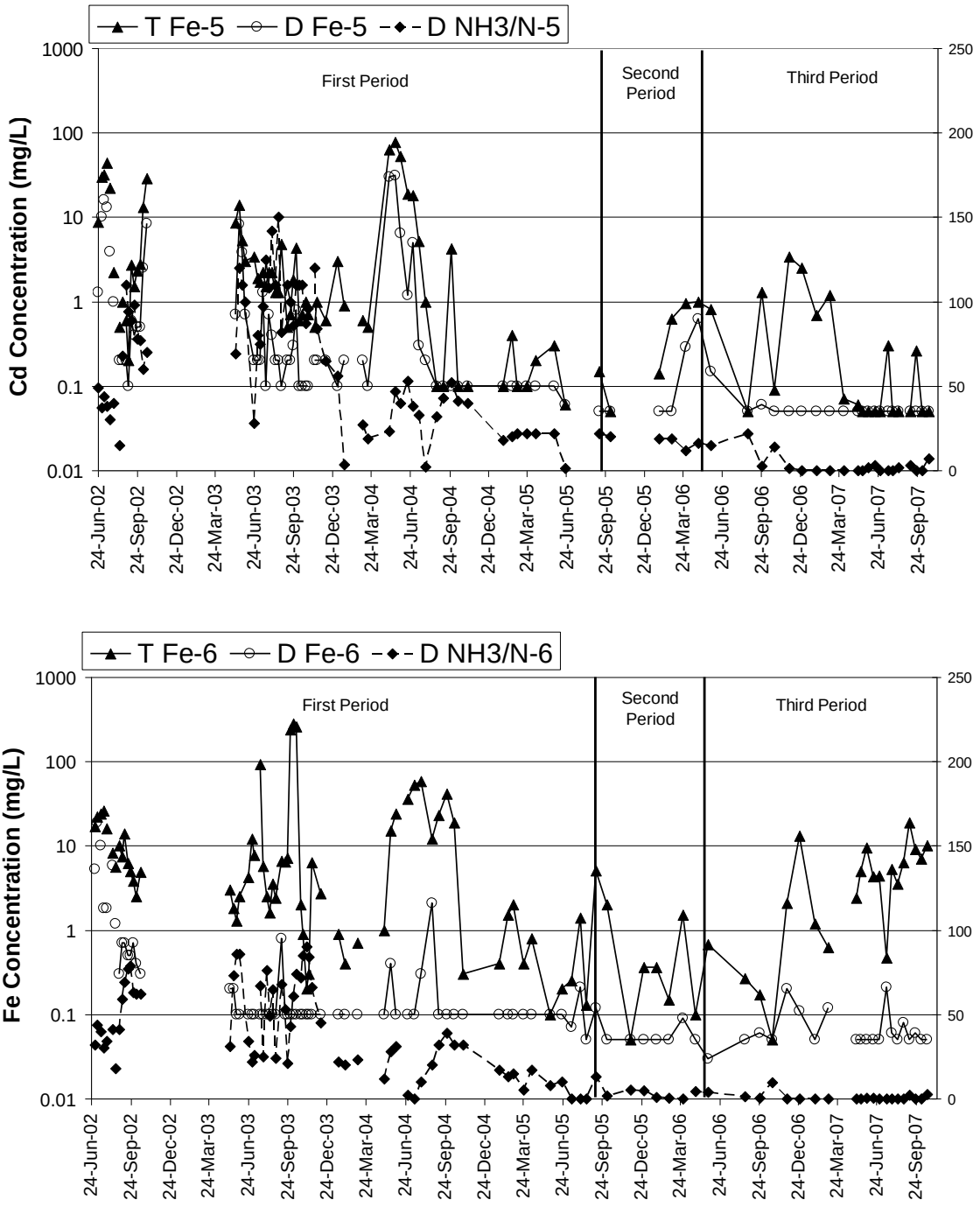


Figure 39 (continued). Concentrations of total and dissolved Fe and dissolved ammonia (mg/L) (left y-axis) versus sample dates (June 24, 2002 to November 10, 2007) for sample points 5 and 6.

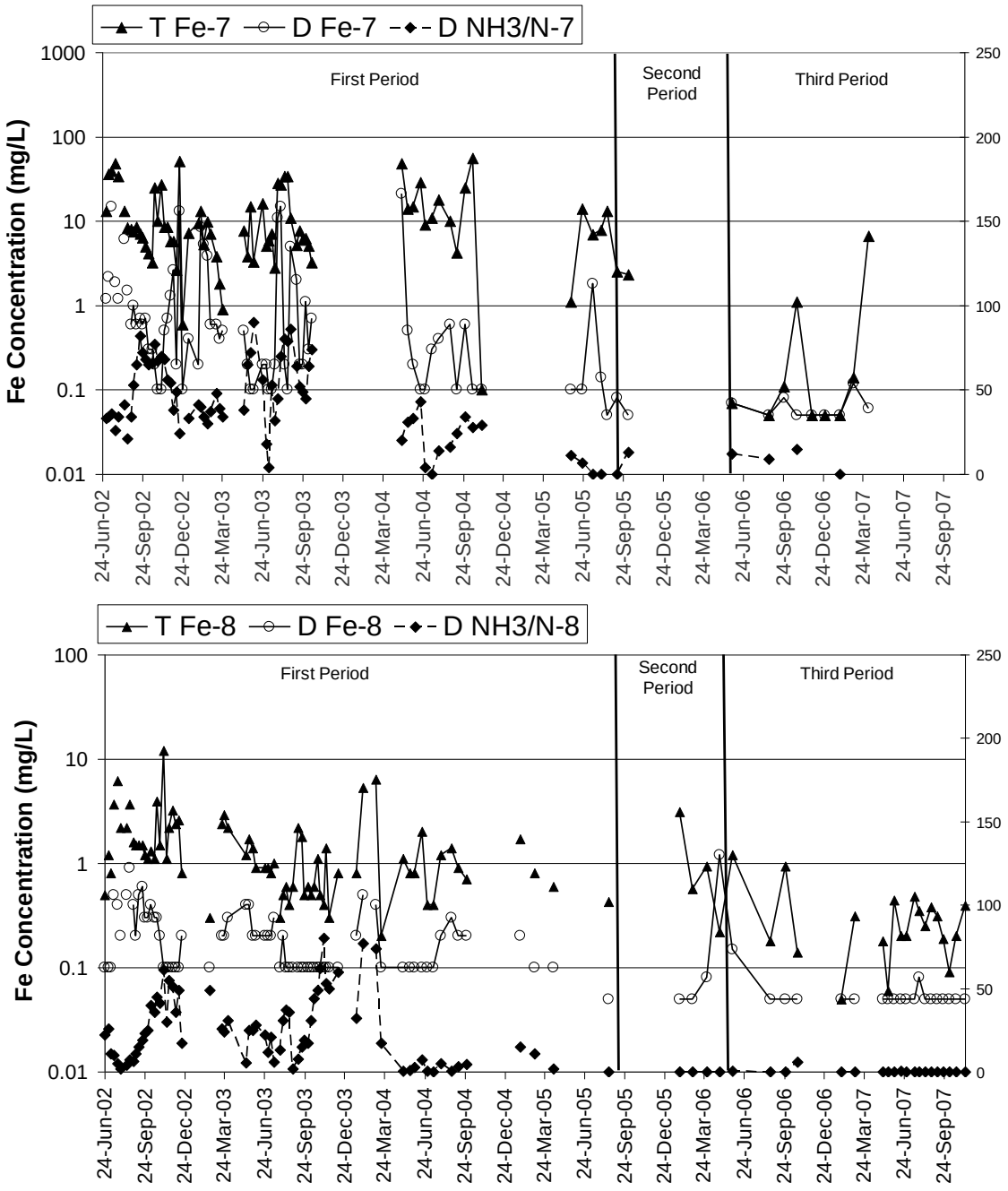


Figure 39 (continued). Concentrations of total and dissolved Fe and dissolved ammonia (mg/L) (left y-axis) versus sample dates (June 24, 2002 to November 10, 2007) for sample points 7 and 8.

was capped. The dissolved Fe concentrations increased through the two anaerobic cells in relation to the total Fe concentrations which are somewhat higher in these two cells compared to the inlet (Table 22; Figure 39). This has been attributed to the iron contained in the sand (Figure 32) used in the substrate of these two cells. The elevated dissolved Fe concentrations track through the 2 plant cells (SP4 and 5) but is dramatically reduced through the *Typha* cell (SP 6) and in the final folding pond (SP8) (Table 22). While mean total Fe concentrations decrease slightly through the first two vegetated cells it increases out of the *Typha* cell and is dramatically reduced in the final holding cell (Table 22), they are quite variable.

After the first anaerobic cell, the dissolved As and Fe concentrations appear to track through the whole system (Figure 37; and Figure 39). Indeed, strong correlations are found especially through the vegetated cells (Table 23) especially the *Typha* cell where both dissolved As and Fe concentrations are low (Table 22). Total As and Fe at SP5 and SP8 are negatively correlated with dissolved oxygen. At the input and SP3 to SP6, the TSS is highly correlated with total and dissolved As and Fe concentrations. Where as out of the first anaerobic cell total As is strongly correlated with dissolved As and total Fe is strongly correlated with dissolved Fe but neither with TSS (Table 23).

Given the inherent variability observed (Figure 36; Figure 37; and Figure 38) and differing hydraulic retention times (HRT) based only on inflow data, the mean concentrations by season (summer and winter) were used to assess the system performance. This reduces the variability and corresponds to periods of plant growth, operational changes to flow rates and temperature differences. During the summer the wetlands experience plant growth and very high evapotranspiration rates. Transpired water is free of metals and during summer periods this has the effect of concentrating the remaining metals in the waters of the plant cells. Indeed in the heat of the summer input flow rates have to be increased to ensure flow reaches the final *Typha* wetland cell. Conversely, in the fall-winter period, plants are senescence with stems and leaves decaying and evaporation rates are low especially with snow cover. As well, summer input flow rates are set higher than the fall-winter input flow rates as higher summer

Table 23. Pearson *r* for TSS, total and dissolved As and Fe and dissolved oxygen for sample points SP1-SP6 and SP8 (all significant at p<0.001 except DO at p<0.02).

Sample Point		TSS	T As	D As	T Fe	D Fe
SP1	**T As**	0.88				
	D As	0.51	0.71			
	T Fe	0.89	0.84	0.51		
	D Fe	-	-	-	-	
	DO	-	-0.25	-	-	-
SP2	**T As**	-				
	D As	-	0.71			
	T Fe	-	-	-		
	D Fe	-	-	-	0.76	
	DO	-	-	-	-	-
SP3	**T As**	0.49				
	D As	-	0.86			
	T Fe	0.55	0.5	0.35		
	D Fe	-	-	0.38	0.65	
	DO	-	-	-	-	-0.28
SP4	**T As**	0.79				
	D As	0.68	0.98			
	T Fe	0.85	0.75	0.7		
	D Fe	0.61	0.63	0.65	0.91	
	DO	-	-	-	-	-
SP5	**T As**	0.7				
	D As	0.54	0.97			
	T Fe	0.94	0.66	0.51		
	D Fe	0.82	0.65	0.53	0.93	
	DO	-0.32	-0.24	-	-0.26	-
SP6	**T As**	0.52				
	D As	-	0.48			
	T Fe	0.96	0.43	-		
	D Fe	-	0.29	0.89	-	
	DO	-	-0.26	-	-	-
SP8	**T As**	-				
	D As	-	0.79			
	T Fe	-	0.26	-		
	D Fe	-	0.4	0.53	0.29	
	DO	-	-0.49	-0.34	-0.28	-0.27

temperatures (7 to +20°C) promote higher biological activity compared to fall-winter temperatures (1 to 10°C).

The overall mean treatment reductions for the total system including the holding cell for As, Cd and Zn is very impressive ranging from 91.5 to 100% for dissolved metals and from 95.8 to 100% for total metals by season (Table 24). The treatment reductions vary by metal and indeed by season with the anaerobic bioreactors responsible for large reductions in all metals concentrations particularly for As and Cd (Table 24). The wetland cells provide an important polishing step especially for Zn (Table 24). The sand filter is effective in reducing total metal concentrations and at this point in the system total metals concentrations are very close to dissolved metals concentrations (Figure 36; Figure 37; and Figure 38). While the final holding pond was placed in the system to provide a reservoir for irrigation and to allow testing prior to release, it also provides a useful polishing function serving as a free water wetland (Figure 36; Figure 37; and Figure 38). Over the years a sediment layer has developed in the holding pond and cattails have colonized the edges of the pond. This pond would provide metals treatment in several ways including evaporation, precipitation and settling of metal sulphides, algal uptake which upon senescence settle to the bottom and its' large storage capacity moderates seasonal and daily upstream treatment variability.

In order to evaluate the system performance over the time frame of 2002–2007, it is useful to consider three separate time periods mentioned earlier. The first is from summer 2002 to summer 2005, second is fall-winter 2005 and the third is summer 2006 to fall-winter 2007 (Table 24; Figure 40). These periods are characterized by differing input metal concentrations (Figure 36; Figure 37; and Figure 38). The changing input concentrations relate to various activities undertaken by Teck to capture and control the seepage and their sources. From 1997 when the initial collection system was installed to 2002 additional seeps were collected increasing the total load to the sump. During 2002 and mid-2003 an engineered cap was installed on the historical landfill which would decrease over time the Zn, Cd, sulphate and ammonia loads from this source. In 2004 any remaining As residues were removed from the As storage pit, the area was then double-

lined with HDPE and As residues placed in the new storage facility. Unfortunately, construction work was not completed before November and the material in the storage area became saturated with water from precipitation. Capping work was completed during in the spring-summer of 2005 with cap in place by July 2005. Additionally, in June 2005 an under-drain from another As storage facility was tied into the collection system thus defining the first period.

During the fall-winter of 2005 the As storage facility was initially dewatered via the seepage collection system causing the "spike" or second period. Additional dewatering events have occurred since but these are less frequent and lower in volume and load. With the source control projects completed, loads to the seepage collection system and the treatment system have been greatly decreased and will likely to continue to decrease over time thus defining the third period (Figure 40).

The first period had mean total and dissolved As concentrations of 91.5 and 38.6 mg/L respectively, total and dissolved Cd concentrations of 4.20 and 2.91 mg/L respectively and total and dissolved Zn of 285 and 204 mg/L respectively (Table 25). This period was characterized by elevated but reasonably stable loads (i.e. the standard deviation is much lower than the second period for all metals; Table 25) where a large portion of the input load is present as dissolved metals from 42% for As to % for Cd and 72% for Zn (Figure 36; Figure 37; and Figure 38). The presentation of means and standard deviations by period (Table 25), while not entirely satisfactory as it does not adequately address HRT (given the limited flow data collected), does provide some overall system trends that help define the periods.

The second period had mean total and dissolved As concentrations of 903 and 91.4 mg/L respectively, total and dissolved Cd concentrations of 20.4 and 0.60 mg/L respectively and total and dissolved Zn of 945 and 43.8 mg/L respectively, where only a small portion of the input load was present as a dissolved fraction – from 2.9% for Cd and 4.6% for Zn to 10% for As (Table 25). Maximum total metal concentrations during

Table 24. Percent reduction of total and dissolved metal concentrations by anaerobic bioreactors (SP1 to SP3), the wetland cells (SP3 to SP6) and overall system performance (SP1 to SP8) by sample periods (S = Summer - May 15 to September 17; FW = Fall Winter - September 18 to May 14).

Season	System	(n)	Total As	Diss. As	Total Cd	Diss. Cd	Total Zn	Diss. Zn
			First or "Stable" Period					
S 2002	Bioreactors	8 (18)*	89.6%	95.3%	98.5%	99.7%	97.7%	97.7%
	Wetlands	11(26)*	-42.0%	-136.9%	84.8%	11.1%	18.8%	42.2%
	Total System	12(29)*	**95.8%**	**96.0%**	**99.8%**	**99.7%**	**98.8%**	**99.5%**
FW 2002	Bioreactors	25	96.7%	99.1%	94.9%	97.8%	81.7%	80.5%
	Wetlands	8	74.2%	-16.5%	91.8%	79.9%	96.6%	97.5%
	Total System	21	**99.4%**	**99.4%**	**99.4%**	**99.5%**	**99.6%**	**99.9%**
S 2003	Bioreactors	12	73.7%	81.8%	84.6%	95.3%	64.3%	73.5%
	Wetlands	11	81.9%	95.9%	95.2%	93.4%	93.2%	95.6%
	Total System	11	**99.4%**	**99.1%**	**99.7%**	**99.7%**	**99.6%**	**99.99%**
FW 2003	Bioreactors	18	94.4%	89.6%	96.1%	95.7%	77.8%	67.9%
	Wetlands	16	51.0%	91.2%	83.3%	86.2%	78.9%	84.9%
	Total System	17	**99.7%**	**99.4%**	**99.5%**	**99.4%**	**97.9%**	**97.5%**
S 2004	Bioreactors	6	97.5%	91.3%	97.6%	99.4%	95.2%	95.3%
	Wetlands	5	-438.8%	-6.5%	40.0%	0.0%	90.1%	99.5%
	Total System	6	**98.9%**	**91.5%**	**99.6%**	**98.9%**	**99.9%**	**99.9%**
FW 2004	Bioreactors	7	99.2%	98.8%	99.7%	99.8%	96.9%	95.6%
	Wetlands	9	-121.8%	56.7%	22.2%	-11.1%	26.3%	26.5%
	Total System	4	**99.5%**	**99.1%**	**99.7%**	**99.8%**	**99.7%**	**99.7%**
S 2005	Bioreactors	5	96.2%	98.7%	97.7%	97.5%	79.8%	76.8%
	Wetlands	5	79.4%	62.5%	92.6%	92.3%	54.8%	56.6%
	Total System	1	**99.7%**	**99.5%**	**99.9%**	**99.8%**	**98.5%**	**98.3%**
			Second or "Peak" Period					
FW 2005	Bioreactors	8	98.3%	89.2%	99.8%	97.1%	98.6%	78.9%
	Wetlands	8	97.3%	97.7%	78.4%	42.9%	21.8%	2.7%
	Total System	5	**99.99%**	**99.9%**	**99.9%**	**98.3%**	**99.7%**	**96.2%**
			Third or "Stabilization" Period					
S 2006		1	*Insufficient data (n=1)*					
FW 2006	Bioreactors	10	94.3%	66.0%	97.8%	97.2%	66.0%	46.7%
	Wetlands	9	42.4%	86.6%	58.3%	23.1%	31.5%	30.7%
	Total System	7	**99.3%**	**96.3%**	**99.1%**	**97.8%**	**96.8%**	**95.8%**
S 2007	Bioreactors	7	57.1%	-100.1%	94.0%	96.5%	76.9%	69.7%
	Wetlands	7	81.1%	96.2%	81.1%	30.0%	-4.6%	-16.5%
	Total System	7	**98.4%**	**89.4%**	**98.9%**	**97.6%**	**99.5%**	**99.6%**
FW 2007	Bioreactors	4	91.4%	79.2%	95.0%	96.7%	80.0%	74.2%
	Wetlands	3	-85.1%	44.6%	66.7%	33.3%	-41.2%	-31.6%
	Total System	4	**98.2%**	**94.2%**	**98.1%**	**97.8%**	**98.7%**	**98.6%**

* where 8 (18) indicates the number of dissolved metal samples = 8 and the total metal samples = 18

this period reached 3,600 mg/L As, 83 mg/L Cd and 3,800 mg/L Zn. This period experienced high and variable (as indicated by the standard deviation values being greater than the mean; Table 25) total metal loads particularly for As and Zn with the total suspended solids concentrations peaking at 6300 mg/L (Figure 36; Figure 37; and Figure 38). These solids are likely mostly $Zn_3(AsO_4)_2$ or Kottigite (Duncan et al 2008).

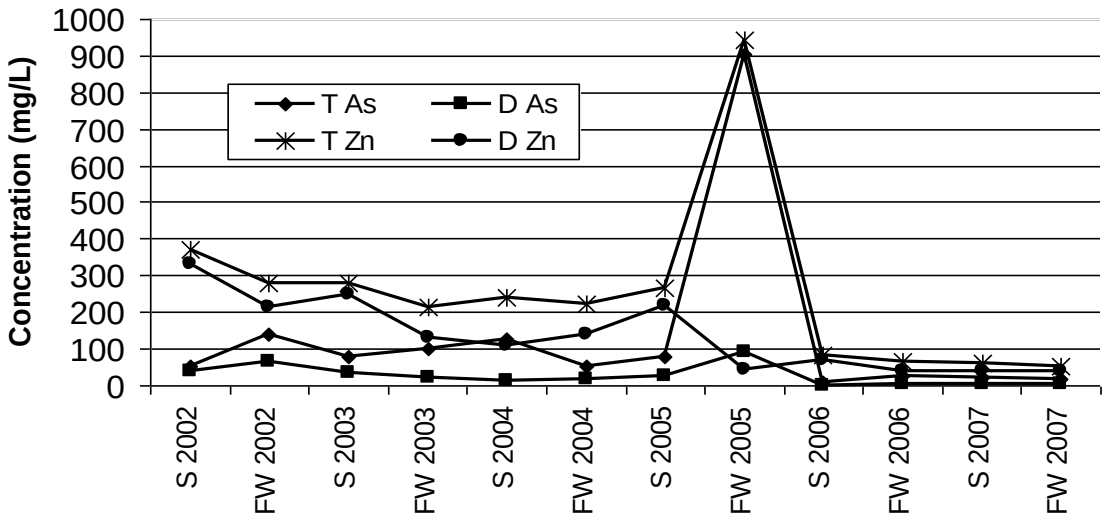


Figure 40. Mean concentrations of total and dissolved As and Zn as mg/L entering the treatment system versus sample periods (S = Summer (May 15 to September 17 each year); FW = Fall Winter (September 18 to May 14 each year)).

The third period had mean total and dissolved As concentrations of 19.1 and 2.87 mg/L respectively, total and dissolved Cd concentrations of 0.97 and 0.52 mg/L respectively and total and dissolved Zn of 64.8 and 46.7 mg/L respectively, and is more comparable to the first period for Cd and Zn where a large portion of the input metal load was as dissolved from 53% for Cd to 72% for Zn. However, only 15% of the As is in dissolved form which is between the first (35%) and second (10%) periods but closer to the second period. The third period is characterized by much lower stable (as evidenced by the lower standard deviations being generally being lower than the mean; Table 25) to

Table 25. Mean and standard deviations of total and dissolved metal concentrations and total sulphate (mg/L) by the three defined sampling periods at sample points 1, 2, 3 6 and 8.

Sampling Point		Total As	Diss. As	Total Cd	Diss. Cd	Total Zn	Diss. Zn	Total SO4
First Period - Summer 2002 to Summer 2005								
SP1	**Mean**	**91.5**	**38.6**	**4.20**	**2.91**	**285**	**204**	**1673**
(n=>91)[1]	*Std Dev[2]*	*77.3*	*38.8*	*2.98*	*2.62*	*121*	*105.5*	*614*
SP2	**Mean**	**23.2**	**9.2**	**0.77**	**0.15**	**76.5**	**50.9**	**1301**
(n=>91)	*Std Dev*	*27.5*	*17.1*	*1.44*	*0.74*	*70.5*	*54.0*	*535*
SP3	**Mean**	**6.54**	**1.99**	**0.18**	**0.07**	**42.3**	**36.9**	**1273**
(n=>80)	*Std Dev*	*11.62*	*5.39*	*0.30*	*0.11*	*46.0*	*40.0*	*546*
SP6	**Mean**	**4.85**	**1.03**	**0.018**	**0.010**	**7.50**	**5.2**	**1260**
(n=>65)	*Std Dev*	*7.06*	*2.23*	*0.028*	*0.002*	*9.84*	*8.4*	*720*
SP8	**Mean**	**1.13**	**0.56**	**0.015**	**0.012**	**2.76**	**1.2**	**1002**
(n=>72)	*Std Dev*	*1.09*	*0.65*	*0.018*	*0.007*	*3.69*	*2.4*	*535*
Second Period - Fall/Winter 2005								
SP1	**Mean**	**903**	**91.4**	**20.4**	**0.60**	**945**	**43.8**	**829**
(n=8)	*Std Dev*	*1490*	*112*	*33.75*	*0.57*	*1532*	*15.7*	*316*
SP2	**Mean**	**32.7**	**14.3**	**0.831**	**0.056**	**50.5**	**20.4**	**829**
(n=8)	*Std Dev*	*41.4*	*18.8*	*0.605*	*0.085*	*34.3*	*26.7*	*355*
SP3	**Mean**	**15.0**	**9.9**	**0.046**	**0.018**	**13.5**	**9.2**	**790**
(n=8)	*Std Dev*	*16.5*	*14.3*	*0.032*	*0.021*	*14.4*	*14.6*	*318*
SP6	**Mean**	**0.40**	**0.23**	**0.010**	**0.010**	**10.6**	**9.0**	**885**
(n=8)	*Std Dev*	*0.52*	*0.39*	*0.000*	*0.000*	*7.8*	*7.7*	*513*
SP8	**Mean**	**0.34**	**0.10**	**0.016**	**0.010**	**3.01**	**1.6**	**648**
(n=5)	*Std Dev*	*0.29*	*0.04*	*0.013*	*0.000*	*2.21*	*1.3*	*107*
Third Period - Summer 2006 to Winter 2007								
SP1	**Mean**	**23.1**	**3.42**	**0.963**	**0.460**	**62.0**	**40.7**	**798**
(n=21)	*Std Dev*	*13.1*	*1.5*	*0.320*	*0.179*	*16.6*	*11.9*	*180*
SP2	**Mean**	**11.5**	**2.09**	**0.255**	**0.035**	**25.7**	**17.9**	**771**
(n=22)	*Std Dev*	*8.4*	*3.9*	*0.273*	*0.052*	*14.0*	*10.2*	*171*
SP3	**Mean**	**4.46**	**2.67**	**0.038**	**0.014**	**16.7**	**15.4**	**808**
(n=22)	*Std Dev*	*8.33*	*6.89*	*0.034*	*0.007*	*7.0*	*7.3*	*256*
SP6	**Mean**	**1.46**	**0.24**	**0.011**	**0.010**	**14.4**	**13.7**	**838**
(n=20)	*Std Dev*	*1.30*	*0.19*	*0.002*	*0.000*	*3.1*	*2.9*	*308*
SP8	**Mean**	**0.28**	**0.23**	**0.011**	**0.010**	**1.01**	**0.78**	**647**
(n=19)	*Std Dev*	*0.11*	*0.11*	*0.005*	*0.000*	*1.51*	*1.30*	*365*

1 indicates that n more total metal samples than dissolved samples were done in this period; 2 calculated in Excel 2003

decreasing metal loads entering the treatment system in response to source control efforts (Figure 36; Figure 37; Figure 38; and Figure 40).

Mean total sulphate inputs decreased from 1673 mg/L in the first period to 829 mg/L in the second period and remain similar in the third period at 798 mg/L (Table 25). As well, the standard deviation decreased by half from the first to the second and half again from the second to third periods.

First Period

Evaluating system performance during the first period for the metal loads for which the system was designed is useful for assessing the system under "stable" conditions, while looking at the system after the "spike event" is instructive on how such a system responds to unusual events of elevated total metals load. The stability of the system is noted as the mean total and dissolved metals' and total sulphate concentrations decrease consistently from the input (SP1) through the cells to the final holding pond (SP8; Table 25). During the first period, total and dissolved As is effectively removed by the bioreactors throughout the year but generally by slightly higher total As reductions (94.4 to 99.2%) in the fall-winter periods with the lower flow rates and temperature versus total As reductions (73.7 to 89.6%) in the summer periods with the higher flow rates and temperatures. Conversely, total and dissolved As reductions are generally lower and much more variable through the wetland cells and indeed As releases have sometimes occurred in the summer periods. Whether this is due to increased summer flow rates or changes in pH and redox conditions mediated by plants in the wetland cells needs further study. Negative correlations of As with DO suggest a change in the redox conditions as being partly responsible. The complete system removes 95.8% to 99.7% of the total As and 91.5% to 99.5% of the dissolved As with higher mean percent As reduction in the winter compared to summer (Figure 40). Note that As removal differs from Cd and Zn being more effectively treated during the fall winter period versus the summer indicative of different removal mechanisms.

For total and dissolved Cd, the main reduction occurs in the anaerobic bioreactors with reductions of total Cd ranging from 84.6% to 99.7% and dissolved Cd from 95.3% to 99.8% with no apparent seasonal trends. While the wetland cells effectively remove much of the remaining total and dissolved Cd, a detailed evaluation is problematic as Cd is at or near detection limit at sample point 3 (Figure 5) as it enters the wetland cells. The

complete system reduces concentrations from 99.4% to 99.9% for total Cd and from 98.3% to 99.8% for dissolved Cd with similar mean percent Cd reduction in the winter compared to summer (Figure 40). This is not surprising given the low levels of Cd input compared to the large system treatment capacity, that CdS is more easily formed then ZnS as pH values increases above 4.5 (Hammock et al 1994; Tabak et al 2003) and Cd has preferentially adsorption to organic matter compared to Zn (Willow and Cohen 2003)

With respect to Zn reductions, while the anaerobic bioreactors decrease the Zn concentrations, the wetland cells assume a much more important role. The percent reductions experienced in the bioreactors ranged from 77.8% to 97.7% for total Zn and from 67.9% to 97.7% for dissolved Zn with no apparent seasonal trends (Table 24). The percent reductions experienced in the wetland cells are more variable ranging from 18.8% to 96.6% for total Zn and from 26.5% to 99.5% for dissolved Zn with generally higher percent reduction in the summer periods. The complete system reduces 97.9% to 99.9% of the total Zn and 97.5% to 99.9% of the dissolved Zn with slightly higher mean percent Zn reduction in the summer compared to winter (Figure 41). As ZnS is more easily formed at pH values greater than 6 (Hammock et al 1994; Tabak et al 2003), the increased biological activity (which elevates pH through the production HCO_3^-) in the wetlands and holding pond during the summer, may explain this increase in percent reduction of Zn.

Second Period

Similar to the first period, the mean total and dissolved metals' concentrations decrease consistently from the input (SP1) through the cells to the final holding pond (SP8; Table 25). However, while total sulphate did decrease slightly through the total system, it was not consistent. Some increases in the first period were noted as water passed through the system (Table 25). Overall percent metal reductions during the second period were good (99.7% or greater) and comparable to the first period for total As, Cd and Zn as well as for dissolved As (Table 24). However, dissolved Cd and Zn at 98.3% and 96.2% respectively are lower than the first period largely due to lower reductions of 42.9% and

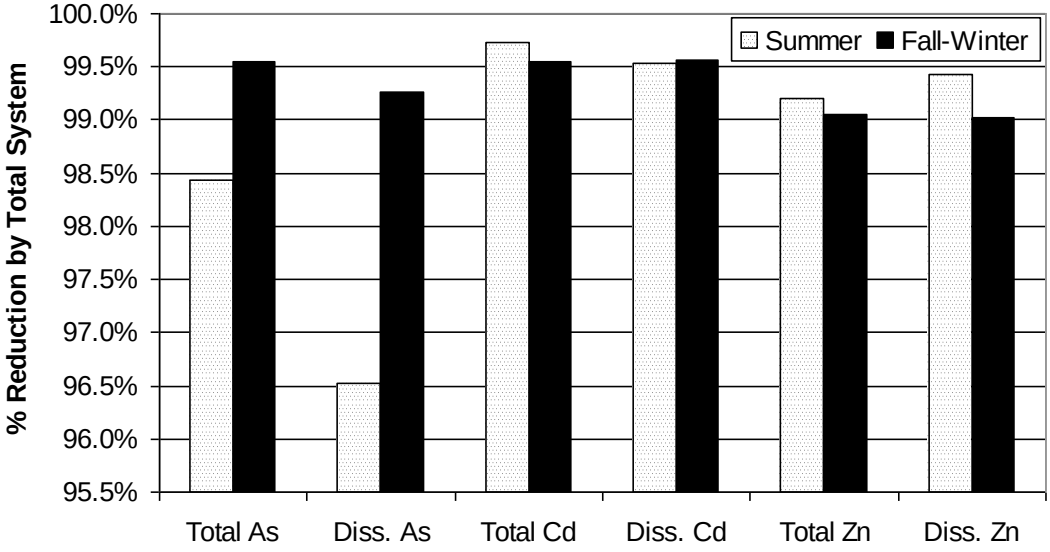


Figure 41. Differences between mean percent reduction of total and dissolved As, Cd and Zn for the total system for the first period (2002-2005) between Summer (n=4) and Fall-Winter (n=3) seasons.

2.7% respectively in the wetland cells (Table 24). Given the large spike event that resulted in threefold higher total Zn concentrations with fourfold lower dissolved Zn concentrations and tenfold higher total As concentrations with threefold higher dissolved As (Figure 40), the system responded well. The high total suspended solids load entering the system during this period is greatly reduced as it passes through the treatment system (Figure 36; Figure 37; and Figure 38). Thus it seems likely the spike was initially treated primarily by filtering of the suspended load (primarily Kottigite - $Zn_3(AsO_4)_2 \bullet 8(H_2O)$), both in the bioreactors and in the wetland cells. The spike event had total Zn and As concentrations (less dissolved) of 57.5 mM Zn and 44.8 mM As resulting in a molar ratio of 1.3 Zn:As which is close to the 1.5 for zinc arsenate, $Zn_3(AsO_4)_2$ but indicating likely that the material was somewhat amorphous and not totally crystalline. Another possibility is the mineral Warikahnite ($Zn_3(AsO_4)_2 \bullet 2(H_2O)$) which has a molar ratio of 38.46% Zn to 29.38% As or 1.3 Zn:As. If all the Zn was tied up with As as Kottigite then 19.2 mM or ~9,100 mg/L of Kottigite would be present in the sample. Given that the total suspended solid load was only measured at 6,300 mg/L, it is likely Zn and As precipitates were close too 100% of the TSS loading. The discrepancy while appearing

large is not surprising giving the difficulty of analyzing a water sample with such high TSS.

The stored material in the cells would be available to react to changing pH, redox (as indicated indirectly by dissolved oxygen concentrations) and over time, to dissolution due to the effects of these abiotic processes (pH and anoxic environment; Duncan et al 2008) and the resultant development of an environment where anaerobic bacteria can thrive that will lead to the bacterially mediated reduction of As. Dissolved metals are much more accessible to bacterially-based removal processes and therefore when the percentages of dissolved metals declines as drastically as it did during the spike events the effects are much more pronounced. While simple filtration processes likely removed the Kottigite, it is not stable under reducing conditions and subsequent release of Zn and As puts an internal load on the treatment system which likely carries over into the third period.

Third Period

The mean total and dissolved As input concentrations are five to eleven-folds lower respectively compared to the first period (Table 25; Figure 40). Similarly, the mean total and dissolved Cd input concentrations are four to six-folds lower respectively compared to the first period (Table 25; Figure 40). The mean total and dissolved Zn input concentrations are both four-fold compared to the first period (Table 25; Figure 40). Despite the lower concentrations in the third period, effective treatment appears to be lower for all the metals (especially dissolved metals) in the period immediately following the spike event (Table 24). Total system percent reductions for dissolved metals were as low as 89.4% for As, 97.8% for Cd and 95.8% for Zn. As well, releases were noted for dissolved As from the anaerobic bioreactors (this was evident from in the mean dissolved As for the whole third period as SP3 at 2.67 mg /L is greater than SP2 at 2.09 mg/L; Table 25) and for total and dissolved Zn from the wetland cells. This is likely due to the release of "stored" Kottigite in the various cells of the treatment system.

In general, the mean dissolved oxygen concentration in the input (SP1) decreases as it passes through the anaerobic bioreactors (SP2 and SP3) and slowly increases as it passes

through the wetlands system (SP4 to SP6) with the highest concentrations in the final holding cell (SP8; Table 26). The median pH values, however, tend in general to be lowest at the input and increase through both the bioreactors and the wetland cells, particularly in the first period and during the spike or second period (Table 26). For the third period, the pH does increase consistently through the bioreactors but is more variable in and between the wetland cells.

The wetlands cells may still be processing the consequence of the spike event as percent reductions are negative for both total and dissolved Zn in the summer of 2007 and the fall-winter of 2007. Thus one can assume a period of stabilization would be required for the treatment system to reach its previous level of performance at the new lower input levels and to treat the large suspended solid load received and filtered during the "spike" event. Another possible explanation or co-factor may be the change that is occurring in the plant population of the third wetland cell where *Phragmites australis* is out-competing the original *Typha latifolia* plantings may result in the release of plaque material (and associated Zn and As) from dying *Typha* rhizomes and/or changing pH values and DO concentrations (Table 26). The median pH for *Typha* wetland cell (SP6) during the first period was 7.06 compared to 6.35 for the third period. While the mean DO was 3.3 mg/L in the first period compared to 3.0 mg/L in the third period. Such Eh and pH changes can affect the solubility of the some of the Zn and As precipitates known to occur in the system (Duncan et al 2008).

Whether the lower percent reductions of Zn in the wetland cells are the result of plant population changes (changing redox and pH conditions thereby affecting treatment rates (Table 26) or the release of previously plaque-bound metals) or is the consequence of a slow release of dissolved Zn from stored Kottigite that exceeds removal capacity as has been proposed is not clear at the present time. The continued operations of the wetlands with additional focused research may address this issue. Indeed the lower percent

Table 26. Median pH values and mean dissolved oxygen (DO) concentrations (mg/L) by sampling points and periods (S = Summer – May 15 to September 17; FW = Fall Winter – September 18 to May 14).

Season		SP1	SP2	SP3	SP4	SP5	SP6	SP8
				First or "Stable" Period				
S 2002	**pH**	5.18	6.22	6.64	6.56	6.85	6.81	7.80
	DO	4.7	2.1	1.8	0.9	1.3	2.1	4.1
FW 2002	**pH**	5.50	6.30	6.70	6.70	6.96	7.20	7.99
	DO	4.5	3.2	4.0	2.6	3.0	3.5	8.1
S 2003	**pH**	5.22	6.32	6.82	6.67	6.64	7.01	7.94
	DO	4.2	2.2	3.2	3.3	3.8	4.1	6.2
FW 2003	**pH**	5.88	6.27	6.52	6.52	6.77	6.81	7.23
	DO	3.7	1.8	2.6	3.0	4.3	4.6	5.8
S 2004	**pH**	6.05	6.36	6.78	6.95	7.00	7.58	7.93
	DO	2.8	1.2	1.2	1.5	1.1	2.5	5.4
FW 2004	**pH**	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
	DO	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
S 2005	**pH**	6.18	6.43	6.70	6.70	6.85	7.10	6.84
	DO	3.6	1.3	2.0	2.3	2.6	3.3	4.9
				Second or "Peak" Period				
FW 2005	**pH**	5.90	6.40	6.69	6.75	6.76	6.79	7.10
	DO	3.4	1.4	2.4	2.0	1.8	3.2	5.3
				Third or "Stabilization" Period				
S 2006	*IS*							
FW 2006	**pH**	6.29	6.58	6.69	6.72	6.93	6.98	7.02
	DO	5.5	2.4	2.4	2.4	2.6	3.8	6.9
S 2007	**pH**	5.85	6.01	6.24	6.32	6.40	6.35	6.82
	DO	5.6	2.6	3.7	2.7	2.9	3.2	7.3
FW 2007	**pH**	6.12	6.22	6.38	6.55	6.37	6.28	6.78
	DO	3.4	2.5	2.2	1.9	1.9	2.3	4.3

ns= not sampled; IS = insufficient sampling

reductions for total and dissolved As in the bioreactors after the spike were noted with the lowest in summer 2007 but this now appears to be recovering in the fall-winter 2007 period (Table 24). The release of dissolved As from the bioreactors in summer 2007 is likely partly due to very low mean dissolved As input (3.03 mg/L) which increased through the bioreactors to 6.07 mg/L. This increase in dissolved As supports the idea that

bacterial dissolution of stored Kottigite may be occurring. In the fall-winter 2007 period, the mean dissolved As input of 4.13 mg/L was decreased through the bioreactors to 0.86 mg/L. A similar depression followed by improvement of percent reductions by the bioreactors during the same periods are noted for total and dissolved Zn as well (Table 24).

If As is removed as a result of the formation of an insoluble thioarsenious compound such as As_2S_3 (orpiment; Newman et al 1997a), it would be expected that as the DO goes down that the As would be removed more effectively. Orpiment is stable at all pH ranges found within the system so pH is not a factor. But if sulphide concentrations get too high the As_2S_3 can be re-dissolved as thioarsenites (Castro et al 1999). Whereas, the release of As from Kottigite is pH dependent at conditions found in the system (Duncan et al 2008). There appears to be evidence of a disconnect between the removal of As and that of Zn (Figure 36; Figure 37; and Table 18).

Summary

The overall mean percent reduction for the total system for As, Cd and Zn is high ranging from 91.5 to 100% for dissolved metals and from 95.8 to 100% for total metals varying by metal and season. The anaerobic bioreactors are responsible for large reductions in all metals concentrations particularly for As and Cd. The vegetated wetland cells provide an important polishing step especially for Zn. The sand filter is effective in reducing total metal concentrations with respect to dissolved metals concentrations. Initially, the final holding pond was just a lined reservoir for irrigation and a failsafe to allow testing prior to release. Over the years, it has transformed into a free water wetland with cattails at the edges and a sediment layer throughout and provides a useful polishing function (particularly for Mn and ammonia). Its' large storage capacity also moderates seasonal and daily upstream treatment variability.

The system has proven resilient to varying inputted metal loads including a large "spike" event caused by the dewatering of the As materials stored in the lined cell in Fall 2005 of zinc arsenate of up to 3,800 mg Zn/L and 3,600 mg As/L. Source control measures implemented between 2002 and 2005 appear to have been successful in

reducing the total metal loads in the collected seepage and since 2006 the decline continued albeit at a slow rate. During the "stable" period, higher mean percent As reductions were noted in the winter compared to summer with releases often occurring from the wetland cells. While slightly higher mean percent Zn and Cd reductions were noted in the summer compared to the winter with the anaerobic bioreactors reducing the bulk of these metals. The high metal reduction rates observed during the spike event are likely due to filtering of zinc arsenate by the anaerobic bioreactors. Lower percent metal reductions were noted after the spike event as the system processed the high load of that event.

Chapter 4 - Wetland Carbon Dynamics – System Lifespan

Introduction

An anaerobic bioreactor designed to passively remove high concentrations of metals from leachate was in operation intermittently for five years since 1998 (Figure 1). High metal removal efficiencies were achieved by metals retention in the bioreactor. Over the period of operation the cell removed combined total metals of 1097 kg or approximately 1 tonne of As, Cd and Zn combined (Table 27; Duncan et al 2004). While the performance of the anaerobic was good, two issues lead to the decision to rebuild the cell. The first issue was the development of short-circuiting which had been fixed to a degree but we wanted to apply the design improvements of the upstream bioreactor into a rebuild. This would require better liner material that could be "welded". The second issue relates to the "success" of the cell and the high metals concentrations that were accumulating in the cell. As noted previously, funding has been limited and the original liner material was an inexpensive geomembrane tarpaulin material. So concerns about the liner integrity into the future came to the forefront. The cell was rebuilt using a 60 mm high density polyethylene liner with new design features incorporated.

The decision to rebuild the cell provided an excellent opportunity to sample the cell from top to bottom in a systematic manner. To the author's knowledge no anaerobic cell of this size has been sampled in such a complete manner. During the deconstruction of the bioreactor, systematic sampling at all depths (layers) of the biological substrate was done to evaluate possible relationships between metals and carbon concentrations as they may impact cell life expectancy.

Table 27. Operating days, total flow and metal removed (kg of As, Cd and Zn) during operating lifespan of deconstructed anaerobic bioreactor.

Year	Operating Days	Total Flow	As (kg) Removed	Cd (kg) Removed	Zn (kg) Removed	Total (kg)
1999	126	1,606,279	99.6	4.6	185	289
2000	261	2,824,000	-3.5	4.6	350	351
2001	342	3,776,806	80.8	15.3	360	456
Total	**729**	**8,207,085**	**177**	**24.5**	**895**	**1097**

Little is known about the potential life expectancy of anaerobic bioreactors. Gusek and Wildeman (1997) suggest that wetlands could have a potential lifespan of 20+ years. The cell lifespan is determined by the remaining carbon that can be used by bacteria and potential for plugging or short-circuiting due to substrate collapse or filling of void spaces by metal precipitates. The opportunity to sample an anaerobic cell of this size at all levels of the cell provides information that can be used to predict the life expectancy of compost bioreactors that are designed to promote SRB to enhance metals' removal by the production of H_2S and subsequent precipitation of divalent metal sulphides.

SRB are not known to use natural polymers such as cellulose and hemicellulose but instead use the soluble fermentation products (e.g., lactose, ethanol, acetate, H_2). It is a complex microbial community that develops in the bioreactors on the solid phase organics with the SRBs at the end of a multiple stage process in the degradation of the complex organic materials into the simpler dissolved compounds. This complexity is shown in a schematic (Figure 42) of the processes adapted from Figueroa et al (2004). Fundamentally, it would be useful to determine the relative ratio of the carbon immediately available to SRBs as compared to the remaining long term carbon available in the cell to the overall bacterial community that supplies the SRBs (above the horizontal dashed line in Figure 42) to determine cell life. The author proposes that Rock Eval 6 may prove to be a useful tool in this regard.

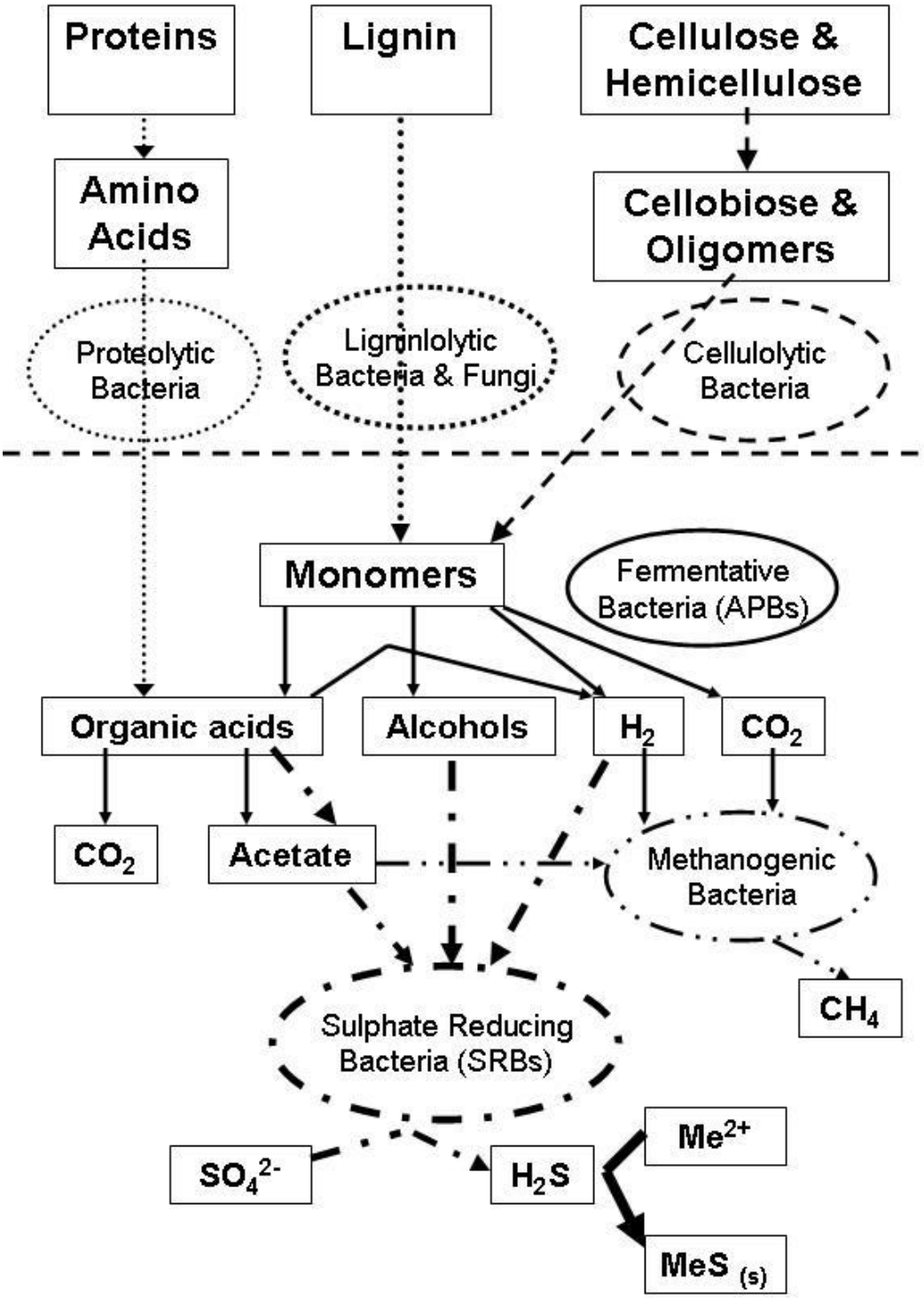


Figure 42. A simple schematic of the anaerobic breakdown of complex organic compounds by bacteria (adapted from Figueroa et al 2004).

Figueroa et al (2004) suggest that the quantitative measures of organic components in agricultural residuals developed by National Renewable Energy Laboratory Biomass to Energy Program to assess its suitability for ethanol production would be useful but overly complex and expensive. The authors went on to define a simpler and less expensive method based on ethanol, neutral detergent and acid solubility extracts. They found that several of the fractions provided good measures of the relative degradation rates of the organic material over the 150 day trial and the sulphate reduction rates in their columns appeared to increase in response to them. As well, they proposed that their method can be used to assess the wide variety of types of organic carbon (e.g., alfalfa, sugar beet pulp, wood chips, walnut hulls, brewery waste, corncobs, etc) for their suitability developing mixtures to fuel anaerobic bioreactors.

Zagury et al 2006 did a simple characterization of several different organic substrates for their suitability for SRB in a 70-day batch experiment with synthetic AMD. Their results did not yield any clear results on the abilities of the substrates to promote sulphate reduction and metal removal. The need to address long term field dynamic flow conditions in flow-through systems was suggested and the authors also concluded that the applicability of batch tests was very limited. A flow-through system would account for the flushing of easily available carbon, nutrients, biomass and other by-products of microbial metabolism. As well, formation of preferential flows, clogging, compaction and subsequent variation in HRT need to be tested to select the best organic carbon mixture.

Subsequently, Neculita and Zagury (2008) again stressed the need for a rigorous and methodological characterization to predict the biodegradability of organic substrates by SRB. They thoroughly assessed four natural organic substrates for a wide variety of elements and biodegradation parameters (e.g., TOC, cellulose, C/N, lignin, etc.) using many techniques. They then ran 150 day batch experiments with varying composition of the four substrates and measured sulphate reduction rates and metal removal. In these batch tests they found C/N and DOC/SO_4^{-2} were associated with better sulphate reducing conditions. They also found higher efficiencies in reactors with 30%

cellulosic wastes compared to those with 2-3% cellulosic wastes. However, the short-term batch experiment did not mimic a flow-through system where DOC may be flushed out of the system as it is produced. Neculita and Zagury (2008) used the best reactive mixture in column bioreactors that they intend to run for over a year at various HRTs. Their work illustrates the complexity of the issue and the goal of determining a cost-effective method of measuring carbon to assess bioreactor performance and predict cell life at the design phase.

Methods - Sampling and Analysis

During cap removal, 25 samples were taken uniformly over the surface from the top layer of the cell. Once the cap was removed, further samples were taken from each subsequent layer systematically (Layers A, B and C respectively with increasing depth). The cell was roughly divided into nine sample quadrats. The top layer immediately below the non-woven geotextile acting as a filter cloth (Figure 1) was designated Layer A. Each of the nine quadrats was sampled at three levels – at the top of the layer (immediately below the non-woven geotextile or RPE) in the middle of the layer, and at the bottom of the layer (immediately above the RPE separation). Samples were placed in labelled 250 ml sample bottles and capped. The B and C layers were sampled in the same way. Samples were stored in a cool place until delivery to GSC – Calgary (Rock-Eval) and Bequerel Laboratories - Mississauga, ON (metals and S). The metals and sulphur were analyzed by ICP-MS and organic carbon by Rock Eval 6 pyrolysis as described by Lafargue et al (1998). A certified reference standard was ran at the start and end of each set of samples ran for Rock Eval 6 pyrolysis for quality assurance purposes.

Rock Eval 6 is an automated commercial two-step pyrolysis and oxidation instrument manufactured by Vinci Technologies of France. This method provides TOC (% total organic carbon) and many other parameters such as hydrogen index, oxygen index, %PC (pyrolizable carbon), %RC (residual carbon), S1 carbon (low molecular weight carbon compounds), and S2 carbon (from thermal cracking) related to the form of carbon present. This detailed carbon analysis may provide useful insights to a cell's life expectancy and be useful in characterizing the suitability of various organic substrates for

their usefulness in anaerobic "compost" bioreactors. Soluble metal ions are reduced to insoluble metal sulphides using carbon as the energy source and deposited in the cell. By comparing TOC and other Rock Eval 6 measures of carbon to metals and to sulphur concentrations it may be possible to elucidate the relationship between carbon consumption by bacteria and metal deposition as sulphides using statistical procedures.

Results and Discussion

Metal and carbon content in the deconstructed cell

In the original construction a 10 cm layer of sand was placed on top of the three layers of the active biological substrate. However, during the second year of operations short circuiting developed with effluent moving along the outside walls of the cell and traveling directly to the filtering layer. This became apparent when rhodamine dye was added to the input of the system to determine hydraulic residence time and the dye quickly appeared on the surface of the cell (Figure 3). Repairs included adding a layer of 60 mm HDPE liner that covered the sand filter layer and that lapped over the sides of the existing cell leaving an open area in the middle of the cell. The entire cell was then capped with a biosolids substrate resulting in another treatment area which comprises the Cap layer.

The added biosolids cap was therefore active for a shorter time period and metals found there are the result of both *in situ* bacterial treatment and metal sulphide migration from lower layers. Sampling attempted to exclude the sand only layer when sampling the Cap layer. However, some extremely low TOC values found in the Cap layer indicate that in some cases the sand layer was inadvertently sampled. These samples were excluded from further statistical treatment as were two samples with anomalous very high TOC values.

Total concentrations of those metals that can form sulphides given the pH conditions of the Trail system include Sb, As, Bi, Co, Cd, Cu, Fe, Pb, Hg, Ni, Ag, Sn, and Zn. The total of all these metal combined is presented for the four layers (Cap, A, B and C) that comprised the cell substrate (Table 28; Duncan et al 2004). The main inflow

contaminants in the system are As, Cd and Zn which taken together comprise 34% of the total potential metal sulphide concentrations in the cell substrate with Fe accounting for over 60%. Almost no Fe was present in the inflow but Fe was present in the sand added to improve the hydraulic conductivity of the bioreactors. Dissolved Fe shows a marked increase in concentration entering to the second anaerobic bioreactor as compared to the collected seepage – an increase from 0.1 to 14.5 mg/L Fe in the first bioreactor (Figure 39). The dissolved iron was likely due to the activities of IRB, which were present in both of the anaerobic cells in relatively high numbers (Table 20). As well, the outflow of the second anaerobic bioreactor shows decreased dissolved Fe as compared to its inflow (from 14.5 to 5.0 mg/L Fe). Iron sulphides will form only when the iron is in the Fe^{+2} oxidation state. Therefore, Fe was included in the total metal sulphide calculations as under the conditions in the bioreactor it could easily precipitate as a sulphide. Metal concentrations assayed in each level in each layer were analyzed for statistical significance using the one-way ANOVA test for variance. Part of this procedure used the quadrat samples as replicates across the three sample layers and across the three layers plus the capping material (Table 28).

The bioreactor system was designed to remove metals by formation of insoluble sulphides. The partially treated effluent enters the cell through the bottom (C) layer and exits through an exit pipe that is buried in the capping layer and it is expected that higher concentrations of sulphides will be seen in the lowest layer. Mean metal sulphides concentrations (Table 28) confirm this assumption with significantly higher concentrations in the bottom (C) layer when compared to A, B, or Cap layers. Indeed, individual sampled quadrats seemed to indicate higher metal concentrations along the short-circuit flow path that developed in the cell. Significant differences are also seen when the three biosolids layers are examined together as well as when all four layers are combined (Table 28).

The reactor is functioning as expected with highest metal sulphides concentrations seen in the C layer and progressively lower amounts seen in layers A and B. The high concentration in the Cap layer is expected since the layer was originally designed as a

filtering mechanism and to treat water short circuiting directly from the C layer that bypassed the A and B layers.

Linear regressions support the model of declining concentrations of TOC and increasing concentrations of S as metal concentrations increase (Table 29). The R^2 values when the four layers combined or when the biosolids only are not high and reflect at best, a weak relationship with the model but the correlations are significant at the 99% level of confidence except when As and TOC in the biosolids layers alone regressions where they are significant at the to 95% level of confidence. The highest R^2 value (0.20) is seen for Cd and S in the four layers combined.

Table 28. Comparison of total potential metal sulphides concentrations in the deconstructed anaerobic bioreactor showing mean metal concentration (mg/kg) across all layers, standard deviation, and results of one-way ANOVA tests across all four layers and between each of the four layers. A separate ANOVA for the three combined biosolids layers (A, B & C) is included.

					One-way ANOVA Results	
Layer	**Cap**	**A**	**B**	**C**	**F**	***P***
Mean Metal Sulphides	20106	23554	23350	26791	4.04	*0.0096* *
St. Dev.	11475	1586	2548	6684		
Cap to A					2.22	*0.14*
Cap to B					1.88	*0.18*
Cap to C					5.66	*0.02***
A to B					0.12	*0.74*
A to C					4.93	*0.03***
B to C					5.20	*0.02***
Biosolids Layers Only					**4.69**	***0.01****

* *Significant at the 99% confidence level*
** *Significant at the 95% confidence level*

When looking at the metals and S linear regressions it is likely that the high concentrations of sulphides found in the capping layer affect the analysis when the four layers combined (i.e. the entire cell) are examined. The designed filtering action results in higher than expected concentrations of metals present in this layer. Although Fe is considered as being present as sulphide, it is not likely that all the Fe found in the system

is present as sulphides and it could be present as siderite ($FeCO_3$) or other stable iron minerals. Since the highest metal concentration seen in the system is Fe by including it in the analysis it strongly affects the results. Examining the R^2 values for the cap layer offers support for this as the results show values of 0.66, 0.58, and 0.73 for As, Cd and Zn respectively and only 0.29 when all sulphides are considered.

When the four layers combined are taken together the effect is less pronounced with R^2 values of 0.19, 0.20 and 0.11 for As, Cd and Zn respectively and 0.11 for all sulphides taken together. When the biosolids layers alone are looked at the regressions for metals and S are 0.18 for As, 0.25 for Cd, 0.25 for Zn and 0.06 for all sulphides.

Examining the individual layers offers additional insights into the operations of the cell. For the bottom or C layer much higher statistically significant correlations are evident for As, Cd and Zn indicating a moderate adherence to the model when regressions for S and metals are examined. For total sulphides, however, the regression is much lower and not significant. When the B and A layers are examined the regressions are neither strong nor significant. In the B layer the As, Cd and total sulphide correlations are negative but the R^2 values are so low and the P values so high that the model is not undermined. Two possible reasons for this are that the sulphides from this area migrated upwards to the filtering area and/or that the short circuit meant that there was a greatly reduced flow of effluent through these layers and therefore a correspondingly lower metal removal. Mean values of sulphides show lower mean concentrations of more than 3000 mg/kg in the A and B layers when compared to the C layer (Table 28).

When TOC regressions are examined the correlations are all negative as expected by the model (Table 29; Duncan et al 2004). But only in the A and Cap layers do the R^2 values for individual metals indicate a moderate to strong relationship with values as high as 0.47 for Zn and TOC in the cap layer and 0.42 in the A layer. The correlations for the

Table 29. Single linear regressions on deconstructed four-layer anaerobic cell where individual metals, As, Cd, Zn were regressed against S and TOC[1].

1st Var.	2nd. Var.	N	R^2	P	1st Var.	2nd. Var.	n	R^2	P
Four layers Combined					**Biosolid Layers Only**				
As	S (+)	92	0.19	0.0000[a]	As	S (+)	75	0.18	0.0001[a]
As	TOC (-)	92	0.09	0.0021[a]	As	TOC (-)	75	0.07	0.0157[b]
Cd	S (+)	92	0.20	0.0000[a]	Cd	S (+)	75	0.25	0.0000[a]
Cd	TOC (-)	92	0.15	0.0002[a]	Cd	TOC (-)	75	0.09	0.0056[a]
Zn	S (+)	92	0.11	0.0006[a]	Zn	S (+)	75	0.25	0.0000[a]
Zn	TOC (-)	92	0.16	0.0000[a]	Zn	TOC (-)	75	0.10	0.0037[a]
Sul.	S (+)	92	0.11	0.0006[a]	Sul.	S (+)	75	0.06	0.0000[a]
Sul.	TOC (-)	92	0.08	0.0037[a]	Sul.	TOC (-)	75	0.21	0.0000[a]
C Layer					**B Layer**				
As	S (+)	25	0.45	0.0002[a]	As	S (-)	23	0.01	0.88
As	TOC (-)	25	0.09	0.1172	As	TOC (-)	23	0.02	0.48
Cd	S (+)	25	0.36	0.0011[a]	Cd	S (-)	23	0.14	0.06[c]
Cd	TOC (-)	25	0.10	0.1296	Cd	TOC (-)	23	0.02	0.47
Zn	S (+)	25	0.46	0.0001[a]	Zn	S (+)	23	0.22	0.02[b]
Zn	TOC (-)	25	0.10	0.1221	Zn	TOC (-)	23	0.09	0.13
Sul.	S (+)	25	0.08	0.1623	Sul.	S (-)	23	0.02	0.41
Sul.	TOC (-)	25	0.39	0.0006[a]	Sul.	TOC (-)	23	0.35	0.0016[a]
A Layer					**Cap Layer**				
As	S (+)	23	0.01	0.7027	As	S (+)	16	0.66	0.0001[a]
As	TOC (-)	23	0.41	0.0006[a]	As	TOC (-)	16	0.43	0.0041[a]
Cd	S (+)	23	0.05	0.2740	Cd	S (+)	16	0.58	0.0003[a]
Cd	TOC (-)	23	0.45	0.0002[a]	Cd	TOC (-)	16	0.32	0.0170[b]
Zn	S (+)	23	.009	0.1412	Zn	S (+)	16	0.73	0.0000[a]
Zn	TOC (-)	23	0.42	0.0004[a]	Zn	TOC (-)	16	0.47	0.0024[a]
Sul.	S (+)	23	0.07	0.1988	Sul.	S (+)	16	0.29	0.0229[b]
Sul.	TOC (+)	23	0.01	0.2313	Sul.	TOC (-)	16	0.21	0.0602[c]

[1] Single regressions were also completed on total potential metal sulphides against both S and TOC. The same regressions were completed on each of the four layers separately, on three biosolid layers together and on four layers combined. Signs in parenthesis indicate either a positive or negative correlation. Abbreviations: Var. is variable; Sul. is Sulphides.

[a] = statistically significant at the 99% level of confidence

[b] = statistically significant at the 95% level of confidence

[c] = statistically significant at the 90% level of confidence

regressions in these two layers are significant at the 99% level of confidence except for Cd in the cap layer where the confidence level drops to 95%. In both these layers the R^2 values for total sulphides are much lower, although it is significant at the 90% level for

the cap layer. In the B layer the only high R^2 value (0.35) is seen in the total sulphide regression a measurement that is significant at the 99% confidence level.

When TOC measurements are regressed against either individual metals or total sulphides for the combined cell or for the biosolids layer only, the results show a weak relationship with R^2 values as low as 0.07 for Cd and TOC for biosolids and as high as 0.15 for Cd and TOC when all four layers are taken together. The correlations are all negative as expected and all are significant at the 99% level of confidence but only the regression for total sulphides against the biosolids layers data points indicates a moderately strong relationship with a R^2 value of 0.21.

The strength of the relationship of metals and TOC is less obvious than for metals and S. This can be explained several ways. First, the starting substrate was not homogeneous in starting TOC concentrations so noise could be expected in the data. Second, not all the metals being treated by the anaerobic cells remain where the TOC was consumed (e.g., Cap has high metal sulphides but some of the lowest TOC values and is likely filtering sulphides produced elsewhere).

Determining system longevity

As the major contaminant entering the system is Zn, one of the main metal sulphides in the anaerobic bioreactor solids is likely ZnS. So a classification scheme (based on Zn concentrations) was developed as follows: 0 – 200, 200 – 400, 400 – 800; 800 – 1600; 1600 – 3200; 3200 – 6400; and 6400 – 12800 mg/L Zn. To reduce the observed variability, 5 outlier samples with less than 1% or greater than 10% TOC were removed (as previously mentioned) and sample means calculated based on Zn concentrations.

Corresponding means for total Zn, Cd and As (mg/kg), S (%), and carbon (TOC as wt%, %PC – pyrolysable organic carbon as wt% and %RC – residual organic carbon as wt%) were calculated for the respective Zn groupings (Figure 43). A relationship between decline in TOC and increase in total metal appears evident. While %RC and TOC appear to track very closely with some minor difference noted in the 0-200 Zn

grouped means, a decrease in %PC compared to %RC and TOC at higher Zn group means seems to indicate the use of available carbon preferentially with increasing total metal. The large increase in of metals when moving from 3200-6400 to 6400-12800 Zn grouped means corresponds to an almost doubling of the percent sulphur from 0.39% to 0.67% (Figure 43), while the percentages Zn and As of the total metals change from 95% Zn and 4% As to 88% Zn and 11% As respectively. This may be indicative of Kottigite being present where higher concentrations of metals exist in the cell and that not all mineralization is as metal sulphides.

These trends are even more evident when the mean ratios of %PC/TOC and %RC/TOC and mean TOC are plotted based on Zn groupings (Figure 44). As TOC trends downward from 6.66% to 2.56% with increasing metal concentrations, the ratio of PC/TOC is also decreasing from 0.37 to 0.26 which is indicative of less of the TOC is readily available. As well, the ratio of RC/TOC is increasing from 0.63 to 0.74 confirming this trend. These results provide support a model where higher metal loads to portions of the cell have reduced the available C. The model assumes where little Zn is found that minimal C processing has taken place (close to time zero), while areas of high Zn concentrations likely due to short-circuiting have had maximal C processing (or figuratively are much older).

The characterization of the carbon is considered a critical design issue especially for long term operation and predicting cell life. The author proposes use of the Rock Eval 6 for this purpose as a quick and cost-effective method for characterizing the amount and type organic carbon in a cell. Further information beyond TOC can be obtained from Rock Eval 6 analysis (Disnar et al 2003; Sanei et al 2005). The S1 curve (mg HC/g sample where HC stands for hydrocarbon) composed of free volatile C compounds of low molecular weight and the types of C being most available to SRB. The S2 curve (mg HC/g sample) is related to the thermal cracking of organic matter and would include

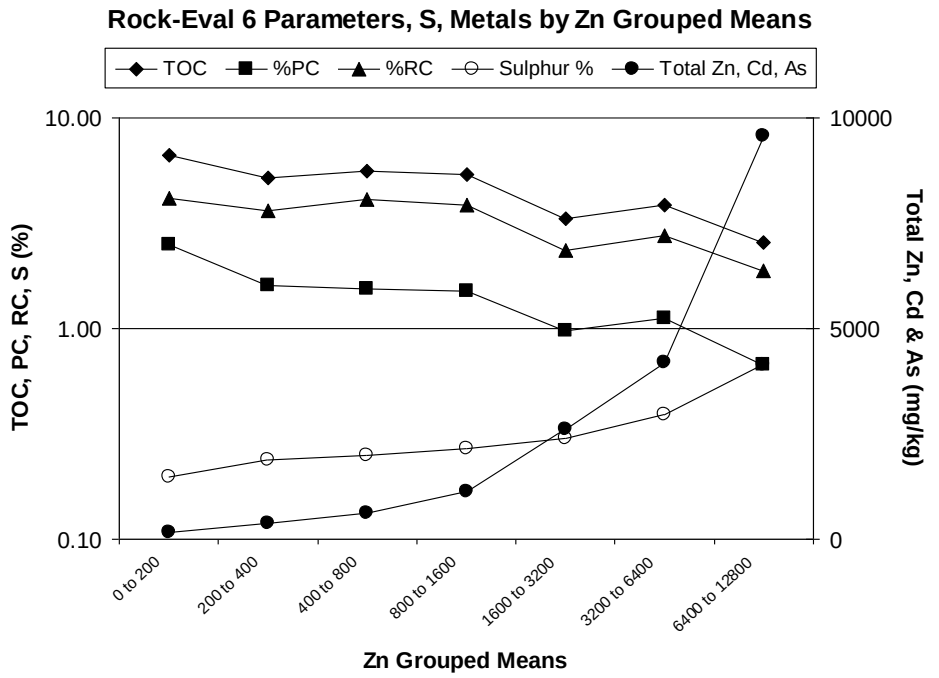


Figure 43. Mean values of TOC (%), %PC, %RC, sulphur (%) and total Zn, Cd and As concentrations (mg/kg) grouped by Zn in deconstructed anaerobic bioreactor.

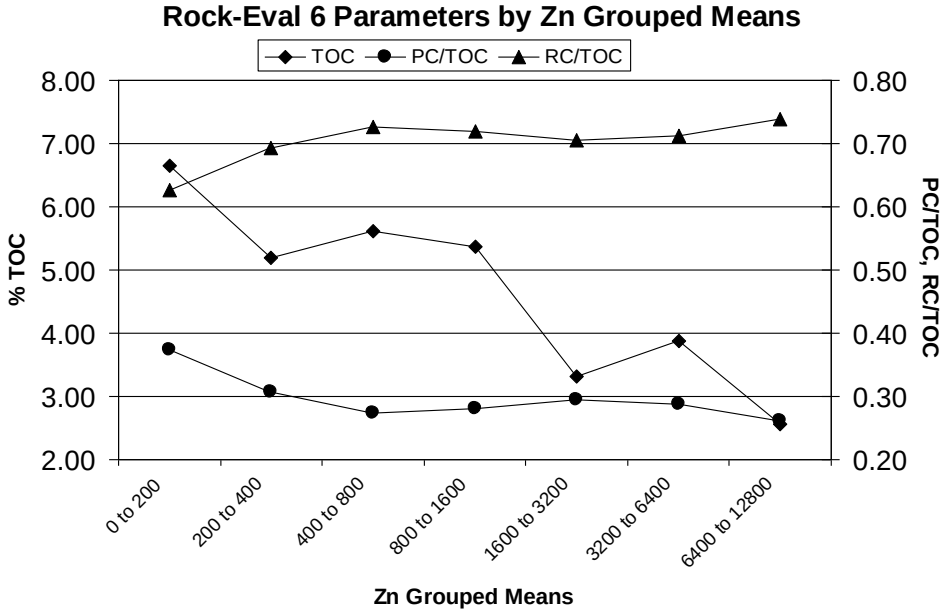


Figure 44. Mean values of TOC, %PC/TOC and %RC/TOC based on Zn grouped means in deconstructed anaerobic bioreactor.

sugars, cellulose and lignin (Sebag et al 2006). Through Gaussian elementary analyses of the S2 curve, a great deal of additional information of the organic matter types present can be determined and this information has been used to characterize the organic matter degradation in soil profiles (Sebag et al 2006). Thus Rock Eval 6 has the potential to be used like other carbon characterization techniques. This thesis will explore only the use of the standard parameters obtained (i.e. TOC, %PC, %RC, S1, S2, HI and OI), where HI stands for Hydrogen Index (as mg HC/g TOC) and OI stands for Oxygen Index (as mg HC/g TOC).

The S1 values decreased from 8.13 to 1.87 mg HC/g sample and the S2 values decreased from 20.34 to 5.68 mg HC/g sample with increasing Zn concentrations (Figure 45). While the ratio S1/S2 from 0.40 to 0.33 indicating a decrease in the lower molecular weight organic compounds compared to the lignin and cellulose in the sample. This may indicate that where the higher metals' concentrations are found that the lower weight organic compounds have been preferentially consumed. The S1/S2 ratio may be useful in determining the rate limiting step of the conversion of cellulose to the low molecular weight carbon after a bioreactor has used the readily available carbon in the source material.

Additional samples for Rock Eval 6 were analysed including raw pulp mill biosolids, well-composted biosolids mixed with sand and samples from the various system cells (Table 30). The raw biosolids are characterized by high TOC content and very high S2 value likely indicating the high cellulose content due to the short wood fibres in the material. The hydrogen index (HI) and oxygen index (OI) are higher than any of the other samples which are similar to freshwater lake sediments (Sanei et al 2000). As the biosolids come for an outdoor aerated lagoon that contains bacteria, algae and zooplankton this result is not surprising. The well-composted biosolids are decreased in TOC both due to degradation of the organic matter during composting but also some dilution by sand addition in this sample. There is a large reduction in the S2 value with a smaller reduction in the S1 value. As well, large reductions in the HI and OI are noted.

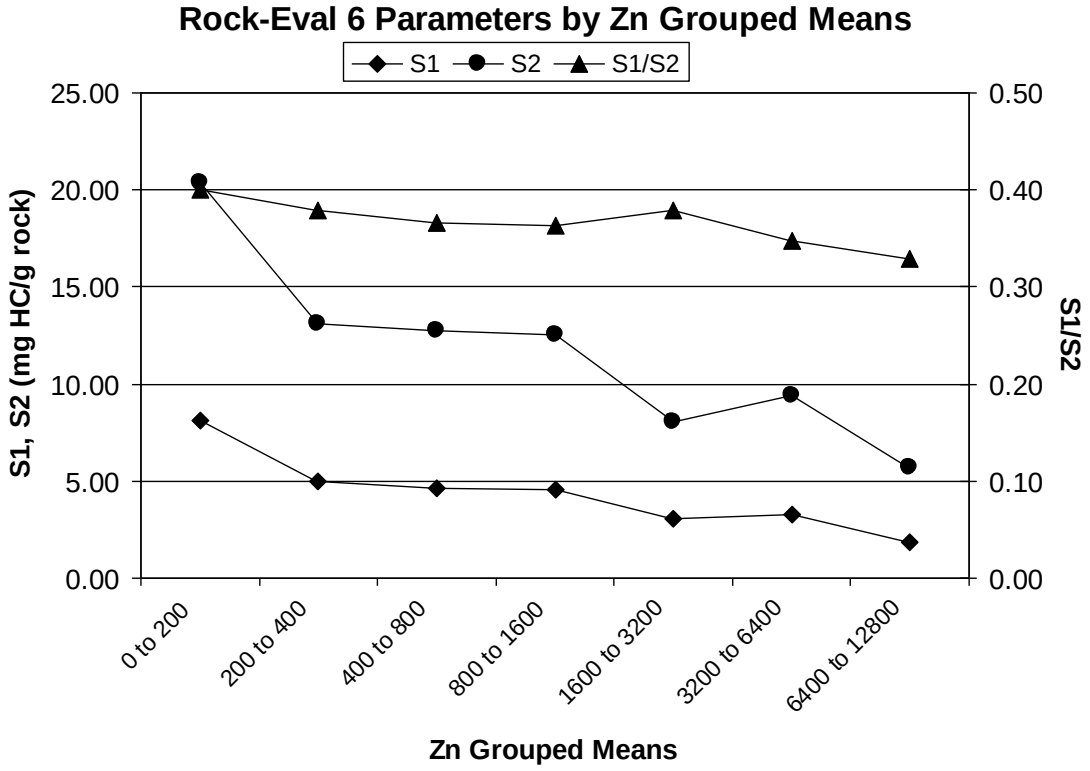


Figure 45. Rock Eval 6 parameters S1 and S2 (as mg HC/g sample) and S1/S2 based on Zn grouped means in deconstructed anaerobic bioreactor.

Two surface cores from the first anaerobic bioreactor displayed quite similar patterns compared to the first two Zn groups (0-200 and 200-400) from the deconstructed anaerobic bioreactor for TOC, PC/TOC, RC/TOC and S1/S2. This may indicate that these 2 cores may not have been on a preferential flow pathway and had not treated much in the way of metals after 5 years of operation. However, the HI and OI are lower for the two cores and indicating some alteration of the organic matter over 5 years.

The presence of organic matter in the gravel matrix of the wetland cells after 10 years of operation is encouraging given that the starting TOC would be essentially nil. This indicates that plant litter is accumulating in the cells and contributing to the anoxic conditions in the deeper portions of the cell. This supports the fact that plant-based

wetlands can have long life expectancy for treating metals and supports the self-sustaining "volunteer" wetland concept discussed by O'Sullivan *et al* (1999). However, vegetative systems are subject to metal toxicity issues at higher metals concentrations thus limiting the range of their usefulness. The S1 and S2 values are quite low compared to the other samples with low S1/S2 ratios and high RC/TOC ratios, which may indicate the presence of a "leached out" litter component.

Table 30. Rock Eval 6 parameters for deconstructed anaerobic bioreactor by Zn grouping, raw and composted (with sand) biosolids and samples from various system cells collected on October 28, 2005 (units are discussed in text).

	TOC	%PC	%RC	PC/TOC	RC/TOC	S1	S2	S1/S2	HI	OI
By Zn Group										
0 to 200	6.66	2.49	4.17	0.37	0.63	8.13	20.34	0.40	305	160
200 to 400	5.20	1.59	3.61	0.31	0.69	4.97	13.11	0.38	252	140
400 to 800	5.62	1.53	4.08	0.27	0.73	4.66	12.74	0.37	227	143
800 to 1600	5.37	1.51	3.86	0.28	0.72	4.56	12.54	0.36	231	146
1600 to 3200	3.32	0.98	2.34	0.29	0.71	3.06	8.06	0.38	244	149
3200 to 6400	3.88	1.12	2.76	0.29	0.71	3.27	9.38	0.35	248	167
6400 to 12800	2.56	0.67	1.89	0.26	0.74	1.87	5.68	0.33	224	191
Biosolids										
Raw	26.4	12.7	13.8	0.48	0.52	18.0	110	0.16	417	258
Composted[a]	11.7	2.40	9.32	0.20	0.80	5.99	21.0	0.29	179	102
Oct 28, 2005										
Anaerobic 1-1[b]	5.90	2.05	3.85	0.35	0.65	5.86	14.18	0.41	240	109
Anaerobic 1-2[b]	6.70	2.41	4.29	0.36	0.64	7.31	17.10	0.43	255	107
Plant 1 - Input	3.07	0.81	2.26	0.26	0.74	1.19	5.80	0.21	189	129
Plant 1 - Output	4.32	1.20	3.12	0.28	0.72	2.14	9.22	0.23	213	119
Plant Cell 2	4.74	0.81	3.93	0.17	0.83	1.25	5.83	0.21	123	97
Typha Cell	3.08	0.88	2.20	0.29	0.71	1.20	6.03	0.20	196	127

a – well-composted biosolids mixed with sand; b – core samples first anaerobic cell.

In a study of a variety of solid C waste materials, Chang *et al* (2000) found that cellulose polysaccharides were the main component consumed by the bioreactors treating AMD. This is consistent with the decreasing S2 values found with increasing metal concentrations (Table 30; Figure 45). The polysaccharide was likely degraded by hydrolytic fermentative anaerobes to fatty acids and alcohols captured by the S1 values. They showed that after depletion of the easily digested material that hydrolytic fermentation was rate limiting for SRBs. The decreasing S1/S2 ratio found at higher metal concentrations is supportive of this concept. Based on the following equation:

$$2CH_2O + SO_4^{2-} \rightarrow 2CO_2 + 2H_20 + S^{2-}$$

Chang *et al* (2000) estimated that 60 g substrate is consumed to remove 96 g of sulphate. Based on landfill work (Ress et al 1998) showing only a quarter of the biomass will be utilized by the bacterial consortium; therefore, they suggested the removal of 1 kg of sulphate will require 2.5 kg of biomass as an aid in designing treatment systems.

Celgar composted biosolids have an average dry bulk density of 820 kg/m^3 and an average organic matter (OM) content of 15.5%, while sand in the area has an average dry bulk density of 1730 kg/m^3 (Fiona Mackay, Zellstoff Celgar, *per com*, 2004), therefore, the dry bulk density of the original biological substrate was estimated to be 1130 kg/m^3 (assuming 65% biosolids to 35% sand by volume). The organic matter content would be at least 7.3% with approximately 82.6 kg OM/m^3. Given that the original anaerobic bioreactor was approximately 1000 m^3, the organic matter in the cell was approximately 82,600 kg organic matter. Using 2.5 kg organic substrate per kg sulphate treated then a total of 33,400 kg or 344,000 moles of sulphate could be treated. The total moles of As, Cd and Zn over 2 years of operating time (from Table 27) was 8,100 moles/year and then rounding that up to 10,000 moles/year to account for the other metals capable of forming sulphides, the project cell life would be in the 34 years. However, if as has been suggested that SRB are only able to utilize 10 to 20% of the available substrate (in Gupta et al 1994) then the cell life would decrease to approximately 21 years ranging from 14 to 27 years.

Another way to predict the cell life is to use the empirical data for metals and Rock Eval 6 collected during the cell deconstruction. Using the complete data set (excluding outliers), the TOC, S1, S2, %PC and %RC were linearly regressed against Zn concentrations in the deconstructed cell (Table 31). The overall cell mean Zn concentration after 2 years of operation was 1034 mg/kg or 517 mg/kg/year of operation. Assuming that metals load remained constant over the period and all carbon could be used up, the predicted total Zn concentration (mg/kg) for each Rock Eval 6 parameter was calculated as carbon went to zero. While this is a simplifying assumption as the decay of the carbon is likely curve linear levelling off to very low carbon degradation

rates as carbon remaining decreases and therefore carbon would not likely go to zero for a long time, the initial linear slope should be useful for predicting the "effective" cell life.

The predicted total Zn concentrations (at C=0) was then divided by a constant metal loading factor of 517 mg/kg/year to estimate cell life. Cell life was predicted to range from 17 to 21 years. However, based on S2 which measures the remaining cellulose polysaccharides, cell life is predicted at 18 years compared to that based on total organic matter above was approximately half of that calculated (34 years based on Chang et al 2000 – 25% usable C) or very comparable (21 years ranging for 14 to 27 years based on Gupta et al 1994 – 10-20% usable C).

Table 31. Cell life predicted based on Rock Eval parameters linearly regressed against measured Zn concentrations (mg/kg) in the substrate as carbon available goes to zero.

Regression Equation	R^2 Value	P Value	[Zn] at C=0	Cell Life (yr)
TOC = 5.92 - 0.00051(Zn)	0.25	<0.001	10,386	20
S1 = 5.37 - 0.00061 (Zn)	0.21	<0.001	8,803	17
S2 = 14.34 - 0.00152 (Zn)	0.21	<0.001	9,434	18
%PC = 1.74 - 0.00019 (Zn)	0.21	<0.001	9,158	18
%RC = 4.19 - 0.00038(Zn)	0.24	<0.001	11,026	21

Summary

With the equivalent of two years of operations (over 4 years of intermittent operations) more than 8 million L of contaminated water were treated and more than 1 tonne or 16,000 moles of the three primary metal contaminants As, Cd and Zn were removed and sequestered in the original anaerobic bioreactor. Metals were present at all depths in the bioreactor with statistically significant differences observed between layers when only metal sulphides are examined.

Linear regressions of As versus S were significant suggesting that the metalloid As is present in close association with sulphur. Under anaerobic conditions with available carbon and sulphate it is likely forming As_2S_3 (orpiment) as a result of dissimilatory

arsenate and sulphate reduction as shown by Newman et al (1997b). Synchrotron analysis on a small number of samples confirmed that As was present as a polysulphides (Duncan et al 2008).

The system operated as designed with higher concentrations of metals seen in the bottom layer when compared to the higher layers. High concentrations of metals were seen in the Cap (filter) layer as designed. Linear regressions suggest that metals are present as sulphides with evidence that lower TOC carbon values are correlated with higher metal concentrations. This relationship existed for S1, S2, %PC and %RC, as well, and was used to empirically estimate the cell life based on Zn treatment rates. Cell life was estimated to be 18 years based on S2 carbon. Based on total carbon biomass cell life was estimated to be from 14 to 34 years depending on the assumed fraction of usable/total carbon.

While the full potential of Rock Eval 6 analysis was not examined in this thesis, future work comparing Rock Eval 6 to other techniques of measuring carbon availability and type is warranted. The use of Gaussian elementary analyses of the S2 curve (Sebag et al 2006) to extract more information from the analysis looks particularly promising. Analysis of the organic materials types used in anaerobic bioreactors and the same material type over time by Rock Eval 6 and other techniques would be required to make Rock Eval 6 a cost-effective tool for designing and assessing the health of anaerobic bioreactors.

Chapter 5 – Cost and Sustainability of Wetlands

Introduction

Passive treatment systems have many advantages over traditional chemical and mechanical systems when applied in the right application. While cost is a key factor when deciding on using a passive versus active system, overall sustainability is now an important consideration in any technological decision and one must assess the economic, environmental and social sustainability of different options. While a quantitative cost comparison of wetland systems to traditional lime-based treatment systems is presented, a set of qualitative sustainability indicators were used to assess the economic, environmental and social factors relevant to wetlands technologies and their overall sustainability. The Trail system, the Park City (in Utah) and Yankee Girl (Ymir, BC) systems (in which the author assisted in design and construction oversight) and various examples from the literature were used in this assessment.

While sustainability has been defined many different ways with little consensus, the goals generally include continued economic well being; environmental protection; the wise use of natural resources; and social equity and progress for individuals, communities and the environment. The concept recognizes the need to design human and industrial systems and processes so that natural resources and their cycles are not disrupted leading to a diminished quality of life through losses in future economic activity or adverse impacts on social conditions, human health or the environment (Muga and Mihelcic 2008). Indicator selection was based on relevancy to the treatment technologies, whether they could show progress to sustainability and was easy to understand; and are presented qualitatively.

Economic and Cost Issues

Various costs to implement a full scale wetlands system or chemically-based treatment system include design and initial monitoring costs; permitting costs; construction costs (capital); ongoing operation and maintenance (O&M) costs; land costs; sludge disposal costs; and system longevity (as it relates to spreading out the capital costs). The initial

design and upfront monitoring of passive wetland systems can be higher than for well understood traditional chemical/mechanical systems (e.g., lime-addition, slow sand filtration). The behavior and performance of lime-based treatment systems are well known, they are reliable and effective and they can generally be designed based on knowing the quality and volume of the effluent to be treated. However, lime-based or alkalinity addition systems require regular access and maintenance to maintain chemical supplies, power, pumps, the sludge handling system and sludge disposal. These requirements often make them impractical for remote, abandoned mine programs (Ziemkiewicz et al 2003).

On the other hand, wetland system performance has been mixed with many failures and successes (Ziemkiewicz et al 2003). In a study of the long-term performance of passive acid mine drainage treatment systems related to pyrytic, coal mining in the Eastern U.S., Ziemkiewicz et al (2003) compared costs of passive systems to chemical systems based on $/tonne of acid load treated/year assuming a 20 year lifespan (or to failure). Active treatment costs were calculated based on the amount of caustic soda (NaOH) to treat a metric ton of acid load ($500/tonne/year). This cost was biased significantly low as it only included the delivered cost of the caustic soda not additional equipment, labour, sludge pond construction, cleaning, etcetera which could double or triple these costs. A total of 137 passive treatment systems were evaluated including aerobic wetland, anaerobic wetland, anoxic limestone drain and vertical flow wetlands. Only 44% of the aerobic wetlands were considered successful with costs ranging from $23 to $1512/tonne/year with construction costs ranging from $1.3k to $15k. Ten of 18 anaerobic wetlands provided positive treatment results, while only 5 provided cost-effective treatment with cost ranging from $138 to $3,912/tonne/year with construction costs from $5k to $550k. Of the 38 anoxic limestone drains, 33 provided positive treatment with 29 below $500/tonne/year with 9 units at <$70/tonne/year with construction costs ranging from $2k to $188k. Of the vertical flow wetlands, 16 of 19 had positive results with 9 units at <$500/tonne/year with construction costs ranging from $11k to $213k. These results indicate that passive systems may not be appropriate in all situations but can and are a very cost-effective solution in many cases.

Wetland systems will react differently depending on the organic substrate used, the metals requiring removal, the climate (e.g., temperature, precipitation) and other factors. Therefore, to ensure a successful system laboratory or pilot-scale systems are generally required. The Trail system (a large scale pilot system treating approximately 20% of collected seepage annually) was based laboratory columns and vegetated mesocosms (77 L Rubbermaid tubs). The Park City wetlands was based on laboratory mesocosms and two subsequent field-based pilot cells trying differing substrate types (first in 2004 and the second in 2006; both were approximately 2 m^3) before the Phase 1 wetland was constructed in 2008 (Fitch and Schoenbacher 2009). The Yankee Girl site was designed on predicted metal concentrations and flows (Tinholt et al 2007) but based heavily on the Trail experience using the same Zellstoff Celgar biosolids. The Lily/Orphan Boy site in Montana (near Helena) used large scale mesocosms seeded with Lily Mine sediments and fed transported Lily Mine water (Nordwick 2008). This led to a field scale demonstration site at the mine. As wetland systems are better understood and more widely applied over a range of conditions, preliminary monitoring and design costs may be reduced.

With any new technologies more effort is required to get regulatory approval and necessary permits. Indeed, in the case of Park City, many public and regulatory meetings over several years were held to obtain necessary public and regulatory approval. So while difficult to quantify, the process was longer requiring the field demonstration biocells to convince city officials and the regulators as opposed to traditional treatment technologies. At the Yankee Girl site a wetland treatment system was requested by the public. The regulators expressed concerns but accepted the wetlands technology as a backup system to traditional liming and encapsulation of the tailings that occurred as part of the remediation of that site. As the system was designed based on the predicted characteristics of runoff from the encapsulated tailings and experience at Trail, no field trial could be completed. Unfortunately, the most cost-effective system was determined to be a buried anaerobic cell and limited funds precluded a final vegetated wetlands polishing system that the public wanted. Numerous discussions with regulators modified the initial approach at Trail, particular with respect to the use of hyper-accumulating

plants. Until wetland technologies become fully understood and their limitations known, they will have to be "sold" at each site.

Construction costs can be higher for wetland systems due to the larger land base requirements, the trucking of all materials required for a long-term system at the front end, the amount of material to be excavated, mixing and placement of substrate material, and if required the lining of the treatment cells (with clay or other liner materials). In a comparison of costs of small scale (3 gallon per minute (gpm)) lime-addition system versus an SRB system (using substrate suspended in the Lily mine shaft, so no land requirements); the initial startup and construction costs were over 3 times higher for the SRB system (Nordwick 2008). The initial estimated costs of the lime-addition were all related to construction and equipment; while the SRB system at Lily/Orphan Boy was about third of the costs ($40K) were related pilot research. But even removing the research costs the initial SRB construction costs were over double at $95k versus $41K for lime-addition.

Ongoing annual operating and maintenance costs at the Lily/Orphan Boy mine were estimated at $50K for SRB treatment compared to a lime addition system at $66k (Nordwick 2008). The SRB had lower labour costs ($12k versus $31K); higher sampling and analysis costs ($35k versus $23k); and lower maintenance and consumable cost ($2k versus $9k – includes lime costs). While the lime addition system had an additional sludge removal cost of $5k. When comparing the two 3 gpm systems using a life-cycle cost analysis over a 30-year period, the net present value (NPV) of costs was lower for the SRB despite its higher capital and startup costs ($1.0 M versus $1.2 M). When scaled up to 100 gpm, the SRB NPV was still lower at $1.5 M versus $1.8 M with the cost per gallon being dramatically reduced in each case. The high upfront SRB costs were diminished over the 30 year period, while the higher operating expenses of lime addition results in its higher costs. However, with operating periods of less than 10 years the lime treatment costs approached the SRB treatment costs. This stresses the need to understand the longevity of these passive treatment systems to understand their cost-effectiveness.

The research and design costs for Park City were valued over $50K with direct costs and in-kind contributions by University of Missouri-Rolla and Nature Works Remediation Corporation. The Park City (50 gpm) system cost $500k to build on 0.52 acres of land owned by the city (valued at $100K). The 6" clay liner required by the public and regulators was a substantial cost to the system. The annual operating Park City costs are $15k for analysis and $10k for labour. The system treats approximately 25% of the peak spring flows (220k gallons per day) and will treat 100% of the winter flows (mid November to February). Results to date have indicated close to 100% removal of Zn and Cd.

The Yankee Girl system had design costs of $45k. Construction and oversight costs were $100K for a 900 m^2 buried anaerobic cell with a volume of 1,600 m^3. There was no charge for pulp mill biosolids except for trucking costs. No land costs were incurred as it was an abandoned site on Crown land and under going remediation by the Crown. The design was based on treating a groundwater flow of 2,300 m^3/yr and a surface runoff flow of 1,300 m^3/yr (approximately 2 gallon per minute). Ongoing monitoring costs are $40k/yr for first 5 years, $15k/yr for years 6 to 10 and $15k/yr every second year thereafter. These high monitoring costs are related to regulator acceptance and the need to learn from this site as it was based on predicted metal concentrations and runoff characteristics. These monitoring costs may be reduced over time depending on the results and the success of the system.

The Trail wetlands system (3-4 gpm) cost over $200k to build and modify from 1997 to reach its current configuration. However, costs are somewhat elevated due to the various retrofits and the multiple cells in the system due to its research purposes. An additional $80k has been spent on maintenance and repair. Annual research, monitoring and operational costs are about $40-50k per annum (excluding internal assay costs). The system has operated more or less continuously from 2002 to 2010 with very successful treatment under varying input conditions that would have been a challenge to address with a lime-based system. Treated water is used to irrigate a tree farm thereby recycling previously toxic water into a productive use.

Life-cycle cost analysis showed the cost-effectiveness of the system to be sensitive to the anticipated life span. Upon closure, the bioreactors could be sealed *in situ* or material dug up and treated by the smelter and the metals recycled. If system performance is decreased due to carbon loss in the substrate (and not plugging) then it would be possible to feed the system (e.g., acetate) to return system performance and incur higher annual operating costs but no new construction costs.

A novel, low cost permeable barrier was installed in Northumberland, UK to treat acidic and metal–rich coal spoil heap drainage (Jarvis et al 2006). The system had operated several years at the time of publication with 50% Fe removal and 40% sulphate removal. Construction costs were considered modest at £60,000 with annual operating costs of <£5,000. They are examining the carbon and sulphur cycling in the system with an objective of predicting the longevity of the system. As this is a buried system, no sludge disposal would be required at end of life.

Large-scale lime-based high density-sludge treatment systems designed to treat high volumes have high initial capital costs as compared to the low volume, simple dosing system covered above. For example, the Trail Smelter Effluent Treatment Plant capable of treating over 10,000 L/s would be upwards of $20 M to replace and annual operating costs of $1.2 M for reagents and $100k for manpower and maintenance. The oxidation and chemical neutralization system employed at Wheel Jane had capital costs of £4.25 M and annual operating costs of £0.76 M (year 2000 costs to treat 300 L/s or 6.8 M gallons/day; Younger et al 2005).

Muga and Mihelcic 2008 compared mechanical (i.e., activated sludge with secondary treatment) to lagoon (facultative, anaerobic and aerobic) technologies for domestic wastewater treatment system treating <5 M gallons per day. They found that construction costs could be up to 5 times higher for mechanical systems ranging from $3 to $11 per gallon/day and lagoon systems ranging from $1 to $4 per gallon/day. Operations and management costs were similarly more expensive for the mechanical system systems

ranging from $0.75 to $2.90 per M gallon/day and lagoon systems ranging from $0.10 to $0.75 per M gallon/day.

Environmental Issues

With respect to sustainability issues, lime-based systems generated large volumes metal hydroxide sludge that requires proper disposal (e.g., landfill). Generally, cost-effective recovery of metals from the sludge is not possible. The lime must be produced by mining limestone, roasting the limestone in kilns to produce lime (CaO) releasing carbon dioxide (a greenhouse gas). More resources are used to grind the CaO and transport it to the site that generates the sludge which may or may be store onsite. As well, energy is required to operate the system on an ongoing basis. Therefore, the overall sustainability of lime-based treatment systems can be questioned but may be the only option that can be applied in high flow situations with limitations on available land.

Passive systems, where they can be successfully installed, rely on available local organic wastes that may have limited other uses or that could be generated from plant biomass close to the site (e.g., chipping trees). The initial transport of organics, gravel/sand and limestone to the site and the actual construction would generate greenhouse gas. But the constructed system does not require ongoing chemicals or energy inputs. Plant-based systems relying on solar energy could theoretically last indefinitely. Whereas, anaerobic or "compost" bioreactors would be expected to become depleted over time.

If a bioreactor becomes carbon depleted, then it may be possible to feed them and extend their lifespan or seal them *in situ* storing the metals permanently onsite. Systems installed in mines would keep metals in their original location (Nordwick 2008). If the site is close enough to a smelter, it could be dug up and treated to recover the metals. If treatment is still required then a new anaerobic bioreactor would need to be built. Given the option, the most sustainable system would be the prevention of the drainage or seepage in the first place currently a large focus of the mining industry. However, numerous abandoned and orphan mine sites creating unacceptable mine drainage exist in

Canada (NOAMI 2009) and world-wide. These sites require long-term cost-effective treatment that is socially and environmental acceptable.

Treated water can become available for other purposes such as irrigation (e.g., Trail) or returned to the environment in a condition that sustains and promotes aquatic life. Indeed, big impetus for the Wheel Jane Project was the large release (25,000 – 50,000 m^3) of metal laden, acid mine water shortly after mine closure to the Carnon River in 1992 which caused a visible orange plume in the estuary (Younger et al 2005). The international news media described the incident as an "environmental disaster".

Energy use by passive systems is much lower than for conventional active systems. Muga and Mihelcic 2008 compared mechanical (i.e., activated sludge with secondary treatment) to lagoon (facultative, anaerobic and aerobic) technologies for domestic wastewater treatment system treating <5 M gallons per day. Lagoon technologies (driven by mechanical aeration) ranged from 5 to 55 x 10^4 kWh/MGD, while mechanical systems ranged from 35 to 235 x 10^4 kWh/MGD with larger systems being more energy efficient. As most of the energy in the lagoons is due to mechanical aeration, total passive wetland technologies (i.e., no pumps, heat-tracing, etc.) would use essentially no energy.

Social Issues

With respect to social acceptability, wetlands (particularly vegetated ones), are well received by the public and to a lesser degree by some regulators. The public find wetlands aesthetically appealing, like the idea of habitat creation for wildlife and like "green" or environmentally friendly technologies. The regulators, while supportive, have a certain sense of caution with new technologies. They have concerns about treatment efficiencies, metal transfer up the food chain, longevity of the systems and end-of-life issues related to maintaining metals in a fixed state. With respect, abandon/orphaned mines where society picks up the costs (via taxes), the regulators are more amenable to a passive approach (e.g. Yankee Girl Mine remediation, Ymir, B.C.). Vegetated wetlands have the potential to sequester carbon (Xiaonan et al 2008) to address greenhouse gas (GHG) issues and could conceivably be used as wetland offset credits in the US

(Robertson 2008). Compared to lime-based systems, passive systems provided they are cost-effective and achieve adequate treatment levels would be more acceptable to the public given their reduced energy and greenhouse gas requirements to build and operate, lack of sludge disposal issue and their habitat creation benefit.

Sustainability of Wetlands

Based on the above discussions on economic, environmental and social issues, a qualitative assessment of the sustainability of low and high flow wetland technologies compared to lime-based technologies (small onsite dosing systems with storage ponds and full scale mechanical plant) for the onsite treatment of mine drainage waters was carried out (Table 32). With respect to costs, the design and research costs are higher for the biological systems given the current state of knowledge. As our knowledge of these systems improves these costs could be reduced in the future. For low volume systems, the construction costs are higher but offset by lower operational and maintenance (O&M) costs, while for larger systems construction costs are high for both types of systems. Land requirements are higher for biological systems but this may not be an issue at remote mine sites with plenty of available land.

With respect to metal concentrations treatable, the chemical systems fare better, as unlike biological systems, they are not subject to toxicity issues. In addition, chemical systems are often more efficient at higher metal concentrations. When operating, designed chemical systems will have a higher treatment reliability compared to biological systems which can be subject to seasonal issues related to winter temperatures and high spring flows. Wetland systems that incorporate a vegetative component have the potential to create high value wildlife habitat compared to *in situ* mine or buried SRB systems or chemical systems. Sludge disposal is an issue of great concern for lime-based treatment systems and generally requires land filling as its potential for metals recycling is low (both due to its low metal concentrations and energy costs required to extract the metals). SRB systems do produce metal sulphides which can more easily be recycled, but recycling may not be economical to recycle depending on proximity to a smelter. The

Table 32. Qualitative sustainability rankings comparing "wetland" systems to lime neutralization systems using a variety of economic, environmental and social indictors.

Indicator	Systems (<100 gpm)				Systems (>>100 gpm)		
	Lime-dosing	SRB in mine	SRB onsite	SRB with Wetlands	Wetlands	SRB	Lime Plant
Economic/Costs							
Design/Research	Nil/Low	Med	Med	Med	Med	Med	Low
Construction	Low	Med	Med	Med	Med	High	High
O and M	Med	Low	Low	Low	Low	Low	High
Land Footprint	Low	Low	Med	Med	High	Med	Low
Environmental							
[Metal] Treatable	High	Med	Med	Med	Low	Med	High
Treatment Reliability	High	Med	Med	Med	Med	Med	High
Habitat Creation	Nil	Nil	Nil	Med	High	Nil	Nil
Sludge Disposal	High	Nil	Nil	Nil	Nil	Nil	High
Energy Use	Med	Nil	Nil	Nil	Nil	Nil	High
GHG - Construction	Low	Med	Med	Med	Med	High	High
GHG - Operational	Med	Nil	Nil	Nil	Nil	Nil	High
Metals Recycle	Nil	Nil	Low	Low	Low	Med	Low
Social							
Aesthetics	Negative	Neutral	Neutral	Positive	Positive	Neutral	Negative
Public Acceptance	Neutral	Positive	Positive	Positive	Positive	Positive	Neutral+
Regulator Accept.	Positive	Positive?	Positive?	Positive?	Positive?	Positive?	Positive
C Sequestration	Neutral	Neutral	Neutral	Positive	Positive	Neutral	Neutral

sulphides produced are sequestered in the SRB system and provided the SRB systems remain anaerobic are safely sequestered in the bioreactor. At end of useful life, the bioreactor could be sealed to provide permanent storage of metal sulphides onsite.

Energy usage and greenhouse gas release are two areas where biological systems are more sustainable than the chemical systems. The lime-based systems have medium to high energy use (depending on system size) and this is on-going for the life of the system. Totally passive SRB compost systems will use no energy but will eventually rundown. Vegetated wetlands (while limited in the metal concentrations they can treat) are essentially solar-powered and can run indefinitely. Numerous examples of natural wetlands removing metals for hundreds of years exist. Greenhouse gas release during construction is an issue for the biological systems due to the fact that all "supplies" are

front-end loaded and they have a larger physical footprint. Construction of a high-flow lime treatment plant can release significant greenhouse gas due to the various construction materials (e.g., concrete, steel) used. The on-going greenhouse gas releases during operation of lime-based systems is very significant due to the ongoing requirement of lime. This impact could be reduced if other waste-generated alkalinity agents (e.g., fly ash) could be used. No new greenhouse gas is released by SRB compost systems as they are recycling waste carbon from other processes. In fact, when used with vegetated wetland systems carbon sequestration can result.

An important factor with respect to public acceptance with wetlands is the aesthetic factor. People like wetlands and the wildlife associated with them (particularly birds). Therefore, the public acceptance of an SRB compost system is greatly improved if it incorporates a vegetative component on top of the SRB or as a separate component. In the experience of the author, the public want to see the vegetation and associated wildlife, even when a buried SRB system is all that is required. So in many instances it is quite beneficial to follow the SRB system with a polishing wetland for both public acceptance and additional treatment capability. The public appreciates "green" technologies and are more willing to accept seasonal fluctuations in treatment levels at a remote, abandoned mine site because they appreciate that the impacts are greatly reduced at a reasonable cost (as it impacts their tax bill). Regulators, while generally positive about "wetland" technologies have more reservations about such systems. They would like high levels of consistent treatment with water bodies always meeting water quality objectives. They express concerns about longevity and "permanent" waste disposal. But it appears that most are intrigued by the possibility of the technology and are willing to try it. Regulators often institute a requirement for higher levels of monitoring as compared to traditional chemical based-systems, therefore adding to the operating costs of a "wetland" system. Future regulatory acceptance of "wetlands" technology will require numerous examples of properly designed and operated systems. System failures due to poor design or construction will reduce regulatory acceptance and therefore should be avoided if possible.

Based on the on the above discussion, the author presents an overall relative ranking of the various systems by assigning numeric values to the indicators in Table 32 and then summing the values by category. For the economic indicators, all values were positive with higher sums indicating higher costs. The values were assigned as follows: Nil=0; Nil/Low=0.5; Low=1; Medium=2 and High=3. For environmental indicators, the values could be positive or negative using the same ranking scale as for costs. The concentration of metals treatable, treatment reliability, habitat creation and the ability to recycle metals were assigned positive values, while sludge disposal, energy use, GHG produced during construction phase and GHG produced during the operational phase were assigned negative values. With respect to the social indicators where the possible direction of change is indicated (Table 32), the direction of change values were arbitrarily assigned as follows: Negative=-3; Neutral=0; Neutral+=1; Positive?=2 and Positive=3 (as opposed to +1, 0 and -1 which would only indicate the direction of the change). This was done solely to make the scales and analysis comparable to the other two categories for graphing purposes.

For small scale systems, while the lime-dosing systems had the lowest costs they did not fare well in the environmental or social categories (Figure 46). In the environmental category high metal concentrations treatable and reliability were positive, the poor ranking arising from sludge disposal, energy use and GHG during the operational phase, while on the social side the only positive factor was regulatory acceptance. The SRB and wetlands had the higher costs but scored well in the environmental and social categories with vegetated wetlands having higher scores on the environmental category due to habitat creation. In the social category, the wetlands scored higher due the aesthetics, high public acceptance and their ability to sequester carbon.

Large scale systems all had similar costs by this ranking method (Figure 47). As more performance and longevity data becomes available, net present value calculations could be done to more accurately determine the costs. However, with these calculations it is not easy to assign dollar values to the more intangible environmental and social indicators

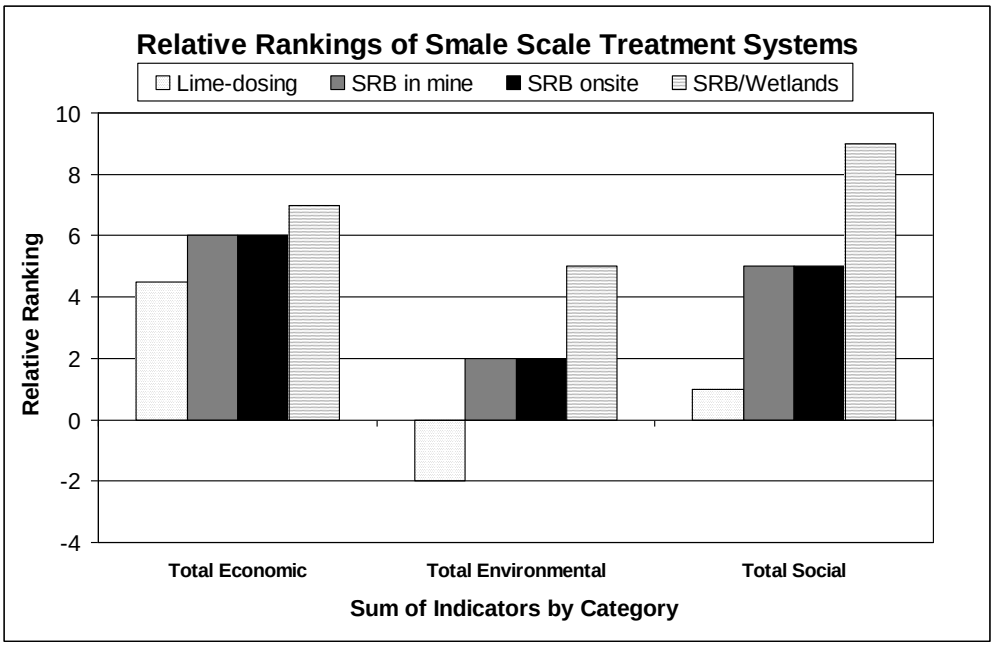


Figure 46. Relative rankings of small scale treatment systems (<100 gpm) by category based on scoring and summing the indicators in each category.

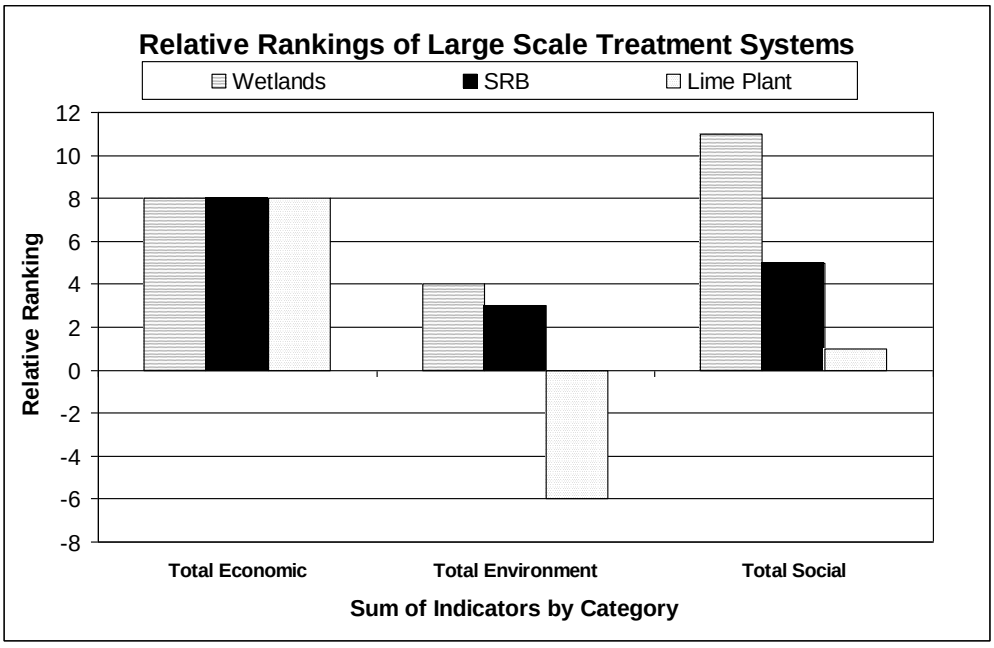


Figure 47. Relative ranking for large scale treatment systems (>>100gpm) by category based on scoring and summing the indicators in each category.

(e.g., habitat creation or aesthetics). Overall the environmental and social categories mirror those observed for the small scale systems, with wetlands having a higher environmental score than SRB due to habitat creation and a higher social score due aesthetics and carbon sequestration.

Overall, the sustainability of wetland systems appears to be relatively high. They have lower costs based on a life span of at least 10 years; they eliminate the need for sludge disposal; they have minimal impact on greenhouse gas release; they can actually sequester C in a vegetated system and they are considered a "green" technology by the public.

Chapter 6 - Anaerobic Bioreactors and Wetlands Design Issues

Flow collection and distribution

Water Delivery

Where possible when situating a biological treatment system, a gravity feed system is preferred. At Trail this was not possible and seepage water was pumped over 1 km and elevated over 100 m from the source sump. This lead to numerous pump failures causing disruptions to seepage input into the treatment system. While water levels are controlled in each stage, a lack of sufficient water in the plants cells occurred during some growing seasons and causing severe plant stress. If pumping is required then a redundant two pump system should be considered. Consistent and even flow with no disruptions is the key to successful operation of a biological system.

Flow disruptions by line freezing or line plugging were also experienced. Frozen lines, generally, put the system out of operation for the rest of the winter season. Delivery lines should be buried sufficiently deep for the climate and cleanout access points adequately insulated. These disruptions caused start-up issues in the spring when the bacterial populations need to re-establish themselves. Lines should be over-sized and have the appropriate slope in the gravity feed system to reduce chances of line plugging. Cleanouts should be installed as well. Bulkhead or French drain collection systems work well but perforated collection pipes should not be wrapped with any type of filter cloth material as fine metal sulphides will plug the cloth very quickly.

The system should be designed for maximum seasonal flow rates likely to be seen using the lower treatment rates experienced at lower winter temperatures. While using this method will provide higher than needed treatment rates in the summer, it should ensure adequate winter treatment rates. A final stage of a large holding pond or open-water wetland that is capable of holding up to a year of seepage flow may be considered to reduce seasonal variability. In some cases an upstream holding pond can be used to reduce seasonal flow variability or store seepage for treatment during the summer only.

Hydraulic Conductivity and Hydraulic Retention Time

Evenly distributed input flow with maximum contact with all available biological substrate material is the desired for maximum treatment efficiency in a biological treatment system. In the first anaerobic cell design, RPE liner was placed horizontally (Figure 1) in an attempt to provide a serpentine path for water flow to increase contact with the substrate. This was ineffective and short-circuiting up the sides occurred. When this cell was re-built a rectangular system of perforated pipe with cross ties was employed over the entire bottom of the cell in a limestone gravel substrate to ensure input distribution across the whole cell. Several cleanout pipes were added that could also be used for feeding the cell. As well, 1 m wide apron of liner material was welded around the edges approximately 1 m above the distribution system to prevent short-circuiting up the sides of the cell.

The biological substrate must have sufficient hydraulic conductivity to allow flow through the cell. At Trail, the biological substrate contained 35% sand for this purposes but with the fine nature of the biosolids care must be used in placing the substrate in the cells to prevent compaction. As well, sufficient non-biological material should be included to support the substrate as carbon is utilized by the bacteria during the life of the cell, otherwise compaction of the substrate can occur and lead to possible plugging or short-circuiting.

Good hydraulic conductivity is critical to developing a stable bacterial community throughout the entire bioreactor. The cell substrate should be well mixed with suitable ratios of organics to solid substrate (e.g., sand, gravel, crushed limestone) to provide suitable and comparable hydraulic conductivity throughout the whole cell. There is a trade off on the size of the solid substrate added for hydraulic conductivity and the surface area available for biofilm attachment. A finer substrate like sand provides large surface for biofilm attachment but has lower hydraulic conductivity than say gravel and would be more subjected to compaction issues (Lyew and Sheppard 1997). Hydraulic conductivity is particularly important in a permeable reactive barrier system to ensure preferential flow through the barrier, so pea gravel was used instead of sand (Ludwig et al

2002). Ludwig *et al* (2002) also suggest that a decrease in hydraulic conductivity (or plugging of the system) should not be a major issue as the low-density organic substrate (specific gravity 1 to 2 gm/cm^3) is replaced with a high-density sulphide precipitate (specific gravity 3 to 5 gm/cm^3). However, based on the author's experience, the metal sulphide precipitates that form will easily plug fine filter cloth materials, so such material should not be used to wrap outlet pipes or as cell capping material prior to any outlet system.

Together with a distributed outlet collection system over the entire cell, all the discussed features combined can prevent short-circuiting and ensure the most effective use of the available cell substrate. As well, the incorporation of an apron near the bottom and around the entire cell in a vertical upflow design is very effective in reducing short circuiting that occurs at the edges of the cell. Another design concept that can reduce flow distribution and short circuiting issues is to build multiple smaller bioreactors (in series and/or in parallel) to handle higher flows and metal loads as opposed to one large bioreactor.

Seasonal changes in flow rates and metal loadings need to be considered in designing a bioreactor especially if a completely passive design. Spring melts or high precipitation events can potentially create higher influent flows to the system. These flows may be cooler; more oxygenated; may have lower pH; and contain higher or lower metal loads. Higher metal loads may occur at the start of the event during the rising limb of the hydrograph (first flush) followed by lower metal loads due to dilution. The higher flows will reduce the HRT in the bioreactor potential causing failure as occurred in Trail in 1999. This can be avoided by having an upstream holding pond or underground reservoir (in a mine situation) or only allowing flows up to maximum design capacity any flows above this are bypassed or diverted around the bioreactor. In some situations this may be acceptable as high dilution in the receiving waters during these situations may produce waters with minimal environmental impacts. In a semi-passive system, say where water is pumped to the bioreactor then the flows can be precisely controlled but a holding pond or reservoir may still be required if the total flows need to be treated.

Biological Considerations

Plant Selection

When considering a vegetation-based wetlands treatment system one needs to determine if the metal concentrations in the input are tolerable to the plants being considered. The plants must also be suitable for the climatic conditions of the site. Over 50 plant species have been investigated in the wetland cells. Perennials are preferred to annuals to reduce ongoing system maintenance. A well established plant community that survives in the high metal environment while at the same time transpires a large volume of water is desired.

Many species of plants have been transplanted into the Trail system and many of these have not survived. Several plants were planted because they grow rapidly and attain a large size with high evapotranspiration rates. This included *Raynotria japonica* an aggressive, rapidly growing plant that, once established, can attain a height of 5 meters in a single growing season but it did not survive. Several grass species including *Andropogon gerardi, Panicum virgatum,* and *Muscanthus sp.* were also tried but did not survive. Several species of *Populous sp.* were transplanted – all hybrids, all capable of sustained high growth rates and but none proved able to handle the high Zn loading.

Since the system was first planted, the focus shifted from plants that are hyperaccumulators of metals to plants that are metal tolerant, do not accumulate metals in above-ground parts and have high rates of evapotranspiration. There are several reasons for the shift from phytoextraction to rhizofiltration. First, most hyperaccumulators are generally annuals and are "weedy" or invasive in nature. Being annuals requires ongoing system maintenance and invasive plants are cause for regulatory concern. Second, the accumulation in metals in above-ground plant parts raised additional regulatory concerns with respect to metals transfer through the food chain through herbivores feeding on the plant tissues. Finally, most hyperaccumulators are small with minimal biomass and low transpiration rates.

Therefore, the focus should be on plants that can survive, remove some metals at their roots and that grow sufficiently aggressively and are large enough that large quantities of water are transpired. Successful species include two species of *Salix*, numerous grasses, *Typha latifolia* and *Phragmites australis*, exhibiting strong and aggressive growth patterns and all capable of attaining sufficient stature and density to ensure large transpiration volume. However, as *Typha latifolia* is native to North America and therefore is preferred over the introduced *Phragmites australis*; but it is quite sensitive to hydrogen sulphide. In general, the grasses produce significant biomass and transpire large volumes of water with minimal metals uptake. As well, the rhizosphere produced by the plants produces and sustains very diverse bacterial communities. The grass-based wetland cells are very effective at rhizofiltration with low potential for metal transfer through the food chain. The grasses that proved successful include *Tripsicum dactyloides, Spartina pectinata,* and *Calamagrostis canadensis*.

Bacterial Requirements

As with any biological system, bacteria have preferred physical, chemical and biological requirements or conditions needed for their optimal growth and productivity. Physical requirements include, for example, substrate type and quality for attachment; flow conditions (as it relates to contact time and delivery of nutrients, food and metals to the bacteria); and temperature. Chemical requirements or factors affecting biological activity include parameters such as dissolved oxygen; redox potential; pH; dissolved concentrations of metals, sulphate, nutrients and carbon (as food source, e.g., organic acids); and other factors. While the biological conditions affecting productivity include the type of bacterial communities present and their processing of the solid carbon substrate; their acclimation to the metals (or other contaminants) present; synergistic/antagonistic effects between bacterial communities; and the response of the desired bacterial community to modifications of the chemical and physical parameters designed to enhance that particular community.

With respect to physical requirements, SRB perform best in a biofilm layer in which they can establish their own microenvironment key to their survival over a wide range of operating conditions (Neculita et al 2007). Biofilms require a solid surface, such as sand

or gravel, to develop. The greater solid surface area available (e.g. sand versus gravel) in a bioreactor will increase the area for biofilm development. However, the finer the solid substrate the more likely for the bioreactor is to be plugged or for short-circuiting to develop. Therefore, there is a trade off between increasing available surface area and the hydraulic conductivity of a bioreactor.

The flow passing over the biofilms or in effect the hydraulic retention time (HRT) of the bioreactor has been shown to greatly affect the efficiency of bioreactors. At Trail, exceeding design capacity greatly reduced treatment capacity which was only restored with reduced flow rates (below design) and feeding of sodium acetate. Others (as cited in Neculita et al 2007) have found that short HRTs may not allow sufficient time for SRB to reduce acidity and precipitate metal sulphides or alternatively flush out biomass and fine metal sulphides out of the system. At Trail in 1999, it appeared that readily available carbon from substrate decomposition was flushed out as well. While a long HRT may result in depletion of sulphate source or available organic carbon and or nutrients in a fed system. At Trail, the sulphate is in large excess to metal loads, therefore, longer retention times do not lead to sulphate depletion. In addition, carbon is provided from the substrate so a longer retention time likely provides more available carbon. HRT should not exceed the bacterial doubling time to prevent flushing out of the bacterial community (Nordwick 2008).

However, to maximize volume treated and prevent sulphate depletion, it is best to run the flow as high as possible without causing system failure. Another potential issue is the accumulation of bacterial biomass and sulphides over time leading to reduced hydraulic conductivity of the bioreactor, increasing the HRT by reducing the flow rate and possibly leading to plugging. This is overcome by having larger void volumes (i.e., larger substrate) which unfortunately reduces solid surface area for biofilm attachment.

With respect to temperature, SRB function optimally above 20 ^{o}C (even as high as 35-40 ^{o}C) in laboratory settings (Holmer and Stockholm 2001) but can tolerate ranges from -5 ^{o}C to 75 ^{o}C (Postgate 1984). It has been shown in this system and others that winter

operation below 5 ^{o}C is possible at reduced treatment rates. Gusek (2005) has shown at a high elevation site where mine effluent being treated was typically less than 5 ^{o}C and treated effluent dropped as low as 0.5 ^{o}C, that sulphate reduction was only reduced to 80% of benchmark rate in this cold-acclimated system. In column studies based on the Trail system at CANMET (Kawaja et al 2006), first columns were operated at 25 ^{o}C for 58 days and then moved to 4 ^{o}C for 84 days. These columns showed reduced zinc removal and lower bacterial numbers. While SRB numbers were reduced from 10^7 to 10^5 MPN the APB numbers reduced from 10^7 to 10^3 MPN. This may indicate that the system impacts may be more to reduced APB activity as opposed to SRB activity. Others have noted that once established SRB are not greatly affected by lower temperatures (Kuyucak et al 2006; Zaluski et al 2006; Tsukamoto et al 2004). Drury (2000) modelled the required HRT for 50% sulphate reduction ranged 8 d at 17 ^{o}C to 41 d at 1 ^{o}C in a passive non-replenished anaerobic bioreactor. This was based on the modelled biodegradability of a complex substrate to produce carbon for the SRB indicating that production of food was the limiting step.

As discussed, the higher the ambient temperature the more efficient sulphate reduction is by SRB. Therefore, a collection or delivery system should be designed to maintain any latent heat in the water source whether it is from underground workings or groundwater. This can be done through insulating or burying pipes, building as close to the source as possible or other techniques. Additionally, the bioreactors can be buried underground or insulated with wood chips or other suitable local materials found on site (e.g., waste rock, till, etc.). This will help maintain the inflow temperature and retain any heat produced by the biological activity. Bioreactors when first commissioned should be started in spring or summer as they will establish faster than ones started in the fall or winter periods. Once the SRB are successfully established, the bioreactor will be more tolerant of temperature changes.

With respect to chemical requirements, pH plays a role in both a preferred range for SRB and solubility of metal sulphides. While optimal growth for most SRB is found in a pH range from 5 to 8, SRB have been found at pH <3 in natural waters (Naculita et al

2007). With respect to Zn removal at Trail, experience has shown that a pH of >7 is desirable as it promotes ZnS precipitation and this is likely related as well to the increased solubility of ZnS at lower pH. Drury (1999) found in their anaerobic solid-substrate bioreactors that the outlet pH in their whey-fed bioreactor was 6.7 with 98% to 80% sulphate removal compared to a pH of 5.5 in a comparable non-fed bioreactor with only 60% to 40% sulphate removal. Similar to Trail results, metals removal was also higher at the higher pH (e.g., 99.7% versus 96.0% for dissolved Zn). Correlation analysis often had high negative correlations of dissolved Zn and Cd concentrations to pH through the system, particularly in the anaerobic cells. At Trail, large reductions of Zn removal efficiency was noted at pH values less than 6.4 with pH higher than 7.0 preferred.

Additional chemical pH control (beyond the alkalinity produced by the SRB) can be incorporated in to the bioreactor design. Once can use crushed and sized limestone in place of gravel as part of the support substrate or as actual treatment layers within the bioreactor provides additional pH control. As well, as part of the overall design, anoxic limestone drains or limestone-lined collection channels can reduce pH of seepage prior to reaching the bioreactor.

Optimal performance of SRB requires an anoxic and reduced microenvironment with a redox potential of < -100 mV (Postgate 1984). However, the Trail system often has dissolved oxygen of 2 mg/L O_2 or more exiting the anaerobic bioreactors indicating positive Eh values. Others (as cited in Naculita et al 2007) have also found positive Eh values at the outlets of their passive bioreactors. However, aqueous outlet measurements do not reflect the anoxic microenvironment in the SRB-containing biofilms. So at best outlet dissolved oxygen (or Eh) provides a relative measure of system performance over time and not the conditions experienced in the biofilms. Additions of readily available carbon such as sodium acetate or fruit sugar (in Trail system) or whey (Drury 1999) are effective in rapidly reducing and maintaining lower Eh values as opposed to relying solely on decomposition of a solid organic substrate. While exposure to oxygen can inhibit SRB metabolism the effect is reversible and some SRB have enzymes related to oxygen tolerance (Naculita et al 2007). However, we found the Trail system, similar to

Willow and Cohen (2003), that pH has a greater impact on bioreactor efficiency than do dissolved oxygen concentrations. However, high dissolved oxygen concentrations in the input will increase the amount of carbon required to establish low oxygen conditions for the SRB.

Therefore, any seepage collection system should minimize the entrainment of oxygen as much as possible. This will reduce the required amount of carbon used by the biological community to produce anoxic conditions in the cell. Techniques are similar to those for retaining heat, such use of pipes as opposed to open trenches and building as close to the source as possible.

While it is obvious that SRB require sulphate, high sulphate concentrations or the build up of reduced sulphur compounds can inhibit sulphate reduction, in particular H_2S. Toxic effects for H_2S were noted at concentrations of 477 to 617 mg/L much higher than those experienced in passive field bioreactors (as cited in Naculita 2007). The production of excess H_2S can be a potential human health concern in bioreactors. But at Trail the high dissolved metal concentrations seem to effectively scavenge any H_2S. However, there could be a concern of H_2S gas release from a system that was treating an effluent with high sulphate and low metal concentrations or systems in a closed space such as a mine adit.

Studies have shown that metals can be stimulatory to SRB at low concentrations, while inhibitory or lethal at higher concentrations with toxic effects being reported at a few mg/L to >100 mg/L (as cited in Naculita et al 2007). The level at which metal toxicity is expressed is difficult to determine as it varies with pH, chelation or CEC sites available, antagonistic/synergistic effects with other metals in solution, hardness and other factors. For example, in laboratory studies without chelators 13 mg/L Zn was toxic to *Desulfovibrio desulfuricans* but with chelators toxicity ranged from 13 to 40 mg/L Zn (Poulson et al 1997). At Trail, the maximum dissolved Zn concentration of 520 mg/L (up to 3800 mg/L total zinc) is well above the results found in the laboratory setting or at other sites. The differences may be due in part to the long period of acclimation to metals

by the bacteria at this site in the seepage and a diverse bacterial community in the bioreactors contributing to the ecological resilience of the biological system.

The SRB require low molecular weight carbon compounds as a food source. They use easily degradable compounds with simple structures such a methanol, ethanol, lactate, polylactic acid, simple carbohydrates (e.g., glucose or sucrose), whey and for some species acetate (as cited in Naculita et al 2007). However, in passive non-fed bioreactors complex organic carbon sources are used to provide a long term source of carbon. A mixture of plant (or cellulose-based) and organic wastes (manures, biosolids – pulp mill or sewage, compost) is often used. The plant material which is harder to degrade provides a long term carbon source while the organic waste is already processed and is more readily degraded. When using complex wastes in a passive system one is trying to promote a consortium of bacteria (a complete ecological community) with the cellulose-degrading bacteria, methanogens and APB producing the simple organic and fatty acids for the SRB and creating the reduced Eh. The anaerobic degradation of complex organic carbon compounds by the APB and cellulose-degrading bacteria may limit the available carbon available to SRB and therefore may be the rate limiting reactions particularly at lower temperatures as described above.

Another consideration in the design and operation of a bioreactor is the inclusion of feeding ports. Although this deviates from the passive design, the ports could be used to facilitate start-up, to address high freshet flows or to recover from upset conditions through feeding the bacterial community low molecular weight carbon sources readily used by SRB and other bacteria, allowing cleaning of piped distribution systems and provide sampling ports. In another context, a smaller bioreactor with seasonal feeding could be designed and built that would trade-off higher operational costs against higher capital costs of a larger bioreactor designed to treat the same flow stream year-round without feeding. Incorporation of feeding ports could be used to extend the life of a bioreactor beyond the life of its contained carbon or fuel source (i.e., after all usable carbon has been depleted then cell could be fed).

Unforeseen increases in metal loads above design capacities with normal flows in 2005 (or high flow overloading in 1999) can be problematic as has been experienced with the Trail bioreactor/wetland system. Under these circumstances the geochemistry of the bioreactor can be changed and while the high metal load may be initially treated (possibly with a lower efficiency) by precipitation and/or filtration, it may not be stable. These interim metal compounds can re-dissolved and further processed as the geochemistry returns to "normal" conditions and metals released in outlets of the bioreactors can be higher than in the input (Duncan et al 2008; Gusek 2005).

While design measures can be incorporated to prevent metal overloading by increased flows (e.g., overflow diversions beyond designed flow rates), it may not be possible to foresee or predict increases in metal concentrations in the source water. If concentrations were expected to increase over time then the bioreactor would have to be designed to treat the anticipated future concentrations. However, the Trail system proved quite resilient and did recover. Multiple bioreactors in series may provide some protection with the first bioreactor taking the brunt of the increase thereby protecting the subsequent bioreactors but this is essentially over-designing the system.

Biological treatment systems, while resilient, operate best under stable conditions. As noted seasonal temperature and flow differences can affect system performance. Some of this is overcome by over-designing for the highest flow rate at winter temperatures. The Trail system has experienced high flows well over design rates with subsequent failure typical of the low HRT issues described above with recovery by decreasing flow (increasing HRT) and feeding (stimulating the SRB directly). Prior to 2002, when the anaerobic bioreactors had open water on the surface, high precipitation rates of low pH rain decreased pH and reduced system performance. Additionally, the high total metal loads experienced in late 2005 also lead to reduced system performance. But the key point is that as stable as possible temperature, pH, flow rates, flow distribution, oxygen input (minimize aeration in collection system) and metals loadings are beneficial to biological treatment systems and methods should be considered when designing a biological system in the field.

The organic materials used to "fuel" the bioreactor are one of the most critical design features to both short and long term success of an anaerobic bioreactor. It is the key design element (along with unique characteristic of a sites effluent stream) that requires *in situ* pilot testing prior to full scale field design being implemented. A wide variety of materials have been used that are wastes or by-products of various processes or industries, such as agricultural, food processing, forestry, composting, sewage treatment, pulp mills and others (Naculita et al 2007). While the organic material may be provided at no or minimal costs, transportation costs particularly to remote sites can be prohibitive. In that case wood chips, sawdust or chopped vegetation may be produced on site. Most designs have included both a readily biodegradable (e.g., animal manures, biosolids, compost) and a more recalcitrant carbon source (e.g., sawdust, wood chips, hay or other high cellulose materials). The readily biodegradable carbon provides both the initial fuel in the short term and in many cases the bacterial inoculums, while the recalcitrant materials provide the long term fuel. Raw wood chips may initially toxic to SRB metabolism due to resin acids but it is rendered non-toxic after the initial period of anaerobic microbial metabolism (Chang et al 2000). This supports the inclusion of readily biodegradable carbon in the matrix to get the process started while the community to degrade the more recalcitrant materials gets established.

One of the key issues is how long will the carbon or fuel last and what tools can be used to assess current cell performance or predict the life span of a passive bioreactor. While Rock-Eval shows great potential to be useful in this regard further research is needed. If using the amount of organic substrate per mole sulphate to be treated as an aid to cell design, the author would recommend that only 10-15% of the material be assumed as usable over the long term.

Multiple Treatment Cells and other Design Issues

Depending on the nature and complexity of the effluent to be treated, multiple contaminant-specific treatments cells in series may provide a very effective overall treatment system. This concept was applied in the Wheal Jane example and to a lesser extent at the Trail system. In this type of system, individual cells in series address certain

contaminants of the effluent stream. The correct placement of the treatment cell in the series is a key design issue. In most cases anaerobic cells would be placed in front of oxic cells, both due to potential toxicity issues to plants and for the production of alkalinity. A critical design flaw of the Wheal Jane system was the upstream placement of the acid-generating aerobic processes upstream of the crucial alkalinity-generating anaerobic processes (Younger et al 2005). In the more oxic conditions downstream, any Fe^{2+} not captured as sulphides with be oxidized at higher pH and precipitate with Mn as hydroxides scavenging any residual metals and As. As well, any ammonia passing through the anaerobic reducing conditions can be removed in a biologically-productive oxic setting.

The addition of an upstream holding pond can be considered to reduce seasonal flow variability or store seepage for treatment during the summer only. If acceptable to regulators a freshet by-pass system could be employed to reduce the size of the treatment system with potential minimal impacts to the aquatic receiving environment. While not always necessary, wetland cells do provide additional treatment (e.g., ammonia, Mn) and filtering capacity not found in the bioreactors which produce fine metal sulphide particles that can pass through the bioreactors. As well, they provide their own carbon source so do not have a finite life. A slow sand filter after a bioreactor could serve this filtering function as well and may be an appropriate stage on its own or prior to wetlands polishing stage. However, slow sand filters add a maintenance component with regular on-going costs and potential disposal issues

A final stage of a large holding pond or open-water wetland that is capable of holding up to a year of seepage flow may be considered to reduce seasonal variability. Mn and ammonia removal is possible if the pond biologically active and has a large surface area for oxygen transfer. Water from upstream cell could be dropped or ran over a riffle surface to enhance oxygen exchange if the topography has sufficient slope.

While it may be beneficial to incorporate feeding ports when designing the an anaerobic cell in case in the future feeding may be required, the concept of an upstream

vegetated cell feeding DOC to a downstream anaerobic cell may have merit in certain situations and increase the life of the anaerobic cell. One possible scenario is low metals surface flow from the mine site is treated in a vegetated wetlands that then flows into an anaerobic cell that is treating high metals mine drainage that would be toxic to vegetation. The issue of variable hydraulic residency time in the anaerobic bioreactor could be addressed only allowing a constant flow from the vegetated wetlands and excluding the majority of the freshet flow which would have low DOC anyways. An over-designed downstream vegetated wetlands may also be beneficial in this regard as well as it may be able to take up additional treatment capabilities as the anaerobic cell reaches the end of its useful life.

Kalin (2001) states the importance of understanding the site geochemistry and weathering rates to predicting the long term contaminant load that needs to be addressed by the wetlands system. As well, as understanding the seasonal release patterns of the contaminants in designing the wetlands treatment system. Many of these issues are addressed by on site pilot treatment systems suggested as crucial by this author. An additional design consideration for constructed wetlands, Kalin (2001) suggests is very important is frost penetration into the wetland sediments. A frozen bioreactor will not treat any seepage, however, if seepage is also frozen at the same time the impact may be minimal. The author recommends burying anaerobic bioreactors or installing permeable reactive barriers as close as possible to the seep to maintain frost-free conditions and keeping ay heat in the seepage or groundwater source.

On still active mining sites, the possibility of designing and building anaerobic treatment cells in the actual mine while all the equipment is on-site. This would drastically lower construction costs and may actually be used to treat mine drainage while the mine is in operation as well. Anaerobic cells and vegetated wetlands can be incorporated in the tailings pond as it is being constructed and filled with tailings again lowering the construction costs. Additionally, the costs are borne as part of the mine operational costs and would reduce final closure costs.

Summary

This large-scale pilot system demonstrates that passive biological treatment systems can effectively treat high concentrations of metals and reduce them to acceptable irrigation standards. Performance of biological systems generally improves over time as the bacterial populations develops and adapts to the input stream. The bioreactors are complex ecological systems composed of many different bacteria that complete different functions. This complete ecological system provides more stability and resiliency than monoculture bacteria treatment systems. The ability of the system to treat "spike" metal loads over four times the designed treatment capacity is remarkable and would not likely be duplicated in chemical treatment systems. However, such events do take a toll on the biological system which then takes some time to recover. In general, biological systems will achieve their best treatment capabilities with stable flows, temperatures and metal loads.

While the longevity of anaerobic or "compost" bioreactors are finite, they can be designed and built with current knowledge to last for over 20 years. Vegetated wetland cells, where they can be used (i.e., low metal toxicity to plants), should be able to last 100's of years with the biggest issue with respect to longevity being the natural ecological succession of wetlands to dry land ecosystems.

Wetland systems can be cost-effective when compared to traditional lime dosing systems. However, they are not a suitable treatment technology in every case, particularly in high flow situations. Where they can be used, they have higher overall sustainability compared to lime-dosing systems with respect to economic, environmental and social sustainability issues.

While "rule-of-thumb" guidelines are useful for design purposes, a pilot scale test at the actual site under year-round ambient conditions testing actual seepage water and organic substrate matrix should be considered to aid in designing the large-scale system. This is especially important where treatment volumes and/or loads are high and will

require very large and expensive construction works. In cases of small volumes or loads, it may be cheaper just to over-build than to do extensive pilot scale work. Based on the author's experiences, a pilot scale test system should have a volume of at least 5 to 10 m^3 to provide meaningful scaling up information to design the full scale system.

However, to assure continued acceptance of this technology, it must be applied only at appropriate sites with sufficient research carried out to ensure successful results. If too many failures of wetland systems occur through inappropriate application, poor design or poor construction and implementation then there will be a loss of acceptance by regulators, mining community and the public. As well, while there are many advantages to wetland systems, the limitations (e.g., seasonal treatment differences, treatment variability) need to be understood and accepted by all involved in the approval process.

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Appendix A – System Evolution 1997 to 2002

Design and Construction History

From 1996 to 2002 the system evolved from a summer only plant-based wetlands to the current year-round treatment system capable of treating full strength seepage for all contaminants present. The wetlands were originally conceived as plant-based horizontal flow wetlands with the idea of increasing the percentage of seepage flow to clean water until plant toxicity was noted. However, construction problems delayed the planting of the wetlands until late in the summer of 1997 and only clean water was fed into the system while the plants took root and started to grow. Subsequent greenhouse experiments using synthetic seepage indicated that plant toxicity would occur as low as 25% seepage strength. So the idea of an anaerobic bioreactor prior to the "wetlands" cell was conceived and built in 1998.

Initially, the system was only operated during the summer with very high treatment efficiencies. The question quickly became how it will operate in the winter and how long such a system would last. The system was winterized in 2000 but many issues arose and successful winterization was completed by 2002. An additional anaerobic bioreactor was added in 2000 for improved treatment of zinc. In 2002 the original anaerobic bioreactor was taken apart, sampled and reconstructed with improved flow characteristics. Many issues were faced and overcome during the "wetlands" evolution.

1997 Wetlands Design and Construction

Although the collected seepage waters contain other metals, the focus was on zinc, arsenic, cadmium and lead due to their relationship to the smelter (Table 1). The wetland cells were designed and built using typical constructed wetland techniques (Kadlec and Knight 1996). The original pilot scale project was designed to treat 10 – 15,000 L of seepage water per day (approximately 13 –20% of total seepage volume). The amount of lead present in the seepage was minimal and therefore not considered in the design of the treatment facility.

Table 1. Total volume of water and mean metal concentrations in Stoney Creek Seepage used for design considerations.

Total Flow (L/day)	Zinc (mg/L)	Arsenic (mg/L)	Cadmium (mg/L)	Lead (mg/L)
~77,000	434	6.0	4.6	0.056

During the summer of 1997, a series of lined (waterproof tarpaulin material – RPE), horizontal sub-surface flow wetland treatment cells were constructed near Topping Creek (locally known as Stoney Creek). A local contractor was hired to build three wetland treatment cells, two aeration cells and a final holding cell. Three RPE-lined gravel-filled wetlands were constructed on a hillside so as to allow passive water flow through the system. Constructed according to standard engineered wetlands principles, they were sized with length at least twice the width. The top two cells (in series) were 5 x 10 meters (50 m^2) and the third cell was 10 x 30 meters (300 m^2) for a total cell area of 400 m^2. Starting from an initial depth of 0.6 meters the bottom of each cell was sloped at 1% as per standard design.

The wetland cells were planted later in the summer and allowed to establish prior on clean water prior to addition of seepage water. The wetlands were planted late in the season with young, vigorously growing grass clumps, *Calamagrostis canadensis*. In addition to the grass species, various members of the *Brassicaceae* and *Helianthus* considered to good metal accumulators. Two 6 m^2 aeration cells were constructed between the wetland cells to aerate the water to assure the wetland plants had sufficient oxygen at their roots. These 2 aeration cells were also used to set water level in the preceding cell and provided convenient sample points. The final lined holding cell was sized to hold three times the volume of water that is contained in the top three cells. This final holding cell allowed for the water to be tested ensuring that it met Provincial guidelines prior to its use as irrigation water on the site or for possible re-treatment, if required.

The system was shut down for the winter in early November 1997, pipes emptied and where necessary, covered with straw for protection from freezing. A mulch layer was applied to those plants thought to be in danger from frost. In addition, the bottom retaining pond cell was drained so that it could collect winter precipitation from the upstream cells.

Winter Bench-scale Testing (1997-1998)

To further test the initial design considerations, bench scale testing over the winter was completed. Four small hydroponics wetlands were built using 77 L plastic tubs filled with a gravel/sand mixture. The bench scale systems were based on a single pass-through of water (similar to the design in Trail and scaled at the same flow rates as the Trail system). The wetlands were planted with young, vigorously growing grass clumps, *Calamagrostis canadensis* and various members of the *Brassicaceae* and *Helianthus*. They were grown in the greenhouse with a 16-hour light, 8-hour darkness cycle and fertilized using commercial fertilizer according to manufacturer's recommendations. A 100 L plastic container served as the reservoir for the manufactured seepage water. Water was delivered as a single pass through to the four wetlands at concentrations of 0, 5, 10 and 40% of the metals present in the Stoney Creek seepage by using calibrated peristaltic pumps and plastic hoses.

This greenhouse research showed that metal concentrations present in the seepage were too high for the chosen plants and would need to be reduced by 50-90% for effective wetlands treatment. While dilution of seepage was possible using the local stream near the pilot scale system, it was not considered a viable option for a full scale treatment system. Therefore, the addition of anaerobic bioreactor prior to the wetlands was evaluated.

During the same period, Dr. Mark Edwards of Teck Cominco Research carried out upflow anaerobic column tests examining the efficacy of various organic materials to remove metals in solution. The organic materials tested were various combinations of Celgar biosolids, wood chips, peat, compost, grass clippings, manure, limestone, sand

including a sugar and sand column only. This work showed that as much as 90% of the metals present in the seepage could be removed using a locally available biosolids product from the Zellstoff Celgar pulp mill in Castlegar, BC. This preliminary study supported the decision to construct an anaerobic bioreactor prior to the wetland cells.

Design and Construction of an Anaerobic Bioreactor in 1998

Design Considerations

Based on the preliminary anaerobic upflow column work by Dr. Mark Edwards and 'rule of thumb' wetlands design factors, an upflow anaerobic bioreactor was designed to potentially treat 20,000 liters per day (13.9 L/min) of collected seepage. The volume sized 'rule of thumb' is the removal of 0.3 mol/(m^3day) of metal where the volume component is the total volume neglecting the pore space and moisture content (Dvorak et al 1991; Hedin et al 1989; Gusek and Wildeman 1997). With metal concentrations of zinc up to 500 mg/L and cadmium up to 10 mg/L, the minimum cell volume was calculated as (155 mol/d)/(0.3 mol/(m^3d)) or 517 m^3. The area based 'rule of thumb' for area loading factors is estimated to be between 10 m^2min/L and 20 m^2min/L (Gusek and Wildeman 1997). This factor is pH dependent with the values presented for pH in the range of 5-7 with lower pH flows requiring higher area loading factors. So assuming the seepage is generally closer to a pH of 5, the calculated cell area was calculated as (20m^2min/L) * (13.9 L/min) or 278 m^2.

Based on the column work by Dr. Edwards, the composition of the material used in the bioreactor was composed of Zelstoff Celgar biosolids (60% by volume), sand (35% by volume) and cow manure (5% by volume). The biosolids was added as the carbon source while the manure acted as bacterial inoculums while providing additional available carbon. Sand was added to assist hydraulic conductivity to the substrate.

Based on these factors and adjusting shape of the bioreactor to fit site topography, a cell was constructed that was 25 m by 18 m at the top with sides that sloped to a bottom area that was 18 m by 10 m with a total depth of 3.5 meters. Giving a total cell volume of approximately 1000 m^3 compared to calculated required volume of 517 m^3. Surface

area of the top of cell is 450 m^2 compared to calculated required area of 278 m^2. This exceeded the design parameters by a considerable margin in terms of both volume (by 100%) and surface area (by 60%).

The vertical upflow design was chosen for several reasons. Firstly, an upflow system has treated water on the surface of the treatment cell with lower metal concentrations in water potentially available to wildlife. Second, as water is pumped under pressure into the system, it was assumed that the water would be forced follow a serpentine path due to the horizontal barriers (baffles) installed to increase the length of the flow path to the surface and residence time. While water delivered to the surface may overflow the cell as opposed to flowing through the biological substrate. Third, water entering under pressure from below would find its way to the top either as planned or by short-circuiting. The cell design provided 1 m free standing water on the surface to provide for winter protection and to help ensure that the biological substrate remained as anaerobic as possible.

Cell Construction

The anaerobic cell was designed to be temporary and therefore an inexpensive waterproof tarpaulin material (RPE) that cannot be welded was used as the liner. To increase flow path length and to prevent failure in the plug flow design the cell was constructed in 3 layers separated by RPE overlapping on the sides of the cell to direct flow through gaps at opposite ends (Figure 1). This design attempted to force water flow in a serpentine pattern up through the cell. In anaerobic bioreactors, it is important that flow contacts the entire available biological substrate with as long as possible residency time.

The entire cell was lined with a single piece of RPE liner which was held in place with a continuous earth berm. A system of PVC feeder pipes were installed across one end consisting of a single supply pipe connected to four 2.4 m perforated pipes (Figure 1). To install the horizontal baffles, the biosolids/sand/manure substrate was placed in the cell to a depth of one meter and a layer of waterproof RPE liner placed on top with an opening

at the far end of the cell away from the water inlet. A second 1 m layer of substrate was added on top with another RPE layer placed over this layer and overlapping 1 m on the closed sides of the cell. Theoretically, this would cause the seepage pumped in to flow in a horizontal zigzag pattern through the cell. A final third 1 m layer of substrate was added over the entire cell. This was then covered by non-woven geotextile filter material and capped with a 10 cm layer of sand to hold the biological substrate in place and to provide a filtering layer. The output collection system consisted of PVC drain tile installed in a gravel substrate behind a stone gabion placed in this final sand layer to collect the treated seepage (Figure 1).

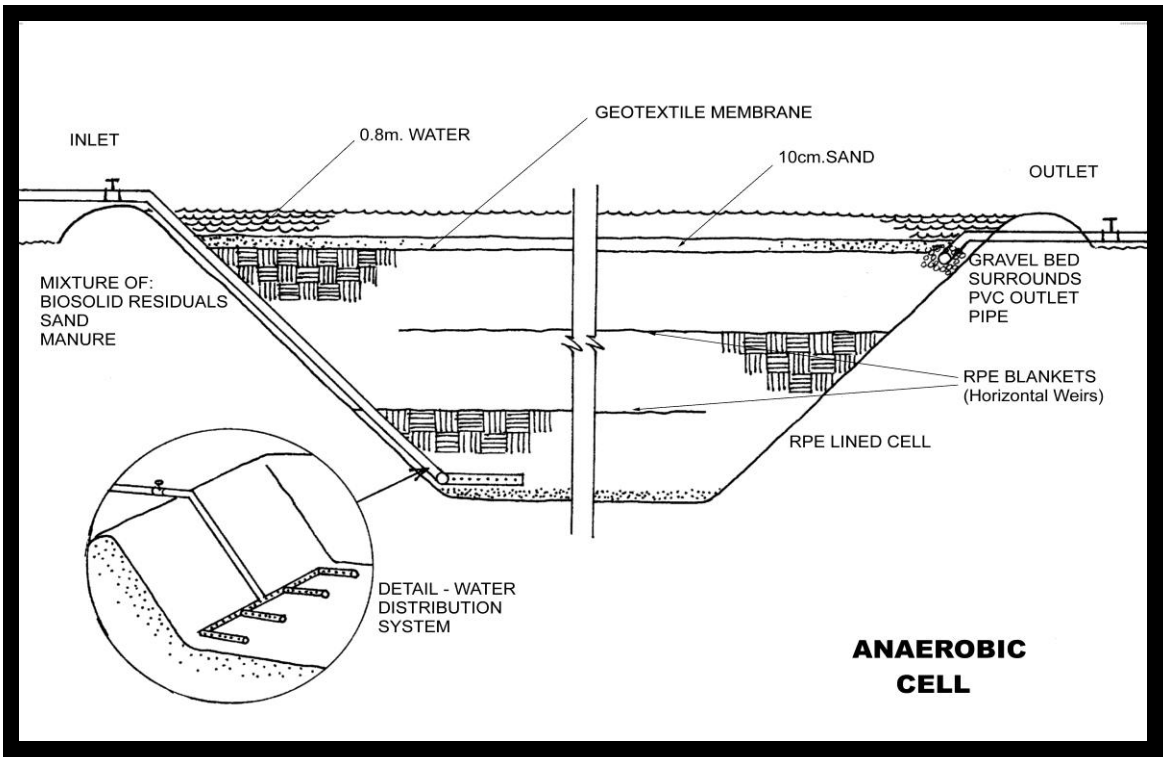

Figure 1. Schematic of anaerobic cell design as conceived showing inlet and outlet structures and horizontal weirs.

The system was completed in June 1998 and initially filled with clean water from the local creek which was allowed to sit for several weeks. This allowed the bacteria an opportunity to develop and upon pumping out, removed excess organic acids from the system (which would complex the metals and carry them out of the treatment system). This water was subsequently pumped out and seepage water pumped into the cell. The

water was pumped from the final seepage collection sump overland in PVC pipe to the treatment system. Approximately 500,000 litres of water were added over five days, allowed to sit for a day then introduced into the plant-based wetlands treatment system.

Additional Spring Planting in the Wetland Cells in 1998

In the first wet cell, 300 perennial *Helianthus* had been transplanted in July of 1997. Due to poor survival, an additional 300 *Helianthus* were subsequently transplanted in the lower west quadrant of the first cell. A grass, *Tripsicum dactyloides,* which had been germinated over winter, was planted in the lower east quadrant of the first cell. This grass has a dense fibrous root structure, grows very aggressively and has shown a high tolerance for the presence of zinc. The previously planted *Brassica* would require ongoing annual seeding efforts so was not replanted. As hybrid poplar and willows were reported to be excellent sinks for zinc, some hybrid poplar trees as well as scrub willow were transplanted into the front half of the first cell.

The front half of the second cell which had been planted with a few clumps of grass, *Agrostis stolonifera,* did well. Additional plantings of the front half of the cell with clumps of *Agrostis stolonifera* taken from the immediate area were done to completely fill in this cell. The grass, *Calamagrostis canadensis,* planted in the lower half of this cell also did well.

The final wetland cell contained *Typha latifolia* and this cell had developed very strongly during the previous year, over-wintered very well and was already showing signs of strong growth during the early spring. There were, however, some holes in the original planting where raccoons had dug up the rhizomes in search of food. Therefore, additional *Typha* rhizomes were located locally and transplanted into the third cell. The system was supplied water from the local creek with some slow release fertilizer (to stimulate plant growth) and calcium in the form of $CaNO_3$ (necessary for strong root development) added.

The total treatment system at the end of summer 1998 consisted of one anaerobic bioreactor, three plant cells and final holding pond (Figure 2). In August 1998, an eight week trial of treating seepage commenced. The system was shutdown in early October prior to freeze up.

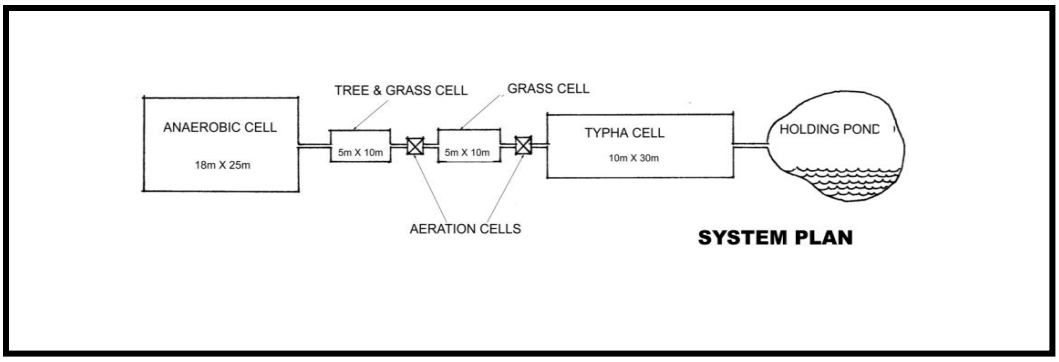

Figure 2. Schematic of the biological treatment system as installed in 1998 (water flows from left to right).

System Issues and Modifications During 1999

Vegetation Issues in 1999

Due to difficulties experienced with plugging in the outflow pipes of the plant cells, the system had been shut down during the summer of 1998 during a time when water supply was critical for the plants. The high temperatures (30-35 ^{o}C) and the loss of water resulted in some trees in the first cell suffering dieback. As a result, it was necessary to re-plant trees in the first cell. Hybrid poplar trees (*Populus deltoides)* and native coyote willows (*Salix exigua*) acquired from the Kalamalka Forest Research Station were planted in the first plant cell. The grass species (*Calamagrostis canadensis* and *Agrostis stolonifera*) had completely filled in the second plant cell, while the *Typha latifolia* had completely covered the third plant cell.

Physical and Flow Issues in 1999

Over the winter, the anaerobic cell experience some erosion and settling of the cell walls, particularly at the bottom corner. In the spring the banks of the anaerobic cell were first repaired by hand shovelling material to fill in the erosion gullies then placing erosion

matting over the entire exposed berm. This was subsequently hydroseeded with a reclamation seed mixture.

Blockages in the plant cell outflows system in 1998 were due to deposition of metal sulphides on the non-woven geotextile filter cloth that had been used to wrap the buried collection pipes. To fix the outlet pipes were dug out and the filter material was removed. A 1 m wide trough was excavated at the lower end of each of the three plant cells using a temporary plywood bulkhead installed to hold back the water-saturated gravel substrate and the filter cloth removed. The temporary plywood bulkheads were then replaced by permanent stone filled gabions in 1999. As well, repairs were made to the outflow pipes and valves installed in the outflow pipes to ensure easier winterizing in subsequent years. An irrigation pump was installed by the holding pond to irrigate the surrounding area or to the anaerobic cell for re-treatment if required. After all the repairs had been made the system ran for a full 18 weeks during the summer of 1999 prior to shutting down for the winter.

Operational Issues and Temporary Shutdown in 1999

The summer of 1999 was atypical for the Trail region with many days of cool rainy weather instead of the normal hot and dry summer months typical of the region. While the system could normally be expected to transpire up to 15,000 L/day, the cool rainy summer meant that this expectation was not fully met. Flow rates were increased to 50% higher than designed rates during July and August to test the system's capacity and to determine if the system could be recovered upon failure. During July and August, input flow rates were as high as 20,000 L/day with total flow rates up to 40,000 L/day due to rain events. The higher flows and higher metal loads induced a failure of the treatment system.

The failure appeared to be due to a lack of biological activity in the anaerobic cell (no bubbles and no H_2S odour observed at the surface of the bioreactor) which resulted in reduced metal removal. Consequently, Zn concentrations out of the anaerobic cell reached levels that were phytotoxic and 10 times higher than in 1998. To prevent the

plants from dying, the plant cells were temporarily switched to clean water feed from the nearby stream. Standing surface water was pumped from the anaerobic cell. The substrate had settled between 0.5 and 0.75 meters and given a surface area of 18 x 25 m; the substrate volume was reduced by over 225 m^3.

Difficulties in passing the design flow through this bioreactor required an examination of the flow patterns in this cell. During re-filling, rhodamine dye was added to the input water and several areas of short circuiting were noted. To remedy the situation the cell was pumped out, new biological substrate material added, 90% of the existing cell covered with geomembrane large enough to lap over the cell edges, and then covered with 10 cm of sand. These modifications returned the biological substrate to its original design volume and minimized short circuits.

When the system had stabilized, water from the final holding pond was pumped back into the anaerobic cell for re-treatment. Also, 150 kg of liquid invert sugar was added to the system as it filled to stimulate bacterial growth. The water from the holding pond was then allowed to remain in the anaerobic bioreactor for a few days and then the entire system was returned to normal operating flow rates within 10 days.

The addition of liquid invert sugar did induce increased bacterial growth and activity. Extensive bubbling at the surface of the anaerobic bioreactor accompanied by the smell of H_2S gas was noticeable after this treatment. The plants previously showing signs of phytotoxicity recovered. As well, flow rates were reduced to be at or below design treatment rates and the system recovered.

The Case for Winterization and Additional Treatment Capacity

Tests during the summers of 1998 and 1999 demonstrated that the system worked well, it could handle the high metal loads and it could be recovered if stressed beyond design capacity. However, to be useful as an industrial treatment option, year-round treatment capability was required. As well, the effect of reduced temperatures and

evapotranspiration rates on the overall treatment capabilities during the winter months needed to be determined. Therefore, it was decided to retrofit the existing system to achieve year-round treatment capability.

As well, an additional bioreactor with limestone would be constructed to optimize Zn removal. Lessons learned from design and construction of the first bioreactor would be incorporated into the design. The addition of limestone would help increase the pH and the new anaerobic cell would also provide additional treatment capacity for winter operations. The need to increase Zn removal rates was essential as higher concentrations of Zn in the collected seepage (as high as 750 mg/L compared to the 400 – 500 mg/L designed for) were observed in 1999. As well, the existing anaerobic bioreactor had not been able to raise pH levels to desired optimal range; therefore a novel hybrid bioreactor that incorporated features of an anoxic limestone drain system with additional sulphide production was designed.

System issues and Modifications in 2000

Winterization

The design of this system is specific for both the terrain and the climate in Trail, British Columbia. Winter weather in this region is usually not typical of most of Canada and although there is some snow cover, ground frost does not penetrate too deep and ice cover on open water bodies is only 5 to 10 cm thick. Due to the general lack of significant snow cover in the valley bottom, the winterization was designed for more severe winter conditions and pipes and control valves were buried to a minimum depth of 0.9 m.

Contaminated water is collected in a main sump and from this source it can be pumped either to the main effluent treatment plant operated by TML or to the biological treatment system. The existing surface pipeline was removed and a new line (2” Sklar) buried in a 1.1 m deep trench from the sump to the treatment site. Where the pipeline was buried under roads, this depth was increased as traffic and snow removal forces frost deeper into the ground. Where the pipeline had to cross Stoney Creek, it was heat traced. At three

places along the length of the pipeline backflow check valves were installed to ensure that start up pressure on the pump was not excessive and to ensure that the line, once filled, would remain filled with water.

Retrofitting the existing wetlands required some innovative site-specific solutions. Numerous items had to be addressed related to the inflow and outflow structures. The input to the surface flow wetland cells utilized a single manifold constructed from 4 inch perforated PVC laid on grade at the upper end of each cell. To winterize, the system was changed to a sub-surface flow wetland by burying the manifold below the surface. Plants were removed from the inflow area and a trench dug in the gravel substrate into which the inlet manifold was laid. To protect against freeing additional material was mounded over this area. Control valves were left at grade and a standpipe was included as part of the design to provide access to these valves.

The original aeration chambers were replaced with a vertical 0.5 m diameter stainless steel pipe sealed at the bottom and buried 2 m in the ground. Water enters through a pipe welded to the column near the bottom into an adjustable standpipe which controls cell water level as above. Water flowing out of the standpipe then drops to the bottom ensuring adequate oxygenation. Water leaves through another welded pipe near the bottom that connects to the next cell. At winter operating levels, the top of the internal standpipe is 0.7 m below grade. For winter operations the standpipe is capped and buried to provide additional protection. The open water areas over the outflow collection pipes in the plant and *Typha* cells were capped with a framed plywood insulated cover. For the holding pond, the overflow drain was connected to a tile irrigation system and a new control valve was installed. Insulated sheds were built at the valve manifolds controlling water flow into and out of the new limestone anaerobic cell and at the outlet of the previously constructed anaerobic cell.

Construction of Anaerobic Limestone Bioreactor

The new anaerobic bioreactor was designed as per ‘rule of thumb’ calculations used in the first design and incorporating lessons learned from the first anaerobic bioreactor. It

was constructed above and upstream of the original anaerobic bioreactor. Due to the topography (on a slope) and geology (bedrock at the top corner) factors, the cell is not rectangular but built as a trapezoid. At the top end it measures 9 m wide and this expands out to 22 m at the down slope end. The length of the cell is 25 m for a total surface area of approximately 400 m^2. The depth changes from 2.8 m at the top end to 4.2 m at the bottom end for a total volume of approximately 1000 m^3 and a treatment volume of 800 m^3 as the biological substrate was filled to 1 to 1.5 m from the top. The cell was lined with 60 mm polyethylene geomembrane that was welded in place to ensure watertight construction. A 1 m wide geomembrane skirt was welded approximately 1 m above the bottom around the whole cell. This was to prevent short-circuiting up the sides. Welded plastic boots were built and installed to house inflow, outflow and emergency, overflow pipes.

A 15 cm layer of limestone was placed on the bottom of the cell. Using 4-inch perforated PVC pipes a circular water distribution system with two cross members was built on top of the limestone layer. The system was designed to ensure a well dispersed vertical upflow across the whole cell. The distribution pipes were covered by an additional 15 cm limestone layer and a layer of cocoa matting to separate the distribution system from the biological substrate and prevent possible plugging during construction. Non-woven geotextile filter cloth is not recommended for this purpose as the fine weave of the fabric results in clogging by metal sulphides and could result in cell failure.

The system was then charged with 2000 L of water to test the permeability of the distribution system. The test indicated adequate permeability in the limestone layer. Subsequently the cell was filled with over 1000 m^3 of biological substrate composed of 65% Celgar biosolids and the remainder equal proportions fine sand and limestone. This mixture was then placed in the cell until it was within 1.5 m of the top edge. The biological substrate was covered with non-woven geotextile filter cloth and then covered by 15 cm of fine sand to ensure that the substrate remained in place and to provide a filtering mechanism for the metal sulphides that are produced.

The anaerobic cell was filled with water available from a nearby stream. This water was amended with ammonium sulphate fertilizer and approximately 150 kg of liquid invert sugar to stimulate suitable conditions for anaerobic sulphate-reducing bacteria. High rates of bacterial activity were obvious as frothing and bubbling occurred over most of the cell surface. After one week, the water was released to the second anaerobic cell and seepage was added in a controlled manner to ensure adequate residency time. Initially, the outflow into the second bioreactor was limited to 15,000 L/day.

Vegetation issues in 2000

In 1999 that *Epilobium grandifolia* was growing in metal containing water and may be a hyperaccumulator. This species is an early succession plant generally found in disturbed soil. In early spring, shoots of this species were transplanted into several locations in the first and second wetland cells.

Most of the poplar trees in the first wetland cell had died and were subsequently removed. Various other plant species were planted to fill in the gaps. Two weeping willow trees seemed to be surviving so two additional species of hybrid willows were planted in the first wetland cell. Other plants transplanted into this cell were two cuttings of bamboo, several root cuttings of *Raynotria japonica*, rhubarb (*Rheum rhabarbarum*) and grass species (*Spartina pectinata, Panicum virgatum, and Andropogon gerardii*).

Assays from 1999 confirmed that *Agrostis stolonifera* (redtop) was not a metal accumulator and likely thrives in the area because it is drought tolerance and appears to have a metals exclusion mechanism. So it was removed from the first half of the second wetland cell and other species transplanted in its place. Fifteen poplar saplings were transplanted to the front of the cell examine poplar's response to lower metal levels in this cell. Eleven rooted hybrid willow cuttings were also transplanted to this cell.

Volunteer *Juncaceae* plants were growing in the *Typha* cell indicating this family's ability to grow in metal contaminated environments. Therefore, several clumps of Juncaceae were transplanted to the second wetland cell. Two species of grasses

(*Miscanthus floridulus* and *Muscanthus sacchariflorus*) and sedge (*Carex muskinguinensis*) were also transplanted. The grasses were chosen, as they are very robust plants with dense root systems and high biomass production. The two grass species were planted near the front, while the *Carex muskinguinensis* was planted in a small area near the back of the cell.

Original Anaerobic Bioreactor Modifications

At the same time other changes were made that included winterizing the input pipe, adding a cell by-pass system and the ability to accept water from other sources and the winterization of the outflow collection system.

System Issues and Modifications in 2001

Typha Cell and Slow Sand Filter

During the summer of 2000 the plants in the *Typh*a cell were growing unevenly throughout the cell with poorer growth in the centre. *Typha* in the center were smaller, less dense and not spreading as the well as at the edges. Additional fertilizer was added during the summer of 2000 and *Typha* roots examined for armouring by iron deposits. There was no evidence of iron deposition and the additional fertilizer did not assist the plant growth. Therefore it was decided to replant the cell. Above ground biomass was cut off and removed, all rhizomes were dug up and examined – those that seemed to be affected (soft tissue and severely branched root hairs) were discarded while healthy rhizomes were washed and ultimately replanted into the cell. Given the large differences between total and dissolved metal exiting the *Typha* cell, it was apparent that this cell was not filtering out treat metal sulphides. Therefore, a slow sand filter was constructed at its output.

Winterization Repairs in 2001

The main pipeline froze during the winter due to improper backfilling of a structure inserted to allow access to backflow valves. This problem was corrected with additional fill. As well, some freezing issues were noted with the structure containing the main control valves, so it was rebuilt.

System Issues and Modifications in 2002

Rebuilding the Original Bioreactor

During 2002, substantial changes were made when the initial 1998 bioreactor was deconstructed and rebuilt. Designed originally as only a temporary installation, a non-permanent liner material was used. In addition, an improved design that assists in better water flow was incorporated in the reconstruction. During deconstruction extensive horizontal and vertical sampling of the biological substrate was done to determine the metal and carbon concentrations at all levels in the cell and bacterial populations present at deeper levels in the cell.

The biological substrate of the anaerobic cell was removed and placed in a lined containment facility. The cell was reshaped and then lined with 60 mm high density polyethylene liner. New input distribution with cleanouts was installed in a limestone base. The original biological substrate was mixed with additional biological substrate (as cell volume was increased) and limestone as the cell was being filled. The pH adjustment with limestone was deemed critical for year-round successful removal of Zn. The cell was capped using pit-run gravels, biosolids and other materials.

Re-construction at this scale created a new issue. Initial water flows through the reconstructed cell were greatly reduced due to compaction by heavy equipment used. This was corrected by feeding the bacteria with sugars, introduced directly to the bottom of the cell through clean-out tubes installed during re-construction. Over time the gases produced by bacterial metabolism effectively lifted the material sufficiently to ensure that water would flow up from the bottom in an even manner. Contributing to this process was a system of controlled direct pumping into this cell directly from the Stoney Creek sump. The pressurized delivery system helped to establish flow pathways in the biological substrate. An emergency overflow collection system was added to each anaerobic cell and tied into the delivery pipeline to the plant-based cells.

Repair of Anaerobic Limestone Bioreactor

Several short circuits occurred that lead to minor overflows in the upstream anaerobic bioreactor. The sloped base of the primary bioreactor cell (due to bedrock encountered during construction) results in a cell where the depth varies from 2.5 m to 7 m along its length. This and the high clay content of the capping material exacerbate the short circuiting. Rather than lateral flow through a subsurface gravel substrate over the entire cell, the impervious clay leads to short circuits in the distribution layer that capped the biological substrate. Considerable retro-fitting was completed in order to ensure adequate flow rates throughout the biological substrate. Initial corrective attempts were made by constructing surface ditches and installing pipes that delivered water to other parts of the cell.

Complete System - Summer 2002

By summer of 2002 the system had evolved to its current configuration (Figure 3). The system is capable of year-round treatment and has operated with minimal issues from 2002 to 2010. It will continue to operate into the foreseeable future. The system has become an acceptable treatment technology and an emergency backup system for treating the Stoney Creek seepages. The possibility of a larger system in the Stoney Creek valley to treat the total seepage volume is being considered by TML.

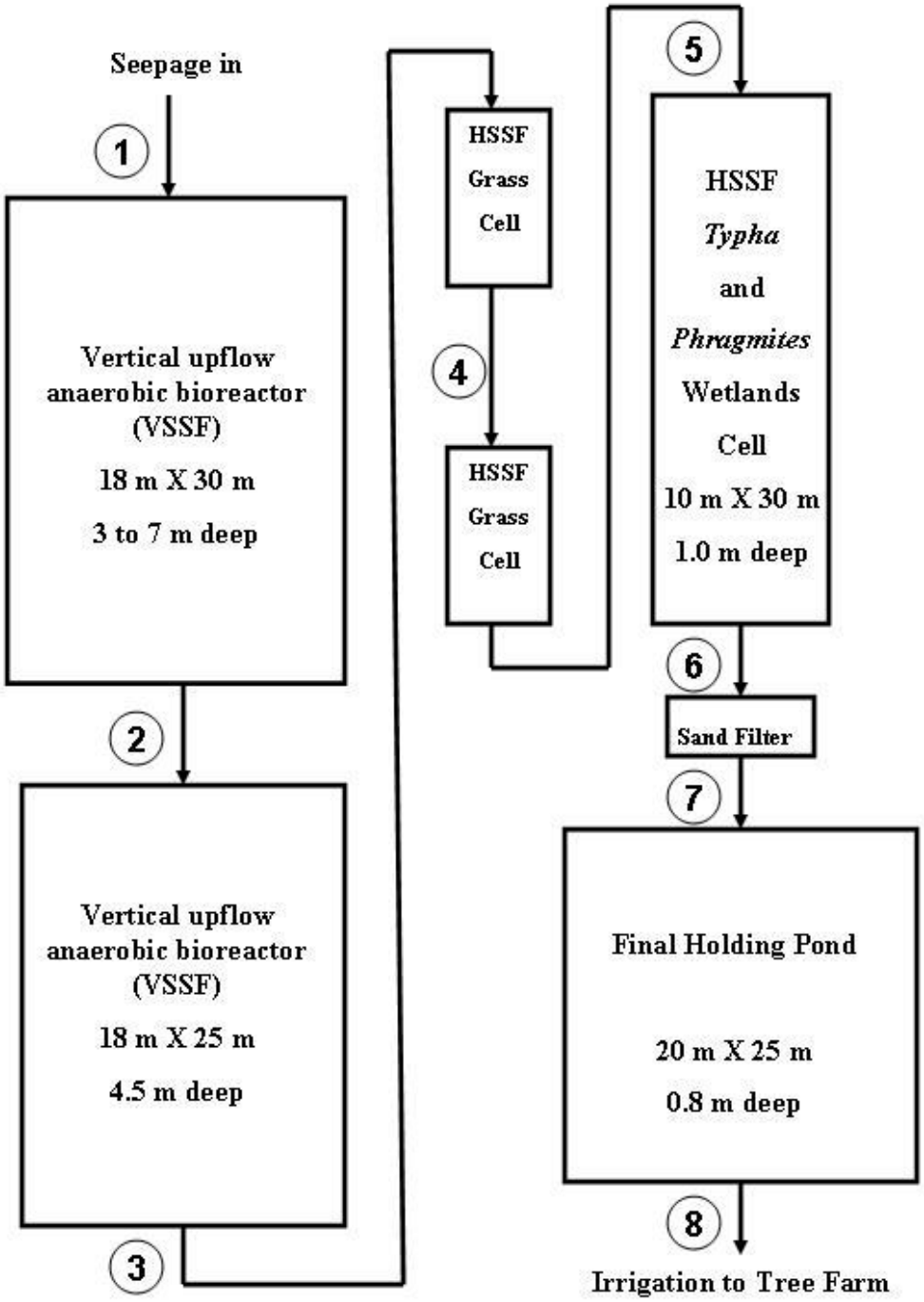

Figure 3. Schematic diagram of the wetlands system (cells are approximately to scale) as of summer 2002 with sampling points indicate in circles.

Appendix B – Microbial Insights, Inc. Data Report

Microbial Insights. Inc.

Sample Name		1nwc1-1	1nwc2-3
Weight of wet sample (g)		50.16	50.20
Moisture content (% water)		33%	34%
Weight of dry sample		33.46	33.36
MI Identifier		1nwc1	1nwc2
Total Picomoles of PLFA[1]		381,702	169,468
Analytical Report			
PLFA Profile (% of total PLFA)	Equivalent Chain Length		
Terminally Branched Saturates (TerBrSats)			
i14:0	13.64	0.8	0.5
i15:0	14.65	3.7	4.8
a15:0	14.74	4.7	3.8
i16:0	15.64	2.5	1.4
i17:0	16.65	1.3	0.7
a17:0	16.74	2.1	0.7
		15.2	11.9
Monoenoics (Monos)			
15:1w6c	14.80	0.4	0.8
16:1w7c	15.80	6.4	14.2
16:1w5c	15.88	1.1	1.6
17:1w8c	16.78	0.0	1.1
17:1w6c	16.81	1.0	3.3
cy17:0	16.86	1.2	2.9
18:1w7c	17.81	26.9	16.0
18:1w5c	17.89	0.4	0.3
cy19:0	18.91	11.3	4.0
		48.8	44.0
Branched Monoenoics (BrMonos)			
br15:1a	14.41	0.0	0.1
br15:1b	14.45	0.1	0.2
i16:1b	15.45	0.1	0.0
i17:1w7c	16.37	0.8	1.4
br19:1a	18.06	0.8	1.8
br19:1b	18.11	1.5	0.0
		3.3	3.6

Mid-Chain Branched Saturates (MidBrSats)			
br14:0	14.52	0.0	0.0
br15:0d	15.48	0.2	0.3
10me16:0	16.45	1.2	0.8
11me16:0	16.47	0.0	0.3
12me16:0	16.53	0.3	0.0
br17:0a	17.07	0.1	0.1
br17:0b	17.14	0.1	0.1
10me17:0	17.43	0.2	0.1
12me17:0/18:2	17.47	0.2	0.2
10me18:0	18.41	0.3	0.0
12me18:0	18.43	0.2	0.0
		2.7	2.0
Normal Saturates (NSats)			
13:00	13.00	0.2	0.3
14:0	14.00	1.0	1.6
15:0	15.00	1.4	3.0
16:0	15.99	12.1	16.4
17:0	17.00	0.9	1.1
18:0	17.99	5.7	2.1
		21.4	24.4
Eukaryotes			
18:2a	17.55	0.8	0.3
18:2b	17.60	0.2	0.2
18:2w6	17.69	1.4	1.9
18:1w9c	17.76	2.9	3.3
20:4w6	19.34	0.3	1.3
20:5w3	19.39	0.4	2.0
20:3w6	19.50	0.1	1.0
20:2w3	19.60	0.0	0.2
20:1w9c	19.68	0.3	0.9
20:1w7c	19.73	0.4	0.9
20:0	20.00	0.4	0.2
22:6w3	21.14	0.1	0.4
22:4w6	21.21	0.2	0.9
22:5w3	21.28	0.1	0.2
22:0	21.99	0.5	0.2
23:0	23.00	0.1	0.0
24:0	24.00	0.5	0.2
		8.6	14.2

Data Summary Sheet

Biomass:		
pmols PLFA/g dry wt.	11,408	5,079
Cells/g dry wt.	2.28E+08	1.02E+08
picomoles prokaryote PLFA	10,432	4,359
picomoles eukaryote PLFA	977	719
ratio prokaryote/eukaryote	11	6
Metabolic Status: (Ratio)		
Growth Phase/Turnover Rate[2]		
cy17:0/16:1w7c	0.19	0.20
cy19:0/18:1w7c	0.42	0.25
Total	0.61	0.45
Environmental Stress[3]		
16:1w7t/16:1w7c	NC	NC
18:1w7t/18:1w7c	NC	NC
Total	0.00	0.00
Community Structure: (% of Total PLFA)		
Gram+/anaerobic Gram - (TerBrSats)	15.2	11.9
Gram - (Monos)	48.8	44.0
Anaerobic metal reducers (BrMonos)	3.3	3.6
SRB/Actinomycetes (MidBrSats)	2.7	2.0
Genera (Nsats)	21.4	24.4
Eukaryotes (polyenoics)	8.6	14.2

[1] method Modified Bligh & Dyer: Detection limit 50 pmoles total PLFA
[2] ratios < 0.1 log phase, 0.1 to 5.0 Stationary Phase, > 5.0 Decline Phase
[3] ratios > 0.1 adapting to environmentally induced stress

NA: Not Analyzed NC: Not Calculated ND: Not Detected