



Semi-passive treatment column
technology to treat mine impacted water
under cold conditions

August | 2022

Project Team

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Acknowledgment

We would like to thank Jennie Gjertsen, Jasmin Dobson and Christian Roldan from Newmont Corporation and Gladys Oka and David Flather from Lorax Environmental Service Ltd. for their support and contribution to this document.

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Abstract

Mining activities have been associated with the release of contaminants into the natural environment. These contaminants can be harmful to the health of ecosystems, wildlife, and humans. Permeable reactive barriers (PRBs) have been used with various components to treat mine impacted water. This study used various proportions of zero-valent iron, woodchips, and gravel in laboratory scale column experiment to treat synthetic mine impacted water. Targeted contaminants were arsenic, uranium and nitrate based on conditions expected at a proposed hard rock mine site. The PRBs successfully removed As and U at rates exceeding 99%. Nitrate removal was minimal until biotic denitrification was supported by the addition of sodium acetate as a soluble carbon source. Uranium removal was negatively impacted by sodium acetate amendment by unknown mechanisms. Cyanide was identified as a potentially problematic by-product generated by the system. Further investigations are required to determine the origin of the cyanide, as well as how to support the simultaneous removal of the three main contaminants of concern.

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List of abbreviations

AMD Acid mine drainage

As Arsenic

DIW Deionized water

HRT Hydraulic residence time

NO₃⁻ Nitrate

ORP Oxidation reduction potential

PRB Permeable reactive barrier

MIW Mine impacted water

U Uranium

ZVI Zero valent iron

1. Introduction

Mining activities can generate and release toxic substances, including heavy metals, into the environment (Thornton 1996; Warhate *et al.* 2003). These substances can have adverse impacts to surface and ground water, soil, biodiversity, and human health (Gray 1997; Johnson 2003;). Acid mine drainage (AMD) has been recognized as one of the mechanisms through which mining impacts water resources (Akcil and Koldas 2006). Acid mine drainage occurs when sulfide-bearing materials are exposed to oxygen and water (Evangelou and Zhang 1997; Akcil and Koldas 2006; Simate and Ndluvo 2014).

Passive and semi-passive bioreactors have been identified as a cost-efficient way to treat mine impacted water. Passive reactive barriers (PRBs) require minimal attention after installment whereas semi-passive bioreactors rely on periodic maintenance and additions of materials such as carbon sources (Trumm 2009; Ness *et al.* 2014). A variety of materials have been employed to treat a range of contaminants in semi-passive bioreactors. Zero valent iron (ZVI) (i.e., Fe^0) is a metallic form of iron which is frequently used in environmental remediation and as a component of permeable reactive barriers (PRBs).

Proponents of a proposed hard rock gold mine have identified arsenic, uranium and nitrate as parameters of potential concern. The objective of this study was to investigate the risks and merits of employing ZVI associated to biotic activities in PRBs to treat contaminated water under cold conditions. First, a literature review was conducted with the objective of collecting information regarding current industry techniques to remediate water contaminated with arsenic, uranium and

nitrate. Second, a column study was conducted to analyze how these techniques may perform under cold conditions mimicking the conditions at a proposed mine site.

2. Literature review

2.1. Arsenic

Arsenic (As) is a common contaminant in the mining industry as the metal is naturally occurring in ore and is released into the environment during mining activities (Williams 2001; Smedley and Kinniburgh 2002; Wang and Mulligan 2006). The most abundant arsenic bearing mineral associated with gold and other precious and base metal ore is arsenopyrite, an iron arsenic sulphide that can react with water and oxygen to contribute to AMD (Rimstidt *et al.* 1994; Natarajan 2008; Corkhill and Vaughan 2009). Arsenic can negatively impact the skin and other organs, enzymatic processes; and immune system of humans and other animals (Pershagen 1983; Duker *et al.* 2005; McCarty *et al.* 2011). The lethal dose, 50% (LD50) for As(III) is 50 μ M and 180 μ M for As(V) (Mochizuki 2019).

Arsenic can be present in the form of As(V), often in oxidizing conditions, or As(III), frequently in reducing environments (Gupta and Chen 1978; Cullen and Reimer 1989; Smedley and Kinniburgh 2002). There is a considerable body of literature supporting the use of ZVI in successfully removing As via PRBs (e.g. McRae *et al.* 2002; Bain *et al.* 2006; Wilkin *et al.* 2009; Gibert *et al.* 2010). ZVI operates by removing As species via adsorption on iron oxides and other corrosion products including Fe(II), Fe(III) and green rusts (Nikolaos *et al.* 2003, Bang *et al.* 2005, Wilkin 2009). Bang *et al.* (2005) determined that dissolved oxygen (DO) concentration has an impact on the formation of iron oxides and subsequent adsorption of As(V) and As(III) when hydraulic residence time (HRT) is low (i.e. $HRT \leq 9$ hr). The authors found that removal of As

species was positively correlated to increased DO concentration (Fig. 1a). In anaerobic conditions, indicated by a negative oxidation-reduction potential, As removal is attributed to adsorption on the surface of ZVI due to corrosion by trace amounts of DO in influent and generally occurs in close proximity to where influent enters the columns (Lackovic *et al.* 2000; Melitas *et al.* 2002; Lien and Wilkin 2004). Bang *et al.* (2005) also found that pH influenced the removal of As, with the highest removal of As in a solution with a pH of 6 and the lowest removal at a pH of 8 (Fig. 1b). In a batch study employing an HRT more relevant to this investigation (i.e., HRT = 3 – 10 days), Farrell *et al.* (2001) also supported the finding that increasing pH may decrease As removal by ZVI. ZVI was employed to remove As in a column study by Zurkan *et al.* (2020) with removal rates greater than 99% during the 28 day experiment with an approximate HRT of 4 days and effluent pH values of 8.06 to 8.86.

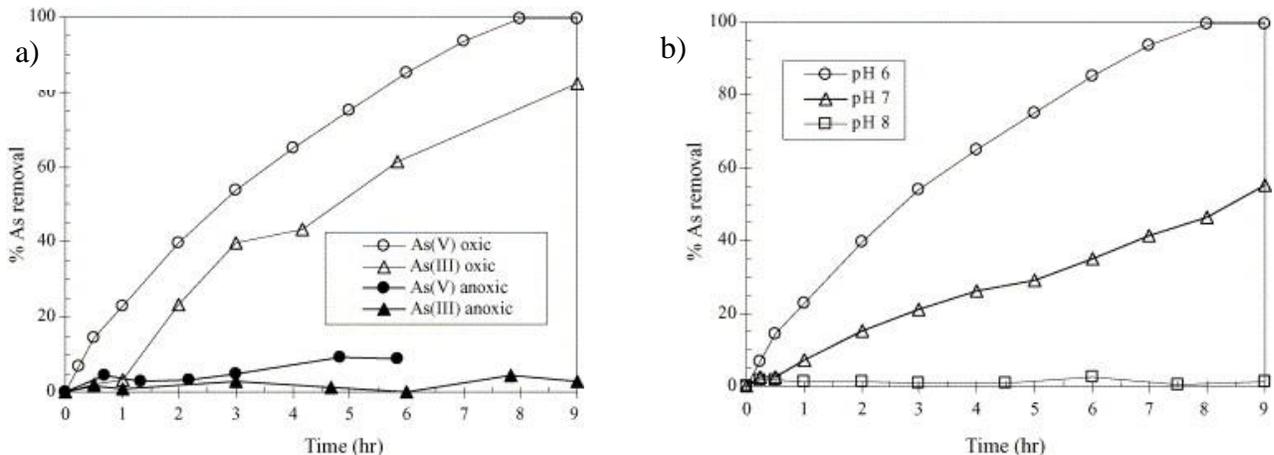


Figure 1. a) Effect of dissolved oxygen on arsenic removal by Fe(0). As(III) and As(V) = 100 mg/L; Fe(0) content = 1 g/L (100 mesh); pH 6. b) Removal of As(V) by Fe(0) at various pH. As(V) = 100 mg/L; Fe(0) content = 1 g/L (100 mesh); mixing in ambient air. (Bang *et al.* 2005).

2.2. Uranium

Uranium (U) is a naturally occurring element that is often associated with gold-bearing ores (Wilde and Bloom 1988; Winde and van der Walt 2004). Uranium can leach from the ore and lead to

adverse health impacts in many organisms including humans, affecting many systems in the body, including the pulmonary, hepatic and neurological systems (Hao *et al.* 2015; Ma *et al.* 2020).

There is a robust body of knowledge supporting the use of ZVI for U removal in contaminated water (Gu *et al.* 1998; Laurent *et al.* 1998; Farrell *et al.* 1999; Morrison *et al.* 2001; Matheson *et al.* 2003; Kornilovych *et al.* 2018). Studies suggest that the mechanism of removal is adsorption of U onto newly generated ZVI corrosion products and subsequent coprecipitation (Noubactep *et al.* 2005). Similar to As removal, U removal by ZVI in anoxic conditions is attributed to adsorption on the surface of the ZVI filings (Fiedor *et al.* 1998; Liger *et al.* 1998; Noubactep *et al.* 2005). Uranium species might also be reduced to less mobile species by corrosion products such as green rusts or iron oxides (Roh *et al.* 2000; Morrison *et al.* 2001; O'Loughlin *et al.* 2003). The long term stability of U species in ZVI PRBs is thus far uncertain, due to the potential of Fe(III) species reoxidizing U into more mobile species (Sani *et al.* 2005). In a performance evaluation of a field scale PRB, Phillips *et al.* (2000) determined that the lifespan of ZVI may be 5-10 years near the inlet portion of the PRB due to increased corrosion, and over 15 years where corrosion occurs more slowly (i.e. further from the inlet). The pH of a system impacts the removal of U in ZVI PRBs, with iron oxides adsorbing U species between 5-10 pH (Hsi and Langmuir 1985; Farrell *et al.* 1999).

2.3. Nitrate

Nitrate may be present at mining sites as a product of nitrogen-based explosives and as a product of the degradation of the cyanide used to leach gold from ore (Akcil and Mudder 2003; Herbert *et al.* 2014; Patel 2016). These industrial activities can release nitrate into the environment in high

concentrations, leading to various negative human health impacts, such as pulmonary issues, and environmental impacts, such as eutrophication (Fan and Steinberg 1996; Patel 2016). ZVI can be used to abiotically remove nitrates in PRBs, as evidenced in many studies (Robertson *et al.* 2000; Klausen *et al.* 2001; Gu *et al.* 2003; Westerhoff and James 2003; Faisal *et al.* 2018). The corrosive nature of the ZVI interactions with As and U is also prevalent in nitrate removal in ZVI PRBs and introduces concerns regarding the longevity of ZVI treatments (Westerhoff and James 2003; Wei *et al.* 2018). Westerhoff and James (2003) found in a pilot scale study that cementation may occur due to the accumulation of iron hydroxides, which can lead to localized decreases in permeability. Henderson and Demond (2007) identified the inhibition of iron corrosion, as well as differences in laboratory versus *in situ* conditions, as causes of reduced PRB longevity. Additionally, ZVI reduction of nitrate to nitrite and the subsequent reduction to ammonium can occur when pH is acidic or near neutral and is generally undesirable (Cheng *et al.* 1997; Huang *et al.* 1997; Hwang *et al.* 2011; Liu and Wang 2019). Nitrate removal decreases as pH increases and removal slows down considerably when influent is alkaline (Klausen *et al.* 2001; Choe *et al.* 2003; Liu and Wang 2019). Additionally, nitrate removal by ZVI in PRBs increases as HRT increases (Westerhoff and James 2003).

2.4. Biotic nitrate removal

Biotic treatment of nitrate contamination has been reported in many studies (Greenan *et al.* 2006; Gibert *et al.* 2008; Robertson *et al.* 2008; Gibert *et al.* 2019; Jansen *et al.* 2019). In anaerobic conditions, heterotrophic denitrifying bacteria use soluble carbon sources, such as sodium acetate, glucose and ethanol, or insoluble carbon sources, such as woodchips, cardboard, and wheat straw, to remove nitrates in PRB systems (Della Rocca *et al.* 2005; Rivett *et al.* 2008). Organic carbon is

often the limiting factor in heterotrophic denitrification, as the denitrifying bacteria use the organic carbon as an electron donor (Rivett *et al.* 2008). There has been considerable research conducted on the use of various carbon sources to support denitrification in PRBs (Della Rocca *et al.* 2007a; Healy *et al.* 2012; Gibert *et al.* 2008; Herbert *et al.* 2014). See Table 1 below for a summary of select literature. Microbial removal of nitrate by bacteria can occur in the presence of ZVI (Till *et al.* 1998; Della Rocca *et al.* 2007b; Shin and Cha 2008; Zhang *et al.* 2019). In batch tests, Zhang *et al.* (2019) found that batches inoculated with microorganisms (i.e. *Methylothermobacter* spp., *Alcaligenes eutrophus*, and *Pseudomonas* spp) removed more nitrate than batches with uninoculated ZVI and batches with inoculum but no ZVI (Fig. 2). In the same study, significant nitrate removal rates were witnessed within pH ranges from 5.8 to 8.4, though researchers did not test nitrate removal at a pH greater than 8.4 (Zhang *et al.* 2019). Microbial nitrate removal without the presence of ZVI was tested in pH from 5 to 10 by Till *et al.* (1998) and it was found that autotrophic nitrate removal was highest when pH was 6 to 9 (Fig. 3). Temperature is another influencing factor on nitrate removal in microbial and ZVI settings (Ginner *et al.* 2004; Peng *et al.* 2015; Kim and Cha 2021). Ginner *et al.* (2004) found that nitrate removal increased as temperature increased from 5°C to 50°C in batch tests with ZVI alone and ZVI inoculated with denitrifying bacteria at a pH of 8.4. In a recent study, Kim and Cha (2021) found that the inhibitory effects of low temperature on nitrate removal was less pronounced in batches with microorganisms and ZVI as opposed to ZVI alone, with a batch study of ZVI and microbes removing 67% of nitrate concentration after 6 days at 3.5°C, with an initial pH of 7.3, increasing to 8.9 by the end of sampling. Kim and Cha (2021) determined that ZVI-supported microbial denitrification is sustainable at low temperatures (e.g., < 25°C) and produces less ammonium than ZVI treatments alone.

Table 1. A review of denitrification performance for various carbon sources reported in the literature. (* carbon to nitrogen ratio of carbon media, n.s = not specified, °Calculated by the authors of the respective studies).

SOURCE OF CARBON	SYSTEM	C:N*	NO3 (PPM)	HRT (DAYS)	TEMPERATURE (°C)	NITRATE REMOVAL YIELD (%)	REFERENCES
WOODCHIPS	Field experiment	n.s.	8	5.3	n.s.	81.25	Jansen et al. 2019
ETHANOL	Field experiment	n.s.	5	0.1	n.s.	100	Jansen et al. 2019
CRAB CHITIN	Batch	n.s.	1.58	9	approx. 20	58.23	Daubert and Brennan 2007
WOODCHIPS	Batch	448.9	7.5	180	20	80.13	Greenan et al. 2006
WOODCHIPS AND SOYBEAN OIL	Batch	795.6	12	180	20	85.41	Greenan et al. 2006
CORN STALKS	Batch	42.6	40.5	180	20	91.75	Greenan et al. 2006
CARDBOARD FIBRES	Batch	280.3	15	180	20	95.8	Greenan et al. 2006
WOODCHIPS	Column	496	19.5 to 32.5	13	10	99.6 ^c	Healy et al. 2012
PINE NEEDLES	Column	46.54	19.5 to 32.6	9.9	10	99.88 ^c	Healy et al. 2012
BARLEY STRAW	Column	65.7	19.5 to 32.7	14	10	99.92 ^c	Healy et al. 2012
CARDBOARD	Column	208	19.5 to 32.8	8.5	10	99.58 ^c	Healy et al. 2012
WHEAT STRAW	Column	134.8	51.8	140	n.s	96.6	Saliling et al. 2007
WOODCHIPS	Column	393.5	51.9	140	n.s	95.9	Saliling et al. 2007
COTTON	Column	n.s.	19	0.16–0.86	27	85-97	Della Rocca et al. 2005
BIOCHAR	Batch	n.s.	30 to 40	0.5 - 1	27	78.2 ^c	Wei et al. 2018
WOODCHIPS AND SODIUM ACETATE	Column	n.s.	20	1	5.5	80 ^c	Roser et al. 2018
WOODCHIPS AND BIOCHAR	Column	n.s.	20	1	5.5	3 ^c	Roser et al. 2018

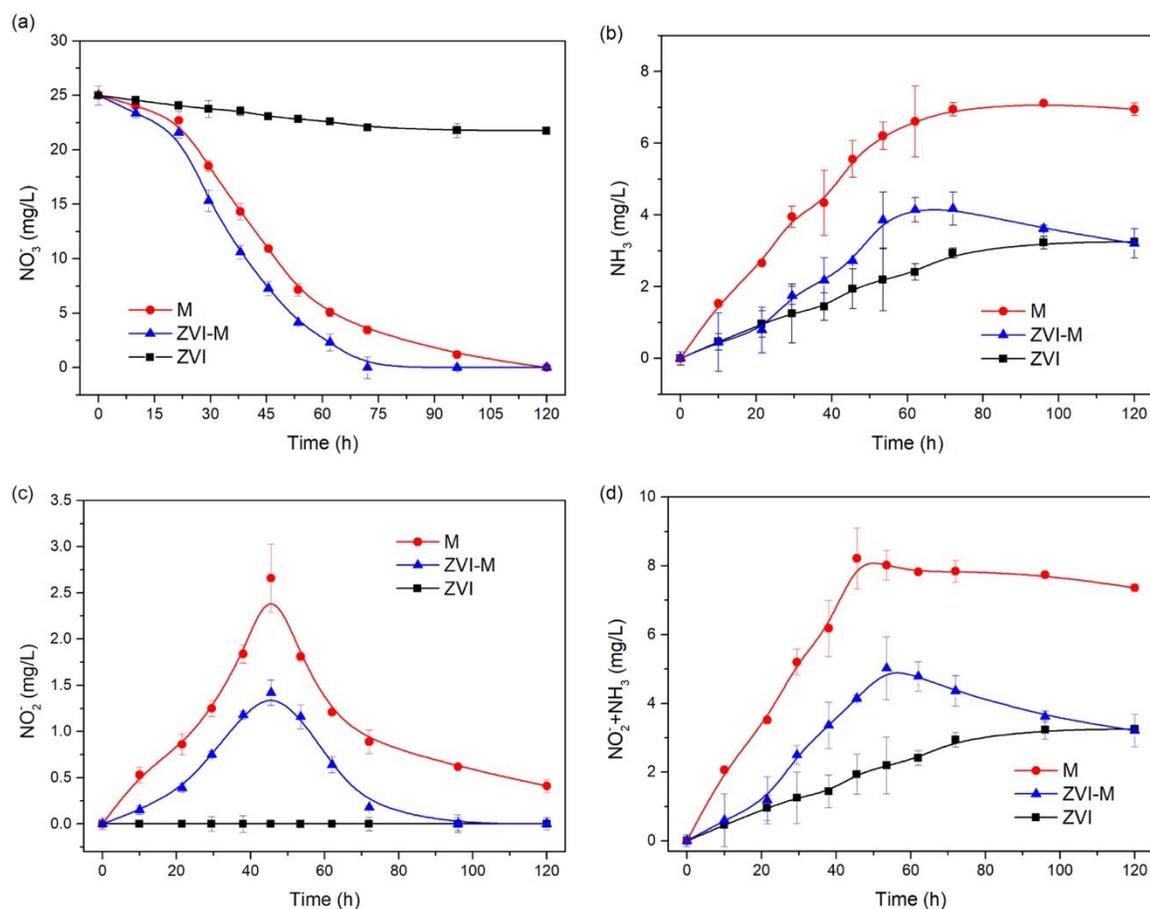


Figure 2. Dissolved N species concentrations in the three experimental systems: Inoculate ZVI flasks (ZVI-M,) uninoculated ZVI flasks (ZVI) and inoculated flasks without ZVI (M) for (a) NO_3^- , (b) NO_2^- , (c) NH_3 , and (d) $\text{NO}_2^- + \text{NH}_3$. Data points and error bars represent the averages of triplicate samples and standard deviations, respectively (Zhang *et al.* 2019).

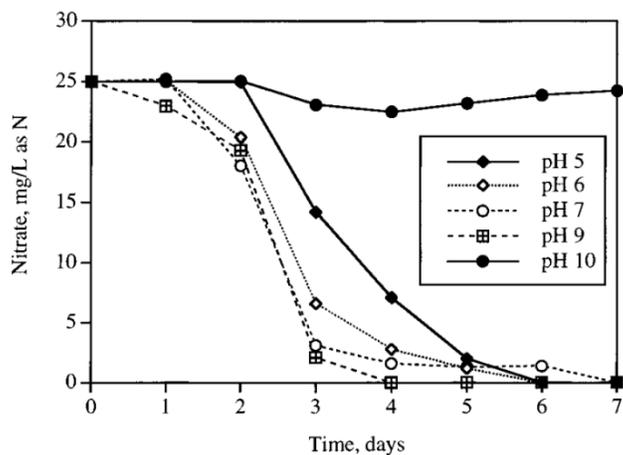


Figure 3. Effect of pH on nitrate removal by *Paracoccus denitrificans*. Reactors were fed H_2 and incubated at 21 °C on a rotary shaker table at 100 rpm (Till *et al.* 1998).

2.5. Permeable reactive barrier components

In addition to the ZVI used in PRBs, other reactive materials and substrates are often employed. Gravel is frequently used as a substrate in PRBs to achieve the desired hydraulic conductivity to support abiotic and biotic remediation efforts (Ludwig *et al.* 2002; Flury *et al.* 2009; Gibert *et al.* 2019). Gravel can vary in characteristics; Conca *et al.* (2002) employed limestone gravel as a reactive material to remediate groundwater contaminated with nitrates, metals, and radionuclides. Gravel with carbonate may raise the pH of the water in PRBs (Benner *et al.* 1997; Turner *et al.* 2005). Carbon substrates are also utilized to influence conditions within PRBs (Roser *et al.* 2018). Woodchips are a common substrate used in denitrifying bioreactors as they are a relatively common forestry by-product (Blowes *et al.* 1994; Saliling *et al.* 2007; Rivas *et al.* 2020). Woodchips are considered to be a longer lasting carbon source than other organic sources, such as cornstalks (Greenan *et al.* 2006). Carbon sources may also be added in soluble forms, such as ethanol, methanol, glucose and sodium acetate (Table 1.) (Her and Huang 1995; Greenan *et al.* 2006; Roser *et al.* 2018). Carbon sources can vary in the bioavailability of carbon and in their performance under cold conditions and various HRT (Healy *et al.* 2012; Nordstrom and Herbert 2017).

3. Methods and materials

3.1. Column materials and construction

The columns were constructed using Plexiglass cylinders. The interior dimensions were 10 cm in diameter and 30 cm in length with a total volume of 2.36 L. The water entered through the inlet at the base of the column and exited at the top of the column to minimize the formation of preferential pathways and to avoid the introduction of oxygen into the system. Three rhizons were installed in each column 6-7 cm apart to allow for sampling throughout the columns. The rhizons are used to

extract small volume of pore of water from soil and sediments, in an easy and non-destructive way. A perforated disk was placed at the bottom of each column, in addition to a sheet of non-reactive polyester fabric and a layer of silica sand, to ensure an even distribution of influent entering the columns. The columns were filled with varying proportions of wood chips, gravel and ZVI (see Fig. 4). Woodchips were sourced from Wiley Mill (Vancouver, BC). Gravel was sourced from the City of Whitehorse gravel pit (Whitehorse, YT). The ZVI was sourced from CONNELLY-GPM, Inc. (ETI CC-1004, 0.150 to 4.750 mm). The gravel grain size varied from 0.6 to 1.3 cm and was rinsed with deionized water (DIW) before being added to columns to remove any residue. Woodchip specifications were 50% wood chunks shredded at a mill and 50% splintered wood. The density of the woodchips, gravel and ZVI was determined to equalate column proportions to substrate mass (see Appendix A).

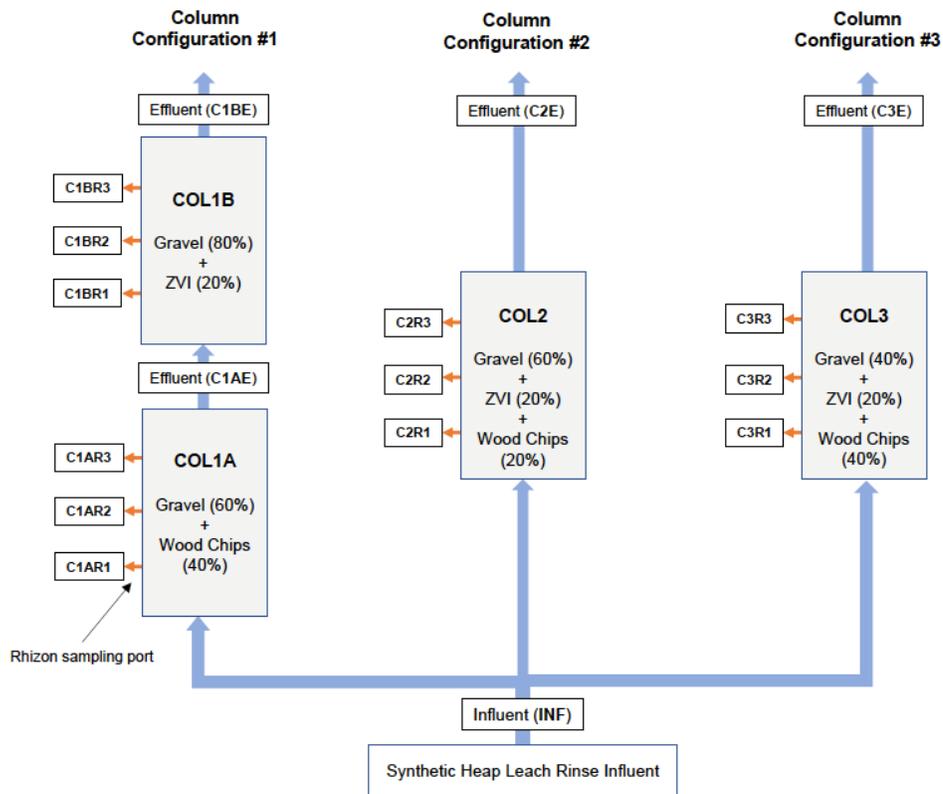


Figure 4. Column configurations and substrate proportions prepared by Lorax Environmental Services Ltd.

The first column (C1) was comprised of two reaction cells, with the first column in the series (C1A) designed to promote denitrification, with the presence of gravel and woodchip to support bacterial growth, followed by U and As removal by ZVI in the second column (C1B). The second (C2) and third (C3) columns were designed as single reaction cells containing all three reactive materials. C2 and C3 varied in their proportions of gravel and woodchips. ZVI proportions were constant between C1B, C2 and C3 at 20%. Substrates were mixed and packed into the column by hand to minimize heterogeneity. The influent used was obtained from a US company that was tasked with mimicking the water that would be produced on site post closure, see table 2 for chemical composition.

Table 2. Concentration in chemical elements and water characteristics targeted in this study to represent mine impacted water.

ELEMENT	TARGETED CONCENTRATION (MG/L)	ELEMENT	TARGETED CONCENTRATION
ALUMINUM	0.015	ZINC	0.007 mg/L
ANTIMONY	0.5	CARBON	5.26 mg/L
ARSENIC	2	SULFATE	2390 mg/L
BARIUM	0.1	PHOSPHOROUS	0.148 mg/L
CALCIUM	270-280	NITRITE	18.6 mg/L
CERIUM	0.0001	NITRATE	329 mg/L
COBALT	0.09	AMMONIA	1.140 mg/L
CUPPER	0.02	pH	6.63
IRON	0.06	ALKALINITY (as CaCO₃)	205 mg/L
MAGNESIUM	145	CONDUCTIVITY	6750 μ S/cm
MANGANESE	0.001		
MOLYBDENUM	0.04		
NICKEL	0.008		
SELENIUM	0.0008		
SILICIUM	10		
SODIUM	1300		
STRONTIUM	0.3		
SULFUR	900		
TUNGSTEN	0.008		
URANIUM	0.45		

3.2. Column operations

Columns were kept refrigerated at 5°C for the duration of the 9-month experiment (Fig. 5). Influent was stored in a secured location and small volumes (approximately 20L) were refrigerated for a minimum of 3 days before being added to the system. This was done to maintain a consistent temperature within the columns and avoid disturbance to microbial populations. The flow rate was controlled by peristaltic pumps (Cole-Parmer, Masterflex L/S, Model No: 07528-30). Flow rate was calculated using the change in influent and effluent volumes which were measured on a weekly basis (Appendix B). Pump rate was adjusted accordingly to reach target hydraulic retention time (HRT) of 4 days.



Figure 5. The columns were maintained at 5°C for the duration of the experiment. Note C1 in the centre, comprised of two columns in series.

3.3. Sampling protocols

The effluent from each column and respective rhizons was widely characterized by analyzing various parameters, such as heavy metals, total organic carbon, sulphate, nutrients and anions (Appendix C). Dissolved oxygen, pH and ORP were performed using a double junction electrode (Eutech Instruments, Oakton, Model No: PCD650). Influent and effluent from the columns were collected, preserved and sent to an off-site lab for the testing of other parameters. Samples were preserved with 18% nitric acid for heavy metals, 37-39% hydrochloric acid for mercury, 49% sulphuric acid for nutrients, 0.2 mL of 6N sodium hydroxide for cyanide. Sulphide samples were preserved with 0.2 mL of 2N zinc acetate and 0.2 mL of 6N sodium hydroxide. For the methodology used in the off-site lab analysis, see Appendix D. Effluent sampling occurred on a weekly basis from weeks 0 to 4, biweekly from weeks 6 to 16, and weekly from weeks 18 to 33 during the sodium acetate amendment period. Final sampling then occurred in week 35 and 36. Full sampling events with rhizons were conducted monthly, with the exception of the two final sampling events in weeks 35 and 36 which were both full sampling events. Sampling error led to C1A not being sampled in the weeks 1 to 3. Presumably, clogging within C1B rhizons 1 and 3 prevented the sampling of these ports in weeks 24, 32, 35 and 36 for R1 and weeks 8, 28, 32, 35 and 36 for R3.

3.4. Sodium acetate amendment

Nitrate removal can be attributed to either biotic and abiotic factors, or a combination of both. After 16 weeks from the start of the experiment, each of the columns saw a nitrate reduction $\leq 5\%$, suggesting abiotic nitrate removal by ZVI was not efficient (see section 4. Results and discussion). Microbial denitrification is a ubiquitous process in natural environments, taking place in both

aquatic and terrestrial ecosystems (Skiba, 2008). Principal components used in the creation of these bioreactors were woodchips and gravel, which were not sterilized prior to installation and so could be considered as microbial carriers containing denitrifying bacteria. Given the environmental conditions in the column, (i.e., low temperature and carbon availability), it was expected that denitrification from microbial activity would be minimal (Martens, 2004). Sodium acetate has been shown to act as an effective carbon source in the growth of nitrifying bacteria (Tam et al., 1992), and was added to the untreated influent between weeks 18 and 33. The target ratio for carbon to nitrogen was 2:1. To calculate this, the influent nitrate concentration from the first 12 weeks of the experiment was averaged. Next, 19.83 g of sodium acetate was added to every 6 L of influent to achieve the 2:1 carbon to nitrogen molar ratio (Appendix E). The addition of sodium acetate was stopped after week 33 to study the effects of its removal on the denitrification process.

3.5. Leach extraction tests

The concentration in cyanide that were detected in the effluent during our study were higher than expected. To identify the source of cyanide, two leaching tests were performed on the ZVI materials used. In addition to the ZVI leaching experiment, the impact of gravel on water chemistry was also investigated.

➤ Cyanide first leach test

Baffled bottom flasks (500 mL) were filled with approximately 50 mL of ZVI (CONNELLY-GPM, Inc. Chicago, IL) and increased to a total volume of 250 mL by the addition of DIW. Resulting mixtures were placed on the agitation table (Eberback Corporation, Model No: E6010.00). One control flask was created with DIW only. Two duplicates of the mixtures were removed from the agitation table at 5 hours, at 24 hours, and at 48 hours. Two samples and the

control flask were removed from the agitation table after 7 days. After they were removed from the agitation table, mixtures were filtered using vacuum filtration with 0.7 μm filters, followed by additional filtration using 45 μm disk filters. For each filtered solution, 38 mL of was dispensed into ALS CN-WAD sampling bottles and preserved with 0.5 mL of NaOH.

➤ Cyanide second leach test

Baffled bottom flasks (500 mL) were filled with 30 mL of the synthetic MIW. To achieve a liquid-to-solid ratio of 0.64, 46.87 g of ZVI was added to the mixture. These adjusted mixtures were agitated on the agitation table. There were duplicates for each mixture and a control flask containing only MIW. Once they were removed from the agitation table, mixtures were filtered using vacuum filtration with 0.7 micron filters, followed by additional filtration using 45 micron disk filters. For each filtered solution, 15 mL was dispensed into ALS CN-WAD sampling bottles and preserved with 0.2 mL of NaOH preservative. Mixtures were sampled after 48 hours of agitation, 4 days, 7 days, 10 days and 14 days. There were also mixtures of ZVI and DIW, sampled after 10 and 14 days so that each time step was sampled for both MIW and DIW.

➤ Gravel leach test

Baffled bottom flasks (500 mL) were filled with 100 mL of DIW. Gravel was added to the mixture to mimic the liquid-to-solid ratio in each column (Table 3).

Table 3. Mass of gravel used in leach test to achieve liquid to solid ratio in columns.

Column	Pore Volume	Gravel % (by volume)	Liquid to Solid Ratio	DI water (mL)	Gravel (g)
C1A	1110	60	0.49	100	205.2
C1B	900	80	0.3	100	333.3
C2	1005	60	0.44	100	227.2
C3	1220	40	0.8	100	125

These mixtures were agitated on the agitation table. There were duplicates for each mixture and a control flask containing only DIW. Once they were removed from the agitation table, mixtures were tested for pH (HANNA Instruments, Model No: HI5522). Mixtures were sampled initially after 20 minutes, then after 24 hours, 48 hours, 72 hours and 7 days.

4. Results and discussion

4.1. Arsenic

The removal of As occurred in all three columns designs (Fig. 6). The first column of C1 (C1A) had As concentrations similar to the influent in all sampling events. The fact that C1A is the only column without ZVI (Fig. 4) suggests that ZVI is the reactive material responsible for the removal of As. By the time the MIW left the second column in the series (C1B), As removal rates were greater than 99% with the exception of weeks 24 to 32 in which As removal rates varied from a low 95.54% in week 30 to a high of 98.99% in week 24. The lowest removal of As in C2 was 99.63% in week 2. The highest removal rate in this column was 99.88% in week 33. The lowest removal rate in C3 was 99.58% in the first week of sampling. The highest removal rate was 99.88% in week 16. The removal of As within columns 2 and 3 does not appear to have been affected by the sodium acetate added to the influent in weeks 20 to 32. However, the lower rate of As removal

in C1B in weeks 24 to 32 may be attributed to changing conditions within the column associated with the amendment, as the As removal rate increases from 96.44% in week 32 (i.e. the last week the sodium acetate was added) to 99.68% in week 33. Another possible explanation may be a problem with flow and hydraulic retention in C1 during roughly the same time period as the reduced As removal rates, though it would be expected that increased HRT would correlate with increased As removal (Fig. 7). C1B is shown in the figure 2 as it was the portion of C1 that had ZVI and therefore featured removal of As . The other columns, C2 and C3 did not experience flow rate issues that were observed in C1. Issues with the flow rate and HRT in C1 coincided with the addition of the sodium acetate. The increase in carbon was intended to support the microbial community within the column and may have led to a rapid increase of microbial populations that could have potentially obstructed the hydraulic pathways within the column. The lowest As removal rates in this column were recorded in weeks 29 and 30 with 95.77% and 95.54% respectively, with week 29 having the highest HRT in the course of the experiment at 14.3 days. The unexpected issues with flow rates in C1 caused HRT to vary greatly from our target HRT of 4 days. Appendix B shows more details on the flow rate issues between weeks 25 and 32.

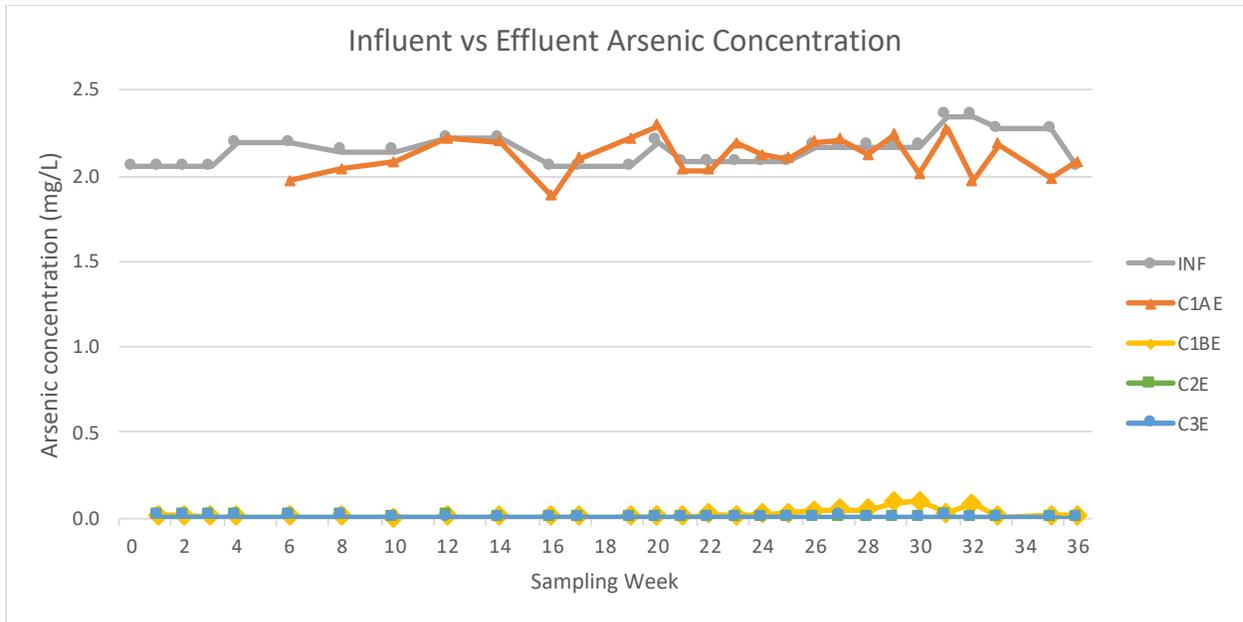


Figure 6. Arsenic concentrations in synthetic mine impacted water influent versus effluents of 3 columns.

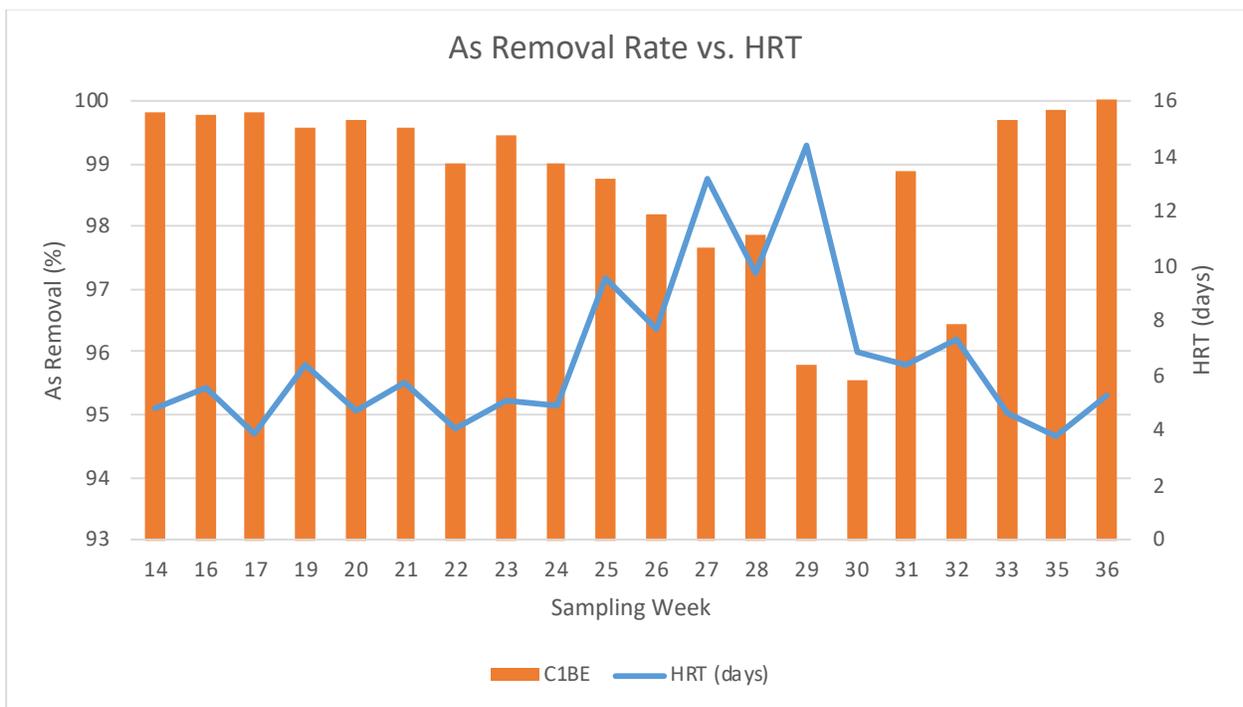


Figure 7. Arsenic removal rates plotted against hydraulic retention time for C1.

The removal of As occurred rapidly through the columns in the three columns with ZVI. The three rhizons in each column allowed for the rates of removal of contaminants to be determined as the water travelled through the columns. C2 had the most rapid removal of As, with the lowest removal rate in the first rhizon being 96.35% in week 4 (Fig. 8b). C1B had the slowest As removal of the columns with ZVI (Fig. 8a). The removal of As near the inlet of each column with ZVI may be attributed to the low concentrations of DO in the influent corroding the ZVI more rapidly where the influent enters the column (Lackovic *et al.* 2000; Melitas *et al.* 2002; Lien and Wilkin 2004). Dissolved oxygen and ORP indicate oxic conditions within the columns, which can increase As removal by ZVI (Fig. 9) (Bang *et al.* 2005).

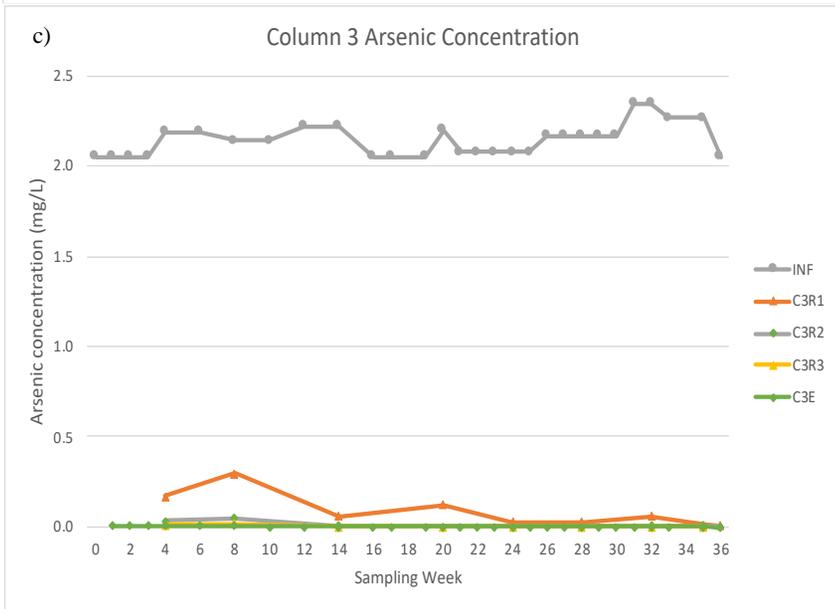
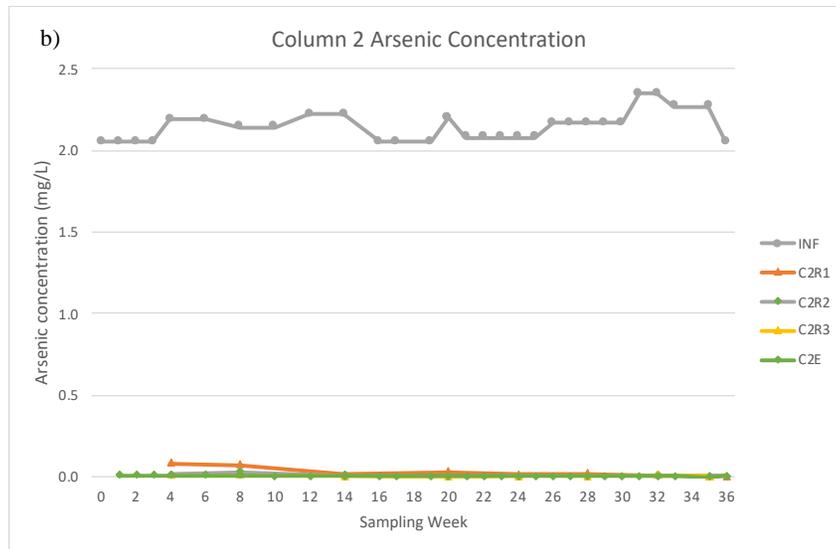
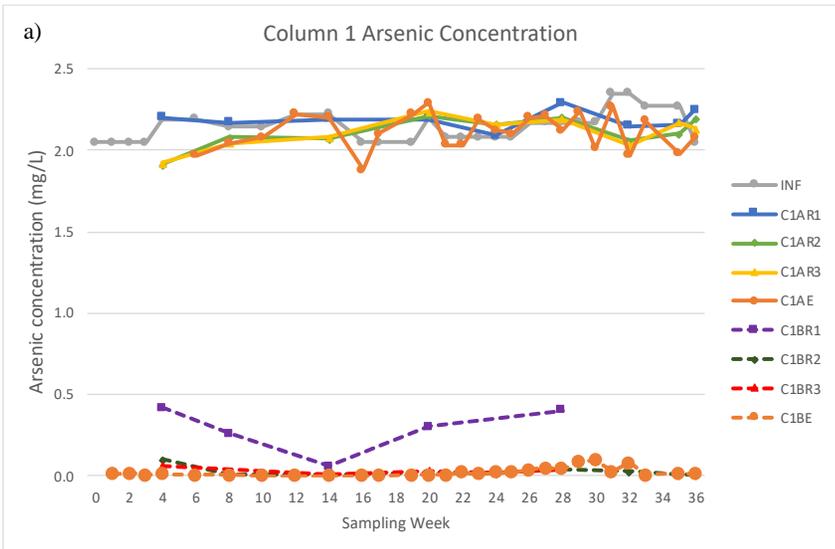


Figure 8. Arsenic concentration in synthetic mine impacted water influent versus effluent in a) C1 b) C2 c) C3.

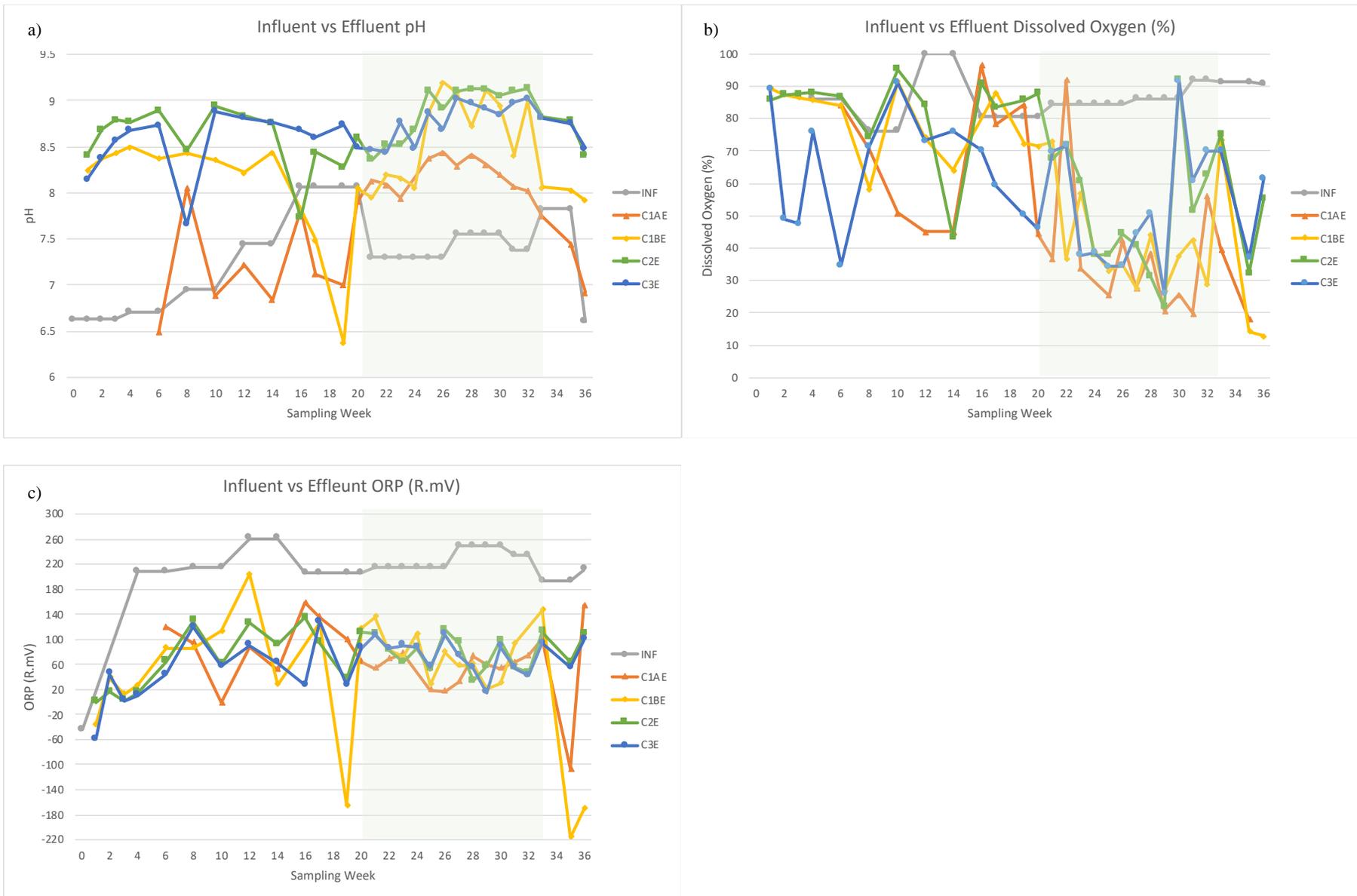


Figure 9. a) pH for in synthetic mine impacted water influent versus effluent of three columns. b) dissolved oxygen (%) in three columns. c) oxidation reduction potential (R.mV) in three columns. Sodium acetate added to influent in weeks 20 to 33 as illustrated with green rectangles.

4.2. Uranium

Following As trends, U concentrations were drastically lowered in the columns containing ZVI, particularly in the first 20 weeks of the study (Fig. 10). This high rate of removal was expected as ZVI is known to remove U in contaminated water (Gu *et al.* 1998; Matheson *et al.* 2003; Kornilovych *et al.* 2018). C3 had the highest removal rate with 99.94% of U removed in weeks 10 to 18 and week 20. C2 had the second highest removal rate with 99.89 at week 16. The highest removal rate in C1 occurred in C1B in weeks 12 to 14 with 99.88% of U removed.

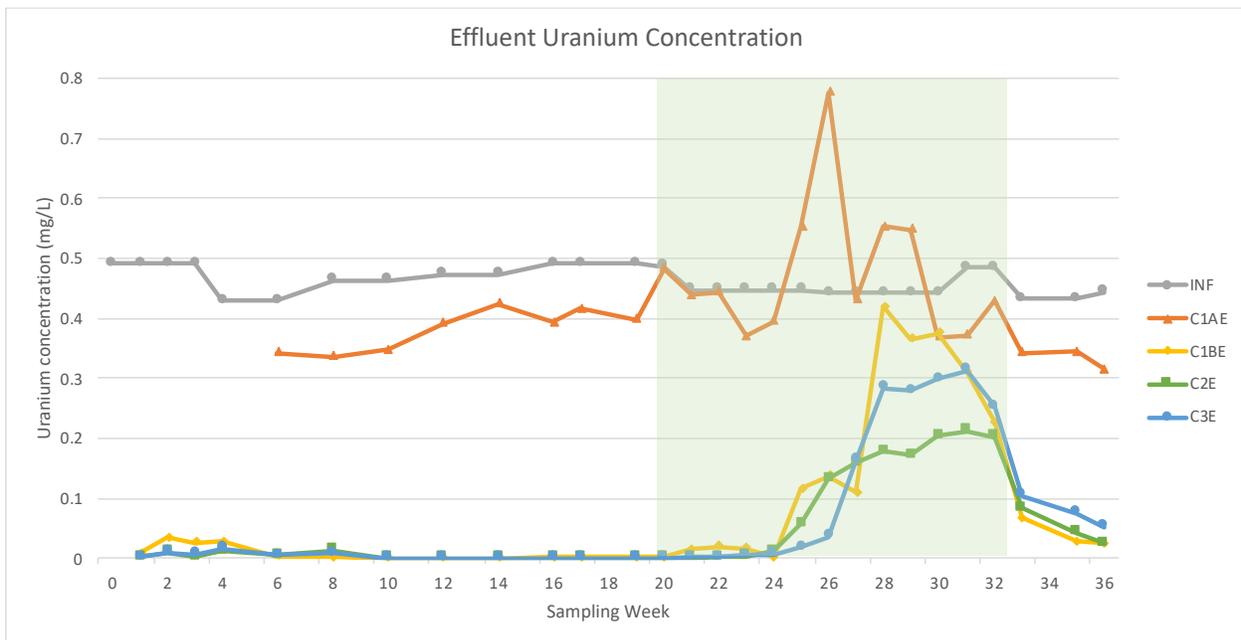


Figure 10. Uranium concentration in synthetic mine impacted water influent versus effluent of three columns. Sodium acetate added to influent in weeks 20 to 33 as illustrated with green rectangle.

Uranium removal does not occur as early in the columns as As (Fig.11). Uranium and As are known to compete for reactive sites on iron, so As may be using more reactive sites at the beginning of the column, with U being removed later in the columns where more ZVI surfaces are available (ITRC 2005). After sodium acetate was added to the influent in week 20 to support conditions for nitrate removal, U concentrations began increasing in all effluent columns. This

may have been related to the changes in the general chemistry in the columns associated with the sodium acetate amendment. Specifically, alkalinity can influence the solubility of U (Nolan and Weber 2015). Alkalinity increased from an influent average of 207.6 mg/L (in CaCO₃) before the amendment, to an average of 843.6 mg/L during the amendment period (Fig. 12). Sani *et al.* (2005) found that Fe(III) species can lead to U instability within ZVI PRBs by remobilizing U species. This suggests that the changing conditions may be influencing the corrosion of the ZVI in a manner that influences the removal of U but does not interfere with the removal of As. The nitrate removal (discussed in section 4.3) that was positively correlated to the carbon increase due to the sodium acetate may have influenced the removal of U. In a meta-analysis of U contamination in aquifers, Nolan and Weber (2015) found that abiotic and biotic nitrate reduction reaction products (i.e., nitrite and nitrous oxides) will abiotically oxidize U(IV) to U(VI). At week 33, the sodium acetate amendment was discontinued to determine if U removal rates would return to previous levels. As a result, alkalinity levels returned to pre-amendment levels immediately following the discontinuation of the amendment (Fig. 12). Uranium concentrations began to decrease in the absence of sodium acetate in all columns in the final weeks of the experiment (Fig. 10). Uranium removal in C2 returned to the highest rate of 94.57% in week 36, from a low of 53.95% in week 30. C1 had a removal rate of 94.36% in week 36, rising from a low of 5.19% in week 28. Removal rates did not recover as well in C3, with 88.06% removal in week 36, up from 32.73% in week 30. It was expected that U removal rates would return to pre-amendment levels, but this did not occur before the experiment had to be terminated due to a shortage of MIW.

These results indicate that the removal of U was more likely attributed to adsorption onto the ZVI rather than reduction. The association of U adsorption and ZVI corrosion products is well

documented (Farrell *et al.* 1999; Scott *et al.* 2005; Chen *et al.* 2017). The increase in U concentration suggests the remobilization of previously adsorbed U back into solution following changes in water chemistry (attributed to the sodium acetate amendment), whereas it would be expected that reduced U species would exit the system. This observation is particularly notable in C1A effluent, in which U concentrations exceed influent concentrations during the sodium amendment period (Fig. 10). The increase in microbial activity following the sodium acetate amendment would have increased reducing conditions within the columns leading to changes in water chemistry and releasing previously adsorbed U. Farrell *et al.* (1999) found that adsorption of U onto ZVI was highly dependent on water chemistry, especially pH.

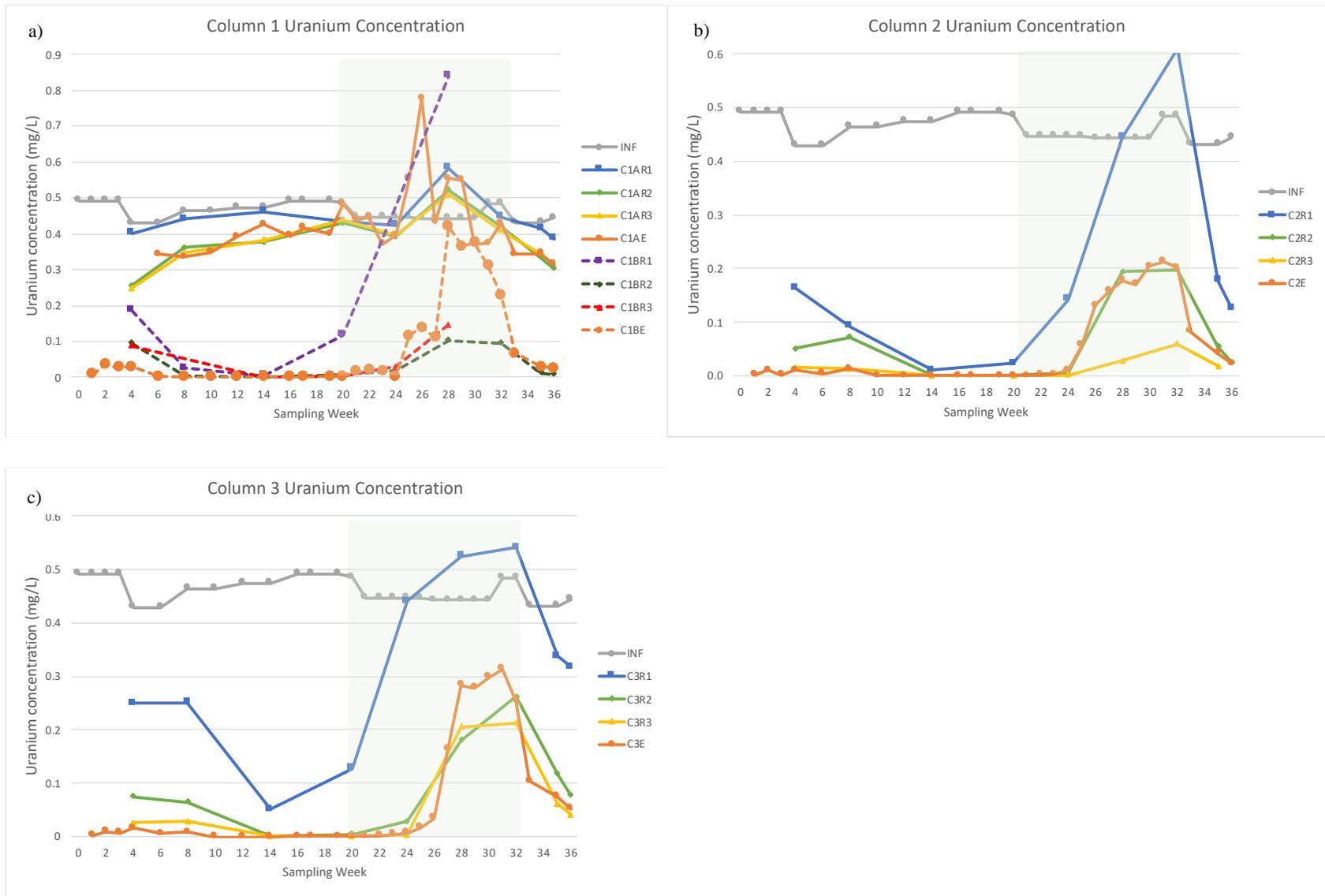


Figure 11. Uranium concentration in synthetic mine impacted water influent versus effluent from three rhizons in a) C1 b) C2 c) C3. Sodium acetate added to influent in weeks 20 to 33 as illustrated with green rectangle.

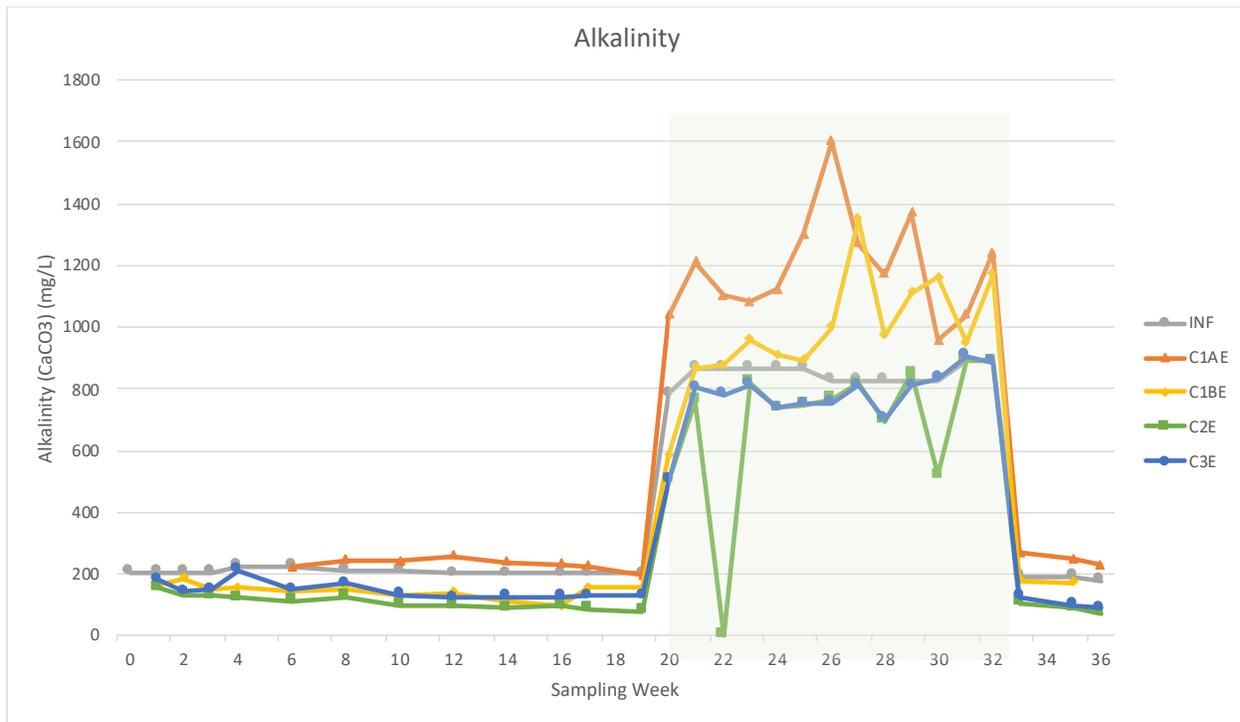


Figure 12. Alkalinity in synthetic mine impacted water influent versus effluent of three columns. Sodium acetate added to influent in weeks 20 to 33 as illustrated with green rectangle.

4.3. Nitrate

In PRBs system, nitrate can be removed both abiotically, by the ZVI, and biotically, through microbial denitrification. A combination of both processes can also occur (see section 2.4), which was expected in this study. However, there were very low rates of nitrate removal in each column in the first 20 weeks of the experiment (Fig. 13). C1A averaged 1.06% removal of nitrate, C1B averaged 5.59%, C2 averaged 6.67% and C3 had an average of 5.81% in weeks 1 to 20. Before week 20, the highest nitrate removal occurred in C1B with 12.57% removal in week 16. C1A had the lowest nitrate removal rates, with the highest being 2.83% in week 14. The positive ORP and DO values do not indicate reduction conditions in the columns that could be supportive of anaerobic nitrification in the studied system (Fig. 9). As stated before, the reduction of nitrate to nitrite by ZVI in the absence of microbial denitrification has been observed (Hwang *et al.* 2011;

Liu and Wang 2019), but the experimental condition of this experiment does not seem to favor the abiotic removal of nitrate by ZVI. Jang *et al.* (2020) found that there was differential denitrification performance among microbes at low temperatures in wood chip bioreactors. Additionally, the consumption of carbon in bioreactors may be slow in cold conditions (Nielsen *et al.* 2018). Thus, to determine if there were the required microbes in the columns, it was decided that sodium acetate would be added to support microbial denitrification, as suggested in the literature (Kozub and Liehr 1999; Roser *et al.* 2018). The sodium acetate concentration was used to achieve a 2:1 carbon to nitrogen ratio.

Once sodium acetate was added to the influent in week 20, nitrate removal increased dramatically in C1A, jumping to 30.32% in week 20 and reaching the highest removal in week 14 at 98.76%. This increased removal rate may also have been influenced by unforeseen flow rate issues in C1 in the weeks 24 to 29, with nitrate removal appearing to be positively correlated to increasing HRT (Fig. 14). The highest nitrate removal rate among all columns was 99.55% in C1B when the HRT was at the equivalent of 21.31 days in week 26. The other 2 columns also experienced increased nitrate removal with the amendment, from 12.93% in C2 in week 4 to 47.05% in week 32 and from 10.00% in C3 in week 17 to 45.58% in week 32. Nitrate removal decreased once the sodium acetate was no longer added as of week 33 (Fig. 13). Our findings mimic the results of a previous experiment by Roser *et al.* (2018), who found that the highest removal of nitrate occurred at the longest HRT tested in that study (80% removal at 24 hrs HRT). That study was conducted under similar cold conditions and employed woodchips and sodium acetate as carbon sources.

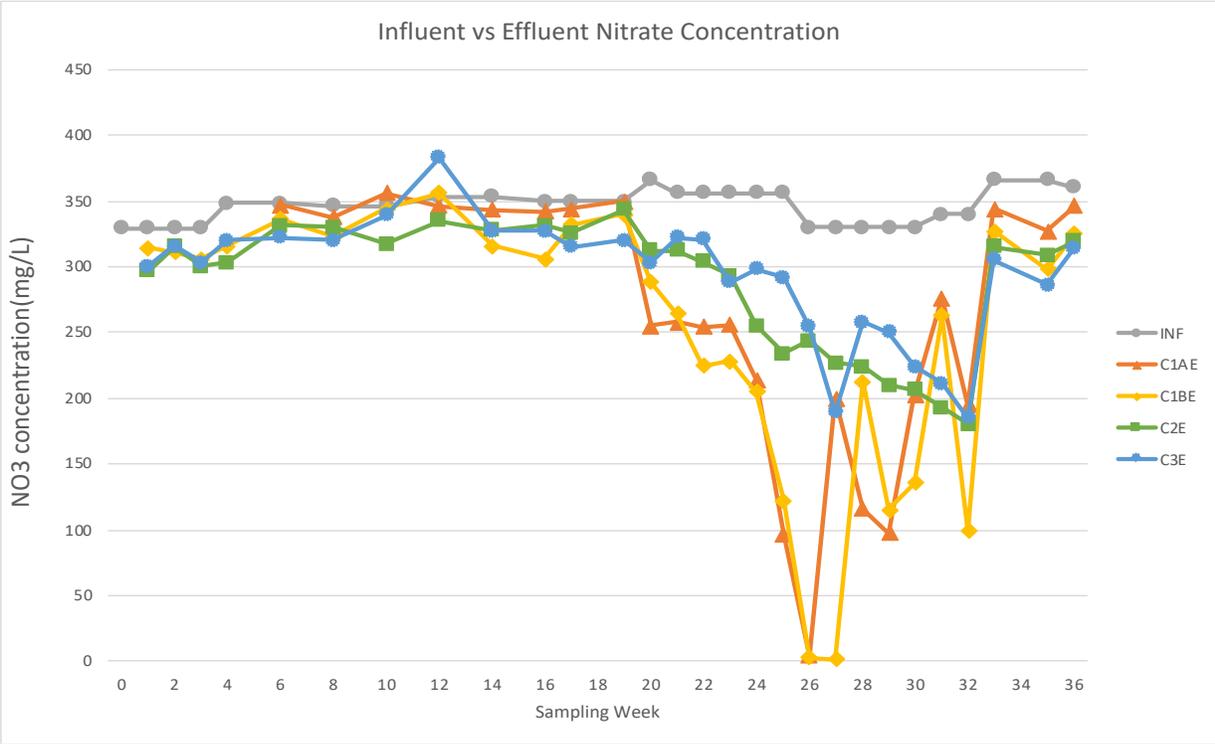


Figure 13. Nitrate concentration in synthetic mine impacted water influent versus effluent of three columns. Sodium acetate added to influent in weeks 20 to 33 as illustrated with green rectangle.

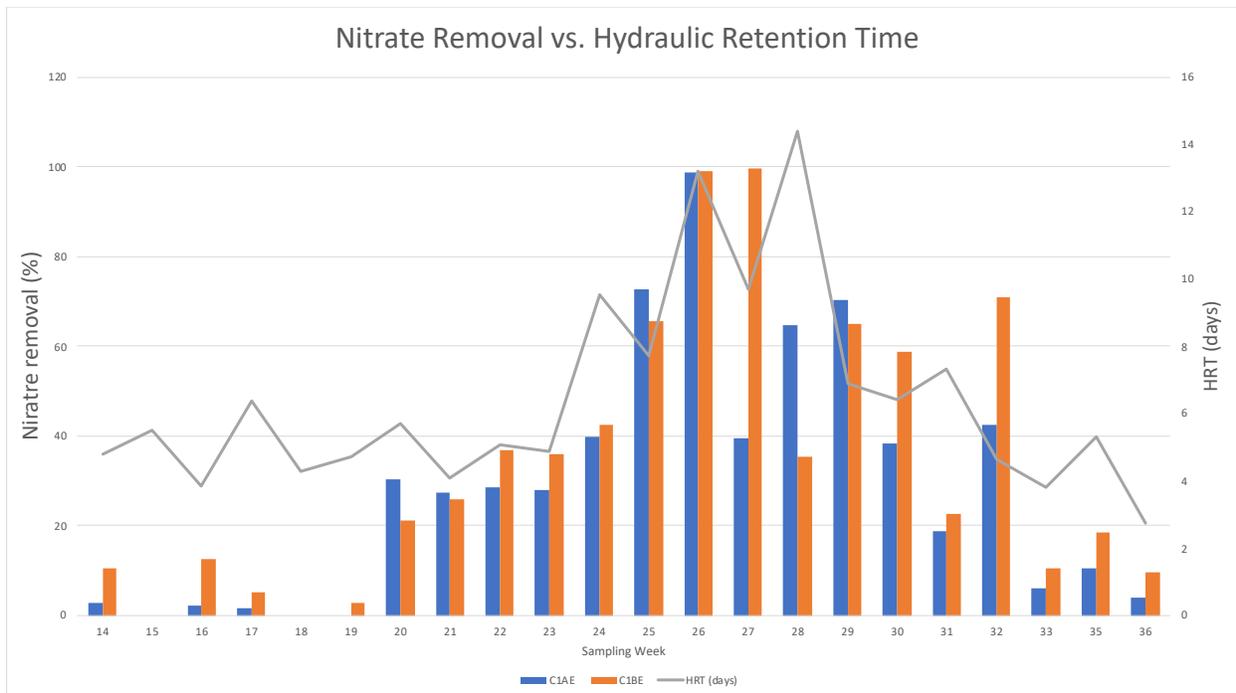


Figure 14. Percentage of nitrate removal plotted against hydraulic retention time in C1.

4.4. Cyanide

Cyanide was below detectable limits in the influent, while concentrations averaged 0.0739 mg/L in the three columns with ZVI (Fig. 15). The elevated cyanide concentration in the effluent was unexpected, considering absence of cyanide in the influent. The cyanide only occurred in the columns that contained ZVI and therefore shake-flask extraction tests were conducted with ZVI, DIW and the synthetic MIW. The agitated shake flask tests revealed that ZVI did not leach cyanide when agitated with DIW for 14 days (Table 4). However, a possible error in testing resulted in a cyanide concentration of 0.0105 mg/L. There was also no cyanide detected after 14 days of agitation with ZVI and MIW. The origin of the cyanide is therefore still unknown. The lack of MIW limited further investigation.

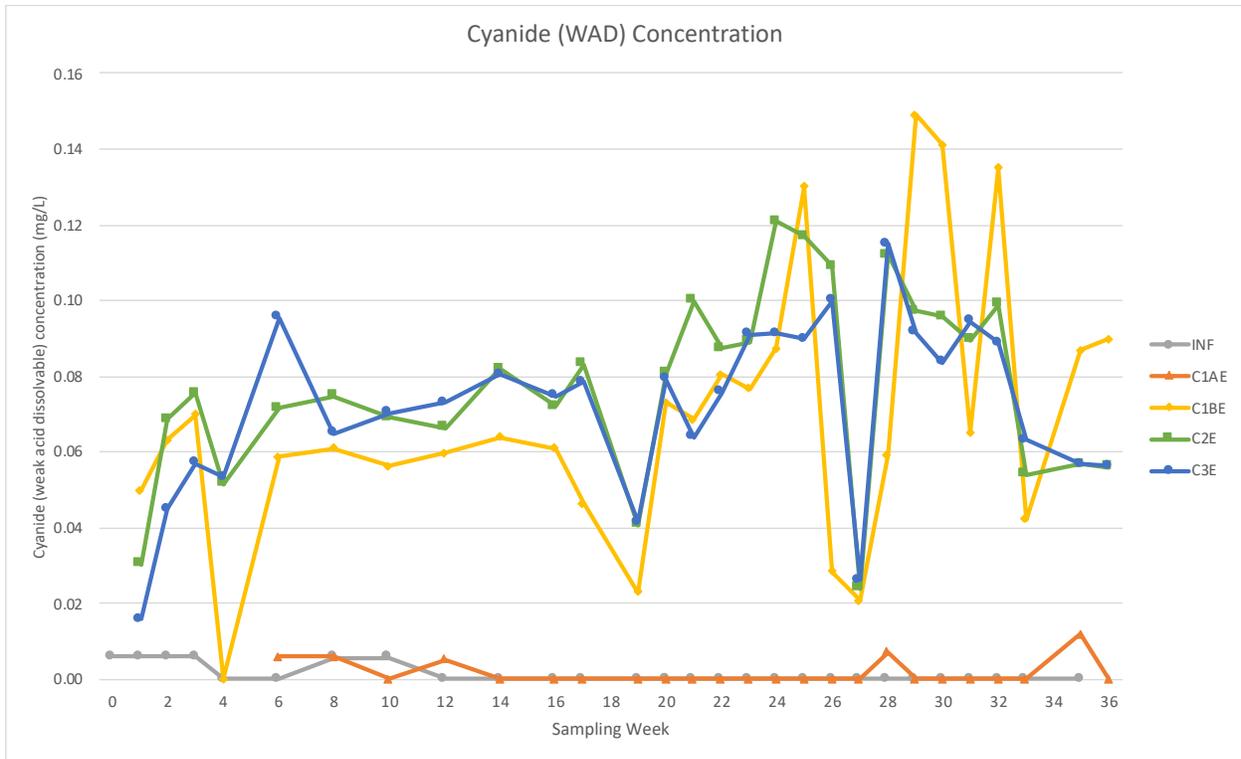


Figure 15. Nitrate concentration in synthetic mine impacted water influent versus effluent of three columns.

Table 4. Results of cyanide leach test using ZVI with MIW and DIW.

Duration	ZVI + MIW	Duration	ZVI + DIW
48 hr	<0.0200	24 hr	<0.0050
4 days	<0.0200	48 hr	<0.0050
7 days	<0.0050	5 days	<0.0050
11 days	<0.0050	7 days	<0.0050
14 days	<0.0050	10 days	<0.0100
14 days – MIW Blank	<0.0050	14 days	<0.0050
		14 days – DIW Blank	0.0105

4.5. Gravel leach test

The type of gravel might impact the conditions in the column (see section 2.5). The gravel leach test revealed that the gravel raised the pH of the DIW from an initial neutral pH to between 8 to 9 pH after 24 hours of agitation (Fig. 16). The pH increase is a good indicator that the gravel used in this study are gravel with carbonate (Benner et al. 1997; Turner et al. 2005). After the first 24 hours, pH change was minimal in each sample. The pH of PRB systems influence removal rates of As, U and nitrate. Therefore, the characteristic of the gravel has implications on the treatment of MIW.

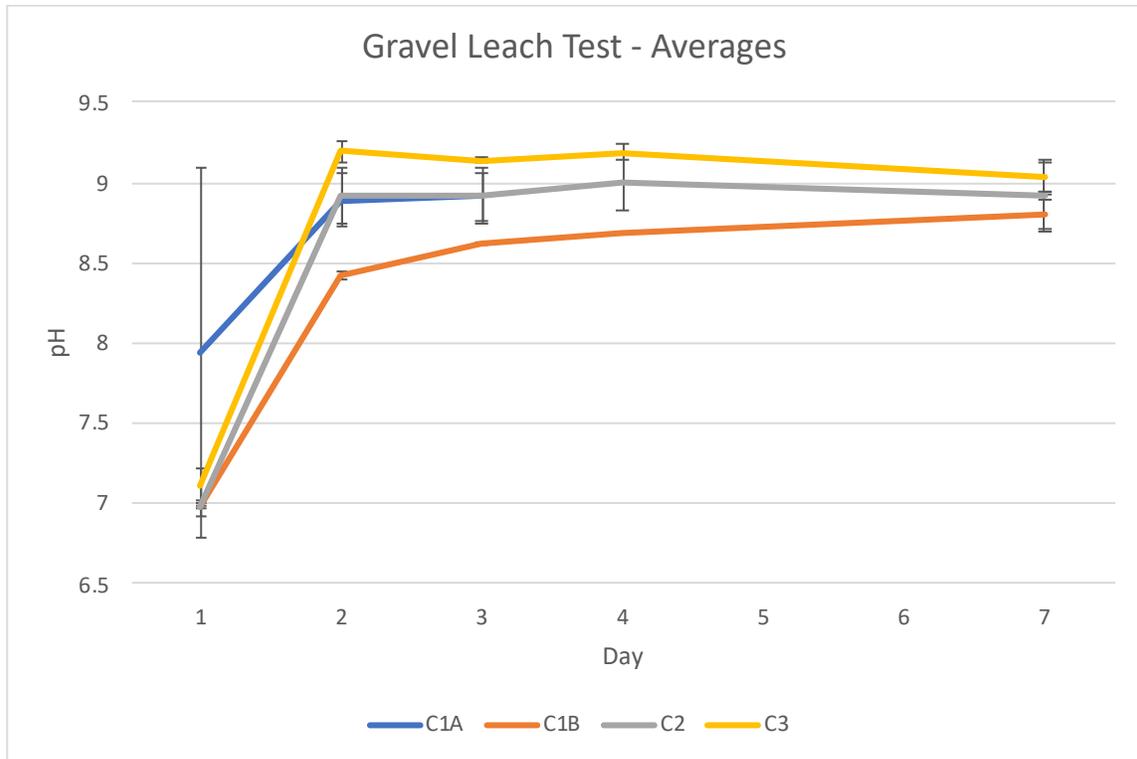


Figure 16. The results of a leaching test using gravel and DIW based on gravel proportions in the 3 columns. Error bars represent the standard deviation in pH between the duplicate samples tested.

5. Conclusion

While the columns were able to remove all contaminants of concern, they were not removed simultaneously. The effluent concentration of As was well below the maximum authorized monthly mean concentration as dictated by the Canadian Federal Metal and Diamond Mining Effluent Regulations (MDMER 2018). The highest concentration of effluent As was 0.0966 mg/L in C1, well below the maximum monthly mean concentration of 0.50 mg/L (MDMER 2018). Hydraulic retention time potentially influenced the rate of removal of As and nitrate and therefore must be considered when designing an *in-situ* bioreactor for mine remediation. The flow rate issues seen in C1 in weeks 25 to 32 suggest that the system may be clogged over time. More research is required to determine the longevity of the system as configured. Cyanide is a potentially

problematic by-product generated in the system and requires further investigation. However, highest cyanide concentration occurred in C1 with 0.149 mg/L, well below the maximum monthly mean concentration of 1.00 mg/L (MDMER 2018). Additional research is required to better understand U speciation in relation to adsorption and reduction mechanisms in bioreactors.

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7. Appendix A

Table 4. Density and mass calculations for column substrates at various proportions.

Density Measurements				
Substrate	Dried mass (g)	Volume (cm³)	Density (g/cm³)	
Woodchips	107.5	600	0.179166667	
Gravel	957.6	600	1.596	
ZVI	103.9	35	2.968571429	
Column	Height (cm)	Diameter (cm)	Volume (cm³)	
	29.7	10.1	2379.51645	
Substrate	Volume (%)	Volume (cm³)	Mass per Configuration (g)	Total Mass
20% ZVI (x3)	0.2	475.9032899	1412.752909	4238.258728
40% Woodchips (x2)	0.4	951.8065798	170.5320122	341.0640244
20% Woodchips	0.2	475.9032899	85.26600611	85.26600611
80% Gravel	0.8	1903.61316	3038.166603	3038.166603
60% Gravel (x2)	0.6	1427.70987	2278.624952	4557.249904
40% Gravel	0.4	951.8065798	1519.083301	1519.083301

8. Appendix B

Table 5. Influent and effluent flow rate calculations for the duration of the experiment.

INFLUENT									EFFLUENT								
AVERAGE	Target			20.9	10.5			12.7		Target			20.9	10.5			12.7
				mL/hr				mL/hr					mL/hr				mL/hr
Week	Week Start	Week End	C1 Target	C1	C2 Target	C2	C3 Target	C3	Week	Week Start	Week End	C1 Target	C1	C2 Target	C2	C3 Target	C3
0	01-Jul	08-Jul	20.9	N/A	10.5	N/A	12.7	N/A	1	01-Jul	08-Jul	20.9	21.7	10.5	9.3	12.7	11.3
1	09-Jul	14-Jul	20.9	23.6	10.5	11.4	12.7	13.2	2	09-Jul	14-Jul	20.9	27.7	10.5	12.2	12.7	14.9
2	15-Jul	21-Jul	20.9	20.7	10.5	9.7	12.7	11.6	3	15-Jul	21-Jul	20.9	20.1	10.5	11.1	12.7	13.0
3	22-Jul	30-Jul	20.9	24.5	10.5		12.7		4	22-Jul	30-Jul	20.9	22.6	10.5	10.8	12.7	12.9
4	31-Jul	05-Aug	20.9	19.3	10.5	11.7	12.7	13.5	5	31-Jul	05-Aug	20.9	22.9	10.5	8.3	12.7	10.2
5	06-Aug	11-Aug	20.9	22.6	10.5	9.9	12.7	11.7	6	06-Aug	11-Aug	20.9	19.0	10.5	9.8	12.7	11.7
6	12-Aug	18-Aug	20.9	18.9	10.5	8.2	12.7	10.0	7	12-Aug	18-Aug	20.9	29.8	10.5	8.2	12.7	10.1
7	19-Aug	25-Aug	20.9	22.3	10.5	13.9	12.7	15.6	8	19-Aug	25-Aug	20.9	25.1	10.5	13.2	12.7	15.1
8	26-Aug	02-Sep	20.9	18.1	10.5	8.1	12.7	9.9	9	26-Aug	02-Sep	20.9	18.6	10.5	8.1	12.7	10.0
9	03-Sep	08-Sep	20.9	18.9	10.5	8.7	12.7	10.3	10	03-Sep	08-Sep	20.9	9.9	10.5	10.0	12.7	12.0
10	09-Sep	15-Sep	20.9	18.9	10.5	8.2	12.7	10.0	11	09-Sep	15-Sep	20.9	18.9	10.5	7.2	12.7	8.9
11	16-Sep	22-Sep	20.9	19.1	10.5	8.9	12.7	10.5	12	16-Sep	22-Sep	20.9	16.0	10.5	7.0	12.7	10.0
12	23-Sep	30-Sep	20.9	18.7	10.5	8.2	12.7	10.0	13	23-Sep	30-Sep	20.9	21.3	10.5	9.7	12.7	10.3
13	01-Oct	07-Oct	20.9	16.7	10.5	8.8	12.7	10.3	14	01-Oct	07-Oct	20.9	22.0	10.5	9.2	12.7	10.6
14	08-Oct	13-Oct	20.9	19.7	10.5	9.5	12.7	11.6	15	08-Oct	13-Oct	20.9	26.7	10.5	9.0	12.7	10.8
15	14-Oct	20-Oct	20.9	13.6	10.5	8.4	12.7	11.2	16	14-Oct	20-Oct	20.9	8.4	10.5	9.3	12.7	11.0
16	22-Oct	27-Oct	20.9	24.3	10.5	9.1	12.7	10.5	17	22-Oct	27-Oct	20.9	24.3	10.5	9.1	12.7	10.2
17	28-Oct	02-Nov	20.9	22.4	10.5	9.1	12.7	8.6	18	28-Oct	02-Nov	20.9	21.7	10.5	11.2	12.7	8.1
18	03-Nov	08-Nov	20.9	21.8	10.5	8.8	12.7	1.7	19	03-Nov	08-Nov	20.9	20.6	10.5	8.4	12.7	6.1
19	09-Nov	14-Nov	20.9	19.9	10.5	10.2	12.7	12.9	20	09-Nov	14-Nov	20.9	18.9	10.5	9.1	12.7	10.3
20	18-Nov	23-Nov	20.9	13.8	10.5	6.6	12.7	8.0	21	18-Nov	23-Nov	20.9	19.0	10.5	10.5	12.7	13.7
21	26-Nov	30-Nov	20.9	29.2	10.5	18.6	12.7	21.8	22	26-Nov	30-Nov	20.9	20.5	10.5	10.3	12.7	13.4
22	01-Dec	09-Dec	20.9	19.7	10.5	11.5	12.7	11.7	23	01-Dec	09-Dec	20.9	12.6	10.5	10.9	12.7	9.6
23	10-Dec	14-Dec	20.9	19.7	10.5	8.8	12.7	12.2	24	10-Dec	14-Dec	20.9	15.8	10.5	0.0	12.7	14.6
24	15-Dec	21-Dec	20.9	8.0	10.5	8.8	12.7	11.5	25	15-Dec	21-Dec	20.9	6.6	10.5	3.3	12.7	13.5
25	22-Dec	29-Dec	20.9	9.6	10.5	14.9	12.7	18.0	26	22-Dec	29-Dec	20.9	3.0	10.5	14.8	12.7	12.8
26	30-Dec	07-Jan	20.9	8.3	10.5	10.0	12.7	8.1	27	30-Dec	07-Jan	20.9	4.1	10.5	9.7	12.7	7.9
27	08-Jan	14-Jan	20.9	17.3	10.5	12.2	12.7	15.6	28	08-Jan	14-Jan	20.9	8.5	10.5	11.6	12.7	15.0
28	15-Jan	20-Jan	20.9	9.6	10.5	12.0	12.7	15.5	29	15-Jan	20-Jan	20.9	8.5	10.5	12.9	12.7	14.9
29	21-Jan	28-Jan	20.9	20.1	10.5	12.9	12.7	13.6	30	21-Jan	28-Jan	20.9	14.3	10.5	11.1	12.7	13.7
30	29-Jan	04-Feb	20.9	22.2	10.5	10.5	12.7	13.4	31	29-Jan	04-Feb	20.9	31.7	10.5	10.6	12.7	13.4
31	05-Feb	11-Feb	20.9	12.5	10.5	10.3	12.7	12.2	32	05-Feb	11-Feb	20.9	12.9	10.5	10.2	12.7	12.4
32	12-Feb	18-Feb	20.9	10.5	10.5	10.4	12.7	12.6	33	12-Feb	18-Feb	20.9	11.6	10.5	10.3	12.7	12.5
33	19-Feb	24-Feb	20.9	38.6	10.5	10.7	12.7	12.5	34	19-Feb	24-Feb	20.9	50.8	10.5	10.4	12.7	12.2
34	25-Feb	04-Mar	20.9	29.0	10.5	12.4	12.7	14.8	35	25-Feb	04-Mar	20.9	21.9	10.5	11.4	12.7	12.7
35	05-Mar	11-Mar	20.9	25.4	10.5	11.8	12.7	13.5	36	05-Mar	11-Mar	20.9	19.8	10.5	11.1	12.7	13.3
36	12-Mar	19-Mar	20.9	20.5	10.5	11.3	12.7	12.9	37	12-Mar	19-Mar	20.9	20.6	10.5	10.3	12.7	12.2

9. Appendix C

Table 6. Sample results template from off-site lab analysis.

Report		C1AE	C1BE	C2E	C3E	C1AR1	C1AR2	C1AR3	C1BR1	C1BR2	C1BR3	C2R1	C2R2	C2R3	C3R1	C3R2	C3R3
ALS WO# WR2001246		Water															
		2020-11-19	2020-11-18	2020-11-18	2020-11-18	2020-11-19	2020-11-19	2020-11-19	2020-11-17	2020-11-18	2020-11-18	2020-11-17	2020-11-17	2020-11-17	2020-11-17	2020-11-17	2020-11-17
		10:00	09:30	14:00	14:00	11:30	14:00	16:00	12:40	12:40	14:00	14:15	09:00	13:30	15:00	09:00	13:30
Anions and Nutrients																	
ammonia, total dissolved (as N)	ammonia, total dissolved (as N)	0.0944	15.3	17.3	21.0												
bromide	bromide	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50	<2.50
chloride	CL-D	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0	<25.0
fluoride	F-D	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00
nitrate (as N)	NO3	255	288	312	303	328	304	263	280	290	310	386	371	317	292	340	349
nitrite (as N)	NO2	10.6	0.804	3.27	0.769	5.18	8.10	12.4	0.929	0.277	0.300	0.268	0.430	0.416	0.297	0.394	0.397
phosphorus, total dissolved	P-TD	0.0718	0.0052	0.0137	0.0286												
sulfate (as SO4)	SO4-D	2070	2230	2240	2180	2360	2260	2140	2110	2220	2220	2290	2300	2270	1970	2250	2460
sulfide, dissolved (as H2S)	sulfide, dissolved (as H2S)	<0.019	<0.019	<0.019	0.210	<0.019	<0.019	<0.019	<0.019	<0.019	0.233	<0.019	<0.019	<0.019	<0.019	<0.019	<0.019
sulfide, dissolved (as S)	sulfide, dissolved (as S)	<0.180	<0.180	<0.180	0.198	<0.180	<0.180	<0.180	<0.180	<0.180	0.219	<0.180	<0.180	<0.180	<0.180	<0.180	<0.180
Cyanides																	
cyanide, weak acid dissociable	CN-WAD	<0.0050	0.0731	0.0806	0.0793												
Dissolved Metals																	
aluminum, dissolved	AL-D	0.0100	0.0052	<0.0050	0.0054	0.0075	0.0070	0.0078	0.0163	0.0052	0.0051	<0.0050	0.0066	<0.0050	<0.0050	0.0075	<0.0050
antimony, dissolved	SB-D	0.506	0.0951	0.0226	0.0336	0.505	0.490	0.498	0.277	0.0950	0.102	0.220	0.0496	0.0108	0.360	0.0966	0.0397
arsenic, dissolved	AS-D	2.29	0.00628	0.00269	0.00262	2.19	2.21	2.24	0.304	0.0237	0.0316	0.0267	0.00371	0.00277	0.123	0.00423	0.00244
barium, dissolved	BA-D	0.0640	0.0236	0.0238	0.0234	0.0706	0.0662	0.0663	0.0327	0.0325	0.0306	0.0327	0.0294	0.0204	0.0430	0.0333	0.0254
beryllium, dissolved	BE-D	<0.000500	<0.000500	<0.000500	<0.000500	<0.000100	<0.000100	<0.000100	<0.000100	<0.000100	<0.000100	<0.000100	<0.000100	<0.000100	<0.000100	<0.000100	<0.000100
bismuth, dissolved	BI-D	<0.000250	<0.000250	<0.000250	<0.000250	<0.000250	<0.000250	<0.000250	<0.000250	<0.000250	<0.000250	<0.000250	<0.000250	<0.000250	<0.000250	<0.000250	<0.000250
boron, dissolved	B-D	<0.050	0.085	0.124	0.118	0.055	<0.050	<0.050	0.052	0.084	0.083	0.068	0.083	0.120	0.068	0.082	0.089
cadmium, dissolved	CD-D	<0.0000750	<0.0000900	<0.0000130	<0.0000125	<0.0000450	<0.0000800	<0.0000700	<0.0000500	<0.0000650	<0.0000800	<0.0000550	<0.0000650	<0.0000700	<0.0000350	<0.0000700	<0.0000800
calcium, dissolved	CA-D	253	181	177	175	258	252	259	187	175	179	236	196	193	245	211	199
cesium, dissolved	cesium, dissolved	<0.000050	0.000138	0.000154	0.000125	0.000075	0.000055	<0.000050	<0.000050	0.000102	0.000121	0.000056	0.000113	0.000147	0.000050	0.000086	0.000144
chromium, dissolved	CR-D	<0.00050	<0.00050	<0.00050	<0.00050	<0.00250	<0.00250	<0.00250	<0.00250	<0.00250	<0.00250	<0.00250	<0.00250	<0.00250	<0.00250	<0.00250	<0.00250
chromium, dissolved	CR-D	<0.00050	<0.00050	<0.00050	<0.00050	<0.00250	<0.00250	<0.00250	<0.00250	<0.00250	<0.00250	<0.00250	<0.00250	<0.00250	<0.00250	<0.00250	<0.00250
cobalt, dissolved	CO-D	0.0910	0.0280	0.0262	0.0250	0.0924	0.0908	0.0914	0.0478	0.0320	0.0277	0.0475	0.0295	0.0252	0.0590	0.0299	0.0252
copper, dissolved	CU-D	0.00420	0.00154	0.00140	<0.00100	0.0121	0.00610	0.00434	0.00241	<0.00100	<0.00100	0.00260	0.00356	<0.00100	0.00310	0.00363	0.00146
dissolved mercury filtration location	dissolved mercury filtration location	Field	Field	Field	Field												
dissolved metals filtration location	dissolved metals filtration location	Field															
iron, dissolved	FE-D	0.059	0.392	0.133	0.121	<0.050	0.055	0.073	2.92	3.34	4.47	7.39	1.78	0.316	8.63	1.97	0.306
lead, dissolved	PB-D	<0.000250	<0.000250	<0.000250	<0.000250	<0.000250	<0.000250	<0.000250	0.000293	<0.000250	<0.000250	0.000883	0.00109	<0.000250	0.000416	0.000820	<0.000250
lithium, dissolved	LI-D	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050	<0.0050
magnesium, dissolved	MG-D	130	136	133	143	132	130	131	115	142	145	135	142	153	140	146	162
manganese, dissolved	MN-D	0.0963	0.247	0.129	0.116	0.0415	0.0761	0.116	0.200	0.286	0.317	0.295	0.212	0.154	0.233	0.283	0.189
mercury, dissolved	HG-D	0.0000152	0.0000128	<0.0000050	<0.0000050												
molybdenum, dissolved	MO-D	0.0432	0.370	0.556	0.552	0.0457	0.0428	0.0424	0.0815	0.315	0.331	0.135	0.317	0.497	0.0881	0.234	0.352
nickel, dissolved	NI-D	0.00342	0.0108	0.00930	0.0280	0.00432	0.00311	0.00354	0.0175	0.0136	0.0157	0.0257	0.0128	0.00756	0.0332	0.0306	0.0272
phosphorus, dissolved	PHUS-D	<0.250	<0.250	<0.250	<0.250	<0.250	<0.250	<0.250	<0.250	<0.250	<0.250	<0.250	<0.250	<0.250	<0.250	<0.250	<0.250
potassium, dissolved	K-D	12.9	13.8	13.1	13.7	12.5	12.9	13.0	11.5	14.1	14.3	12.9	13.6	14.6	12.8	13.4	14.2
rubidium, dissolved	rubidium, dissolved	0.00541	0.00547	0.00561	0.00577	0.00573	0.00613	0.00484	0.00496	0.00598	0.00576	0.00631	0.00644	0.00610	0.00602	0.00594	0.00619
selenium, dissolved	SE-D	0.000850	0.000430	0.000351	0.000346	0.000587	0.000710	0.000858	0.000521	0.000712	0.000480	0.000612	0.000592	0.000543	0.000903	0.000811	0.000466
silicon, dissolved	SI-D	9.32	1.90	1.53	1.45	9.72	9.34	9.27	3.47	2.12	2.11	2.97	1.78	1.58	4.31	1.91	1.50
silver, dissolved	AG-D	<0.000050	<0.000050	<0.000050	<0.000050	<0.000050	<0.000050	<0.000050	0.000109	<0.000050	<0.000050	0.000206	0.000377	<0.000050	0.000230	0.000414	<0.000050
sodium, dissolved	NA-D	1700	1460	1470	1480	1780	1740	1720	1370	1580	1490	1800	1770	1670	1850	1710	1620
strontium, dissolved	SR-D	0.344	0.270	0.319	0.360	0.314	0.325	0.350	0.254	0.286	0.273	0.316	0.311	0.370	0.310	0.353	0.377
sulfur, dissolved	sulfur, dissolved	907	898	910	889	949	912	908	766	900	892	877	885	882	901	879	883
tellurium, dissolved	tellurium, dissolved	<0.00100	<0.00100	<0.00100	<0.00100	<0.00100	<0.00100	<0.00100	<0.00100	<0.00100	<0.00100	<0.00100	<0.00100	<0.00100	<0.00100	<0.00100	<0.00100
thallium, dissolved	TL-D	<0.000050	<0.000050	<0.000050	<0.000050	0.000129	0.000060	<0.000050	0.000629	<0.00166	0.000186	0.000737	0.00220	<0.000050	0.000747	0.00206	<0.000050
thorium, dissolved	thorium, dissolved	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050
tin, dissolved	SN-D	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050	<0.00050
titanium, dissolved	TI-D	<0.00150	<0.00150	<0.00150	<0.00150	<0.00150	<0.00150	<0.00150	<0.00150	<0.00150	<0.00150	<0.00150	<0.00150	<0.00150	<0.00150	<0.00150	<0.00150
tungsten, dissolved	W-D	0.00276	0.00086	0.00130	0.00205	0.00735	0.00384	0.00296	0.00179	0.00081	0.00084	0.000320	0.00125	0.00105	0.00518	0.00217	0.00164
uranium, dissolved	U-D	0.483	0.00258	0.000696	0.000281	0.433	0.429	0.440	0.118	0.00814	0.0						

10. Appendix D

Table 7. Summary of analytical methods used in off-site lab analysis.

Summary of Analytical Methods

Parameter Group	Standard Method	Method Description
Dissolved Metals	APHA 3030B/6020A (mod)	Analysis by CRC ICP-MS.
Dissolved Mercury	APHA 3030B/EPA 1631E (mod)	Cold-oxidation using bromine monochloride prior to reduction with stannous chloride, and analysis by CVAAS or CVAFS.
Anions (nitrate, nitrite, sulphate, chloride, bromide, fluoride)	EPA 300.1 (mod)	Ion chromatography with conductivity and/or UV detection.
Cyanide (WAD only)	APHA Method 4500-CN I	In-line sample distillation with final determination by colourimetric analysis.
Sulphide	APHA 4500 -S E-Auto-Colorimetry	A continuous flow manifold adds HCl to the sample which converts sulphide to a gas, then the sulphide is separated from the flow using a gas dialysis membrane. A Colorimetric reaction produces a methylene blue compound which is measured at 660 nm.
Dissolved Organic Carbon (DOC)	APHA 5310B TOTAL ORGANIC CARBON (TOC)	High temperature combustion to convert carbonaceous material to CO ₂ which is then analyzed by IR spectroscopy.
Ammonia (NH ₃)	J. ENVIRON. MONIT., 2005, 7, 37-42, RSC	Flow-injection analysis with fluorescence detection.
Total Dissolved Phosphorus (TD-P)	APHA 4500-P Phosphorous	Persulphate digestion followed by colourimetric analysis .

11. Appendix E

Carbon Calculations:

Initial Influent $[\text{NO}_3] = 340 \text{ mg/L}$

Moles of N = $24.28571429 \text{ mmol/L}$ (with $M(\text{N}) = 14 \text{ g/mol}$)

Target $[\text{C}] = 48.57142857 \text{ mmol/L}$ or 582.86 mg/L (with $M(\text{C}) = 12 \text{ g/mol}$)

2 moles of C per one mole $\text{C}_2\text{H}_9\text{NaO}_5$

$[\text{C}_2\text{H}_9\text{NaO}_5] = 24.28571429 \text{ mmol/L}$ or 3304.8 mg/L (with $M(\text{C}_2\text{H}_9\text{NaO}_5) = 136.08 \text{ g/mol}$)