

Multi-stage Biological Treatment System for Removal of Heavy Metal Contaminants

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ABSTRACT

A multi-stage treatment system to treat landfill leachate containing high levels of heavy metals has been constructed in Trail, British Columbia. The site is in close proximity to the Teck Cominco lead zinc smelter and treats 12-15,000 L a day of landfill leachate emanating from a historic capped landfill site. Zinc levels as high as 651 ppm, As levels to 285 ppm and Cd to 1 ppm are each reduced to levels of <0.5 ppm (<0.02 ppm for Cd). The treatment system includes a two-stage anaerobic digestion system that includes an anoxic limestone drain configuration to assist in pH elevation. The two anaerobic digesters are constructed using vertical sub-surface wetlands design criteria.

Following the bacterial-based treatment system the partially treated leachate flows into a series of plant-based cells that includes a number of plants known for their ability to withstand the presence of heavy metals for both metal tolerance and sequestration potential. Detailed measurement of plant uptake over a 3-year period demonstrates both metal tolerance and limited sequestration potential.

The system was originally designed as a prototype system capable of treating metals during the summer growing season but it has been upgraded and is currently operating year-round. Results from wintertime operations indicate that removal efficiencies remain as high for Cd and As but fall for Zn. For best removal of Zn as a sulphide a pH of between 7.2 and 7.6 is required. When our system failed to do this the levels of Zn concentration in the final effluent rose dramatically. Ensuring appropriate pH levels is, therefore, of prime concern when complete Zn removal is required.

The critical microorganisms in successful operation of the system are sulphate-reducing bacteria (SRB), which produce alkalinity and precipitate metals as their sulphides. Three groups of bacteria were enumerated at various points within the treatment system, the SRB, iron reducing bacteria (IRB) and fermentative bacteria which provide the carbon substrates (simple organic acids and alcohols) that are used by the SRB. The highest numbers of bacteria were found in the lower anaerobic cell with SRB numbers as high as 10^7 bacteria/g of the pulp mill biosolids. The lower cell was the first to be constructed and has had time for bacterial populations to develop. The role of the IRB in this particular system is unclear. Future work will be directed towards characterizing the reactions of As in anaerobic cells.

INTRODUCTION

Water and soil affected by long-term smelting and mining activity can be ubiquitous in areas in close proximity to smelting operations. In Trail, British Columbia, smelting operations have been in operation since 1890, initially including open-pit smelting. After

more than a century of such activity, there are many places where water quality can be affected by contact with high levels of metals and returned to the environment. Teck Cominco have embarked on a major remediation program to treat this water by collecting it at source. Current methodologies are used to remove metals before release. Additionally, the company has supported research in the development of technologies using biologically-based treatment systems capable of removing metals sufficient to meet current regulatory guidelines.

A historical vegetation-capped landfill, near the Teck Cominco smelter in Trail BC, produces a leachate that contains toxic metals. Prior to 1997, rainwater percolating through the landfill dissolved metals and traveled down through a deep sand layer to bedrock. It then moved laterally, eventually reaching Stoney Creek a Columbia River tributary. A seepage collection system was built in 1997. This collection system funneled the seepage water into a system of collection sumps from which it was pumped to a lime-based Effluent Treatment Plant until the prototype biologically based treatment system was completed.

To evaluate an alternative treatment technology Nature Works Remediation Corporation was contracted by Teck Cominco to design and build a prototype biological treatment system. The first phase was constructed in 1997 to remove high concentrations of Zn, Cd and As from this leachate. Following the successful operations during the 8-week trial period (1998) the system was prepared for a full summer (18 weeks) of operations in 1999, operated from June 8, 2000 to Jan. 20, 2001 and continuously since April 10 2001.

INITIAL SYSTEM DESIGN, CONSTRUCTION & TESTING

In 1997 a series of self-contained sub-surface flow wetland cells were constructed. The site was topographically ideal and had a stream of clean water. A local contractor was hired to build three treatment cells and a holding pond. The cells were designed and built using typical constructed wetland techniques (Kadlec and Knight, 1996).

The first cell (50 m²) included *Brassica*, a species reported to be able to withstand heavy metals and *Helianthus annuus*. The second cell, (50 m²), contained *Calamagrostis canadensis* and native grasses transplanted from the immediate area. The third treatment cell, (300 m²) contained *Typha latifolia*. Cells were initially watered (1st year) using the local stream while root growth was established. The holding pond built as the last stage retains water for final testing before being used for irrigation.

Bench-scale Testing

Models of the system were constructed at the University of Guelph and planted with the species being investigated. Large 77 L containers, each containing the suite of metals in the leachate, were used to provide water at a rate based on field-scale system design parameters. Four systems were constructed containing 5, 10 and 40% of the metals present in the leachate and a control that used deionized water only.

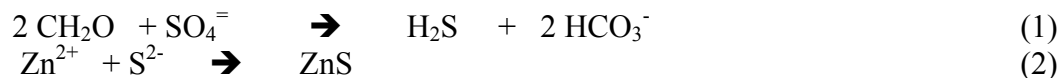
This greenhouse research showed that the metal concentrations in full-strength leachate were too high for the chosen plants – those tested in 40% leachate were severely affected.

Metal levels would need to be reduced by 50 - 90% for wetland treatment to be successful. During the same period Teck Cominco research personnel examined the efficacy of various biomass materials to remove metals in an anaerobic environment. This work showed that as much as 90% of the metals present in the leachate could be removed using a locally available residual biomass product from a nearby pulp mill. For the field scale prototype both dilution and pre-treatment using an anaerobic digester were considered. The second option was chosen as it would provide complete treatment and allow the pilot plant to be scaled up to treat the seepages in situ. During the early summer of 1998 a large, 500m³ anaerobic digester was built to provide a complete treatment system.

Microbiological Operations

In anaerobic zones in wetlands, or bacterial digesters, sulphate-reducing bacteria (SRBs) can decontaminate mine drainage, if it is moderately acidic or already neutralized with low acidity. The SRBs are able to eliminate metals such as Zn, Cu and Cd from wastewaters passing through them, as well as to increase alkalinity and lower sulphate concentrations. The SRBs use low molecular weight carbon compounds as electron donors and reduce sulphate ions to produce sulphide ions. The sulphide ions react with metals to produce metal sulphides, which precipitate or are filtered out.

The SRB-mediated reactions in the anaerobic zone of a wetland cell or in an anaerobic Vertical Sub-Surface Flow (VSSF) wetland cell may be represented by:



Where CH₂O represents the carbon source and zinc is used to represent a typical metal cation in mine drainage.

An adequate supply of sulphate is necessary in the system for the reactions to work. The carbon source may be any type of carbonaceous material (e.g., sawdust, wood) submerged in a wetland cell's water, the decaying roots/detritus of the wetland plants growing in the wetland cell, or a layer of microbially-available carbonaceous material such as municipal compost or pulp mill biosolids in a bioreactor.

Anaerobic Cell Design

Three main factors are crucial to the design and sizing of an anaerobic cell. These include the volume of water to be treated, the concentration of metals present and the treatment area required to do so, and the composition of the organic biomass.

The potential to treat 20,000 L per day (13.9 L/min) was used to estimate cell area. The volume sized 'rule of thumb' used in calculation based on metal concentrations is the removal of 0.3 mol/(m³d) 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). Zinc content can range up to 500 mg/L, Cd up to 10 mg/L, and As up to 45 mg/L. Therefore:

$$\text{Cell Volume} = (155 \text{ mol/d}) / ((0.3 \text{ mol}/(\text{m}^3 \text{d})) = 517 \text{ m}^3 \quad (3)$$

While the area based 'rule of thumb' is estimated to be between 10 m²min/L and 20 m²min/L (Gusek and Wildeman, 1997). This factor is pH dependent in the range of 5-7 with higher pH values requiring lower loading factors. Therefore:

$$\text{Cell Area} = (20 \text{ m}^2 \text{min/L}) \times (13.9 \text{ L/min}) = 278 \text{ m}^2 \quad (4)$$

The composition of biomass used was 60% pulp mill biosolids, 35% sand and 5% cow manure. Based on these parameters and allowing for adjustments due to site characteristics the initial anaerobic cell was constructed.

Additional Construction

During the first full summer's operations (18 weeks, summer, 1999) results were good although problems were experienced in the original plug flow design. Metal removal efficiencies were high and biological processes elevated leachate pH, but efficient Zn removal requires a pH between 7.2 and 7.6 and these levels were not being reached in the system. Additionally, it was learned (Al Wildman, personal communication) that for best Zn removal where originating levels are high, better results are obtained with a two-stage anaerobic system. Therefore, a second modified anaerobic cell was constructed (spring 2000) that, in addition to adding further treatment capacity, included design components of an anoxic limestone drain. Total biomass of the two cells is approximately 1220 m³. During construction of the second cell additional construction was undertaken to achieve year-round operations (modifications in surface flow wetlands design, construction of a buried pipeline delivery system and construction of winterized control structures).

TESTING

Operational Parameters

An extensive program of regular sampling was established. For the past three years (1999, 2000, 2001) samples are taken from 7 points in the system (input to the system and output from each of the five cells and the final holding pond) and delivered to the Teck Cominco assay lab for ICP-MS assays. Assays include metal levels (total and dissolved) for Zn, As, Cd, and Fe, as well as levels of NO₃ and SO₄. Additional measurements include temperature, pH, and dissolved oxygen. Flow rates were originally measured using a battery powered impellor type accumulating flow meter or manually using stopwatch and measured container. Weather data were collected for two years (hours of sunlight, evaporation pan measurements, rainfall accumulation) to determine evaporation dynamics.

Plant Uptake and Sequestration

Plants have been harvested and assayed for metals content during three summer's operations. The first year (1999) only a few plants were assayed, but during 2000 and 2001 those plants that had initially shown either tolerance or sequestration ability were extensively sampled. Individual plants were marked, sampled monthly, labelled, washed with distilled water to remove surface dust accumulation, bagged, dried then submitted to

a private lab for ICP-AES metal assays. Only leaves are taken since field preparation of root specimens for metal accumulation is more difficult and possibly less accurate due to the potential for conflicting data between metals removed and stored in roots, metals adhering to the root hairs and metals that are accumulated in the rhizosphere of individual plants. Plants tested during 2000 and 2001 were: *Spartina pectinata*, *Rheum rhaponticum*, *Epilobium grandifolia*, *Deschampsia caespitosa*, *Salix* (ssp. streamco), *Salix* (ssp. Native), *Helianthus annuus*, *Carex marteninsei*, *Tripsicum dactyloides*, *Juncaceae*, *Typha latifolia*, and *Calamagrostis canadensis*.

Microbiological Sampling and Testing

Samples were taken from the two anaerobic cells by driving 2.5 cm PVC pipes into the cell. The pipes were then removed, sectioned, sealed with saran wrap and placed in storage at 4°C until they could be processed. Four samples were taken from each of the upper and lower anaerobic cells. Four liquid samples were taken from the lower cells (plant cells, *Typha* cell and holding pond). Four biosolids samples were taken from the surface of the first anaerobic cell and three were taken from the surface of the second anaerobic cell.

The sulphate reducing bacteria were grown in a modified Postgate medium C in 20 mL serum bottles (Postgate, 1984). Preparation of the medium is described in Benner et al. (2000). The tubes taken from the anaerobic cells were sectioned in the anaerobic chamber and 1 g of sediment was added to each of five replicate serum bottles. Inoculated samples were sequentially diluted and incubated in the anaerobic chamber for 30 days. Positive growth of SRB was indicated by the precipitation of Fe-sulphides. Values are reported as most probable determinations (MPN) (Cochran, 1950). The IRB were grown in a medium with the following composition in g/L: NaHCO₃, 2.5; NH₄Cl, 1.5; NaH₂PO₄, 0.6; CaCl₂•2H₂O, 0.1; MgCl₂•6H₂O, 0.1; MnCl₂•4H₂O, 0.005; Na₂MoO₄•2H₂O, 0.001; peptone, 1.5; and ferric EDTA, sodium salt, hydrate, 1.84. The final medium was adjusted to pH 7.0 and was prepared as described for the SRB. Samples from the cells were inoculated and diluted as previously described for the SRB. After 30 days the serum bottles were injected with a 0.1% ferrozine solution, (Sorensen, 1982). A positive result was indicated by a purple complex formed between ferrozine and ferrous iron. Bacterial numbers were calculated from an MPN table (Cochran, 1950).

RESULTS

Results are available for the summer operations during 1998 (12 weeks) and the 18 weeks that the system operated during 1999, but they will not be provided in this paper. Once the second biological treatment cell (combined anoxic drain and anaerobic digester) was constructed the system was re-filled and re-started on June 6, 2000. It operated successfully until late January of 2001 when a delivery pipe in the underground pipeline froze. It was re-started in April of 2001 after repairs. Results for the period June 6, 2000 to January 20, 2001 will be reported as one year's operations and results from late May, 2001 to January 20, 2002 as a second year's operation.

Flow rates 2000

Starting June 6, 2000, the anoxic cell added to the system was charged (172,388 L). By October 30 total input was 2,526,597 L, a treatment rate of 16,348 L/d. Mean volume recorded into the holding pond by October 30 was 1,797,590 L.

The calculated total for water evaporated and transpired is the difference between the input to the system less system charge less system outflow:

$$2,526,597 - (172,388 + 1,797,590) = 556,619 \text{ litres.} \quad (5)$$

During 161 operating days, 556,000 L were evaporated or transpired. Daily records of levels in the evaporation pan were recorded and from these we can calculate that 1850 L/d (214,650 L total) were evaporated from the largest (450 m²) open anaerobic pond and 160,000 L from the smaller second pond – total evaporation 375,000 L. The 181,000 L not accounted for in these calculations can be considered as water lost to transpiration from vegetative cells. Evaporated and transpired water is considered as free of metals.

During winter, flow rates are reduced to 6000 L/d, an additional 486,000 L treated (81 days X 6000 L/d). Sampling frequency was reduced to once a week from 4 sample points (the plant based cells were not sampled). For the full operating period the total volume of water treated is 2,840,209 L (2,526,597 + 486,000 less system charge of 172,388). Assays showed that metal reduction rates, until the system froze, continued to be within the summer operational range.

Flow Rates 2001

Measurement showed a total of 2,824,000 L through the system in 2001, a mean measured daily treatment rate of 15,327 L/d. Flow rates were reduced from Nov 1 to 6000 L/d. An additional 486,000 L was treated. Weekly assay results showed a marked change in Zn removal efficiency with substantially higher concentrations of Zn present in each stage of the system and in the final holding pond (gradually reaching a maximum of 125 ppm in the final holding pond) although assay results for Cd and As remained excellent.

Manual measurements of outflow from the anoxic/anaerobic cell of 2,080,736 L. were recorded. Detailed evaporation pan measurements were not carried out during 2001 but by calculation we arrive at a figure for evaporation during the period May 1 to October 20 of 570,876 L (2,824,000 input less 2,080,736 output, 172,388 system charge).

Mean flow rates are substantially the same for the two years with a larger total for 2001 due to a longer operational period (Table 1). Daily rates during 2002 were lower due to efforts made to more accurately determine the system's operational limits, to reduced flow rates at start-up and to reduced flows when pH changes resulted in lower Zn removal efficiencies.

Table 1. Comparison of flow rates during similar operating periods for 2 years (2000, 2001) showing total volume treated, daily treatment rate during summer and amount evaporated over summer period, total volume treated during winter and total for period. Volume in litres.

Time Period	Total Flow	Mean Daily Flow	Total Evaporated	Winter Flow	Year's Total
07/06/2000	2,354,209	16,348	556,619	486,000	2,840,209
01/05/2001	2,615,612	15,327	570,876	486,000	3,101,610

pH and Dissolved Oxygen Levels

These two important parameters are measured three times a week at 8 points in the system and levels are used as an indication of system functioning. Proper system operations should show an increase in pH as leachate moves from cell to cell and a decline in dissolved oxygen. The system operated as expected during 2000 but changes were noted in 2001 (Table2).

Table 2. Showing mean pH and dissolved oxygen levels in each of the treatment cells during the two-year reporting period. Data shown are the results of three times weekly measurements during summer months followed by weekly measurements during winter. For 2001 data are given until August 8, when the pH adjustment mechanism began to fail.

Period	Parameter	System Input	Anaerobic 1 Out	Anaerobic 2 Out	1 st Plant	2 nd Plant	3 rd Plant
07/06/2000 to 20/01/01							
n= 74	pH	5.13	6.76	6.94	6.86	6.78	6.83
	D.O.	6.24	3.0	2.10	1.82	1.85	2.99
01/05/2001 to 15/10/01							
n =55	pH	5.52	6.80	7.12	7.04	7.12	7.28
	D.O.	3.88	2.44	3.06	1.40	1.80	3.65

During summer 2001 a change was observed in pH adjustment taking place in the first anoxic/anaerobic cell. After August 8, a marked change was noted (data not shown). Prior to this date all pH values were above 7, ranging from 7.05 to 7.46. Post August 8, the pH of the anoxic cell output dropped below 7 and ranged downwards from 6.84 to 6.7. In the 1st plant cell the pH dropped from 7.06 (July 23) to a range of 6.57 to 6.99. A similar change was observed in the 2nd plant cell. In the *Typha* cell the pH drop did not occur until September 17 when it changed from < 7 to a low of 6.25. In the previous year, pH remained high in this cell and it was capable of removing the last of the metals present in the system.

Dissolved oxygen data are collected together with pH measurements and the two analyzed together. The level leaving the first anaerobic (limestone drain) cell is lower than that entering although the drop is not as steep as expected. The first two plant cells are definitely anaerobic with D.O. levels below 2 (1.40 and 1.80 respectively). During summer 2001, water was allowed to remain on the surface of the *Typha* cell, as part of

our investigations into why the plants in this cell were not growing evenly. This could explain the higher D.O. reading observed during the summer of 2001, 3.65 vs. 2.9, (Table 2). Uneven water flow, lack of oxygen or a lack of nutrients were each investigated as causative factors for the poor growth but no conclusion was reached and *Typha* continues its uneven growth pattern.

Metals Removed

The mean concentration of Zn found in the input effluent from June 6 to November 17, 2000, was 395.95 ppm in a range from 6.9 – 710 (n = 56, Standard deviation = 197). A reduction in the level of Zn was evident in each of the five cells that comprise the treatment system. Each cell removes a percentage of the Zn present in its input, and this is shown (Table 3).

Table 3: Metal concentrations and percentage removal during operating period 07/05/00 to 20/01/01. Percentage removed at each stage is calculated as the amount removed during each stage based on values at input and outflow from specific stage. Concentrations are from output of each stage. Levels reported as ppm (n=56).

Assay Point	Arsenic Level	% Reduction	Cadmium Level	% Reduction	Zinc Level	% Reduction
Input	39.4		5.3		395.9	
Anaerobic 1	9.2	76.5	1.9	63.2	226.3	42.9
Anaerobic 2	10.5	+ 13.6	0.3	83.2	102.6	54.7
Cell 1	8.4	19.8	0.2	21.2	63.8	37.8
Cell 2	8.3	1.3	0.2	23.1	53.6	16.1
Cell 3	1.9	76.8	0.03	85.0	14.2	73.4
Final Pond	1.6	15	0.02	33.4	11.4	19.9
Total %		95.80		99.50		97.10

For Cd and Zn the reduction in each cell is as expected – lower concentration of each metal at each stage. For As, the changes are variable and there is an increase in As levels seen as a result of activity in the 2nd anaerobic cell (Table 3). Concentrations do not return to the initial levels of approximately 40 ppm but the increase is noticeable and it is only rectified in the third plant cell. Metal removal of Zn and Cd also takes place in this final cell and removal rates are high in absolute percentage terms. The large size of this treatment cell with a correspondingly deep matrix means a larger anaerobic zone. With low D.O. levels, high pH and a larger anaerobic zone this cell functions effectively as a final polishing cell.

When total metal loads are examined the combined concentrations of metals that flowed into the system during summer 2001 was slightly reduced from the previous year 418.8 ppm versus 440.7 ppm (total number of samples = 62). Assays were taken at the same points as previously and assayed using the same facility. The mean concentrations of the three most important contaminating metals at the testing points are reported below (Table 4). Removing assay results prior to June 7 (construction and bacteria stabilization period)

results in even lower mean concentrations in the holding pond (As of 0.39 ppm, Cd. 02 ppm, and Zn 8.04 ppm - data not shown).

Table 4: Metal concentrations and metal removal during operating period 14/05/01 to 20/01/02. Percentage removed at each stage is calculated as the amount removed during each stage based on values at input and outflow from specific stage. Concentrations are from output of each stage. Levels reported as ppm (n=72).

Assay Point	As Total	% Removal	Cd Total	% Removal	Zn Total	% Removal
Input	99.6		4.9		314.3	
Anaerobic 1	28.2	71.4	2.03	59.2	129.0	58.9
Anaerobic 2	5.7	79.9	0.4	81.8	99.6	22.8
1st Plant	1.6	80.4	0.04	89.2	47.01	52.8
2nd Plant	1.1	30.2	0.04	0	39.9	15.2
Typha	1.04	6.7	0.04	0	25.4	36.4
Holding	0.95	8.6	0.04	0	18.7	26.3
Total % Removed		99.0		99.2		94.0

Between the two years differences can be noted in final metals concentrations (Tables 3 & 4). Initial concentration of As is higher (and with a greater range) in 2001/02 than the previous year with levels more than 250% higher. Concentrations of Cd and Zn are lower with concentration variability reduced for Zn. Removal of As is better in the second year than the first with a mean level in the final holding pond of 0.95 ppm as compared to a mean level of 1.6 during the previous year. Cd removal appears less efficient. This may be attributed to assay results reported in more general terms (<0.01) as opposed to more accurate results obtained previously.

The Zn removal efficiency is much less than in the first year with absolute levels remaining in the holding pond and removal efficiency expressed as percentage showing poorer results. If results prior to the change in pH elevation are examined separately, the results are better with concentrations in the final holding pond showing a mean level of 0.62 ppm (May 14 to Sept. 8) – 99.81% removal efficiency. The removal efficiency for As and Cd remained unaffected by the pH adjustment problem. The decrease in pH is likely the major contributing factor to a decreased level of Zn removal. For Zn to be effectively removed as a sulphide a pH of 7.2 – 7.6 is optimal (Gusek and Wildman 1997).

The modified anaerobic cell built to assist in pH adjustment operated well initially, but during the summer of 2001 it became less so. The limestone in the system may be becoming armoured. Concomitant with the observed reduction in pH a rise in Zn concentration in the final holding pond was observed. The increase was gradual at first but increased rapidly after Sept 5 when it rose suddenly from 1.6 to more than 16 with increases continuously accelerating until by Nov 14 the level of Zn was 143 ppm. It remained at or near this level until January of 2002 when dolomite was added to the input collection system to assist the pH adjustment mechanism. Following this addition, there

was a decline in Zn concentration but the concentrations did not return to its previous low levels. Ensuring appropriate pH levels is, therefore, of prime concern when complete Zn removal is required.

Bacterial Removal and Production of Sulphides

The effectiveness of the bacterial activity in metal removal can be established by examining the metal removal from the first two cells – the primary bacterial reactors (Table 5). Bacteria are active in the following plant-based cells as well and may serve as the primary metal removal mechanism. The role of plants may be to evapotranspire clean water, and possibly to sequester metals. Evapotranspiration may also serve an important function by increasing metal concentration for further bacterial treatment.

Levels of As are reduced by 71% in the first anaerobic (limestone drain) cell and the remaining total is reduced by a further 80% in the second anaerobic cell. Total reduction in the two cells is 94%. For Cd the numbers are 61 and 83% respectively with a total reduction of 93%. For Zn, however, the reduction is much less and we can see that the first anaerobic (limestone drain) reduces the level by only 59% with a further 23% reduction in the second anaerobic cell. The two cells combined reduce the Zn levels by a combined 68%.

Table 5: Concentrations of metals in leachate entering and leaving the two anaerobic cells. Results are from operating period 14/05/01 to 20/01/02. Levels reported in ppm

Cell	As Total	% Removed in Cell	Cd Total	% Removed in Cell	Zn Total	% Removed in Cell
In Anaerobic	99.6		4.9		314.3	
Out Anaerobic	28.2	71	2.03	60	129.0	59
Out Anaerobic 2	5.7	80	0.4	82	99.6	23
Total % Removed		94		93		68

Microbiology of the system

Three functional groups of bacteria were enumerated, the sulphate reducing bacteria (SRB), the fermentative bacteria, and the iron reducing bacteria (IRB). The SRB are critical to the operation of the anaerobic cell as they generate alkalinity and precipitate metals and the fermentative bacteria produce the substrates (electron donors and carbon source) for the SRB. Fermentative bacteria include obligate anaerobes such as the *Clostridia* and also a number of facultative anaerobes (grow either in the presence or absence of oxygen). SRB are obligate anaerobes and can only use simple carbon compounds such as formic, acetic and lactic acids, ethanol, as well as hydrogen as

electron donors. Most of the IRB are facultative and can use ferric iron as a terminal electron acceptor in the absence of oxygen. The role of the IRB in these anaerobic cells is less clear than the other groups.

Appreciable numbers of all three groups of bacteria were found in the upper cell (Table 6), which indicates that the operation of the cell was satisfactory. High numbers of SRB e found in the surface sediments 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 6). The higher numbers in the second cell may be due to the fact that it has been in operation for three years longer than the first cell and at least a year is required for bacterial populations to reach optimal numbers. Anaerobic bacteria (SRB and IRB) were also detected in the plant cells and the Typha cell, which indicates that these cells also have anaerobic zones (Table 6). It is likely that metal removed in the plant cells may occur by both filtration (removal of suspended solids) and also by precipitation as metal sulphides.

Table 6: Microbial populations in the anaerobic cells, plant cells and holding pond.

Location	Microbial Populations, #/g or #/ml* (Average values)		
	Fermentative Bacteria	Sulphate Reducing Bacteria	Iron Reducing Bacteria
First cell: surface	1.3×10^4	1.3×10^6	5.1×10^4
First cell: 5-20 cm	2.2×10^6	7.4×10^5	6.3×10^4
Second cell: surface	6.4×10^4	8.2×10^6	2.1×10^6
Second cell: 5-20 cm	1.1×10^6	1.7×10^7	4.1×10^6
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
Holding pond *	n.d.	2.7×10^5	3.3×10^5

n.d. - not done.

Metal Removal by Plants

When examining the roles of plants in the system it is necessary to examine their function as evapotranspirators as well as their role in actively sequestering metals. The volume of water transpired is described above and the concentrations of metals in sampled tissues of different plant species is shown (Table 7). Only those plants tested where mean values over 5 harvests exceeded 1000 ppm are reported. It is clear from this data that none of the plants that are able to grow in the system show significant abilities to accumulate metals – certainly none of those tested approach the hyperaccumulation standard of > 1% total metals by dry weight (Baker and Brooks, 1989; Baker et al., 1991).

The role of the cells where plants are present is not limited to the active role played by the plants – their ability to take up and sequester metals and their transpiration function. It must also include the metal removal capability of the cell matrix itself as reflected by the concentrations of metals in water entering and exiting. It is likely that the removal mechanism in each of the plant cells is a reflection as much of the bacterial activity as the

active role that plants play in the system. Since each plant-based cell includes an anaerobic zone and dissolved oxygen levels in the cells are generally low, the role of anaerobic bacteria is possibly of prime importance. The reduction of metal levels in the final *Typha* cell that includes a large anaerobic zone due to its size and design would support this idea.

Table 7. Mean metal uptake in selected plants (all cells combined) for all harvests in summer of 2001. Metal levels for Pb are included for information purposes only as it is not a primary contaminant in the collected leachate. All values are in ppm.

Plant Species	N =	Zn	As	Cd	Pb	TOTAL
<i>Epilobium grandifolia</i>	63	2249	157	13	74	2493
<i>Rheum rhaponticum</i>	19	2281	130	14	33	2459
<i>Deschampsia</i>	6	1747	61	11	65	1884
<i>Salix</i> (ssp.streamco)	24	1021	9	5	19	1054
<i>Salix</i> (spp. native)	16	951	18	12	21	1002
<i>Typha latifolia</i>	46	1240	29	10	35	1313
Controls						
Grasses (various)	19	370	6	1	6	385
<i>Epilobium grandifolia</i>	24	351	5	1	6	363
<i>Salix</i> (native)	43	361	6	1	6	374
<i>Typha latifolia</i>	86	361	6	1	6	374

Re-examining metal removal data from the complete system illustrates the role of plant cells in metal removal (Tables 4 & 5). For example in 2001/02 (Table 5) Zn concentrations are reduced from 99.62 ppm at the input to the first plant-based cell to 18.67 in the final holding pond a reduction of 81.3%. Corresponding concentrations for Cd are 0.37 at input to 0.04 (91.89%) and for As 5.67 at input to 0.95 (92.95%). Similar reductions are seen in 2000/2001 (Table 4). Some of this is due to metal sequestration but as seen from our work these levels are not high and no plant can be considered a hyperaccumulator (Table 7). The reduction in metal concentrations is likely due to plant sequestration in part and as well to bacterial activity in the plant rhizosphere as well as SRB activity in the deeper anaerobic areas of each of these cells. Which of these three potential removal mechanisms is primary has yet to be determined.

CONCLUSION

We have effectively demonstrated that a biologically based treatment system to remove metals present at initial high levels can operate successfully and can do so through winter conditions. The problems that have surfaced have each been addressed and we have made further changes in our design such that we believe that the current configuration will ensure year-round levels of operations that exceed those we have demonstrated to date. Specifically, we expect to overcome the problem with pH adjustment so that with reduced flow rates and increased insulation of the cells as a result of capping procedures, Zn removal will return to its previous high level of efficiency and remain there.

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