Summer 8-15-2023

Approved Butte Reduction Works (BRW) Smelter Area Mine Waste Remediation and Contaminated Groundwater Hydraulic Control Site Quality Assurance Project Plan (QAPP) for Microbial Analysis and Biotreatability; Request for Change BRW-2023-02

Josh Bryson

Atlantic Richfield Company

Josh Bryson
Liability Manager

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August 15, 2023

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RE: Approved Butte Reduction Works (BRW) Smelter Area Mine Waste Remediation and Contaminated Groundwater Hydraulic Control Site Quality Assurance Project Plan (QAPP) for Microbial Analysis and Biotreatability; Request for Change BRW-2023-02

Agency Representatives:

I am writing you on behalf of Atlantic Richfield Company to submit the approved Request for Change (RFC) BRW-2023-02 to the *Butte Reduction Works (BRW) Smelter Area Mine Waste Remediation and Contaminated Groundwater Hydraulic Control Site Quality Assurance Project Plan (QAPP) for Microbial Analysis and Biotreatability Study* (BRW Biotreatability Study QAPP). The RFC was approved by Agencies on August 2, 2023.

If you have any questions or comments, please call me at (406) 723-1834.

Sincerely,

Josh Bryson, PE, PMP
Liability Manager
Remediation Management Services Company
An affiliate of **Atlantic Richfield Company**

Cc: Chris Greco / Atlantic Richfield – email
Josh Bryson / Atlantic Richfield – email
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Robert Bylsma / UP – email
John Gilmour / Kelley Drye – email
Leo Berry / BNSF – email
Robert Lowry / BNSF – email
Brooke Kuhl / BNSF – email
Lauren Knickrehm / BNSF – email
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Josh Vincent / WET – email
Scott Bradshaw / W&C – email
Emily Stoick / W&C – email
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Scott Juskiewicz / Montana Tech – email

File: MiningSharePoint@bp.com - email
BPSOU SharePoint - upload

RESPONSE TO AGENCY COMMENTS
FOR THE
BUTTE PRIORITY SOILS OPERABLE UNIT (BPSOU) BUTTE REDUCTION WORKS (BRW) SMELTER AREA MINE WASTE REMEDIATION AND CONTAMINATED GROUNDWATER HYDRAULIC CONTROL SITE
QUALITY ASSURANCE PROJET PLAN FOR MICROBIAL ANALYSIS AND BIOTREATABILITY STUDY; REQUEST FOR CHANGE BRW-2023-02
DATED MAY 15, 2023

General Document Comments

EPA General Comment 1:

The study is generally well designed, except for the compositing of two test pits with different hydrocarbon mixes. For example, the PAH mix for BRW21-TP76 (8.5-9.0) is dominated by naphthalene, fluorene, and acenaphthalene, while BRW21-TP78 (0.0-0.5) is dominated by pyrene. By compositing these two test pit samples variability will be introduced into the treatability testing which may make it harder to determine if an apparent decrease in the concentration of a compound is due to biotransformation or variability. Despite the best efforts to composite samples, heterogeneity is inevitable, especially when the subsample samples are composed of different grain sizes. The heterogeneity within a single sample is bad enough, but when combined with compositing can be extreme. It will help that the testing will be done in triplicate, but better results would be achieved using single samples. Please consider using single samples or adding an upfront test to evaluate the effectiveness of the compositing. For example, an upfront test might consist of analyzing each test pit sample separately in triplicate as well as the final composited sample (which is already planned).

Atlantic Richfield Company Response:

The goal of this study is to help determine if landfarming is a feasible treatment option for hydrocarbon-impacted soils removed from the Butte Reduction Works Site as part of the Remedial Action (RA). As a result, this study was designed so that the samples sent to AECOM are representative of the soils that may potentially be treated in a landfarm. During the RA, hydrocarbon-impacted soils will be excavated with large construction equipment; stockpiled for sampling; and then loaded, hauled and redistributed at a landfarm (if treatment is necessary) or the designated repository. This process will result in the soil with differing hydrocarbon mixes being handled and mixed together multiple times.

Atlantic Richfield Company does not believe any additional testing is necessary to achieve the objective of this study because AECOM will be submitting a baseline sample as part of the study to determine the soil conditions post-compositing. However, an additional step has been added to the Request for Change where field screening will be done for the soil sample from each individual test pit as well as the composite sample submitted to AECOM.

Specific Document Comments

EPA Specific Comment 1:

2nd page, 1st paragraph following bullet list – SOP-S-06 Test Pit Sampling has different subparts depending on the type of sampling to be performed. The VOC test pit sampling does not specify mixing, but the inorganic procedures do. Please clarify which procedure will be used, and if the inorganic procedure is to be used, briefly discuss why potential loss of VOCs is not a concern or is preferable to using heterogeneous soils. Do the previous test results suggest that VOCs are not present (Table 1) because they have already biodegraded or that the VOCs were lost during sampling/mixing? Please discuss.

Atlantic Richfield Company Response:

Samples will be collected in accordance with the general procedures in Standard Operating Procedure (SOP) SOP-S-06 for inorganics. This has been clarified in the text.

Some volatiles may be lost during the test pit excavations and sample mixing; however, the loss of volatiles through mixing of the soil is acceptable to meet the primary objective of this study. The goal of this study is to help determine if landfarming is a feasible treatment option for hydrocarbon-impacted soils removed during waste excavation and not to document the *in-situ* conditions of the soil. Previous site investigations have characterized the extent and concentrations of soil impacted with hydrocarbon compounds within the site. Therefore, this work is focused on the treatability of the soil post-removal, and it is acceptable for some loss of volatiles during the sampling process to achieve this objective because volatiles will be lost during the excavation and handling of the hydrocarbon-impacted soils during the RA.

End of Comments.

ATLANTIC RICHFIELD COMPANY

RFC - REQUEST FOR CHANGE

DATE July 13, 2023	RFC NO. RFC-BRW-2023-02	CONTRACTOR Pioneer Technical Services, Inc.	RFP NO.
CONTRACT DESCRIPTION: Butte Reduction Works Smelter Area Site		ATTENTION OF: Josh Bryson Liability Manager	
SUBJECT: Installation of Additional Geotechnical Boreholes and Collection of Geotechnical Data ❍ ELECTRICAL ❍ MECHANICAL ❍ CIVIL ❍ STRUCTURAL/ARCHITECTURAL ❍ INSTRUMENTATION ⊗ ENVIRONMENTAL			
OPERABLE UNIT: Butte Priority Soils Operable Unit MAJOR WORK TASKS: Enhanced Biotreatability Study		REFERENCE DWG., P.O., TAG, SPECIFICATION NO. (FOR DEVIATIONS OR DEFICIENCIES) ETC.: Final Butte Reduction Works (BRW) Smelter Area Mine Waste Remediation and Contaminated Groundwater Hydraulic Control Site Quality Assurance Project Plan for Microbial Analysis and Biotreatability Study.	

PROBLEM DESCRIPTION:

This request for change (RFC) to the *Final Butte Reduction Works (BRW) Smelter Area Mine Waste Remediation and Contaminated Groundwater Hydraulic Control Site Quality Assurance Project Plan (QAPP) for Microbial Analysis and Biotreatability Study* (BRW Biotreatability Study QAPP) (Atlantic Richfield Company, 2021) provides the procedures and protocols necessary for Atlantic Richfield Company (Atlantic Richfield) to complete an enhanced biotreatability study as initially presented in Section 4.5.3.4 of the BRW Biotreatability Study QAPP.

An initial biotreatability study was completed by AECOM Technical Services, Inc. (AECOM) in 2022 per the protocols and procedures outlined in the BRW Biotreatability Study QAPP. Five test pits were excavated to collect soil samples (Figure 1). Field testing and screening, including photoionization detectors (PIDs), a Hanby Soil Test Kit, and an X-ray fluorescence (XRF) field unit, was used to determine the appropriate soil interval to be sent for laboratory analysis (Figure 1). Samples were then sent to the respective laboratories. The initial characterization results are shown in Table 1.

AECOM completed a soil slurry analyses for a sample from each test pit to help quantify microbial activity. Batch test reactors (soil slurry samples) were prepared using a 5:1 water to soil volumetric ratio to improve mixing and contact between the heavy metals, hydrocarbons, oxygen, and the native bacteria in the soil. The contents of the reactors were mixed using a magnetic stir bar and plate for 24 hours to establish baseline conditions, which were followed by sampling and analysis. The oxygen uptake rate (OUR) and total and dissolved adenosine triphosphate (ATP) measurements were performed to assess the microbial activity of the soil bacteria. Microbial analysis to characterize the bacteria present using their CENSUS-qPCR method was subcontracted by AECOM to Microbial Insights. The initial biotreatability study came to the following general conclusions:

- Parameters measured in the laboratory [i.e., ATP, OUR, and carbon dioxide (CO_2) production] provided the evidence needed to establish differences in biomass activity. Therefore, ATP, OUR, and/or CO_2 production can be employed as proxy measurements for biomass activity and health.
- Based on the “total eubacteria” count, all soils have a relatively high concentration of total bacteria. Therefore, metals are not inhibiting general biological growth within the soil at the BRW Site.
- Overall, the soils showed signs of healthy, broad microbial populations but the potential for aerobic biodegradation of organic pollutants appears limited due to the low counts of oxygenase enzymes. Results from functional genes quantification (quantitative polymerase chain reaction [qPCR] biomarkers) indicates a lack of detection of alkane monooxygenase (alkB) and monooxygenase (almA) enzymes in most of the soil analyzed. These oxygenases are enzymes involved in the degradation of hydrocarbons via aerobic respiration; they are a key factor in introducing oxygen atoms to the hydrocarbon structures, which opens the pathway for further biodegradation.

Additional details on the results and conclusions from the AECOM work are included in AECOM's Bench-Scale Treatability Study Report (Attachment A). This RFC focuses on the results from the samples submitted to AECOM, because AECOM completed the initial biotreatability study. Additional results from other work detailed in the BRW Biotreatability Study QAPP (i.e., results of the total oxidant demand test) will be provided in the revised BRW Pre-Design Investigation Evaluation Report to be submitted to Agencies prior to the BRW 60% Remedial Design Report.

Based on the results from initial microbial analyses, an enhanced biotreatability study is needed to further answer the main principal study question presented in the BRW Biotreatability Study QAPP: Is landfarming a feasible treatment option for the hydrocarbon compounds within the soil at the BRW Site? An enhanced biotreatability study, where different treatment scenarios are evaluated (e.g., addition of moisture, nutrients, etc.) over a longer period of time would be helpful to further evaluate biodegradation (i.e., landfarming) as a viable process for treating hydrocarbon-impacted soils removed from the BRW Site as part of the Remedial Action. To help determine if landfarming is a feasible treatment option, the proposed enhanced biotreatability study will collect data to specifically answer the following questions:

- Can a landfarm setting improve the occurrence of hydrocarbon-degrading bacteria (i.e., alkB and almA enzymes)?
- What impact does the addition of nutrients and a hydrocarbon degrading inoculum have on occurrence of hydrocarbon-degrading bacteria (i.e., alkB and almA enzymes)?
- What is the estimated biodegradation rate of total petroleum hydrocarbons (the sum of volatile petroleum hydrocarbons plus total extractable hydrocarbons) if BRW soil is treated in a landfarm?
- What impact does the addition of nutrients and a hydrocarbon degrading inoculum have on the biodegradation rate?

Atlantic Richfield proposes to collect soil from the general area and depths indicated on Figure 1, from test pits BRW21-TP76 and BRW21-TP78. These two locations were selected for additional study, because they generally had the highest concentrations of hydrocarbon-compounds based on data collected from the initial biotreatability study; it is anticipated that enough sample volume is available from these two locations for the proposed enhanced biotreatability study. While initial results from BRW21-TP77D showed high concentrations of some polycyclic aromatic hydrocarbons, it took multiple test pits during the initial biotreatability study field work for the field staff to locate this impacted soil. Therefore, it is assumed that the volume of this impacted soil is minimal. If additional sample volume is required, soil will be collected from BRW21-TP75. The final number and locations of test pits will be determined by the Field Team Leader and Contract Project Manager (CPM) in consultation with the Contractor Quality Assurance Officer (QAO). Considerations that will impact the decision on sampling locations include location of utilities, infrastructure and land use in the area due to ongoing Butte-Silver Bow operations, safety concerns, and equipment access.

Field team members will collect soil from BRW21-TP76 and BRW21-TP78. The soil will be collected from approximate locations and depths sampled during the initial biotreatability study, following these general procedures:

- The test pits will be excavated and logged in accordance with the procedures in Section 4.4.2.1 of the BRW Biotreatability QAPP.
- During excavation of the test pit, visual and olfactory observations (sight and smell), and two PIDs (9.8 eV and 10.6 eV lamps) will be used to identify sources of hydrocarbons following the procedures in Section 4.4.2.3 and Section 4.4.2.4 of the BRW Biotreatability QAPP.
- For each test pit, once the anticipated depth is reached the Field Team Leader will visually inspect the soil to determine if the anticipated lithological layer and soil type(s) are present (Figure 1).
- If the visual inspection confirms the anticipated lithological layer and soil type, samples from each test pit will be collected for field screening to verify the targeted soil conditions are present (i.e., conditions present during the initial biotreatability study). Field screening will be conducted following the general procedures below:
  - Use two PIDs, one with a 9.8 eV lamp and another with a 10.6 eV lamp, to screen for any petroleum compounds via the headspace method. The procedures for using the PID units are detailed in the BRW Biotreatability QAPP.
  - Use a Hanby Soil Test Kit (or similar test kit as determined by field personnel) to screen for hydrocarbon compounds. The general procedures for using the field test kit are detailed in the BRW Biotreatability QAPP.
  - Use field XRF analysis to screen the soil for metals contaminants of concern concentrations. Use a Niton™ XL3 XRF Analyzer (XL3), or equivalent, and follow the procedures outlined in the BRW Biotreatability QAPP.
- Once field screening has been completed and the results confirm the anticipated soil conditions (i.e., soil type and hydrocarbon-compound concentrations) are present, a sample will be collected from each test pit (BRW21-TP76 and BRW21-TP78).
- If the anticipated soil conditions for a planned test pit location (i.e., BRW21-TP76 and BRW21-TP78) are not present, the Field Team Leader and CPM in consultation with the Contractor QAO will determine the appropriate action, which may include excavating another test pit within the same Enhanced Biotreatability Sample Area (Figure 1) and/or collecting additional soil from near BRW21-TP75.

The samples from each test pit will be thoroughly mixed together as one sample that will be submitted to AECOM. After the sample is thoroughly mixed, field screening will be conducted following the general procedures above to document the soil conditions of the composite sample. The sample will then be placed in a 1-gallon resealable plastic bag and packed in a cooler with ice to preserve organic compounds at a temperature less than 6 °C and shipped to AECOM.

Samples will be collected in accordance with the general procedures in Standard Operating Procedure (SOP) SOP-S-06 for inorganics (Appendix A of the BRW Biotreatability QAPP). The field team will record the information on the Test Pit Log form provided in Appendix B of the BRW Biotreatability QAPP. The field team will also record the resource-grade Global Positioning System coordinates of all test pits. Additionally, all relevant and applicable SOPs in Appendix A of the BRW Biotreatability Study QAPP will be followed.

The soil sample will be sent to AECOM to complete the enhanced biotreatability study. The general steps, provided by AECOM, for the soil slurry analyses are detailed below:

- After receipt of the composite soil sample collected and further homogenization in the laboratory, AECOM will submit untreated sample aliquots to Pace Analytical Services, Inc., for initial characterization. In addition to baseline chemical analyses, a microbial characterization of this baseline sample will be conducted by Microbial Insights. Microbial characterization will consist of total bacteria and functional genes quantification via CENSUS-qPCR. Additionally, AECOM will conduct in-house analyses for soil baseline characterization. Proposed analyses and sample schedule are shown on Table 2 and Table 3, respectively.
- Upon receipt of baseline analytical results, AECOM will prepare test trays in the laboratory with the composite soil sample that will contain a representative soil concentration of hydrocarbons. These trays will contain approximately 1 to 1.5 inches of soil thickness, whereas the length and width will be adjusted to ensure available soil volume for the proposed characterization.
- Four test scenarios will be set up: 1) as-received soil with no moisture added; 2) moisture added to soils with a target moisture content in the range of 60% to 70%; 3) biostimulated soils with moisture and nutrients added; and 4) bioaugmented soils with moisture, nutrients, and an inoculum of hydrocarbon-degrading bacteria added. Three trays (triplicates) will be set up for each scenario.
- AECOM will periodically collect samples from these batch experiments to conduct in-house analyses and for further subcontracted analyses. It is anticipated that the samples will be collected every 3 weeks for 3 months from start of study. The anticipated sampling schedule along with analytical tests is shown on Table 3.

It is anticipated that the enhanced biotreatabiltiy study will take 3 months. The sampling and/or analyses may be altered by the CPM, in consultation with the Contractor QAO. Agencies will be notified of any significant changes to the proposed approach.

All additional quality control details for this project, including documentation; quality assurance/quality control samples, instrument/equipment testing, inspection, maintenance, and calibration; data management procedures; assessment and oversight; and data validation and usability are included in the BRW Biotreatability Study QAPP. Additionally, the health and safety concerns and project responsibilities are included in the BRW Biotreatability Study QAPP. An updated project organization chart is shown on Figure 2.

References:

Atlantic Richfield Company, 2021. Silver Bow Creek/Butte Area NPL Site Butte Priority Soils Operable Unit Draft Final Butte Reduction Works (BRW) Smelter Area Mine Waste Remediation and Contaminated Groundwater Hydraulic Control Site Quality Assurance Project Plan (QAPP) for Microbial Analysis and Biotreatability Study. Prepared by Pioneer Technical Services, Inc. Revision 0. November 2021.

Figures:
Figure 1. Site Investigation Locations for the Biotreatability Study
Figure 2. Project Organization Chart

Tables:
Table 1: Preliminary Biotreatability Analytical Results
Table 2: Sample Collection, Preservation, and Holding Times
Table 3. Proposed Sample Schedule

Attachments:
Attachment A: Bench-Scale Treatability Study Report, Evaluation of Bioactivity of Indigenous Bacteria in Soils Impacted with Hydrocarbons and Heavy Metals, AECOM Technical Services.

	❍Design Deficiency ❍ Engineering Change Request ❍ Agency Directive ❍ Construction Deficiency ❍ Schedule	❍ Material Substitution ❍ Vendor Material Deficiency ⊗ Scope ⊗ Additional Data Collection ❍ Clarification/Information ❍ Other	

RESPONSE/DIRECTIVE

1. Approve the installation of additional geotechnical boreholes and collection of additional geotechnical data.

Project Manager Karen Helfrich Date 07/13/2023.

Atlantic Richfield Co. Representative [signature] Date 07/13/2023.

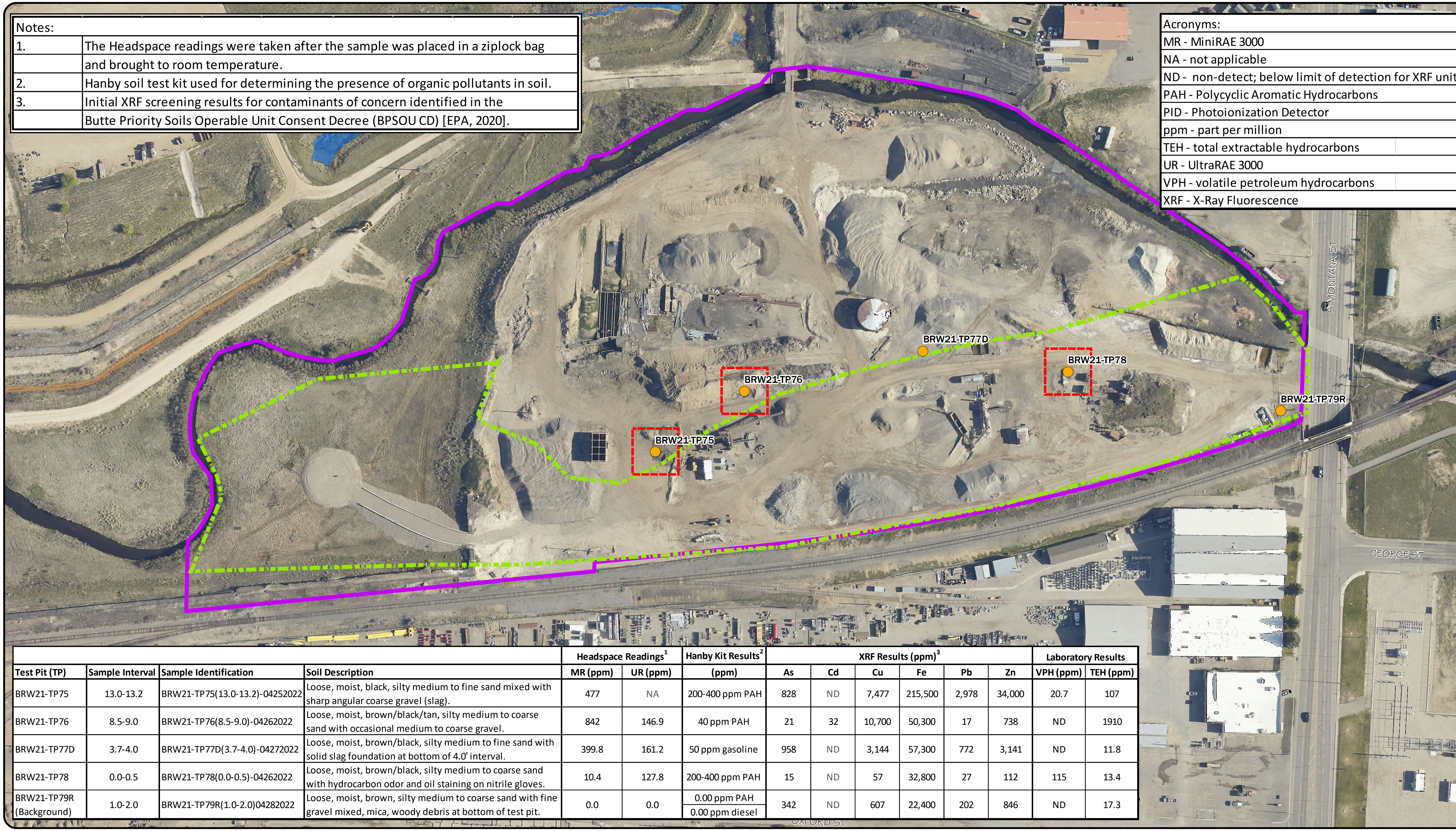
EPA Representative NIKIA GREENE Digitally signed by NIKIA GREENE Date: 2023.08.02 10:07:45 -06'00' Date ______.

DEQ Representative [signature] Date 8/2/2023.

CC: See Cover Letter

Notes:	
1.	The Headspace readings were taken after the sample was placed in a ziplock bag and brought to room temperature.
2.	Hanby soil test kit used for determining the presence of organic pollutants in soil.
3.	Initial XRF screening results for contaminants of concern identified in the Butte Priority Soils Operable Unit Consent Decree (BPSOU CD) [EPA, 2020].

Acronyms:
MR - MiniRAE 3000
NA - not applicable
ND - non-detect; below limit of detection for XRF unit.
PAH - Polycyclic Aromatic Hydrocarbons
PID - Photoionization Detector
ppm - part per million
TEH - total extractable hydrocarbons
UR - UltraRAE 3000
VPH - volatile petroleum hydrocarbons
XRF - X-Ray Fluorescence



				Headspace Readings[1]		Hanby Kit Results[2]	XRF Results (ppm)[3]						Laboratory Results	
Test Pit (TP)	Sample Interval	Sample Identification	Soil Description	MR (ppm)	UR (ppm)	(ppm)	As	Cd	Cu	Fe	Pb	Zn	VPH (ppm)	TEH (ppm)
BRW21-TP75	13.0-13.2	BRW21-TP75(13.0-13.2)-04252022	Loose, moist, black, silty medium to fine sand mixed with sharp angular coarse gravel (slag).	477	NA	200-400 ppm PAH	828	ND	7,477	215,500	2,978	34,000	20.7	107
BRW21-TP76	8.5-9.0	BRW21-TP76(8.5-9.0)-04262022	Loose, moist, brown/black/tan, silty medium to coarse sand with occasional medium to coarse gravel.	842	146.9	40 ppm PAH	21	32	10,700	50,300	17	738	ND	1910
BRW21-TP77D	3.7-4.0	BRW21-TP77D(3.7-4.0)-04272022	Loose, moist, brown/black, silty medium to fine sand with solid slag foundation at bottom of 4.0' interval.	399.8	161.2	50 ppm gasoline	958	ND	3,144	57,300	772	3,141	ND	11.8
BRW21-TP78	0.0-0.5	BRW21-TP78(0.0-0.5)-04262022	Loose, moist, brown/black, silty medium to coarse sand with hydrocarbon odor and oil staining on nitrile gloves.	10.4	127.8	200-400 ppm PAH	15	ND	57	32,800	27	112	115	13.4
BRW21-TP79R (Background)	1.0-2.0	BRW21-TP79R(1.0-2.0)04282022	Loose, moist, brown, silty medium to coarse sand with fine gravel mixed, mica, woody debris at bottom of test pit.	0.0	0.0	0.00 ppm PAH 0.00 ppm diesel	342	ND	607	22,400	202	846	ND	17.3

LEGEND
- Test Pit Locations
- Waste Removal Corridor
- BRW Site Investigation Boundary
- Enhanced Biotreatability Sample Areas (Proposed)

N

DISPLAYED AS:
PROJECTION/ZONE: MSP
DATUM: NAD 83
UNITS: INTN'L FT
SOURCE: PIONEER/QUANTUM 2020

0 75 150 300
Feet

FIGURE 1

PIONEER TECHNICAL SERVICES, INC.

SITE INVESTIGATION LOCATIONS FOR THE BIOTREATABILITY STUDY

DATE: 5/12/2023

Path: Z:\Shared\Active Projects\ARCO\BPSOU\BRW\GIS\Z_EnhancedBiotreatability_QAPP\BRW_EB_QAPP_001_LocsBio_23.mxd

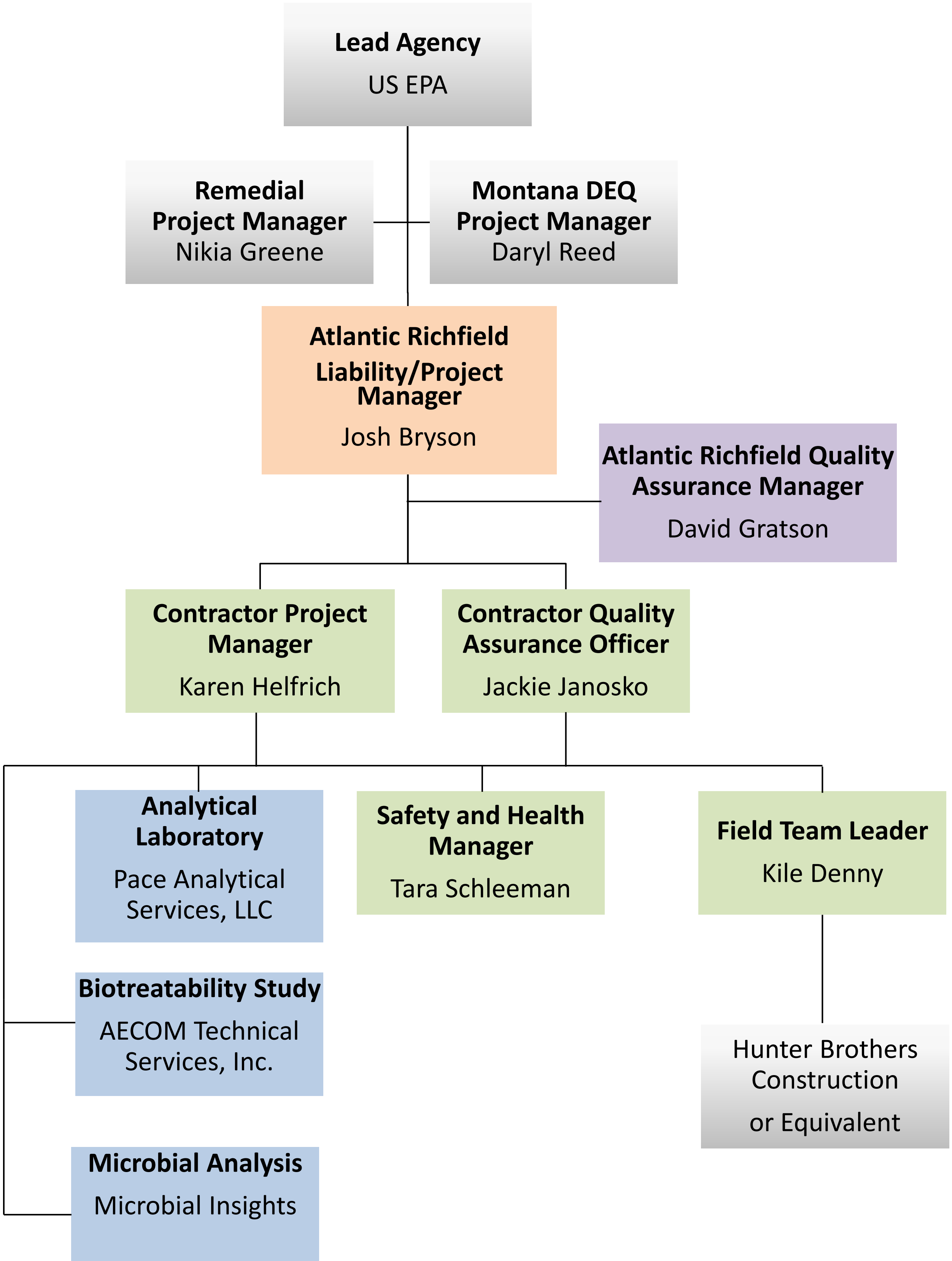


Figure 2

PIONEER TECHNICAL SERVICES, INC.

Project Organization Chart

Table 1: Preliminary Biotreatability Analytical Results

		Sample Identification				
Analyte	**Units**	**BRW21-TP75 (13.0 - 13.2)**	**BRW21-TP76 (8.5 - 9.0)**	**BRW21-TP77D (3.7 - 4.0)**	**BRW21-TP78 (0.0 - 0.5)**	**BRW21-TP79R (1.0 - 2.0)*** **
pH	standard units	8.95	7.49	7.32	7.4	7.51
Specific Conductance	uS/cm	555	279	915	279	917
Total Solids	Percent (%) Weight	98.8	94.1	91.7	93.9	91.9
Total Organic Carbon	mg/kg	3,400	3,620	4,740	49,700	4,620
Alkalinity as CaCO3*	mg/kg	103	< 91.5	197	97.3	104
Nitrogen**	mg/kg	169	53.9	160	347	129
Nitrate	mg/kg	1.17	0.959	2.08	0.984	0.696
Nitrogen, TKN	mg/kg	173	< 57.4	238	366	138
Sulfate	mg/kg	101	104	389	< 13.7	661
Sulfur (S)	mg/kg	5,490	251	1,870	568	390
Arsenic (As)	mg/kg	554	16.3	1,360	14	238
Cadmium (Cd)	mg/kg	3.7	33.5	9.7	0.35	1.5
Copper (Cu)	mg/kg	4,180	1,490	2,390	47	322
Iron (Fe)	mg/kg	51,400	13,500	32,000	15,300	13,900
Lead (Pb)	mg/kg	1,370	5.8	1,490	32.1	215
Manganese (Mn)	mg/kg	22,800	96.5	20,600	712	1,740
Zinc (Zn)	mg/kg	16,500	602	4,510	131	422
Mercury (Hg)	mg/kg	0.13	0.06	13.9	0.053	0.53

Comparison of Solids to SPLP Concentrations for Hydrocarbons	**Solids** mg/kg	**SPLP** ug/L	**Solids** mg/kg	**SPLP** ug/L	**Solids** mg/kg	**SPLP** ug/L	**Solids** mg/kg	**SPLP** ug/L	**Solids** mg/kg	**SPLP** ug/L
Volatile Petroleum Hydrocarbons (VPH)										
Benzene	< 0.0855	< 1.67	< 0.0941	< 1.67	< 0.0986	< 1.67	< 0.472	< 8.35	< 0.0983	NA
Ethylbenzene	< 0.0855	< 1.67	< 0.0941	< 1.67	< 0.0986	< 1.67	< 0.472	< 8.35	< 0.0983	NA
m-&p-Xylenes	< 0.171	5.89	< 0.188	< 3.33	< 0.197	< 3.33	< 0.941	< 16.7	< 0.197	NA
Methyl-tert-butyl ether	< 0.0855	< 1.66	< 0.0941	< 1.66	< 0.0986	< 1.66	< 0.472	< 8.3	< 0.0983	NA
o-Xylene	< 0.0855	< 1.67	< 0.0941	< 1.67	< 0.0986	< 1.67	< 0.472	< 8.35	< 0.0983	NA
Toluene	< 0.0855	2.98	< 0.0941	< 1.67	< 0.0986	< 1.67	< 0.472	< 8.35	< 0.0983	NA
Xylenes, Total	< 0.255	5.89	< 0.281	< 1.67	< 0.294	< 1.67	< 1.4	< 8.35	< 0.293	NA
Aliphatic (C5-C8)	< 1.71	< 33.3	< 1.88	< 33.3	< 1.97	< 33.3	< 9.41	< 167	< 1.97	NA
Aliphatic (C9-C12)	15.6	96.1	< 1.88	53.3	2.31	37.5	71.2	315	2.2	NA
Aromatic (C9-C10)	5.1	< 33.3	1.96	< 33.3	< 1.97	< 33.3	44.1	< 167	< 1.97	NA
Total Purgeable Hydrocarbons	30.4	105	7.62	< 66.7	5.54	< 66.7	350	< 333	4.97	NA
Total VPH	20.7	102	< 3.76	< 66.7	< 3.94	< 66.7	115	< 333	< 3.93	NA
Extractable Petroleum Hydrocarbons (EPH)										
Aliphatic (C19-C36)	7.23	< 200	6.39	< 200	5.7	< 210	5.87	1080	10.6	NA
Aliphatic (C9-C18)	85.9	< 200	1630	< 200	< 1.83	< 210	< 1.79	1060	< 1.83	NA
Aromatic (C11-C22)	14.2	< 200	277	< 200	< 7.27	< 210	7.59	< 200	< 7.26	NA
EPH Screen	173	122	2450	< 100	27.1	< 105	34.6	7080	32.9	NA
Total Extractable Hydrocarbons	107	< 200	1910	< 200	11.8	< 210	13.4	2900	17.3	NA
Total Petroleum Hydrocarbons	107	< 200	1910	< 200	< 7.27	< 210	13.4	2140	10.6	NA
Polycyclic Aromatic Hydrocarbons (PAH)										
Acenaphthene	0.0073	0.021	0.175	0.016	< 0.0022	0.013	< 0.0042	< 0.084	< 0.0022	NA
Acenaphthylene	0.0112	< 0.0062	< 0.0012	< 0.0064	0.0143	< 0.0072	< 0.0023	< 0.074	0.011	NA
Anthracene	0.0086	0.0087	0.0135	0.0063	0.0267	< 0.0064	< 0.0019	< 0.065	0.0115	NA
Benz(a)anthracene	0.0173	0.015	0.0049	0.011	0.0874	0.011	0.0314	< 0.11	0.0265	NA
Benz(a)pyrene	0.0472	< 0.009	0.0039	< 0.0093	0.0847	< 0.011	0.0531	< 0.11	0.0247	NA
Benz(b)fluoranthene	0.0446	< 0.0097	0.0036	< 0.01	0.154	< 0.011	0.11	< 0.12	0.0426	NA
Benz(g,h,I,)perylene	0.114	< 0.0083	<0. 002	< 0.0085	0.0824	< 0.0097	0.181	< 0.099	0.036	NA
Benz(k)fluoranthene	0.0142	< 0.0084	< 0.0011	< 0.0087	0.0547	< 0.0099	0.0269	< 0.1	0.016	NA
Chrysene	0.0404	0.015	0.0039	0.011	0.143	< 0.011	0.159	< 0.11	0.0356	NA
Dibenz(a,h)anthracene	<0.0012	< 0.0081	< 0.0013	< 0.0084	0.0212	< 0.0095	< 0.0025	< 0.097	< 0.0013	NA
Fluoranthene	0.0269	0.015	0.0084	< 0.015	0.21	< 0.017	0.368	0.78	0.052	NA
Fluorene	0.007	0.012	0.231	0.017	0.0052	< 0.0072	< 0.0024	< 0.074	0.0044	NA
Indeno(1,2,3-cd)pyrene	0.0405	< 0.011	< 0.00086	< 0.012	0.0853	< 0.013	0.0769	< 0.14	0.0284	NA
Naphthalene	0.007	< 0.009	0.119	0.016	0.0265	0.012	0.094	< 0.11	0.0657	NA
Phenanthrene	0.0227	0.026	0.0523	0.02	0.14	< 0.015	< 0.0017	< 0.15	0.0419	NA
Pyrene	0.0571	0.014	0.0097	0.011	0.173	< 0.012	1.83	2.4	0.048	NA

*CaCO3-Carbonate Alkalinity.
**Nitrogen - Total Nitrogen Calculation.
****BRW21-TP79R (1.0 - 2.0)* is the background sample. SPLP analysis was not requested for this reason.
<X = Value less than detection limit (value in cell (X) is the method detection limit).

mg/kg - milligram per kilogram
uS/cm - microsiemens per centimeter
TKN - Total Kjeldahl Nitrogen (total concentration of organic nitrogen and ammonia)
NA - Not applicable. Analysis was not requested.
SPLP-Synthetic Precipitation Leaching Procedure

Table 2. Sample Collection, Preservation, and Holding Times

Analytical Lab/Company[1]	Analyte	Analytical Method	Lab Reporting Limit *(CRQL for Data Validation Purposes)*	Lab Method Detection Limit[2]	Holding Time	Container Size	Preservation[3]	Justification
Groundwater Field Parameters (if encountered and feasible to sample)								
Pioneer	Temperature (°C)	NA	NA	NA	NA	NA	NA	Determine general parameters of groundwater encountered during test pit excavation, if feasible.
	pH							
	Specific Conductance (SC)							
	Oxidation Reduction Potential (ORP)							
	Dissolved Oxygen (DO)							
Soil Field Readings								
Pioneer XRF	Arsenic (As)	NA	NA	NA	NA	NA	NA	Field screening to confirm preferred soil conditions are present.
	Cadmium (Cd)							
	Copper (Cu)							
	Lead (Pb)							
	Zinc (Zn)							
	Mercury (Hg)							
Pioneer PIDs MiniRAE (PID MR) - 10.6 eV lamp UltraRAE (PID UR) - 9.8 eV lamp	Volatile Organic Compounds BTEX (Benzene, Toluene, Ethylbenzene, and Xylenes)	NA	NA	NA	NA	NA	NA	
Hanby Field Soil Kit	BTEX (Benzene, Toluene, Ethylbenzene, and Xylenes) Semi-volatile Organic Compounds Polycyclic Aromatic Hydrocarbons	NA	NA	NA	NA	NA	NA	
Enhanced Biotreatability Study - Lab Samples/Readings								
Pace Analytical Services, LLC	Total Organic Carbon	Walkley Black	100 mg/kg	25.5 mg/kg	28 Days	2-4 oz. amber glass jars	None	Monitor progress of biotreatability study. Samples will be sent from AECOM to Pace during biotreatability study. It is anticipated that the samples will be collected at 2-weeks, 1-month, 2-months, and 3-months from start of study.
	TKN, Nitrogen	EPA 351.2	20 mg/kg	4.48 mg/kg	28 Days			
	Total Phosphorus	EPA 6010	100 mg/kg	1.86 mg/kg	180 Days			
	Ammonia	EPA 350.1	10 mg/kg	7 mg/kg	28 Days			
	Fertilizer Recommendation Analysis				48 Hours After Extraction	Gallon Zip Lock Bag (100 g of soil)	None	
	Nitrogen-Nitrate	ASA 33-3.2	0.1 mg/kg	0.056 mg/kg				
	Potassium	ASA 13.3.5.2	0.05 mg/kg	0.01 mg/kg				
	Phosphorus	ASA 33-3.2	2 mg/kg	0.88 mg/kg				
	Volatile Petroleum Hydrocarbons (VPH)	MTVPH			7 Days	4-oz. amber glass container	None	
	C5 to C8 Aliphatics (unadjusted & adjusted)		5 mg/kg	1.665 mg/kg				
	C9 to C12 Aliphatics (unadjusted & adjusted)		5 mg/kg	1.665 mg/kg				
	C9 to C10 Aromatics		5 mg/kg	1.665 mg/kg				
	Volatile Petroleum Hydrocarbons		10 mg/kg	3.335 mg/kg				
	Benzene		0.001 mg/kg	0.00046 mg/kg				
	Toluene		0.005 mg/kg	0.0013 mg/kg				
	Ethylbenzene		0.0025 mg/kg	0.00074 mg/kg				
	Total Xylenes		0.0065 mg/kg	0.00088 mg/kg				
	Methyl-tert-butyl ether (MTBE)		0.001 mg/kg	0.00047 mg/kg				
	Extractable Petroleum Hydrocarbons (EPH) Fractionation	MTEPH			14 Days	4-oz. amber glass container	None	
	C9 to C18 Aliphatics		20 mg/kg	3.3 mg/kg				
	C19 to C36 Aliphatics		20 mg/kg	3.3 mg/kg				
	C11 to C22 Aromatics (unadjusted & adjusted)		20 mg/kg	3.3 mg/kg				
	Total Extractable Hydrocarbons		10 mg/kg	NA[5]				
	Total Petroleum Hydrocarbons		50 mg/kg	NA[5]				
	Polycyclic Aromatic Hydrocarbons (PAHs)	EPA 8270SIM						
	1-Methylnaphthalene		0.02 mg/kg	0.00449 mg/kg				
	2-Methylnaphthalene		0.02 mg/kg	0.00427 mg/kg				
	Acenaphthene		0.006 mg/kg	0.00209 mg/kg				
	Acenaphthylene		0.006 mg/kg	0.00216 mg/kg				
	Anthracene		0.006 mg/kg	0.0023 mg/kg				
	Benzo(a)anthracene		0.006 mg/kg	0.00173 mg/kg				
	Benzo(a)pyrene		0.006 mg/kg	0.00179 mg/kg				
	Benzo(b)fluoranthene		0.006 mg/kg	0.00153 mg/kg				
	Benzo(g,h,i)perylene		0.006 mg/kg	0.00177 mg/kg				
	Benzo(k)fluoranthene		0.006 mg/kg	0.00215 mg/kg				
	Chrysene		0.006 mg/kg	0.00232 mg/kg				
	Dibenzo(a,h)anthracene		0.006 mg/kg	0.00172 mg/kg				
	Fluoranthene		0.006 mg/kg	0.00227 mg/kg				
	Fluorene		0.006 mg/kg	0.00205 mg/kg				
	Indeno(1,2,3-cd)pyrene		0.006 mg/kg	0.00181 mg/kg				
	Naphthalene		0.02 mg/kg	0.00408 mg/kg				
	Phenanthrene		0.006 mg/kg	0.00231 mg/kg				
	Pyrene		0.006 mg/kg	0.002 mg/kg				
AECOM[4]	Adenosine Triphosphate (ATP)	LuminUltra reactant kit and a luminometer.	5 pg ATP/mL	3 pg ATP/mL	7 days	15 mL	Proprietary preservative.	Monitor progress of biotreatability study.ATP counts indicate the biomass health and can be used to make relative comparisons among the different scenarios.
	pH	HACH Benchtop meter HQ 430d	NA	NA	As soon as possible.	NA	None	pH measurement range is from 0 to 14 S.U. and the accuracy is +/- 0.05 S.U.
	Percent Moisture	ASTM D2216	0.005%	0.005%	As soon as possible.	NA	None	
Microbial Insights	Microbial Analysis (Microbial Insights)	CENSUS-qPCR Method	500 to 5,000 cells/sample	100 cells/sample	1-2 days	10 grams of soil	Temperature ≤ 4°C	The concentrations of specific microorganisms and functional genes provides a line of evidence for biodegradation of petroleum hydrocarbons, and thus, native bacteria metabolism.

[1] Atlantic Richfield may choose to use a different laboratory based on project needs. Regardless of the laboratory chosen, Atlantic Richfield will ensure the necessary reporting limits, required methodology, and the specified quality assurance/quality control and data validation requirements are followed as detailed in the BRW Biotreatability QAPP. Agencies will be informed of any changes in the reporting limits, methodology, or the quality assurance/quality control and data validation procedures.

[2] Pace Analytical Services, LLC will report results to the method detection limit. The analytical lab's reporting limits and detection limits are subject to change as these values are updated periodically to reflect analytical sensitivity and capability. Atlantic Richfield will ensure that any updates to the reporting limits or detection limits do not affect the ability for the Data Quality Objectives to be met and the updates will be specified in the Phase III Data Summary Report.

[3] In addition to the preservation listed, all samples will be cooled to <6 °C. Not all analyses require this but because multiple containers will be collected at most sample areas, all samples will be cooled.

[4] Pioneer will supply AECOM with enough soil to complete the identified analyses. All samples will be placed in 1-gallon ziplic bags and packed in a cooler with ice to preserve organic compounds at a temperature < 6 °C and shipped to AECOM.

[5] There is no method detection limit due to the result not being a direct analysis, the result is calculated.

Units: mg/kg - milligram per kilogram
mL - milliliter

Table 3. Proposed Sample Schedule

Analytical Lab/Company[1]	Sampling Event[2,3]				
	Baseline	3-Week	6-week	9-week	12-week
Enhanced Biotreatability Study - Lab Samples/Readings					
Pace Analytical Services, LLC	Total Organic Carbon	Total Organic Carbon	Total Organic Carbon	Total Organic Carbon	Total Organic Carbon
	TKN, Nitrogen	Not Analyzed	TKN, Nitrogen	Not Analyzed	TKN, Nitrogen
	Total Phosphorus	Not Analyzed	Total Phosphorus	Not Analyzed	Total Phosphorus
	Ammonia	Not Analyzed	Ammonia	Not Analyzed	Ammonia
	Fertilizer Recommendation Analysis (N, K, P)	Not Analyzed	Fertilizer Recommendation Analysis (N, K, P)	Not Analyzed	Fertilizer Recommendation Analysis (N, K, P)
	Volatile Petroleum Hydrocarbons (VPH)	VPH	VPH	VPH	VPH
	Extractable Petroleum Hydrocarbons (EPH) Fractionation	EPH Fractionation	EPH Fractionation	EPH Fractionation	EPH Fractionation
	Polycyclic Aromatic Hydrocarbons (PAHs)	PAHs	PAHs	PAHs	PAHs
AECOM	Adenosine Triphosphate (ATP)	ATP	ATP	ATP	ATP
	pH	pH	pH	pH	pH
	Percent Moisture	Percent Moisture	Percent Moisture	Percent Moisture	Percent Moisture
Microbial Insights	Microbial Analysis (CENSUS-qPCR)	Not Analyzed	Microbial Analysis (CENSUS-qPCR)	Not Analyzed	Not Analyzed

[1]Atlantic Richfield may choose to use a different laboratory based on project needs. Regardless of the laboratory chosen, Atlantic Richfield will ensure the necessary reporting limits, required methodology, and the specified quality assurance/quality control and data validation requirements are followed as detailed in the BRW Biotreatability QAPP. Agencies will be informed of any changes in the reporting limits, methodology, or the quality assurance/quality control and data validation procedures.

[2] Measurements/analyses will be done in triplicate.

[3]The planned sample schedule may be altered by the Contractor Project Manager, in consultation with the Contractor Quality Assurance Officer. Agencies will be notified of any significant changes to the proposed schedule.

BENCH-SCALE TREATABILITY STUDY REPORT

EVALUATION OF BIOACTIVITY OF INDIGENOUS BACTERIA IN SOILS IMPACTED WITH HYDROCARBONS AND HEAVY METALS

PIONEER TECHNICAL SERVICES, INC.

Project number: 60683150

September 9, 2022

Delivering a better world

Quality information

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Acronyms and Abbreviations

%	percent
AECOM	AECOM Technical Services, Inc.
alkB	alkane monooxygenase
almA	monooxygenase
ATP	adenosine triphosphate
BNSF	Burlington Northern Santa Fe Railway Line
BPSOU	Butte Priority Soils Operable Unit
CO_2	carbon dioxide
DNA	Deoxyribonucleic acid
EPH	Extractable Hydrocarbons
EBAC	Total Eubacteria
ID	Identification
L	liter
mg/kg	milligrams per kilogram
mg/L	milligrams per liter
OUR	oxygen update rate
Pioneer	Pioneer Technical Services
qPCR	quantitative polymerase chain reaction
Site	Butte Reduction Works Smelter Area Mine Waste Remediation and Contaminated Groundwater Hydraulic Control Site
TKN	total Kjeldahl nitrogen
TOC	total organic carbon
TPH	total petroleum hydrocarbons

1. Introduction

AECOM Technical Services, Inc. (AECOM) was retained to perform a bench-scale biotreatability study on behalf of Pioneer Technical Services (Pioneer) to evaluate the toxicity of heavy metals on native bacteria in hydrocarbon-impacted soils from the Butte Reduction Works Smelter Area Mine Waste Remediation and Contaminated Groundwater Hydraulic Control Site (Site), located in Butte, Montana.

The site covers approximately 24 acres and is located west of Montana Street between Silver Bow Creek and the Burlington Northern Santa Fe (BNSF) Railway line in Butte, Montana. Since 1885, multiple industries have operated at the site, which include a copper smelter, a zinc concentrator, manganese production, and phosphate production. These previous activities have left behind a variety of solid materials (including slag, tailings, manganese waste, demolition debris, foundations, etc.) as well as soil and groundwater impacted with metals and hydrocarbons. The site is part of the Butte Priority Soils Operable Unit (BPSOU) Consent Decree, which requires soil and groundwater impacted with organic pollutants within the Site to be properly managed in agreement with the remedy.

Because the soils in the site area are impacted with heavy metals due to its industrial history, it is suspected that the native soil bacteria, and thus the potential for bioremediation, may be affected by the presence of heavy metals. Heavy metals toxicity on soil bacteria has been demonstrated and studied in several laboratory scale experiments. Various methods have been used to determine toxicity effects in bacteria; these include bacteria respiration quantification via oxygen consumption, DNA quantification via quantitative polymerase chain reaction (qPCR), and thymidine incorporation, among others. This bench-scale biotreatability study was part of an evaluation of soil characteristics, such as microbial activity, that could provide evidence for the viability of soil remediation options such as natural attenuation.

2. Treatability Test Goals

The goal of this study was to evaluate the overall bacteria biomass health in different soil samples collected from the site, which are known to be impacted with various heavy metals and hydrocarbons. The biomass activity of these soils was compared against a soil sample impacted with a significantly lower concentration of heavy metals.

This study focused on oxygen uptake rate (OUR), adenosine triphosphate (ATP), carbon dioxide (CO_2) and qPCR measurements as the metrics to evaluate overall bacteria activity and metabolism in site-collected soil samples. The overall biomass health was determined through OUR, ATP, CO_2 and qPCR measurements on bench-scale slurry reactors.

3. Methodology

3.1 Preliminary Activities

Pioneer performed characterization of split soil samples collected in the field. This characterization consisted of analysis of heavy metals, hydrocarbons, moisture, nitrate, nitrite, sulfate, total organic carbon (TOC), organic carbon (Walkley-Black), total Kjeldahl nitrogen (TKN), and pH. Total oxidant demand was conducted in one soil sample identified as BRW21-

TP78 (TP78) which was collected at a depth interval of 0.0-0.5 feet. Split soil samples were sent to the AECOM Process Technologies Treatability Laboratory in Austin, Texas for further testing (AECOM Treatability Lab).

3.2 Sample Collection

Five samples were collected in the field from April 25th to April 28th, 2022. The samples were taken from the following locations:

- BRW21-TP75 (13.0-13.2)
- BRW21-TP76 (8.5-9.0)
- BRW21-TP78 (0.0-0.5)
- BRW21-TP77D (3.7-4.0)
- BRW21-T79R (1.0-2.0)

The soil samples were collected in doubled-bagged Ziploc bags and shipped to the AECOM Treatability Lab. The samples were clearly labeled, with the name of the sample, and the time and date of sample collection. Upon receipt, AECOM laboratory staff inspected the samples for leaks or any other anomalies. The samples were logged and stored under refrigeration.

3.3 Experimental Procedures

AECOM performed an initial baseline characterization test that served as a preliminary screening of the microorganisms' health in the five soil-composite samples impacted with heavy metals and hydrocarbons. This screening employed OUR, ATP, CO_2, and qPCR measurements to determine biomass activity.

Table 1. Experimental design for the baseline characterization of slurry reactors prepared with composite soil samples.

Slurry Reactor	Sampling Time
Composite Soil 1	24 h
Composite Soil 2	24 h
Composite Soil 3	24 h
Composite Soil 4	24 h
Composite Soil 5	24 h

Batch test reactors were prepared as water-soil slurries at a water to soil volumetric ratio of 5:1 to improve mixing and contact between the heavy metals, the hydrocarbons, oxygen, and the native bacteria in the soil.

For all soils, the natural organic matter and existing hydrocarbons are considered as the only carbon substrate available for the native bacteria. Each reactor consisted of a 1-liter (L) glass media bottle containing 0.5 L of slurry capped with a porous foam plug to allow the exchange of gases such as carbon dioxide and oxygen, while preventing contamination by external bacteria. The reactors' contents were mixed using a magnetic stir plate and stir bar for 24 hours to establish baseline conditions, which were followed by sampling and analysis.

3.4 Analytical Methods

Oxygen uptake rate, ATP, CO_2, and qPCR parameters were measured to provide evidence of native bacteria activity and quantify their relative performance among soils impacted with various concentrations of metals and hydrocarbons.

Table 2. Parameters measured for Task 1.

Parameter	Number of Samples Per Site Test	Performed by
OUR	1 event x 5 slurries = 5	AECOM
ATP	1 event x 5 slurries = 5	AECOM
CO_2	1 event x 5 slurries = 5	AECOM
CENSUS-qPCR	1 event x 5 slurries = 5	Microbial Insights Inc.

Oxygen consumption was monitored as the OUR by transferring the slurry contents into a bottle in which a luminescent/optical dissolved oxygen probe (Hach® Intellical™ LDO101) monitored the oxygen uptake for 15 to 20 minutes. OUR measurements were performed in triplicate for each slurry reactor at the end of the 24-hour cycle.

Adenosine triphosphate measurements were also performed in triplicate at the end of the 24-hour cycle as a proxy measurement of bacteria presence and activity. ATP measurements included one ATP standard check at the beginning and end of a sample batch and every 10 measurements. ATP was measured as total and dissolved, with the latter reflecting the ATP signal from bacteria cells that have undergone lysis (death), using an ATP test kit (LuminUltra) and luminometer (PhotonMaster™). Therefore, the ATP measurements provided information on the ratio of active and inactive biomass cells (biomass stress index). It is expected that higher levels of toxicity will have a negative impact in the total counts of ATP and increase the biomass stress index (i.e., more ATP from inactive cells). All these parameters mentioned have been shown to correlate with microbial activity in batch bioreactors; however, their sensitivity is dependent on the biomass concentration in the soil samples.

Carbon dioxide generation was measured in the composite soils as a line of evidence for microbial metabolism as CO_2 is a common product of microbial respiration. Dissolved CO_2 measurements were performed using a CO_2 titration test kit (Hach® Model CA-23) with a concentration range between 5 and 100 mg/L of CO_2.

Microbial analysis of CENSUS-qPCR used for bacteria population quantification was performed by Microbial Insights, Inc. This analysis consisted of extracting DNA from 10 grams of soil from each slurry reactor and amplifying the target DNA strands using qPCR and qPCR primers designed for a specific biomarker. The biomarkers analyzed via qPCR were "Total eubacteria" (EBAC), which quantifies the totality of bacteria cells in a sample, and the biomarkers for alkane monooxygenase (alkB) and monooxygenase (almA) enzymes. Oxygenases are enzymes involved in the degradation of hydrocarbons via aerobic respiration; they are a key factor in introducing oxygen atoms to the hydrocarbons structure, which opens the pathway for further biodegradation. The biomarker alkB is associated with the biodegradation of most alkanes with 5 to 16 carbons (C5-C16) in length, whereas almA has been linked to biodegradation of C20-C32 hydrocarbons. The purpose of these microbial analyses was to find out the relative differences among the composite soils regarding total bacteria count and specific biomarkers linked to hydrocarbons biodegradation.

4. Results

The bacteria biomass health varied among the five tested soils. The biomass activity was measured by ATP, OUR and carbon dioxide measurements after 24 hours of each reactor being set up. The TP78 soil showed the highest biomass activity based on the results. TP 79 had the second highest biomass activity but was significantly less than TP78. TP75, TP76 and TP77 showed the least biomass activity and therefore poorer biomass health.

ATP and ATP Stress Index

The total ATP, which is representative for the overall size of the bacterial population, for soil TP78 was over 18,000 picograms of cellular ATP/mL of sample as shown in Figure 1. The cellular ATP is calculated from subtracting the dissolved ATP from the total ATP values; this ATP indicates biomass activity. The total ATP represents the total population of bacteria by measuring the cell walls present within a sample. The dissolved ATP represents only the dead or severely compromised bacteria within a population.

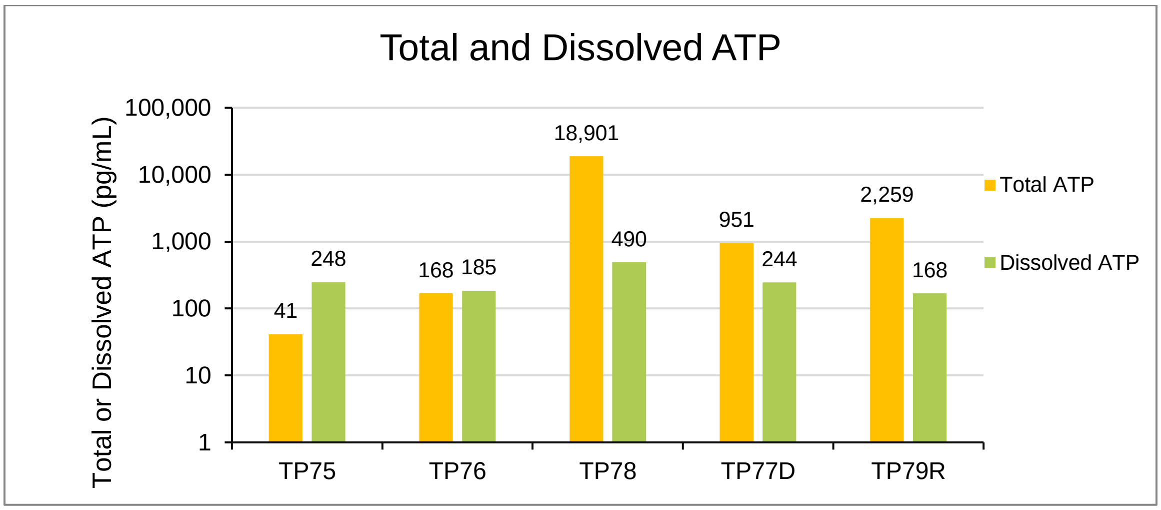


Figure 1. Total and dissolved ATP of the reactors after 24 hours

Note: For low levels of ATP, dissolved ATP measurements can result in higher values compared to total ATP measurements. All measurements were performed in triplicate with less than 5% relative percent difference. Differences in the dissolved and total ATP measurements are likely due to analytical variability when collecting sample aliquots.

Based on the calculated biomass stress index, TP78 also has the healthiest population of bacteria out of all the reactors. The ATP stress index is calculated by dividing the dissolved ATP by the total ATP, and then convert that result into a percentage. TP78's stress index was 2.6 percent (%) (Figure 2). A healthy bacterial population is considered to be one with a stress index under 50%.

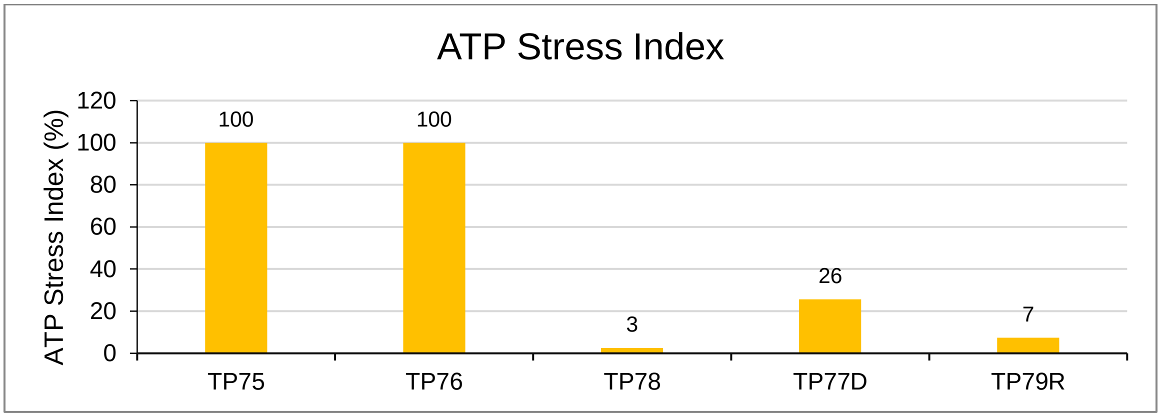


Figure 2. ATP stress index of the reactors at 24 hours

Total Bacteria

The ATP results were confirmed by CENSUS-qPCR analysis, which provides a count of the population of eubacteria within a sample (Figure 3). The TP78 eubacteria count was almost an order of magnitude higher than that of the other four samples. In general, total bacteria counts above 10^5 cells/g indicate a healthy environment for biological processes to occur. Therefore, all soils have a relatively high concentration of total bacteria.

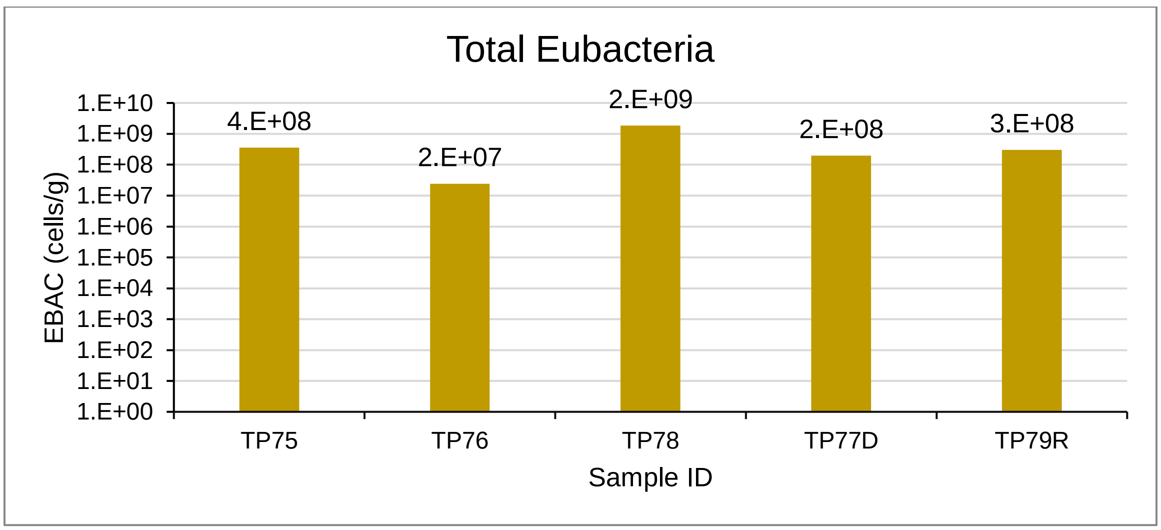


Figure 3. Total eubacteria population of the reactors after 24 hours

OUR

The OUR further corroborates that the TP78 soil has the highest level of biomass as shown in Figure 4. The slope values are taken from the OUR data found in Appendix 6.1 and the plotted graphs in Appendix 6.2. The OUR for each reactor was measured in triplicate after 24 hours of set up.

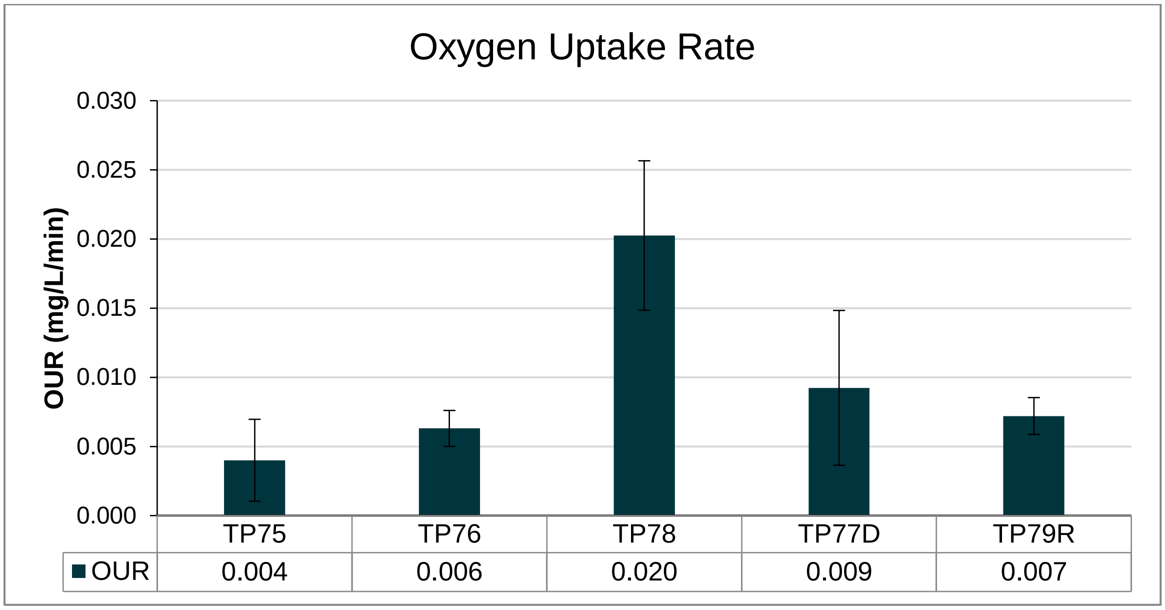


Figure 4. Average Oxygen Uptake Rate of each reactor at 24 hours. The OUR values are reported in mg/L of oxygen consumed per minute

Carbon Dioxide

The carbon dioxide results were not as conclusive as the ATP and OUR results, but still pointed to the TP78 sample as having the healthiest bacterial population as it is an indicator of bacterial respiration (Figure 5).

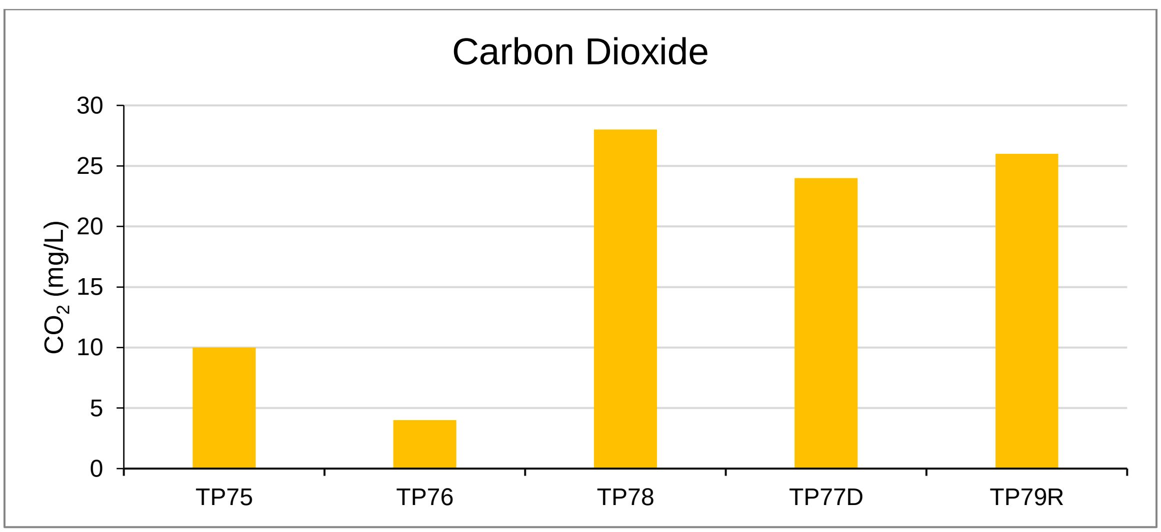


Figure 5. Carbon dioxide concentration of each reactor at 24 hours

Nitrogen and Organic Carbon

Simultaneous to the 24-hour reactor test, the five soil samples were also sent off for analysis for general chemistry and hydrocarbon and metals content. The five soils had similar general chemistries. TP78 had the highest TKN value at 366 milligrams per kilogram (mg/kg) whereas the second highest was 238 mg/kg in TP77 (Appendix 6.3). Due to the high TKN result, the total nitrogen value was the highest in the TP78 sample. The TP78 sample also had the highest total organic carbon content of 49,700 mg/kg by an order of magnitude compared to the second highest sample. Both the high nitrogen content and total organic carbon indicate an environment suitable for biological activity within the soil.

Hydrocarbons

The TP78 soil had the most Total Petroleum Hydrocarbon in the C05-C12 range (total petroleum hydrocarbons [TPH] C05-C12) and Total Volatile Hydrocarbons present at 350 and 115 mg/kg, respectively (Appendix 6.4). However, TP75 had a higher level of Total Extractable Hydrocarbons (EPHs) than TP78, TP77D, and TP79R with 173 mg/kg which was only surpassed by TP76 at 2,450 mg/kg.

Functional Genes

Results from the functional genes qPCR biomarkers indicate a lack of detection of alkB and almA in most of the soils (Figure 6 and Figure 7). For alkB, only soils TP75 and TP78 showed detection, whereas for almA, only TP75 showed detection.

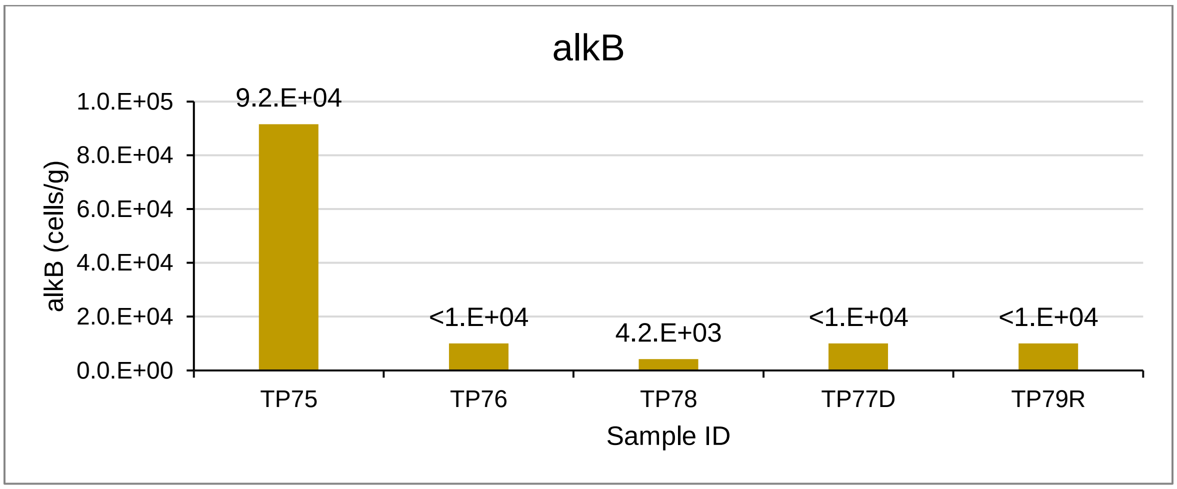


Figure 6. alkB enzyme biomarker concentration of each reactor at 24 hour

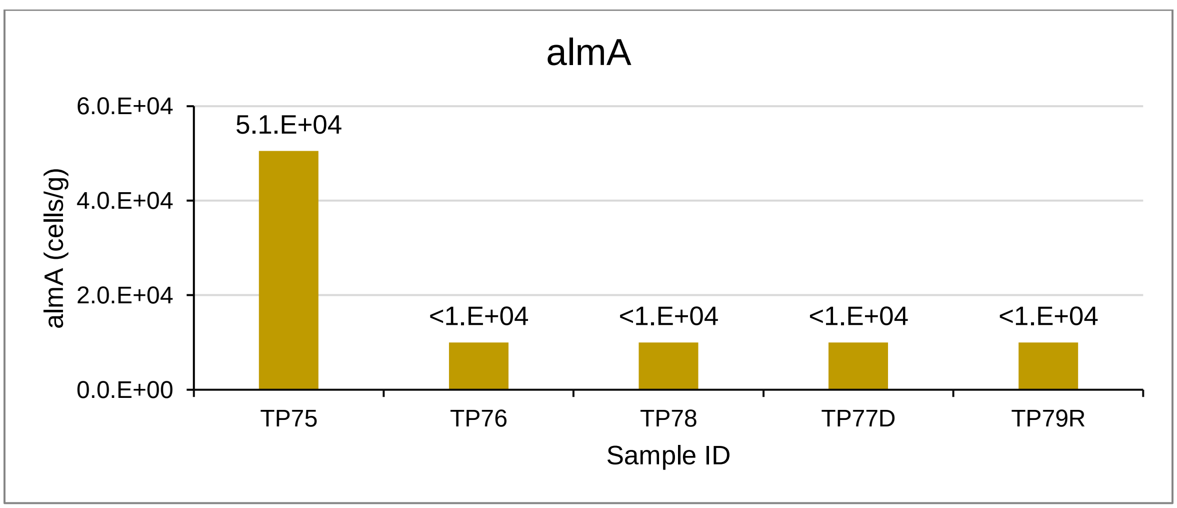


Figure 7. almA enzyme biomarker concentration of each reactor at 24 hours

To put these results in context, the qPCR data base from Microbial Insights was utilized to estimate the percentiles for the biomarkers tested. These percentile values indicate if the biomarkers were present at the highest end of concentrations (near 100th percentile) or fell to the low end (near 0th percentile) based on all the data collected from Microbial Insights for those biomarkers. To aid the database calculator provide an accurate estimate of the percentiles, data regarding TOC, pH, nitrate, sulfate, and alkalinity was entered. Figure 8 illustrates how the detected biomarkers compare against Microbial Insight's database percentiles. Note that the biomarker almA did not have a percentile value due to the insufficient number of samples in soil matrices for this target in Microbial Insight's database.

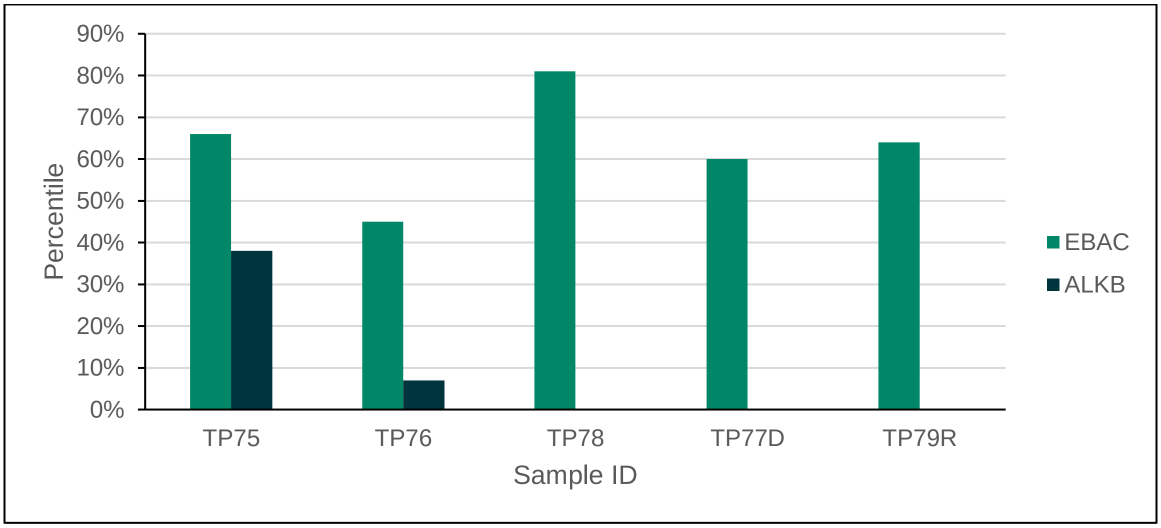


Figure 8. Biomarker targets percentiles for the five soil slurry samples evaluated. Data points are only shown for biomarkers detected

Comparing the concentrations of total ATP, dissolved ATP, stress index, carbon dioxide, total eubacteria, alkB and almA with the metals' concentrations showed a slight correlation on the effect of metals and bacteria on one another (Appendix 6.7). The biomarkers alkB and almA showed an increasing positive correlation with total metals concentrations as both enzyme biomarkers increase with higher metals concentration. TP75, which has the highest metals concentration, has the highest alkB and almA enzyme concentrations (Fig. 6 and 7) and a high concentration of aliphatic hydrocarbons and EPHs. This suggests that biomarkers such as alkB and almA are more strongly correlated to the presence of hydrocarbons than the presence of heavy metals. In addition, this particular soil may have a stronger presence of hydrocarbon degrading bacteria. Nevertheless, TP75 showed one of the highest ATP stress indices, the second lowest CO_2 production, and the lowest OUR, indicating poorer overall biomass activity.

Overall, moderate trends were observed in the metals analysis between the five soils. TP78 had the second lowest total metals concentration at 16,237 mg/kg (Appendices 6.5 and 6.6) and showed the highest concentration of bacteria, lower ATP stress index, highest CO_2 production, and highest OUR. In addition, the least healthy soils, TP76 and TP75, were collected from the deepest locations at 8.5-9.0 and 13.0-13.2 depth intervals, whereas the healthiest soil, TP78, was the shallowest. This suggests that biomass health is correlated to soil depth.

The moderate to high TPH concentrations and the relatively low heavy metals concentrations, in conjunction with strong bacteria metabolism markers, indicate that the TP78 soil presents the highest bacteria activity and highest potential for biodegradation. Therefore, the strong markers for bacteria health correspond to the soil with high hydrocarbons and low heavy metals concentrations.

5. Conclusions

This bench-scale treatability test demonstrated the use of several parameters as proxy indicators of bacteria activity and health for soils containing petroleum hydrocarbons and heavy metals. The test achieved its objective of demonstrating the usability of these parameters and correlate them to the soil's properties. In conclusion:

- Parameters such as ATP (and ATP Stress Index), OUR, CO_2 production, and total bacteria (via qPCR) correlate well to indicate general biomass activity; functional genes alkB and almA did not correlate well to the other parameters.
- Functional genes correlated best with the concentrations of EPHs and aliphatic hydrocarbons.
- The TP78 soil sample showed the best results for ATP, OUR, CO_2 production and total bacteria, while having a high TPH (C05-C12) concentration and the second lowest heavy metals concentrations.
- The TP75 showed the lowest results for ATP, OUR, and CO_2 production, while showing the highest functional genes biomarkers.
- Overall biomass activity correlates to high TOC, high nitrogen concentration, low heavy metals concentration, and high TPH (C05-C12) concentration, while negatively correlating with soil depth.

Based on the results of this bench-scale test, the parameters measured in the laboratory provided the evidence needed to establish differences in biomass activity and therefore it is recommended that ATP, OUR, and CO_2 production can be employed as proxy measurements for biomass activity and health.

In a broader perspective, biodegradation could be a suitable option for soils like TP78 and, to a lesser degree, the rest of the soils. TP78 showed evidence of strong, healthy biomass and one functional gene (alkB) associated to hydrocarbons biodegradation. Other soils like TP77D and TP79R showed high outputs of CO_2 and low ATP stress indices, both signs of a healthy biomass. More time is needed to improve the evaluation of biodegradation in soils; if the incubation of slurry reactors is increased from 24 hours to longer periods of time (28 days for example), soil biomass metabolism will acclimate and change, most likely increasing the concentration of the proxy parameters and the qPCR counts of total bacteria, if oxygen and organic carbon are present to sustain this growth. With the caveat of the short incubation period in this experiment, it can be concluded that soils TP75, TP77D, and TP79R showed signs of healthy broad microbial populations, while TP75 showed a less healthy microbial population with potential for biodegradation of hydrocarbons, and soil TP76 had the lowest potential for biodegradation and the poorest overall biomass health.

6. Appendices

6.1 Oxygen Uptake Rates Tables

Control

	Run 1		Run 2		Run 3	
	Time (min)	DO (mg/L)	Time (min)	DO (mg/L)	Time (min)	DO (mg/L)
	0.00	8.57	0.00	9.11	0.00	8.89
	0.25	8.55	0.25	8.79	0.25	8.66
	0.50	8.54	0.50	8.66	0.50	8.63
	0.75	8.53	0.75	8.61	0.75	8.61
	1.00	8.51	1.00	8.59	1.00	8.61
	1.50	8.51	1.50	8.57	1.50	8.59
	2.00	8.49	2.00	8.55	2.00	8.58
	2.50	8.48	2.50	8.53	2.50	8.57
	3.00	8.47	3.00	8.51	3.00	8.57
	4.00	8.49	4.00	8.51	4.00	8.56
	5.00	8.48	5.00	8.50	5.00	8.56
	6.00	8.48	6.00	8.51	6.00	8.56
	7.00	8.48	7.00	8.50	7.00	8.56
	8.00	8.48	8.00	8.50	8.00	8.56
	9.00	8.48	9.00	8.50	9.00	8.55
	10.00	8.48	10.00	8.50	10.00	8.55
SLOPE (OUR)	**-0.003**		**-0.010**		**-0.006**	
Average OUR	**-0.006**					
St Dev	**0.003**					

Soil TP78

	Run 1		Run 2		Run 3	
	Time (min)	DO (mg/L)	Time (min)	DO (mg/L)	Time (min)	DO (mg/L)
	0.00	8.42	0.00	8.70	0.00	8.62
	0.25	8.34	0.25	8.52	0.25	8.39
	0.50	8.33	0.50	8.19	0.50	8.16
	0.75	8.32	0.75	8.02	0.75	7.95
	1.00	8.32	1.00	7.99	1.00	7.93
	1.50	8.30	1.50	7.96	1.50	7.88
	2.00	8.30	2.00	7.95	2.00	7.89
	2.50	8.29	2.50	7.91	2.50	7.86
	3.00	8.27	3.00	7.91	3.00	7.83
	4.00	8.25	4.00	7.89	4.00	7.78
	5.00	8.24	5.00	7.88	5.00	7.76
	6.00	8.23	6.00	7.85	6.00	7.75
	7.00	8.23	7.00	7.83	7.00	7.74
	8.00	8.21	8.00	7.80	8.00	7.72
	9.00	8.19	9.00	7.79	9.00	7.71
	10.00	8.18	10.00	7.76	10.00	7.72
SLOPE (OUR)	**-0.016**		**-0.025**		**-0.025**	
Average OUR	**-0.020**					
St Dev	**0.005**					

Soil TP75

	Run 1		Run 2		Run 3	
	Time (min)	**DO (mg/L)**	**Time (min)**	**DO (mg/L)**	**Time (min)**	**DO (mg/L)**
	0.00	8.81	0.00	8.90	0.00	8.83
	0.25	8.82	0.25	8.77	0.25	8.76
	0.50	8.83	0.50	8.74	0.50	8.70
	0.75	8.78	0.75	8.70	0.75	8.69
	1.00	8.77	1.00	8.71	1.00	8.63
	1.50	8.77	1.50	8.69	1.50	8.64
	2.00	8.77	2.00	8.69	2.00	8.62
	2.50	8.77	2.50	8.67	2.50	8.62
	3.00	8.76	3.00	8.65	3.00	8.61
	4.00	8.77	4.00	8.65	4.00	8.61
	5.00	8.76	5.00	8.68	5.00	8.62
	6.00	8.74	6.00	8.69	6.00	8.60
	7.00	8.72	7.00	8.70	7.00	8.59
	8.00	8.73	8.00	8.70	8.00	8.60
	9.00	8.73	9.00	8.69	9.00	8.60
	10.00	8.72	10.00	8.70	10.00	8.60
SLOPE (OUR)	**-0.007**		**0.001**		**-0.006**	
Average OUR	**-0.004**					
St Dev	**0.004**					

Soil TP79R

	Run 1		Run 2		Run 3	
	Time (min)	**DO (mg/L)**	**Time (min)**	**DO (mg/L)**	**Time (min)**	**DO (mg/L)**
	0.00	8.94	0.00	9.20	0.00	9.23
	0.25	8.71	0.25	8.76	0.25	8.74
	0.50	8.64	0.50	8.64	0.50	8.66
	0.75	8.60	0.75	8.60	0.75	8.62
	1.00	8.59	1.00	8.59	1.00	8.60
	1.50	8.56	1.50	8.56	1.50	8.58
	2.00	8.55	2.00	8.55	2.00	8.57
	2.50	8.54	2.50	8.54	2.50	8.56
	3.00	8.53	3.00	8.54	3.00	8.56
	4.00	8.53	4.00	8.53	4.00	8.55
	5.00	8.53	5.00	8.52	5.00	8.54
	6.00	8.52	6.00	8.52	6.00	8.53
	7.00	8.52	7.00	8.51	7.00	8.53
	8.00	8.52	8.00	8.51	8.00	8.53
	9.00	8.53	9.00	8.51	9.00	8.52
	10.00	8.53	10.00	8.51	10.00	8.53
SLOPE (OUR)	**-0.006**		**-0.008**		**-0.009**	
Average OUR	**-0.007**					
St Dev	**0.001**					

Soil TP77D

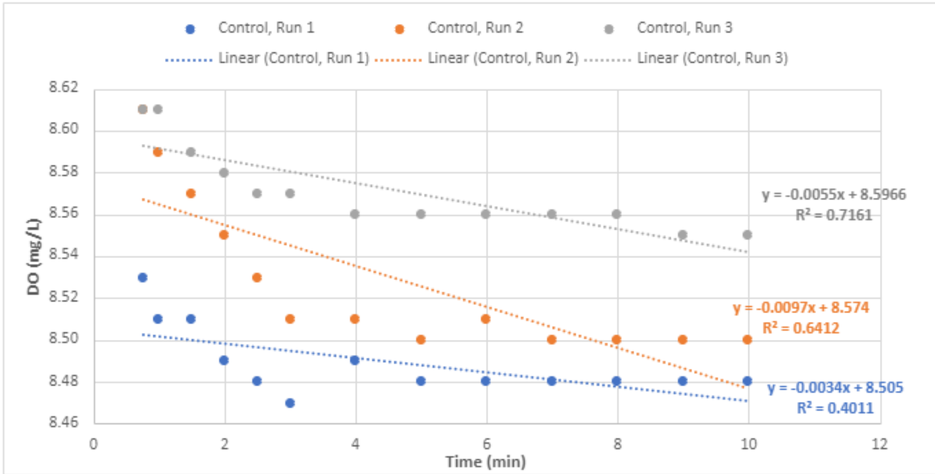
	Run 1		Run 2		Run 3	
	Time (min)	**DO (mg/L)**	**Time (min)**	**DO (mg/L)**	**Time (min)**	**DO (mg/L)**
	0.00	8.92	0.00	9.14	0.00	9.03
	0.25	8.69	0.25	9.05	0.25	8.83
	0.50	8.72	0.50	8.92	0.50	8.79
	0.75	8.75	0.75	8.86	0.75	8.77
	1.00	8.75	1.00	8.82	1.00	8.73
	1.50	8.72	1.50	8.78	1.50	8.70
	2.00	8.73	2.00	8.75	2.00	8.69
	2.50	8.73	2.50	8.74	2.50	8.69
	3.00	8.73	3.00	8.74	3.00	8.69
	4.00	8.73	4.00	8.72	4.00	8.68
	5.00	8.73	5.00	8.71	5.00	8.65
	6.00	8.72	6.00	8.72	6.00	8.64
	7.00	8.72	7.00	8.72	7.00	8.63
	8.00	8.72	8.00	8.71	8.00	8.63
	9.00	8.71	9.00	8.71	9.00	8.63
	10.00	8.72	10.00	8.70	10.00	8.62
SLOPE (OUR)	**-0.003**		**-0.012**		**-0.013**	
Average OUR	**-0.009**					
St Dev	**0.006**					

Soil TP76

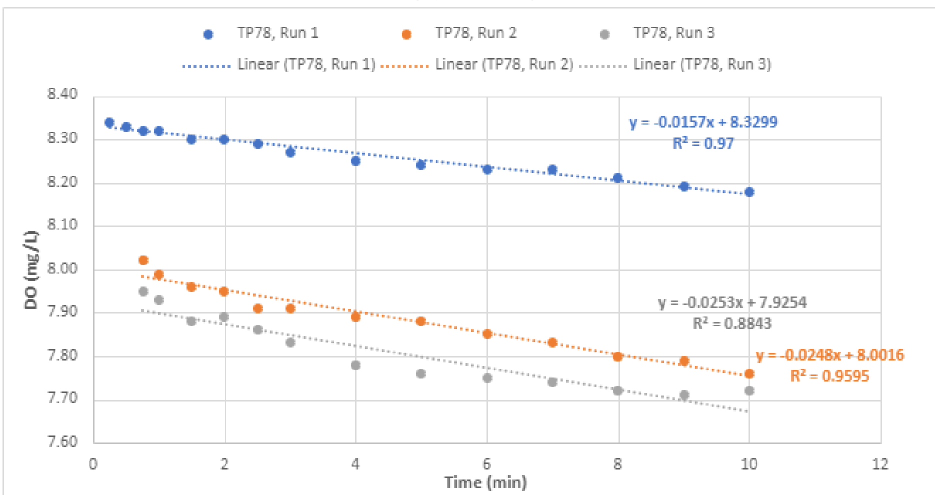
	Run 1		Run 2		Run 3	
	Time (min)	**DO (mg/L)**	**Time (min)**	**DO (mg/L)**	**Time (min)**	**DO (mg/L)**
	0.00	9.16	0.00	8.92	0.00	9.07
	0.25	8.78	0.25	8.66	0.25	8.69
	0.50	8.74	0.50	8.62	0.50	8.64
	0.75	8.70	0.75	8.61	0.75	8.61
	1.00	8.68	1.00	8.60	1.00	8.59
	1.50	8.65	1.50	8.59	1.50	8.58
	2.00	8.64	2.00	8.59	2.00	8.56
	2.50	8.63	2.50	8.58	2.50	8.55
	3.00	8.63	3.00	8.57	3.00	8.55
	4.00	8.62	4.00	8.56	4.00	8.55
	5.00	8.61	5.00	8.57	5.00	8.54
	6.00	8.61	6.00	8.57	6.00	8.54
	7.00	8.61	7.00	8.57	7.00	8.54
	8.00	8.61	8.00	8.56	8.00	8.54
	9.00	8.62	9.00	8.56	9.00	8.54
	10.00	8.61	10.00	8.56	10.00	8.54
SLOPE (OUR)	**-0.007**		**-0.006**		**-0.004**	
Average OUR	**-0.006**					
St Dev	**0.001**					

6.2 Oxygen Uptake Rates Plots

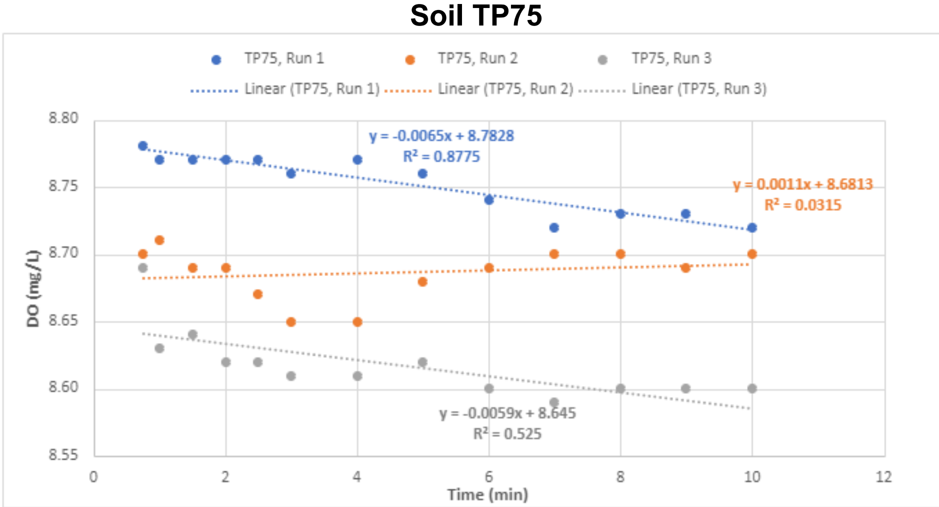
Control

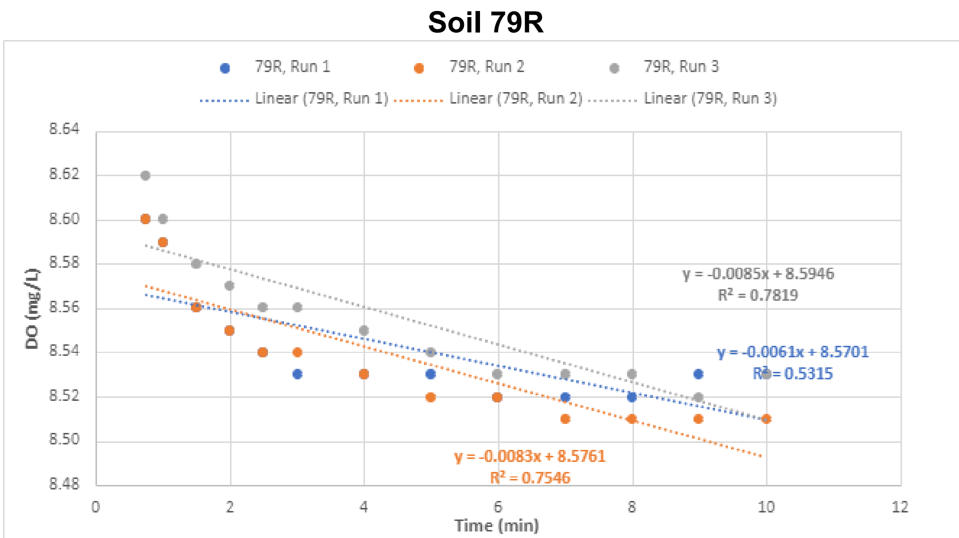


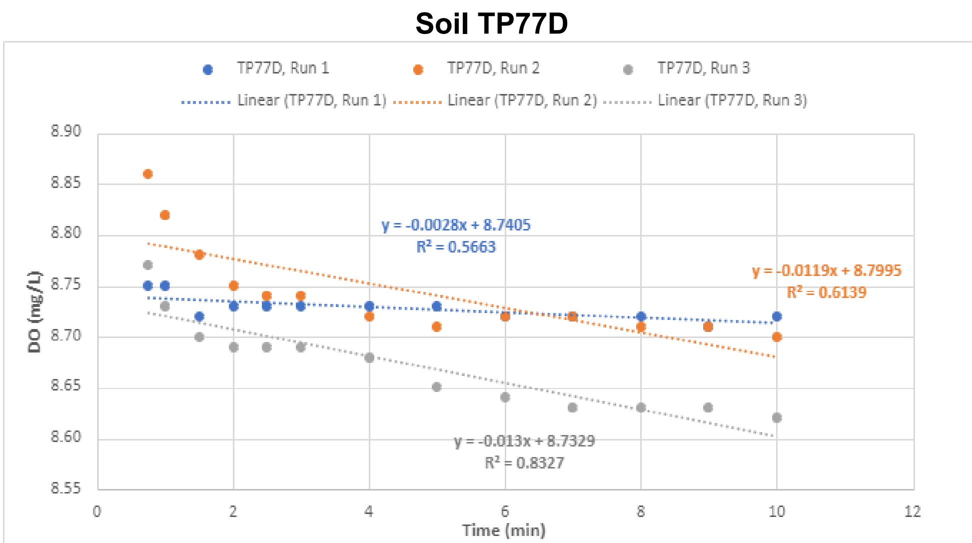
Soil TP78

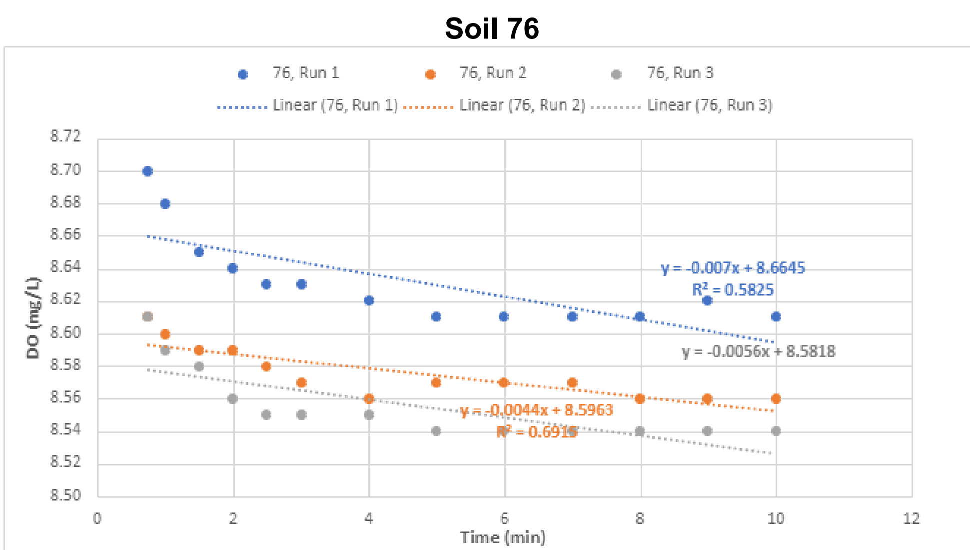


Soil TP75




Soil 79R
79R, Run 1
79R, Run 2
79R, Run 3
Linear (79R, Run 1)
Linear (79R, Run 2)
Linear (79R, Run 3)
DO (mg/L)
Time (min)
y = -0.0085x + 8.5946
R² = 0.7819
y = -0.0061x + 8.5701
R² = 0.5315
y = -0.0083x + 8.5761
R² = 0.7546


Soil TP77D
TP77D, Run 1
TP77D, Run 2
TP77D, Run 3
Linear (TP77D, Run 1)
Linear (TP77D, Run 2)
Linear (TP77D, Run 3)
DO (mg/L)
Time (min)
y = -0.0028x + 8.7405
R² = 0.5663
y = -0.0119x + 8.7995
R² = 0.6139
y = -0.013x + 8.7329
R² = 0.8327


Soil 76
76, Run 1
76, Run 2
76, Run 3
Linear (76, Run 1)
Linear (76, Run 2)
Linear (76, Run 3)
DO (mg/L)
Time (min)
y = -0.007x + 8.6645
R² = 0.5825
y = -0.0056x + 8.5818
y = -0.0044x + 8.5963
R² = 0.6916

6.3 Baseline General Chemistry Results

	TP75 (13.0-13.2)	TP76 (8.5-9.0)	TP77D (3.7-4.0)	TP78 (0.0-0.5)	TP79R (1.0-2.0)
pH	8.95	7.49	7.32	7.4	7.51
Specific conductivity (umhos/cm)	555	279	915	279	917
Total Solids (%)	98.8	94.1	91.7	93.9	91.9
TKN (mg/kg)	173	<57.4	238	366	138
Nitrite (mg/kg)	<0.9	<0.98	<0.91	<0.97	<0.93
Nitrate (mg/kg)	<0.9	<0.98	1.2	<0.97	<0.93
Nitrogen as NO_3 (mg/kg)	1.17	0.96	2.08	0.98	0.70
Nitrogen (NO_2+NO_3) (mg/kg)	<0.9	<0.98	1.2	<0.97	<0.93
Total Nitrogen (mg/kg)	169	53.9	160	347	129
TOC (mg/kg)	3400	3620	4740	49700	4620
Sulfate (mg/kg)	101	104	389	<13.7	661
Sulfur (mg/kg)	5490	251	1870	568	390
Alkalinity, Carbonate (mg/kg)	<86.9	<91.5	<89.6	<85.2	<93.0
Total Alkalinity (mg/kg)	103	<91.5	197	97.3	104

These results are preliminary and have not gone through Pioneer's data validation process.

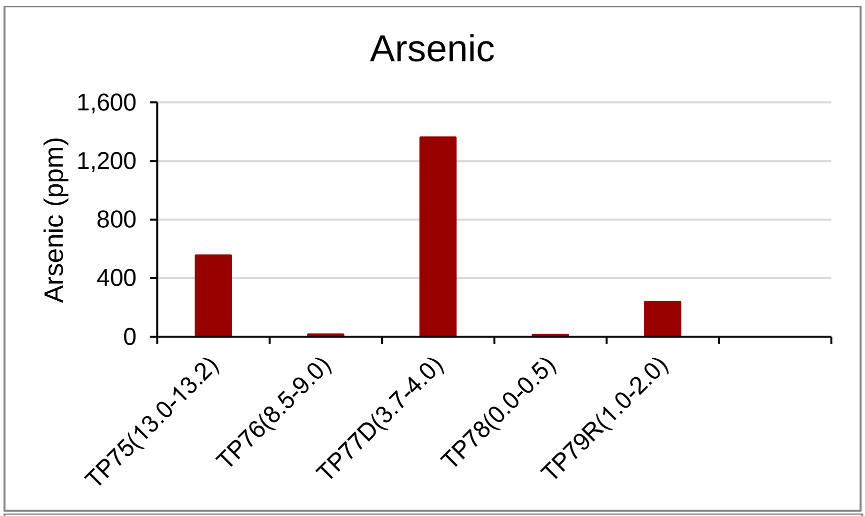
6.4 Baseline Hydrocarbons Results

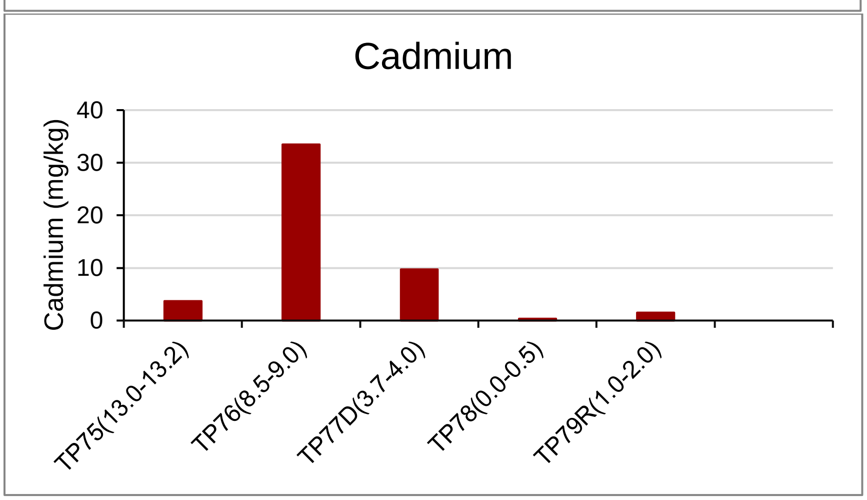
	TP75 (13.0-13.2)	TP76 (8.5-9.0)	TP77D (3.7-4.0)	TP78 (0.0-0.5)	TP79R (1.0-2.0)
Aliphatic Hydrocarbons C5-C8, Unadjusted (mg/kg)	<1.71	<1.88	<1.97	<9.41	<1.97
Aliphatic (C05-C08), Adjusted (mg/kg)	<1.71	<1.88	<1.97	<9.41	<1.97
TPH (C05-C12) (mg/kg)	30.4	7.62	5.54	350	4.97
Aromatic (C09-C10), Unadjusted (mg/kg)	5.1	1.96	<1.97	44.1	<1.97
Aliphatic (C09-C12), Adjusted (mg/kg)	15.6	<1.88	2.31	71.2	2.2
Aliphatic C9-C12, Unadjusted (mg/kg)	15.6	<1.88	2.31	71.2	2.2
Aliphatic Hydrocarbons C9-C18 (mg/kg)	85.9	1630	<1.83	<1.79	<1.83
Aromatic (C11-C22), Adjusted (mg/kg)	14.2	277	<7.27	7.59	<7.26
Aromatic Hydrocarbons C11-C22, Unadjusted (mg/kg)	14.2	277	6.03	7.59	6.76
Aliphatic Hydrocarbons C19-C36 (mg/kg)	7.23	6.39	5.7	5.87	10.6
EPH Screen (mg/kg)	173	2450	27.1	34.6	32.9
Total Extractable Hydrocarbons (mg/kg)	107	1910	11.8	13.4	17.3
Total Petroleum Hydrocarbons (mg/kg)	107	1910	<7.27	13.4	10.6
Total VPH (mg/kg)	20.7	<3.76	<3.94	115	<3.93

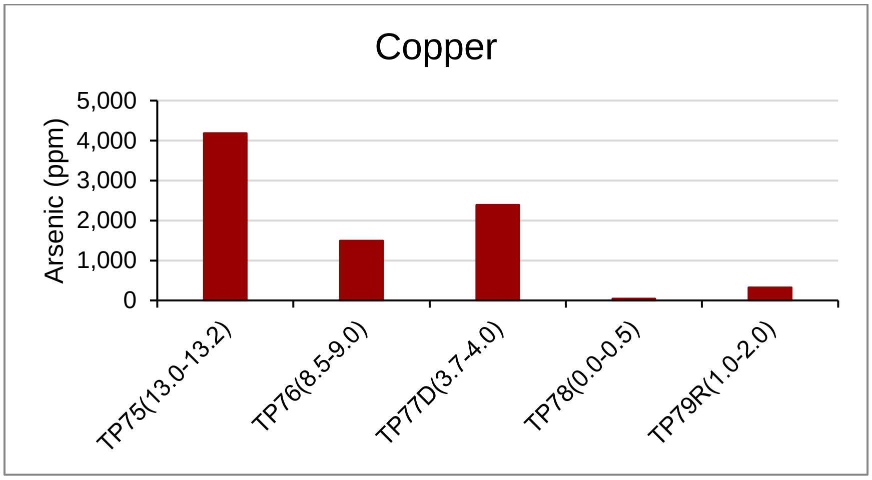
6.5 Baseline Metals Results

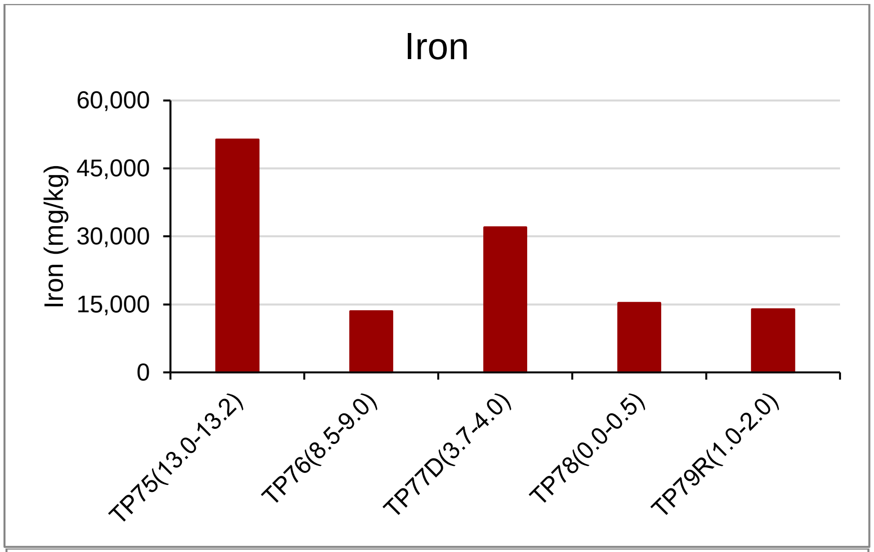
	TP75 (13.0-13.2)	TP76 (8.5-9.0)	TP77D (3.7-4.0)	TP78 (0.0-0.5)	TP79R (1.0-2.0)
Method	**6020B**	**6020B**	**6020B**	**6020B**	**6020B**
Arsenic (mg/kg)	554	16.3	1360	14	238
Cadmium (mg/kg)	3.7	33.5	9.7	0.35	1.5
Copper (mg/kg)	4180	1490	2390	47	322
Iron (mg/kg)	51,400	13,500	32,000	15,300	13,900
Lead (mg/kg)	1370	5.8	1490	32.1	215
Manganese (mg/kg)	22,800	96.5	20,600	712	1740
Mercury (mg/kg)	0.13	0.06	13.9	0.053	0.53
Zinc (mg/kg)	16,500	602	4510	131	422

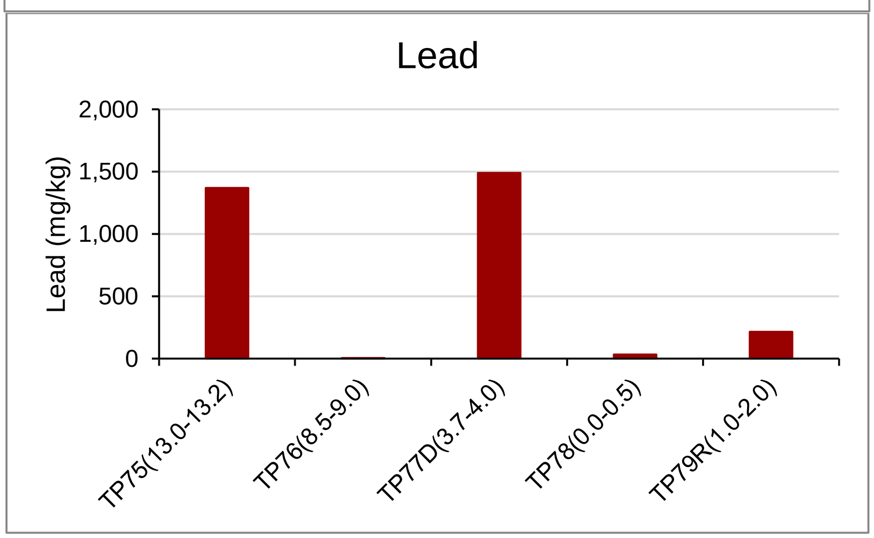
6.6 Baseline Metals Graphs by Method 6020B

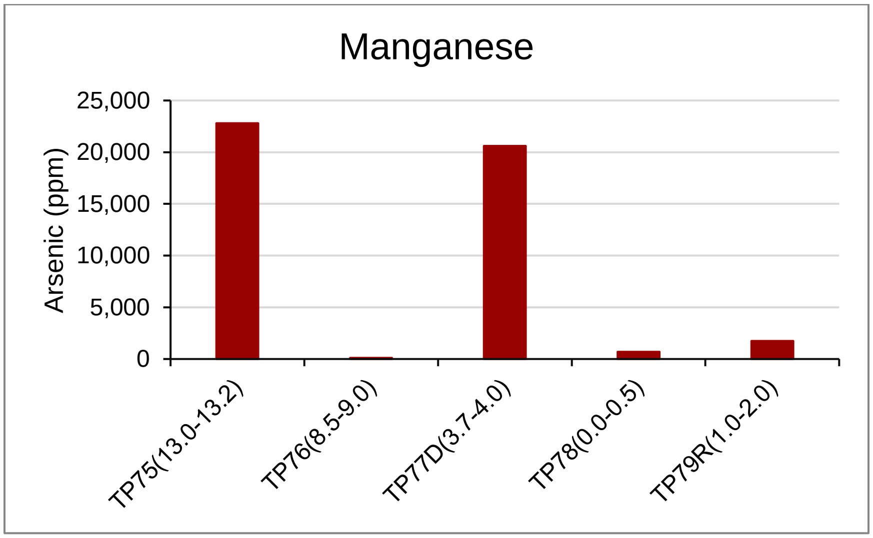


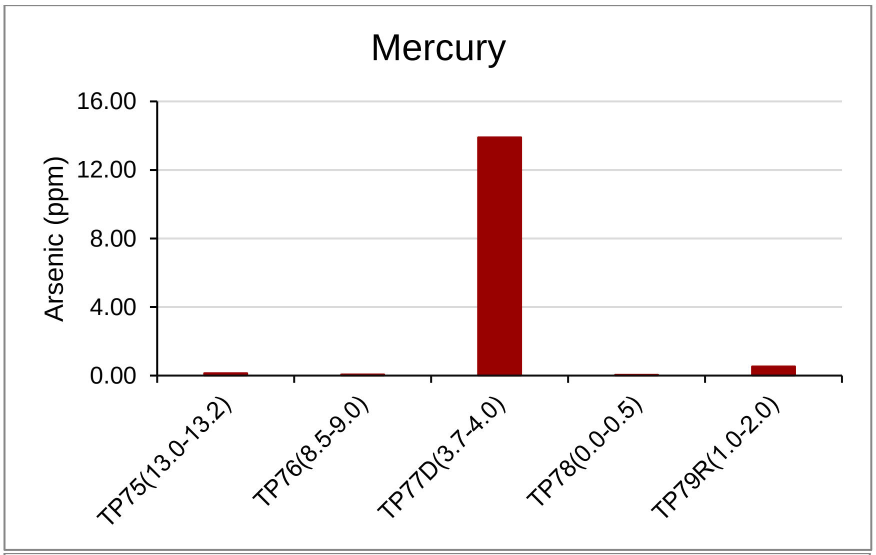


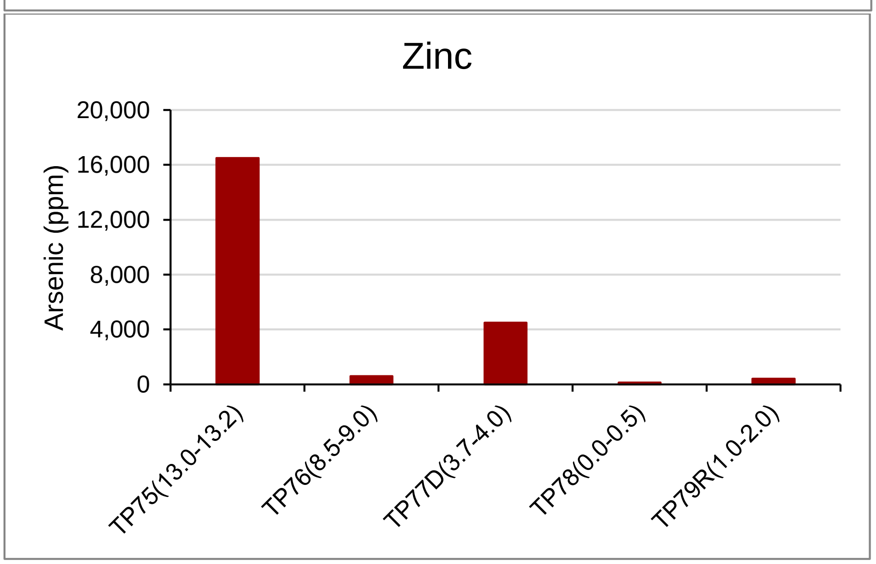

Copper
Arsenic (ppm)
5,000
4,000
3,000
2,000
1,000
0
TP75(13.0-13.2)
TP76(8.5-9.0)
TP77D(3.7-4.0)
TP78(0.0-0.5)
TP79R(1.0-2.0)


Iron
Iron (mg/kg)
60,000
45,000
30,000
15,000
0
TP75(13.0-13.2)
TP76(8.5-9.0)
TP77D(3.7-4.0)
TP78(0.0-0.5)
TP79R(1.0-2.0)

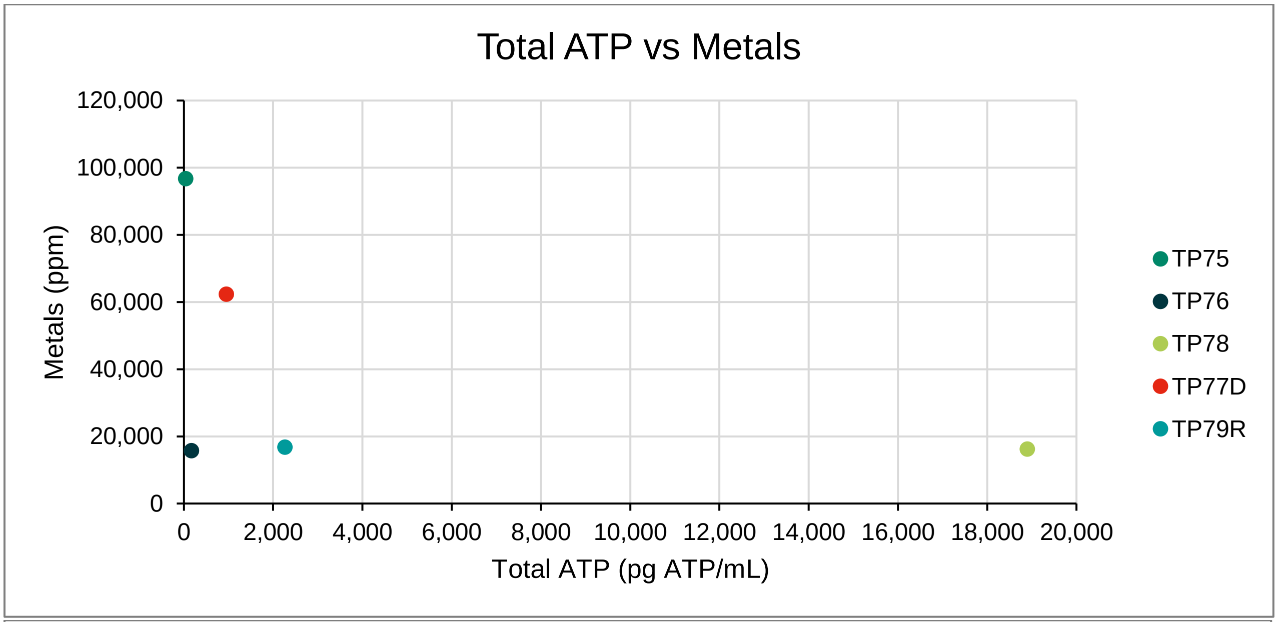

Lead
Lead (mg/kg)
2,000
1,500
1,000
500
0
TP75(13.0-13.2)
TP76(8.5-9.0)
TP77D(3.7-4.0)
TP78(0.0-0.5)
TP79R(1.0-2.0)

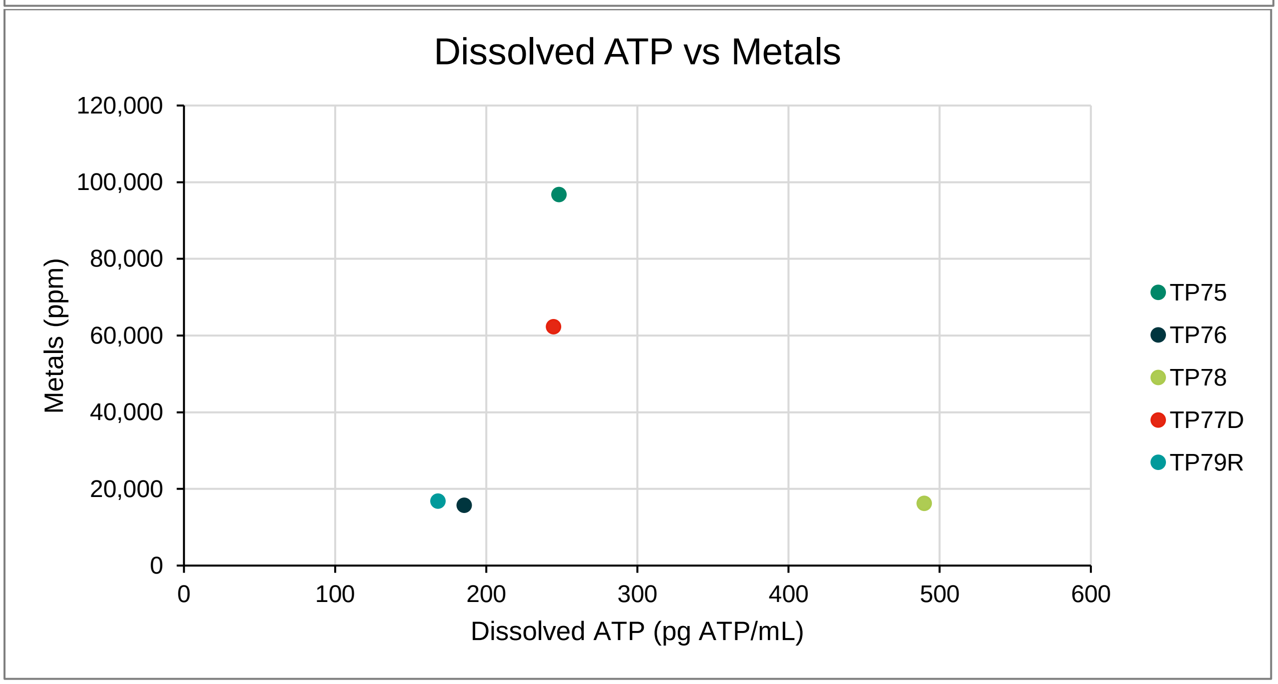

Manganese
Arsenic (ppm)
25,000
20,000
15,000
10,000
5,000
0
TP75(13.0-13.2)
TP76(8.5-9.0)
TP77D(3.7-4.0)
TP78(0.0-0.5)
TP79R(1.0-2.0)

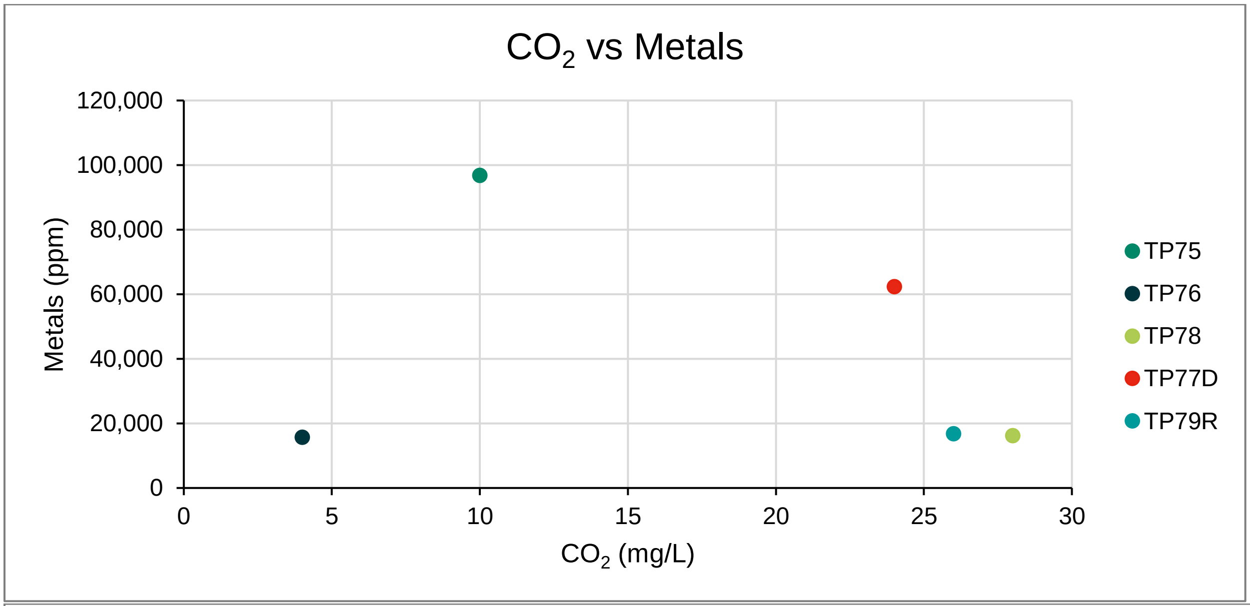

Mercury
Arsenic (ppm)
16.00
12.00
8.00
4.00
0.00
TP75(13.0-13.2)
TP76(8.5-9.0)
TP77D(3.7-4.0)
TP78(0.0-0.5)
TP79R(1.0-2.0)

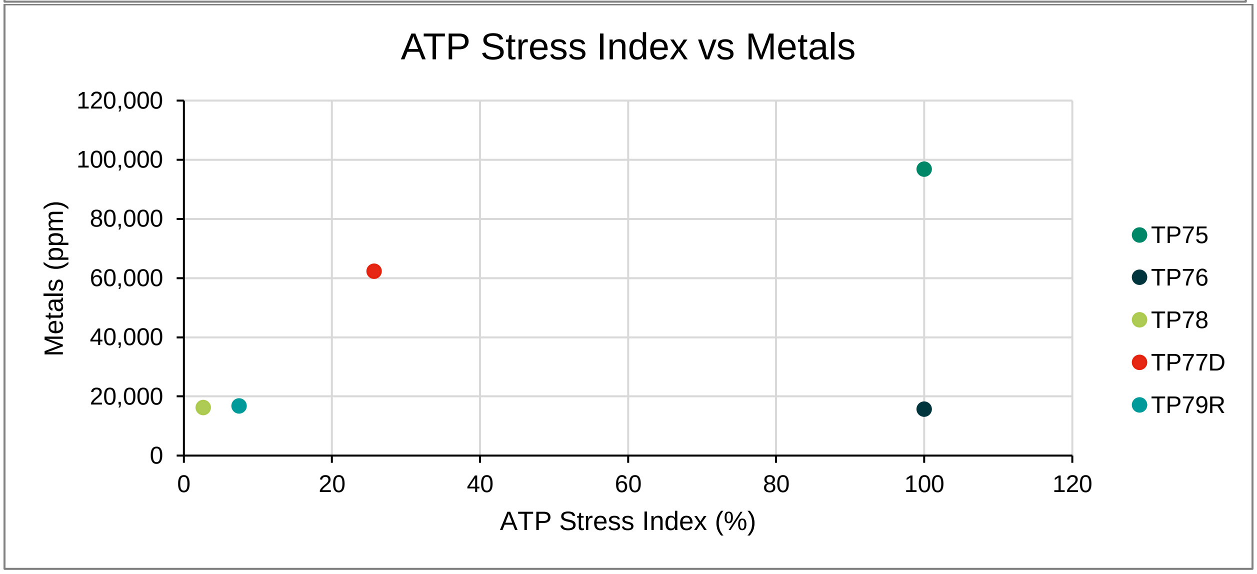

Zinc
Arsenic (ppm)
20,000
16,000
12,000
8,000
4,000
0
TP75(13.0-13.2)
TP76(8.5-9.0)
TP77D(3.7-4.0)
TP78(0.0-0.5)
TP79R(1.0-2.0)

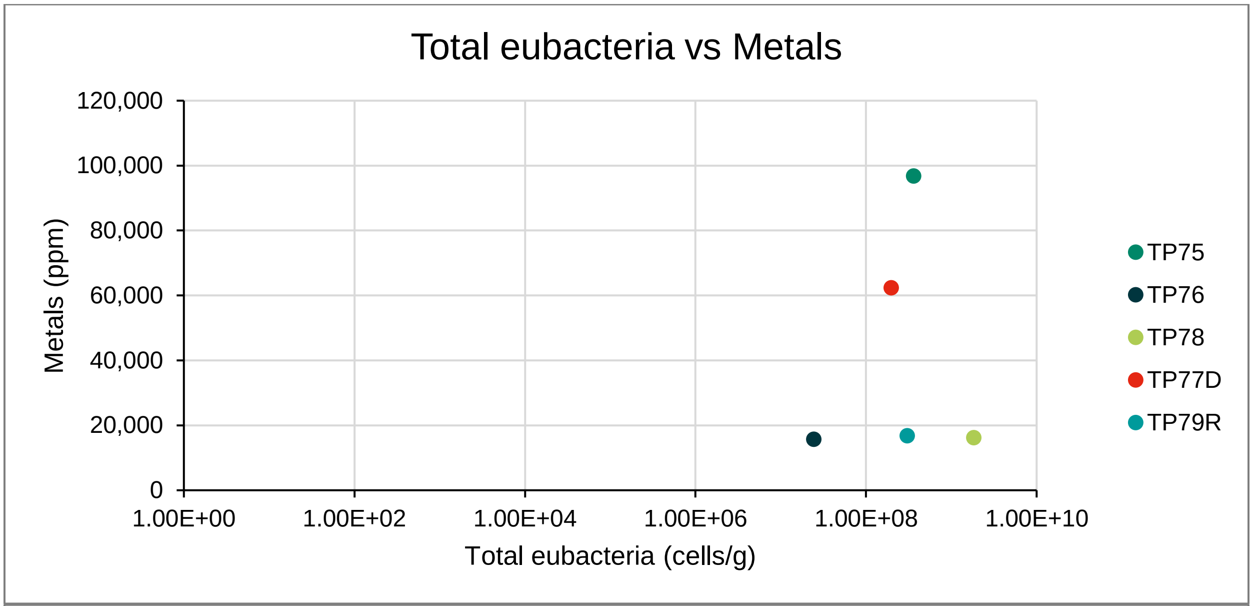
6.7 Analytes vs Metals Concentrations

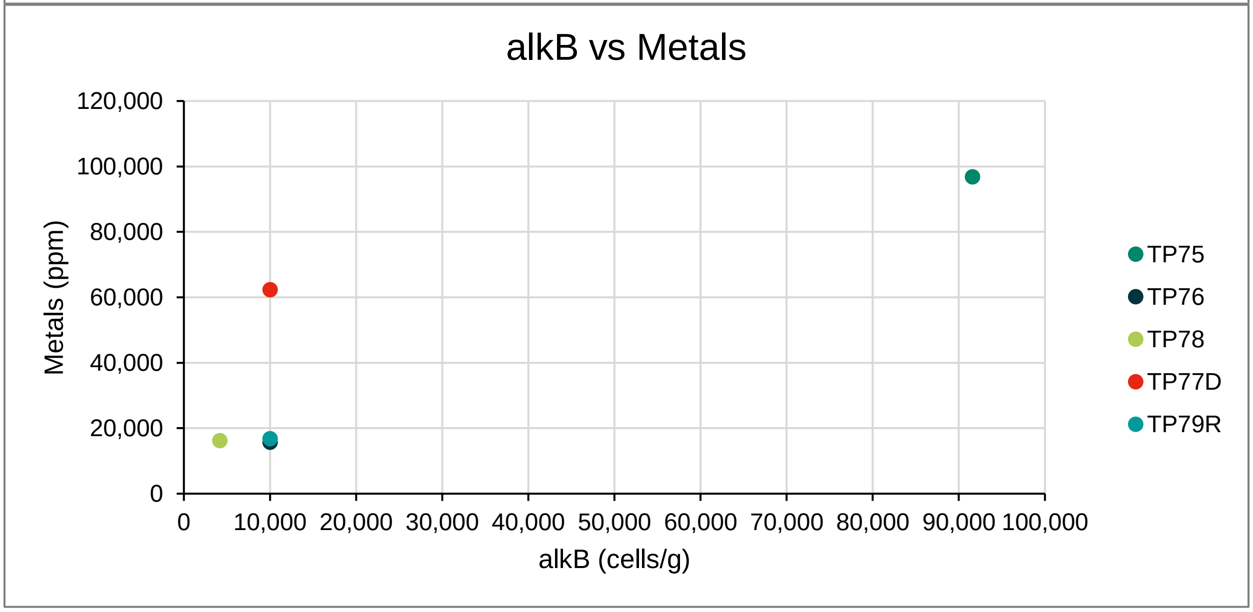


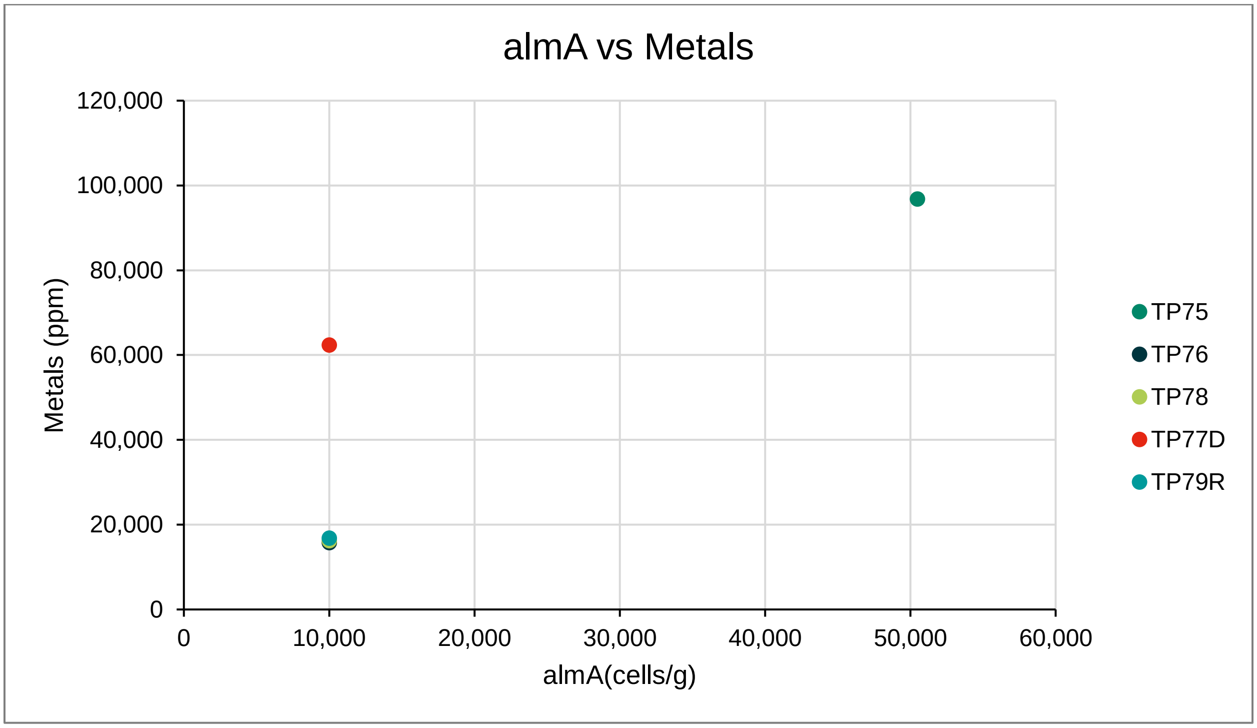



CO_2 vs Metals
Metals (ppm)
120,000
100,000
80,000
60,000
40,000
20,000
0
0
5
10
15
20
25
30
CO_2 (mg/L)
TP75
TP76
TP78
TP77D
TP79R


ATP Stress Index vs Metals
Metals (ppm)
120,000
100,000
80,000
60,000
40,000
20,000
0
0
20
40
60
80
100
120
ATP Stress Index (%)
TP75
TP76
TP78
TP77D
TP79R


Total eubacteria vs Metals
Metals (ppm)
120,000
100,000
80,000
60,000
40,000
20,000
0
1.00E+00
1.00E+02
1.00E+04
1.00E+06
1.00E+08
1.00E+10
Total eubacteria (cells/g)
TP75
TP76
TP78
TP77D
TP79R


alkB vs Metals
Metals (ppm)
120,000
100,000
80,000
60,000
40,000
20,000
0
0
10,000
20,000
30,000
40,000
50,000
60,000
70,000
80,000
90,000
100,000
alkB (cells/g)
TP75
TP76
TP78
TP77D
TP79R


almA vs Metals
Metals (ppm)
120,000
100,000
80,000
60,000
40,000
20,000
0
0
10,000
20,000
30,000
40,000
50,000
60,000
almA(cells/g)
TP75
TP76
TP78
TP77D
TP79R