MEMO

Date	**April 8, 2019**
To	**Nevada Environmental Response Trust**
From	**John Pekala, Scott Warner, and Chris Ritchie**
Copy to	**Nevada Division of Environmental Protection**
	United States Environmental Protection Agency
Subject	**In-Situ Bioelectrochemical Laboratory-Scale Treatability Study Monthly Progress Report**

TASK PROGRESS UPDATE: JANUARY 2019

At the direction of the Nevada Environmental Response Trust (NERT or Trust), Ramboll US Corporation (Ramboll) has prepared this memorandum which summarizes Ramboll's progress during January 2019 toward successfully implementing the In-Situ Bioelectrochemical Laboratory-Scale Treatability Study for the remediation of perchlorate in water.

TASK M24 – IN-SITU BIOELECTROCHEMICAL LABORATORY-SCALE TREATABILITY STUDY

- Task Leaders – Scott Warner / Chris Ritchie
- Current Status
  - Microcosm testing using samples of site soil were run for approximately two months to evaluate electron acceptor preferences (and removal order) with respect to the following electron donor conditions: 1) no electron donor to act as a control, 2) hydrogen only, 3) hydrogen and acetate, and 4) acetate only. The results are summarized in Figure 1. All three microcosms containing electron donor(s) showed removal of all contaminants including chlorate and perchlorate to below 0.5 mg/L (the current limit of method detection), but not until days 57 and 63, respectively. The microcosms containing acetate rapidly consumed (within 4 days) nitrate and nitrite, which was in contrast to the presumed autotrophic microcosms containing only hydrogen as an electron donor, which took until day 57 to reduce nitrate and nitrite. These results suggest that an extended lag phase may be required for autotrophic microorganism growth. These batch tests further suggest chlorate and perchlorate removal is achievable in the presence of electron donor—whether hydrogen only, hydrogen and acetate, or acetate only—after such a lag phase. Additional testing is planned (see below) to evaluate the cause of the observed lag and what impacts it may have with respect to potential field implementation. It is currently hypothesized that once growth of a critical mass of autotrophic perchlorate reducers is achieved that sustainable perchlorate reduction can be maintained.
  - A second round of batch microcosm testing will be conducted to further assess the effect of pH and presence of perchlorate-reducing bacteria on perchlorate removal and the associated lag in response. For these microcosms, both electrolyzed groundwater (pH

~11, simulating "direct" electrochemical treatment) and non-electrolyzed groundwater (pH~8.5, simulating "indirect" electrochemical treatment) will be used with a high perchlorate/low nitrate concentration and have excess electron donors (either acetate and hydrogen, or hydrogen only). For each treatment, both native soil and bioaugmented microcosms will be prepared. Additionally, a no electron donor control set of microcosms will be prepared. These additional batch tests will be used to better understand how conditions effect lag time and will inform column operation (e.g., the effect of competing electron acceptor concentrations and bioaugmentation) and future field testing (e.g., direct or indirect electrochemical treatment of groundwater).

- Microbial DNA was extracted from the microcosms to quantify the abundance of perchlorate-reducing bacteria in each microcosm. Prior to quantifying the perchlorate-reducing bacteria through an assay known as quantitative polymerase chain reaction (qPCR), a comparative standard curve must be generated. Development of an accurate standard curve required preparation of two pure cultures of bacteria known to reduce perchlorate (*Dechloromonas agitata* and *Dechloromonas aromatica*) using inoculations from freezer stocks. *D. agitata* is actively growing and reducing perchlorate and is being utilized as a positive control for qPCR screening and inoculum for upcoming bioaugmentation tests. *D. aromatica* is exhibiting extremely low growth kinetics and has, therefore, not yet been utilized for assays. Using the standard curve generated by the *D. agitata* the preliminary results indicate perchlorate-reducing bacteria were present in low abundance in the microcosms, but more accurate quantification is currently underway. As perchlorate-reducing bacteria produce the enzymes responsible for the biotic degradation of perchlorate, sustained and abundant concentrations of perchlorate-reducing bacteria are critical. Further, samples from the microcosms were preserved for perchlorate reduction gene expression analysis; RNA will soon be extracted for reverse transcription qPCR (RT-qPCR) to evaluate the activity of genes known to be involved in the perchlorate reduction pathway. This is a method that can demonstrate and quantify the level of active perchlorate reduction occurring in a system. Additional perchlorate-reducing bacteria quantification information will be provided as it becomes available.
- Three experimental flow through soil columns started on October 31, 2018 and remain in operation to assess perchlorate and competing electron acceptor (i.e. nitrate, nitrite, and chlorate) removal. The columns operate under the following electron donor conditions: 1) no electron donor to act as the control, 2) electrochemically-produced hydrogen and acetate to simulate a direct treatment and 3) hydrogen only to simulate an indirect treatment. Figure 2 shows the average influent and effluent concentrations of electron acceptors in each of the three columns. Substrate consumption in the two columns receiving electron donors has been established, as shown in Figure 3. Figure 4 provides a "snapshot" of column performance for the columns receiving electron donors. Overall, both the hydrogen and acetate and hydrogen-only columns indicate substrate consumption is relatively consistent with electron donor removal at variable levels. Microbial DNA was collected from the columns and will be screened for perchlorate-reducing bacteria abundance using qPCR. In addition, nitrate reducing bacteria and eubacteria (potential predatory microbes) will be quantified. After additional column sampling events to further establish nitrate and nitrite removal characteristics in the

original soil columns, the operation of the columns will be modified to assess heterotrophic/autotrophic perchlorate/chlorate removal in the presence of less competing electron acceptors. After column sampling has been completed and performance under these new operating conditions has been established, if perchlorate removal remains minimal, bioaugmentation of known perchlorate-reducing bacteria in the columns will be considered. More information on these continuing tests will be provided in subsequent progress reports.

- Schedule and Progress Updates
  - A revised schedule for the bench-scale testing has been developed given the positive results demonstrating that this technology can reduce perchlorate under simulated site conditions in microcosms albeit it with a significant lag period. This lag period is expected to effect the duration of subsequent testing. Considering the effect of lag time, bench-scale testing is now expected to continue into June 2019.
  - A bench-scale treatability study report is anticipated to be submitted in Q3 2019. A work plan addendum proposing a field test will be submitted as an attachment provided that, at the completion of laboratory testing, the data support moving forward with a field test. Updates will be forthcoming in subsequent progress reports.

- Health and Safety
  - There were no safety incidents during January 2019.

ATTACHMENTS

Figure 1: Electron Donor Preferences in Batch Microcosms

Figure 2: Average Influent and Effluent Concentrations in Soil Columns

Figure 3: Average Substrate Consumption in Soil Columns

Figure 4: Soil Column Performance

In-Situ Bioelectrochemical Laboratory-Scale Treatability Study Progress Update

Nevada Environmental Response Trust Site
(Former Tronox LLC Site)
Henderson, Nevada

Nevada Environmental Response Trust (NERT) Representative Certification

I certify that this document and all attachments submitted to the Division were prepared at the request of, or under the direction or supervision of NERT. Based on my own involvement and/or my inquiry of the person or persons who manage the systems(s) or those directly responsible for gathering the information or preparing the document, or the immediate supervisor of such person(s), the information submitted and provided herein is, to the best of my knowledge and belief, true, accurate, and complete in all material respects.

Office of the Nevada Environmental Response Trust

Le Petomane XXVII, not individually, but solely in its representative capacity as the Nevada Environmental Response Trust Trustee

Signature: Jay A Steinberg, not individually but solely as President, not individually, but solely in his representative capacity as President of the Nevada Environmental Response Trust Trustee

Name: Jay A. Steinberg, not individually, but solely in his representative capacity as President of the Nevada Environmental Response Trust Trustee

Title: Solely as President and not individually

Company: Le Petomane XXVII, Inc., not individually, but solely in its representative capacity as the Nevada Environmental Response Trust Trustee

Date: 4/9/15

In-Situ Bioelectrochemical Laboratory-Scale Treatability Study Progress Update

Nevada Environmental Response Trust Site
(Former Tronox LLC Site)
Henderson, Nevada

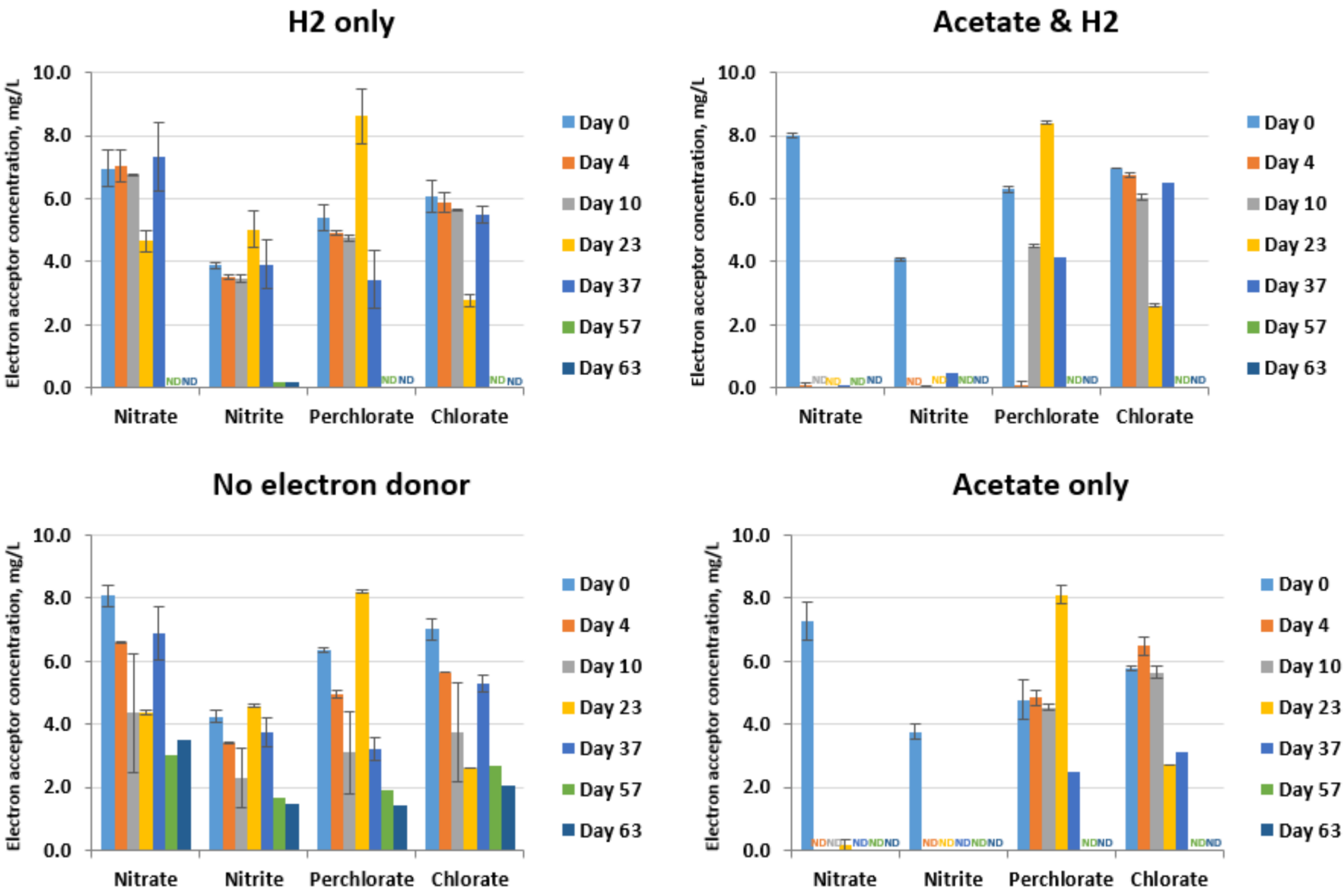
Responsible Certified Environmental Manager (CEM) for this project

I hereby certify that I am responsible for the services described in this document and for the preparation of this document. The services described in this document have been prepared in a manner consistent with the current standards of the profession, and to the best of my knowledge, comply with all applicable federal, state, and local statutes, regulations, and ordinances. I hereby certify that all laboratory analytical data was generated by a laboratory certified by the NDEP for each constituent and media presented herein.

Description of Services Provided: Prepared In-Situ Bioelectrochemical Laboratory-Scale Treatability Study Progress Update, Nevada Environmental Response Trust Site, Henderson, Nevada

April 8, 2019
Date

John M. Pekala, PG
Principal
Certified Environmental Manager
Ramboll US Corporation
CEM Certificate Number: 2347
CEM Expiration Date: September 20, 2020

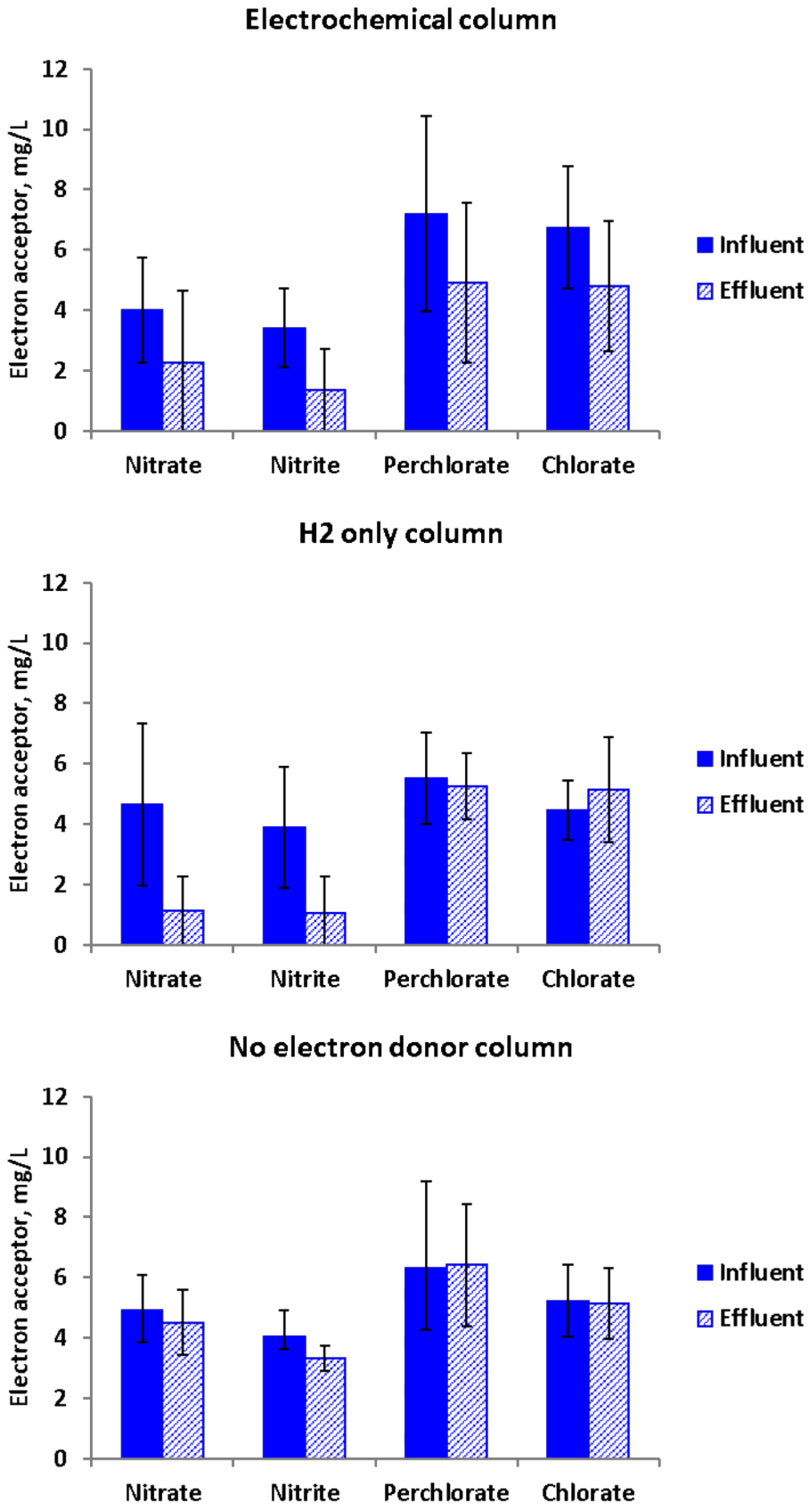


RAMBOLL

Electron Donor Preferences in Batch Microcosms
Nevada Environmental Response Trust
Henderson, Nevada

Figure
1

Drafter: JH	Date: 02/12/19	Contract Number: 1690011200	Approved:	Revised:



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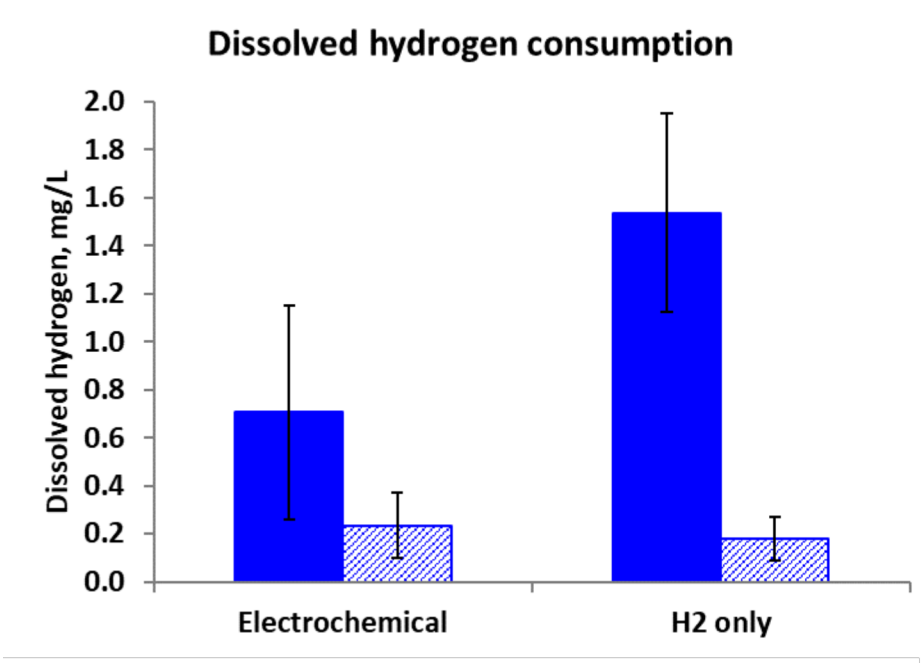
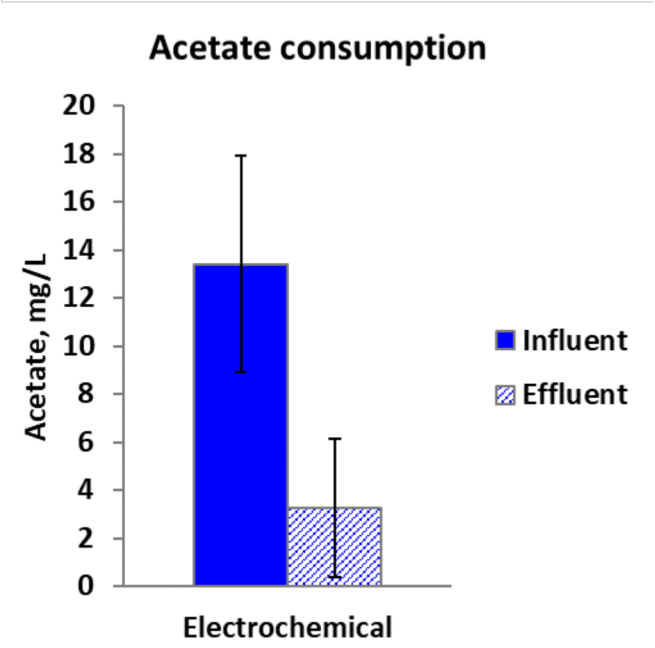
Average Influent and Effluent Concentrations in Soil Columns
Nevada Environmental Response Trust
Henderson, Nevada

Figure
2

Drafter: JH	Date: 02/12/19	Contract Number: 1690011200	Approved:	Revised:





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Average Substrate Consumption in Soil Columns
Nevada Environmental Response Trust
Henderson, Nevada

Figure
3

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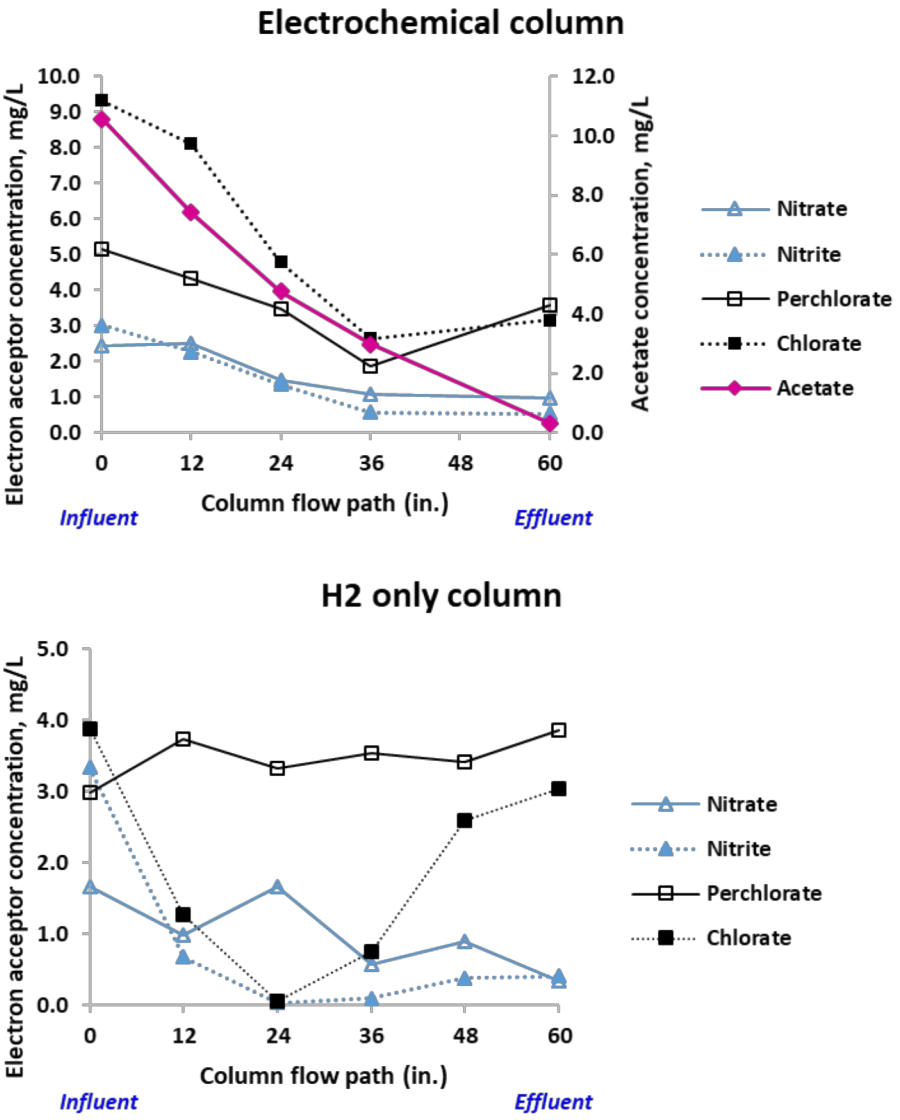

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Soil Column Performance
Nevada Environmental Response Trust
Henderson, Nevada

Figure
4

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