2022 Water Mining Research Report

Dec 2023

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INTRODUCTION

This report summarizes progress for research projects that were active in 2022 related to improving the use and management of water by the Mining Subcommittee of Canada's Oil Sands Innovation Alliance (COSIA) Water Environmental Priority Area (EPA).

Please contact the Industry Champion identified for each research project if any additional information is needed.

The COSIA Water EPA Mining Subcommittee participants during the period of this report were: Canadian Natural, Imperial, Suncor Energy, Syncrude Canada Ltd. and Teck Resources Limited.

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- Canadian Natural
- Cenovus Energy Inc.
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- Imperial Oil Ltd.
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- Syncrude Canada Ltd.
- Teck Resources Limited

December 2023

Cover - Syncrude Research Associate Warren Zubot and Senior Technology Development Engineer Gail Buchanan

DEDICATION

The 2022 edition of the annual Water Mining Research Summary is dedicated to our friend and colleague Warren Zubot who passed away unexpectedly in late March 2023. Warren, who as a Senior Environmental Associate spend almost 20 years working on mine-water management and treatment at Syncrude Research and Development, retired in December 2022. In addition to being a good friend with a sharp wit and great sense of humour, he is remembered as a strong advocate for the application of sound science in assessing and tackling the environmental impacts of oil sands mining. He supplemented his education in chemistry and civil engineering with a Master's degree in Environmental Engineering under Dr. Mohamed Gamal El Din at the University of Alberta. As a founding member of the Water Environmental Priority Area of COSIA shortly after it was formed in October 2012, he was a passionate and vocal contributor to strategic planning and to the development and execution of water research projects for the oil sands mining sector. Outside of work, Warren enjoyed international travel, annual motorcycle trips to the southwestern United States, whitewater rafting and long walks in the evening after work. He also loved to share stories with friends and colleagues over a beer.

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NATURAL AND ANTHROPOGENIC INPUTS TO THE ATHABASCA WATERSHED

WE0057 - Metals versus Minerals: impacts of atmospheric dust deposition on the speciation of trace elements in snowmelt and peatland surface waters

COSIA Project Number: WE0057

Research Provider: University of Alberta

Industry Champion: Canadian Natural (CNRL)

Industry Collaborators: Imperial Oil Ltd., Suncor Energy Inc., Syncrude Canada Ltd., Teck Resources Limited

Status: Year 5 of 7

PROJECT SUMMARY

Open pit bitumen mining in northern Alberta generates considerable volumes of dust. The dusts are derived not only from the mines, but from wind erosion of dry tailings and gravel roads, construction activities, and quarries, in addition to natural sources such as river banks and sand bars. The dusts themselves consist mainly of mineral particles, some of which are chemically reactive (e.g., calcite, a calcium carbonate) whereas others are effectively insoluble (such as quartz, a silicate). There are also ongoing concerns about potentially toxic trace elements (TEs) being released to the atmosphere from bitumen mining and upgrading, but the extent of these emissions and their ecological significance is unclear. Most environmental impact studies to date have not clearly distinguished between TEs (such as cadmium and lead) from the combustion of fossil fuels needed for bitumen upgrading and TEs that are hosted within the crystal lattice of the mineral particles themselves. Trace elements that are emitted to the air during combustion at high temperatures tend to be very small (< 1 micron) and in soluble form (such as oxides), whereas mechanically-generated mineral dusts tend to be rather large (10 to 100 microns) and much less soluble (e.g., silicate minerals such as quartz and feldspar). Very small, soluble, metal-containing particles such as those from combustion may represent a threat to biota, depending on the pH of soil and water, and other factors, but large, insoluble particles such as mechanically-generated mineral dusts most likely do not. The main goal of this study is to clearly distinguish between these two sources of TEs to the air, using size-resolved analyses of snow and Sphagnum moss from bogs. The secondary objective is to understand what impact, if any, the two sources of TEs may have on the chemical composition of meltwater and peatland surface waters from bogs that drain into the Athabasca River.

PROGRESS AND ACHIEVEMENTS

Snow and dust (Dr. Fiorella Barraza)

Snow samples had been collected from five ombrotrophic peat bogs (MIL, JPH4, McK, McM, ANZ) within the industrial zone the Athabasca Bituminous Sands (ABS) region, and at a control site (UTK). These samples were used to develop an analytical method for the determination of TEs in dusty snow by Javed et al, 2022 (10.1039/d1ea00034a). To characterize the spatial variation in atmospheric deposition of TEs, snow was also collected along the Athabasca River and its tributaries (Firebag River, MacKay River, Muskeg River, Steepbank River, Clearwater River). In 2016, 25 sites were sampled (20 Athabasca River sites and five tributaries); in 2017, 14 sites (11 Athabasca River sites and 3 tributaries). In addition, snow was collected from three very remote locations: Athabasca Glacier, Maligne Lake and Rock Lake.

The industrial materials (e.g. coke and dry tailings) and natural materials (e.g. soil and glacial till) provided by our partners are potential sources of airborne dusts. These samples have been already processed (physical, mineralogical, and chemical analyses) by the Bioaccessibility project team.

Between March 2022 and February 2023 more analysis of the snow samples were done, including: (i) SEM-EDS analysis of the acid-insoluble fraction (>0.45 µm, after allowing melted snow to react with acid followed by filtration), (ii) quasi-total and «dissolved» (<0.45 µm) TEs in bulk snow, determined by ICP-MS, and (iii) total TEs in the particulate fraction (>0.45 µm), determined by ICP-MS and ICP-OES.

Chemical reactivity of dust in snow: acid-soluble and insoluble fractions (winter 2017)

The chemical reactivity and behaviour of the different TEs is variable. Compared to their quasi-total concentrations, the acid-soluble fraction ranged from 0.1% to 67% for the lithophile elements (including «mobile» elements such as Sr), from 1 to 20% for elements enriched in bitumen, and from 3 to 70% in the case of potentially toxic chalcophile elements. Cadmium is exceptional, given that the concentrations in the acid-soluble fraction were almost 100% of the total concentrations. Sites closest to industry did not always reflect the greatest extent of dust dissolution.

Selected filters containing insoluble dust particles were analyzed using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). Particles of various shapes and varying in size from less than 10 to approximately 200 µm, were identified. Large particles were more abundant in samples collected closer to the midpoint between upgraders than downstream of industry. The elemental composition provided by the EDS analyses suggests the presence of mineral hosts such as quartz, feldspars, micas, and clays. Taken together, the minerals which were readily identifiable are those which dominate the composition of the mineral fraction of the ABS. Of these minerals, quartz was most abundant at sites where the concentrations of TEs were very low (e.g. the sites downstream of industry). Sulfur was also found in samples collected close to the upgraders and this finding may be related to the piles of petcoke and elemental sulfur found in the area.

Quasi-total and «dissolved» concentration of TEs (winter 2016)

For the quasi-total concentration of TEs, unfiltered snow was digested with HNO3 at high pressure and temperature. The «dissolved» fraction was obtained after filtration of melted snow and acidification with HNO3.

Conservative, lithophile elements (Al, La, Th, Y) which serve as dust indicators, elements enriched in bitumen (Ni, Mo, V) as well as the potentially toxic chalcophile elements (As, Cd, Pb, Sb, Tl) were overwhelmingly found in the particulate fraction (70-100%): this component is operationally defined as the quasi-total concentration minus the «dissolved» fraction.

The «dissolved» concentrations were compared with the corresponding values for the waters sampled (in earlier studies) on the main stem of the Athabasca River in the spring, when snowmelt exerts its greatest influence. Overall, the concentrations of all TEs were less abundant in the snow compared to the river, except V at some sampling sites.

Total concentration of TEs in the particulate fraction (winter 2016 and 2017)

Filters containing the particulate fraction were digested with HNO3 and HBF4 as described by Javed et al, 2022.

Work with these samples is ongoing, and has been slow because of the very small size of the samples on the filters.

Major elements such as Al, Na, Ca, Mg, K, P, and S were analyzed using ICP-OES and we expect to receive this data soon. SEM and X-ray diffraction analysis will be performed in selected samples collected in 2016 to properly identify the mineral hosts of TEs and to compare them to the acid-insoluble dust particles.

Peat bog waters (Sundas Butt, candidate)

Moss porewaters, extracted from the upper 2-5 cm layer of Sphagnum moss (i.e. the green, living layer), and peat porewaters, collected at the surface of the bogs (JPH4, McK, McM, ANZ) from excavated pits (30-40 cm deep), were analyzed for TEs, over three consecutive years (2019-2021). Increases in dissolved concentration of TEs in peat bog waters relative to the decrease in distance from the mining area (both moss and peat porewaters) are seen mainly in regard to easily mobilized lithophile elements such as Li and Fe, providing evidence of the dissolution of dust. However, the presence of elevated concentrations of conservative lithophile elements such as Y and Th in waters near industry is surprising, given that they mainly occur in extremely stable minerals such as zircon and monazite which are not expected to dissolve in bog waters. The abundance of these elements in the dissolved fraction of peat bog waters near industry suggests the possibility of their existence as nano-sized dust particles.

To understand the distribution of TEs within the «dissolved» fraction (i.e. < 0.45µm) among ions or small molecules (i.e., «truly-dissolved» species (< 1 kDa)) and colloidal species (> 1 kDa), size-resolved TE analyses were performed using AF4-UV-ICPMS (Asymmetric flow-field flow fractionation coupled to a UV/Visible diode array detector and ICPMS). Peat bog waters used for this study were collected in autumn (2021) and kept unacidified.

«Truly-dissolved» fraction (<1 KDa)

Some TEs are primarily present in the "truly-dissolved" fraction of peat bog waters (moss and peat porewaters). These include some mobile lithophile elements (e.g. Li and Mn), bitumen-enriched elements (V and Ni), and potentially toxic TEs (As and Cd). Between 70-100% of the dissolved (<0.45 µm) concentration is present in this fraction (<1 KDa).

«Colloidal» fraction (>1 KDa to 0.45 µm)

In moss porewaters, the majority of TEs (such as Al, Ba, Pb, Sr, Th, V, Y) were predominantly associated with large (>600 kDa) and small inorganic colloids (5-100 kDa). The presence of large inorganic colloids in waters near mining activities may be an indication of nanosized dust particles. Conversely, in the peat porewaters, ionic species (Ba, Sr, V) and DOM-associated species (Al, Pb, Th, Y) were more common. The DOM-associated species include “truly dissolved” organic-TE complexes (< 1 kDa), colloidal complexes of Fe and Al (1– 1.5 kDa), and organo-mineral colloids (2–5 kDa). The differences in TE size distributions between moss porewaters and peat bog porewaters suggest that some fraction of dust dissolves in these acidic waters, resulting in the release of ions to the solution which may remain as ions in true solution, or form metal-organic complexes ranging in size from «truly dissolved» to colloidal species.

Overall, an increase in the concentration of the most lithophile elements (e.g. Li and Fe) in the «truly dissolved» fraction of peat bog waters is likely a reflection of dust dissolution. At sites near industry, As -a chalcophile element- also showed elevated concentrations in the «truly dissolved» fraction of moss porewaters, relative to the control site. However, the concentrations found (162-763 ng/l) are so low that they are unlikely to have any direct ecological relevance.

Sphagnum moss (Na Chen, M.Sc. thesis defended in April 2022)

The relative abundance of TEs found in the coarse and fine aerosol fractions was estimated by comparing TE concentrations in bulk Sphagnum moss and the acid soluble ash (ASA) fraction. An independent confirmation of the spatial trends observed (increasing concentrations towards the industry) was done by analyzing the acid insoluble ash (AIA) fraction. This work was completed between August and October 2022, after the M.Sc. thesis defense.

Sample collection and analysis

Sphagnum mosses had already been collected from 30 ombrotrophic (rain-fed) bogs within the ABS region in the autumn of 2015, prepared for chemical analysis, and frozen. Additional samples were collected in 2019 and 2020, from the five bogs where snow samples had been collected, as well as UTK (the reference site). Total concentrations of TEs in moss were determined by digesting dried samples using a mix of concentrated HNO3 and HBF4, following the method developed in our lab (Javed et al, 2020: dx.doi.org/10.1139/cjss-2020-0001). The ASA fraction was obtained by leaching ash samples in high purity, 2 % nitric acid (pH 0.5) for 15 minutes before filtering through a 0.45 µm PTFE filter. The AIA fraction was obtained by digesting the residue remaining on the PTFE filters using the above-mentioned method. All TE concentrations were obtained using ICP-MS.

Results

The ash content of Sphagnum moss clearly increased towards industry, consistent with our previously published work on the same topic. In the present study, as expected, total concentrations of conservative, lithophile elements such as Al, Y and Th increased towards industry, along with metals found in bitumen (V, Ni, and Mo) and elements of concern (Pb, Sb, and Tl). With the exception of Mo, the concentrations of these elements in the ASA fraction also showed obvious or slightly increasing trends with distance toward industry. Within this acid-soluble fraction, these elements correlated strongly or moderately with acid-soluble Y, suggesting a connection to mineral dissolution. The proportion of TEs that were acid soluble is dichotomous: the soluble proportion was high for Al, Y, V, and Ni, but low for Th, Mo, Pb, Sb, and Tl. This indicates that the former elements might be largely contributed by ultrafine clay minerals such as kaolinite and illite which react in acid because of their very small size, while the latter perhaps occur in larger mineral grains such as feldspars and heavy minerals (e.g., monazite, zircon) which are more stable. Silver and Cd exhibit behaviours more like micronutrients such as Cu and Zn, suggested that these were impacted by plant uptake in addition to mineral dust deposition.

Comparing the ASA (surrogate for fine aerosols) and AIA fraction (representing coarse aerosols) from the four bogs near industry it is clear that some elements such as Al, Y, V and Ni were more abundant in the ASA fraction. By contrast, Mo, Pb, Sb, Tl, Ag, and Cd were more abundant in the AIA fraction. Most of the elements are less abundant in the AIA fraction than typical rocks at the surface of the earth i.e. the concentrations in AIA are below the average values for the Upper Continental Crust (UCC). In other words, a large part of the coarse dust being generated by industry is actually “clean” in regard to TEs. The exceptional elements are Pb and Sb (similar or slightly enriched, relative to the UCC), as well as Ag and Cd. Silver and Cd are enriched in the AIA by 100x or more. However, the anomalous behaviour of these two elements, and their considerable enrichment in ancient (pre-anthropogenic) aerosols, relative to crustal rocks, is well known.

Additional research on higher plants

Trace elements were also investigated in the leaves of the Pitcher Plant which has been identified as culturally important by Indigenous communities across Canada. Collected from fens and bogs in the ABS region, concentrations of potentially toxic TEs were low and not indicative of any toxicological concerns. However, lithophile elements provided clear dust signatures in plant tissues related to distance from roads and surface mines, even after the leaves were washed. Elements associated with fugitive dust and bitumen extraction (e.g. V and Ni) declined exponentially with increasing distance from a surface mine, following a well-established regional pattern. The study illustrates the importance of including both chalcophile as well as lithophile elements in any effort to document anthropogenic inputs to terrestrial ecosystems. (Dennett et al, submitted).

LESSONS LEARNED

Snow

The results obtained for the acid-soluble fraction of snow (winter 2017) do not reflect the chemical reactivity that would occur in nature when the dusts are in contact with far less acidic solutions. For example, the surface waters of the AR exhibit pH values which are mainly between 7.2 (in autumn) and 8.4 (in spring). In contrast, the pH of the nitric acid leaching solution was <1. Despite this huge difference in pH (more than 6 orders of magnitude), we found that: (i) in the autumn, Mo is more abundant in the dissolved fraction of the AR, compared to the acid leachate, (ii) As and Sb are similar to the river water values, (iii) several TEs of environmental concern are actually more abundant in the dissolved fraction of the AR: for example, Cd and Ni are approximately 3x more abundant in the river, Tl 6x, and V 20x, compared to the acid leachate. Therefore, even if dusts were to be leached with strong mineral acids not found in nature, even then they are unlikely to contribute significantly to the burden of dissolved As, Cd, Ni, Sb, and Tl in the river.

Lead in the acid soluble fraction of snow is 100 x more abundant than the dissolved fraction of the river (Barraza et al, in preparation), and this indicates the importance of undertaking some leaching experiments using a more realistic pH range (e.g. pH 4 as in bog waters).

Peat bog surface water

This study illustrates the importance of quantifying TEs in the «truly dissolved» fraction of water, as it provides direct evidence of dust dissolution and also identifies the bioavailable forms of elements released. In the ABS region, dust dissolution increases the concentration of lithophile elements in the «truly dissolved» fraction of peat bog waters, some of which are essential micronutrients (i.e., Mn, Mo, Ni) for plants.

Sphagnum moss

The study found that the ASA fraction of moss ash, taken to represent the fine aerosol fraction, contained two classes of elements: those exhibiting a large proportion in acid-soluble forms (e.g. Al, Y, V, and Ni), and those with a small proportion in such forms (e.g. Th, Mo, Pb, Sb, and Tl). Thus, the relative bioaccessibility of elements of concern (namely Pb, Sb and Tl) is much lower than that of the elements representing aluminosilicates (i.e. Al and Y) or bitumen (i.e. V and Ni).

Taken together, these studies present a clear illustration of the need to determine not only the concentrations of TEs in surface waters, including peatland waters, but also (and perhaps especially) the chemical reactivity of TEs in aerosols and dusts, to thoroughly evaluate their associated health risks for living organisms.

PRESENTATIONS AND PUBLICATIONS

Journal Publications:

Published

Shotyk, W., 2022. Environmental significance of trace elements in the Athabasca Bituminous Sands: facts and misconceptions. Environmental Science: Processes & Impacts 10.1039. D2EM00049K. https://doi.org/10.1039/D2EM00049K

Submitted, under review

Dennett, J.M., Dersch, A., Chipewyan Prairie First Nation, Barraza, F., Shotyk, W., Nielsen, S.E. Trace elements in the culturally significant plant Sarracenia purpurea in proximity to dust sources in the oil sands region of Alberta, Canada. Science of the Total Environment (revised version, in review) on February 28, 2023.

Shotyk, W., Barraza, F., Cuss, C.W, Grant-Weaver, I., Germani, C., Javed, M.B., Hillier, S., Noernberg, T. Natural enrichment of Cd and Tl in the bark of trees from a rural watershed devoid of point sources of metal contamination. Environmental Research (submitted March 13, 2023).

In preparation

Barraza, F., Javed, M.B, Noernberg, T., Schultz, J., Shotyk, W. Evaluating the chemical reactivity of dusts from mining and upgrading of the Athabasca Bituminous Sands (ABS) in Alberta, Canada using trace elements in snow. Environmental Pollution.

Barraza, F., Noernberg, T., Schultz, J., Shotyk, W. Temporal and spatial variation of acid-soluble trace elements from dust deposition in snow within the Athabasca Bituminous Sands (ABS) region in Alberta, Canada.

Barraza, F., Noernberg, T., Schultz, J., Pelletier, R., Shotyk, W. Metals vs Minerals: Trace elements in particulate and sub-micron aerosols deposited on snow near the Athabasca Bituminous Sands (ABS) region in Alberta, Canada.

Barraza, F., Zheng, J., Krachler, M., Krebs, M., Chad W. Cuss, Oleksandrenko, A., Shotyk, W. Uptake of cadmium by plants and natural enrichment in soils and aerosols derived from them.

Barraza, F. et al. Size-resolved fractionation of dissolved trace elements (< 0.45 µm) into colloidal (1 kDa to 0.45 µm) and ionic (< 1 kDa) forms in snow from the Athabasca Bituminous Sands (ABS) region in Alberta, Canada.

Chen, N., Barraza, F., Belland, R., Javed, M.B., Grant-Weaver, I., Chad, C.W., Shotyk, W. Trace elements in the acid soluble ash fraction of Sphagnum moss: Surrogate for atmospheric deposition of sub-micron aerosols within the Athabasca Bituminous Sands region.

Environmental Science, Atmospheres (rejected by ES&T, the paper is now being revised for this new RSC journal).

Butt, S., Barraza, F., Devito, K., Frost, L., Javed, M.., Oleksandrenko, A., Shotyk. W. Butt, S., Barraza, F., Frost, L., Javed, M.B., Oleksandrenko, A., Shotyk, W. Spatio-temporal variations in the dissolved concentration of trace elements in peat bog porewaters impacted by dust inputs from open-pit mining activities in the Athabasca Bituminous Sands (ABS) region.

Shotyk, W., Barraza, F., Butt, S., Chen, N., Cuss, C.W., Devito, K., Frost, L., Grant-Weaver, I., Javed, M.B., Noernberg, T. Trace elements in peat bog porewaters: indicators of dissolution of atmospheric dusts and aerosols from natural and anthropogenic sources.

Environmental Science, Water Research and Technology (rejected by ES&T, the paper is now being revised for this new RSC journal).

Conferences, presentations, and workshops:

Presented

Barraza, F., Krebs, M. 2022. Trace element bioaccessibility and bioavailability in the environment of the ABS region: insights from the acid-soluble fraction of snow, and experimental studies with synthetic gastric fluids. 2022 COSIA Mine Water Management Science Workshop. Edmonton, Canada. October 18-19, 2022 (in person, invited).

Barraza, F. 2022. Workshop: Trace element analysis of soil, water, and plants: challenges and strategies. 46 National Congress of Innovation in Soil Sciences and Healthy Soils for Sustainable Development. Saltillo, Mexico. October 3-4, 2022 (online, invited).

Barraza, F., Chen, N., Javed, M.B., Noernberg, T., Shotyk, W. 2022. Evaluating the chemical reactivity of dusts from mining and upgrading of the Athabasca Bituminous Sands (ABS) in Alberta, Canada using trace elements in moss and snow. https://doi.org/10.46427/gold2022.10642

Goldschmidt 2022. Session 13f: «Geochemical processes related to mined, milled, or natural metal deposits in a rapidly changing global environment». July 10-15, 2022 (online).

Barraza, F., Shotyk, W. Workshop: Trace element analysis of soil, water, and plants: challenges and strategies. 2022 Joint Canadian Society of Soil Science (CSSS) Annual Meeting-Alberta Soil Science Workshop (ASSW). Edmonton, Canada. May 23-27, 2022 (in person, invited).

Butt, S., Barraza, F., Frost, L., Javed, M.B., Oleksandrenko, A., Wang, Y., Shotyk, W. Impact of dust dissolution on the dissolved fraction (<0.45 µm) of trace elements (TEs) and their size-based distribution in peatland waters in the Athabasca Bituminous Sands Region (ABSR). ALES (Agricultural, Life & Environmental Sciences) Research Symposium. University of Alberta Graduate Students' Association. March 28 and 29, 2023. Edmonton, Canada. Oral presentation

Shotyk, W. Trace element bioaccessibility and bioavailability in the environment of the ABS region: perspectives from the acid-soluble ash (ASA) fraction of Sphagnum moss and peat bog surface waters. 2022 COSIA Mine Water Management Science Workshop. Edmonton, Canada. October 18-19, 2022 (in person, invited).

Shotyk, W. Trace Elements in the Lower Athabasca River Watershed: Facts and Misconceptions. Alberta Institute of Agrologists, Annual General Meeting, Banff Springs Hotel, October 31 - November 1, 2022.

Shotyk, W., Barraza, F., Butt, S.A., Chen, N., Cuss, C.W., Devito, K., Frost, L.V., Grant-Weaver, I., Javed, M.B. and Noernberg, T. Trace elements in peat bog surface waters and Sphagnum moss porewaters: indicators of dissolution of atmospheric dusts from open pit mining and related industrial activities. Goldschmidt Hawaii Conference, July 15, 2022.

Shotyk, W. Soil: the key to understanding trace elements in air, water, and plants, from source assessment to ecological significance. Canadian Soil Science Society, Edmonton, May 24, 2022. Plenary Lecture.

Accepted abstracts

Barraza, F., Javed, M.B., Schultz, J., Noernberg, T., Shotyk, W. Trace elements in particulate and sub-micron aerosol fractions in snow from the Athabasca Bituminous Sands (ABS) region in Alberta, Canada. 1st Joint International Conference of 16th International Conference of Biogeochemistry of Trace Elements (ICOBTE) and 21st International Conference of Heavy Metals (ICHMET). Special Session 6 «Critical Metals in Mine Wastes, Soil and Water». September 6-10, 2023. Wuppertal, Germany. For poster.

Shotyk, W., Barraza, F., Butt, S., Chen, N., Dennett, J., Frost, J., Oleksandrenko, A., Wang, Y., Grant-Weaver, I., Noernberg, T., Javed, M.B., Cuss, C.W., Devito, K. Trace elements in peat bog surface waters and plant fluids: indicators of dissolution of natural and anthropogenic dusts and aerosols. 1st Joint International Conference of 16th International Conference of Biogeochemistry of Trace Elements (ICOBTE) and 21st International Conference of Heavy Metals (ICHMET). Regular Session 9, Sub-session: «Atmospheric deposition and its role over the centuries in soil pollution with potentially toxic elements». September 6-10, 2023. Wuppertal, Germany. For Keynote speaker (invited), to be presented by Barraza, F.

RESEARCH TEAM AND COLLABORATORS

Institution: University of Alberta

Principal Investigator: William Shotyk

Name	Institution or Company	Degree or Job Title	Degree Start Date (For Students Only)	Expected Degree Completion Date or Year Completed (For Students Only)
Fiorella Barraza	University of Alberta	PhD, Postdoctoral Fellow		
Sundas Butt	University of Alberta	PhD candidate	January 2020	April 2024

WE0072 - Bioavailability and bioaccessibility of trace elements in natural and industrial particles of the Lower Athabasca River watershed

COSIA Project Number: WE0072

Research Provider: University of Alberta

Industry Champion: Canadian Natural (CNRL)

Industry Collaborators: Imperial Oil Ltd., Suncor Energy Inc., Syncrude Canada Ltd., Teck Resources Limited

Status: Year 3 of 3

PROJECT SUMMARY

There is ongoing concern regarding the potential environmental impacts and ecological significance of trace elements (TEs) from open pit bitumen mining and upgrading. Total concentrations of TEs in environmental media have often been interpreted as having biological significance, regardless of the chemical form of the element. This has led to many misunderstandings and concern that may often be exaggerated. The overall objective of this work is to examine, understand, and communicate the risks to the health of aquatic ecosystems represented by TEs associated with industrial particles. This includes by-products and residua of bitumen mining and upgrading (dry tailings, coke, soil, and overburden) that may be added to the Athabasca River by wind and water erosion. The particles will be reacted in synthetic gastrointestinal fluids to estimate bioaccessibility and bioavailability to invertebrates, fish and other aquatic life. As a control for this approach, the gradient in bioavailability and bioaccessibility of TEs represented by natural particles suspended in the Athabasca River will be examined at selected locations. We hypothesize that bioavailability of TEs is constant, because there is no significant change in pH along this gradient. In contrast, bioaccessibility of TEs increases with distance downstream because of the increasing abundance of small particles. To provide additional perspective and context for the main thrust of the study, selected samples representative of urban runoff, as well as the dominant rock-forming minerals of the earth's crust (plagioclase feldspar, potassium feldspar, mica, quartz, and calcite) will also be analyzed.

PROGRESS AND ACHIEVEMENTS

Sample preparation and characterization

Particle size is an important factor influencing the dissolution rate of minerals; in general, the dissolution rate increases with a decrease in particle size. Specifically, the dissolution rate is proportional to the surface area of the mineral. Furthermore, clays tend to concentrate TEs due to their negative surface charge and high surface area. The materials provided by our industry partners, including soils, tailings, etc., have thus been separated according to their size by wet sieving into four different size fractions: medium sand (+250 µm), fine sand (+64 µm), silt (+10 µm), and silt/clay (-10 µm) using in-house designed and fabricated, acid-cleaned plastic sieves. Minerals have been characterized using scanning electron microscopy (identification of sand- and silt-sized particles) and x-ray diffraction (identification of clay-sized minerals).

The results show that quartz is most often the dominant mineral, followed by feldspars and mica. For the clay-sized fraction, clay minerals make up a significant portion.

In vitro digestive fluid extraction – bioaccessibility determination

While there is a considerable literature on bioaccessibility of TEs in particulate matter that have employed synthetic gastrointestinal fluids, most studies to date have focused on human bioaccessibility. While the gastrointestinal (GI) tract of humans and other vertebrates, including fishes, share many similarities there are also important differences. Therefore, to ensure that our experimental design is applicable to aquatic life, a comprehensive literature review on fish physiology, fish digestive processes and enzymes, and in vitro digestive fluid extraction of fishes was performed. Based on this review, the following experimental parameters have been selected: 1) two simulated gastric fluid (SGF) formulations were selected, HCl and HCl + pepsin, as these occur in the GI tract of aquatic organisms and have low background levels of TEs which allows the determination of the maximum number of elements (including major elements which allow the nature of the dissolution reactions taking place in the GI tract of aquatic organisms); 2) two experimental pH values, 2 and 4, as the pH of gastric fluid in fish typically ranges between 2 and 4; 3) two temperatures, including 8 °C (to mimic fall and spring) and 20 °C (to mimic summer), with TE bioaccessibility expected to increase with temperature; 4) durations of 16 hours (at 20 °C) and 36 hours (at 8 °C) to reflect differences in gastric residence time, which is highly dependent on temperature; and 5) two different solid:fluid ratios that represent different fish species (1:60 for carnivorous fish that generally ingest sediment incidentally and a ratio of 1:10 for benthic fish that take up significant amounts of sediment with their food). It is important to note that a large number of fish species (~20-25%) are agastric, i.e. they lack a true stomach. Consequently, these fish do not secrete HCl and any experiments carried out with SGFs do not apply. This is an important consideration for the Athabasca River which has two abundant agastric, benthic species: the Longnose Sucker and the White Sucker. To account for these and other agastric species as well as invertebrates, which also lack a stomach, experiments using simulated intestinal fluids (SIFs) will also be carried out. For the SIFs, the final formulations are still to be determined as preliminary experiments have shown relatively high background levels of TEs.

To date, all materials have been leached in in vitro lab experiments using dilute HCl as the SGF and a pH of 2 at ambient temperature for 16 hours using a solid:fluid ratio of 1:60. A subset of samples has also been leached using HCl + pepsin as the SGF instead, and another using a solid:fluid ratio of 1:10. To determine the "quasi total" concentrations of TEs in the starting materials, all samples of particulate matter were also digested in concentrated nitric acid using the elevated temperatures and pressures provided by the Ultraclave microwave digestion system. "Quasi total" concentrations differ from total concentrations in that no HF is used in the digestion procedure: thus, the most refractory silicate minerals are not dissolved. Using the data obtained from in vitro experiments and the "quasi total" concentrations obtained from digestion in concentrated nitric acid and elevated T and P, bioaccessibility can be calculated for each element as
Bioaccessibility (%) = (extractable TE concentration/"quasi total" TE concentration) x 100.

The results of the in vitro experiments of the bulk samples (< 2 mm) show a wide range in TE concentrations and bioaccessibility: both the materials studied and the elements analyzed show very different degrees of reactivity. Lead, for example, yielded bioaccessibility values ranging from 1 – 22%. This variability is also apparent in the "quasi total" concentrations of TEs in the materials investigated. For most materials, the "quasi total" concentrations are below the average crustal concentrations. Both the bioaccessible fractions and "quasi total" concentrations tend to increase with a decrease in particle size. Experiments with HCl + pepsin as SGF have yielded results similar to those with HCl alone as SGF. Using higher solid:fluid ratios resulted in significant shifts to higher pH values and consequently lower TE concentrations. The pH change in this case reflects acid neutralization via mineral dissolution, and we assume the pH rise leads to the hydrolysis of metal ions and loss from solution through their precipitation, illustrating the importance of pH control during the experiments.

LESSONS LEARNED

Thoughtfully designed experiments under carefully controlled laboratory conditions, combined with high quality analyses point toward limited bioaccessibility of TEs in gastric fluids. The wide range in bioaccessibility for the different materials and elements obtained in this study is further evidence of the disconnect between total concentrations and environmental risk of TEs in the lower Athabasca River watershed, reinforcing the need to consider bioaccessibility in any risk assessments. Our results include three main findings. First, ”quasi total” concentrations of TEs in the investigated by-products and residua of bitumen mining and upgrading, such as dry tailings, are generally below or at crustal levels, for all particle sizes. Second, *in vitro* experiments show that bioaccessibility is independent of particle size. Third, samples leached *in vitro* have low concentrations of TEs, regardless of particle size. Thus, for the experiments to succeed, it is essential to have low background levels of TEs in the simulated digestive fluids. Further, it is vital to obtain experimental data on the bioaccessibility of as many trace elements as possible, for two reasons. First, some TEs have indirect toxicity due to bioaccumulation, making their analytical determination important regardless of the concentrations found after leaching *in vitro*. Second, the greater the number of TEs quantified, the easier it will be to constrain the nature of the dissolution reactions taking place in the digestive tract. Therefore, we are currently working on a method to purify the reagents used in the preparation of the simulated digestive fluids.

PRESENTATIONS AND PUBLICATIONS

Journal Publications:

Published

Shotyk, W. (2022) Environmental Significance of Trace Elements in the Athabasca Bituminous Sands: Facts and Misconceptions. Environmental Science: Processes and Impacts. Environ. Sci.: Processes Impacts, 24, 1279-1302. Invited paper.

Shotyk, W., Bicalho, B., Cuss, C.W., Donner, M.W., Grant-Weaver, I., Javed, M.B. and Noernberg, T. (2021) Trace elements in the Athabasca Bituminous Sands: a geochemical explanation for the paucity of environmental contamination by chalcophile elements. Chem Geol 581, 120392. Invited paper, Special Issue in honour of Prof. J.D. Kramers. https://doi.org/10.1016/j.chemgeo.2021.120392

In preparation

Krebs, M.Y. Application of in vitro fluid digestion methods for estimating the bioaccessibility of sediment-associated contaminants to aquatic organisms: A review.

Conferences, presentations, and workshops:

Presented

Barraza, F. & Krebs, M.Y. Trace element bioaccessibility and bioavailability in the environment of the ABS region: insights from the acid-soluble fraction of snow, and experimental studies with synthetic gastric fluids. COSIA Mine Water Management workshop, Edmonton, Alberta, Oct 18-19, 2022, Oral presentation.

Shotyk, W. Trace element bioaccessibility and bioavailability in the environment of the ABS region: perspectives from the acid-soluble ash (ASA) fraction of Sphagnum moss and peat bog surface waters.

2022 COSIA Mine Water Management Science Workshop. Edmonton, Canada. October 18-19, 2022 (in person, invited).

Shotyk, W. Trace Elements in the Lower Athabasca River Watershed: Facts and Misconceptions. Alberta Institute of Agrologists, Annual General Meeting, Banff Springs Hotel, October 31 - November 1, 2022.

Shotyk, W., Barraza, F., Butt, S.A., Chen, N., Cuss, C.W., Devito, K., Frost, L.V., Grant-Weaver, I., Javed, M.B. and Noernberg, T. Trace elements in peat bog surface waters and Sphagnum moss porewaters: indicators of dissolution of atmospheric dusts from open pit mining and related industrial activities. Goldschmidt Hawaii Conference, July 15, 2022.

Shotyk, W. Soil: the key to understanding trace elements in air, water, and plants, from source assessment to ecological significance. Canadian Soil Science Society, Edmonton, May 24, 2022. Plenary Lecture.

RESEARCH TEAM AND COLLABORATORS

Institution: University of Alberta

Principal Investigator: William Shotyk

Name	Institution or Company	Degree or Job Title	Degree Start Date (For Students Only)	Expected Degree Completion Date or Year Completed (For Students Only)
Mandy Krebs	**University of Alberta**	**Postdoctoral Fellow**		
Judy Schultz	**University of Alberta**	**Research Associate**		

OIL SANDS PROCESS-AFFECTED WATER CHEMISTRY AND TOXICITY

WJ0116 - Development of Microbial Fuel Cell Biosensor for Detection of Naphthenic Acids

COSIA Project Number: WJ0116

Research Provider: University of Alberta

Industry Champion: Imperial Oil Ltd.

Industry Collaborators: None

Status: In progress

PROJECT SUMMARY

Naphthenic acids are considered as major contributors to acute toxicity in oil sands process-affected water (OSPW). Monitoring NAs in water samples from tailing ponds, nearby groundwater wells, and surrounding surface water is essential due to their potential seepage from oil sands tailings ponds into surrounding water sources. Current analytical techniques for NA quantification, such as Fourier transform infrared spectroscopy, gas chromatography-mass spectrometry, and high-performance liquid chromatography-mass spectroscopy, are time-consuming, resource-intensive, and expensive, requiring samples to be sent to a lab for analysis. Consequently, this project aims to develop a microbial electrochemical biosensor that can quickly detect NAs in water samples on site. Briefly, microbial electrochemical biosensors use electroactive bacterial biofilms serving as biosensing elements to produce an electrical signal in response to an analyte, which can be a target environmental contaminant. The electrical signal can be linked to the concentration of the target analyte. In recent years, different configurations of microbial electrochemical biosensors have been demonstrated for various environmental contaminants. With its low fabrication costs, simple configuration, and rapid response time, a microbial electrochemical biosensor can provide a potential solution for the on-site measurement of NAs in water samples.

In this project, Dr. Dhar's lab at the University of Alberta has been developing a microbial electrochemical biosensor for detecting NAs in OSPW. These specific goals and timelines of the project are as follows:

- Conducting proof-of-concept tests using microbial electrochemical biosensors to detect model NAs in water samples (2017-2018, completed)
- Understanding the impact of various environmental factors such as salinity, temperature, and petroleum hydrocarbon compounds on the biosensor's response with model NA compounds (2018-2020, completed)
- Investigating different electrochemical methods to improve calibration precision using cyclohexane carboxylic acid and commercial NAs (2020-2021, completed)
- Exploring the possibility of using an exogenous quorum sensing (QS) autoinducer to enhance the biosensor's sensitivity to detect commercial NAs (2021, completed)
- Investigating the potential of the microbial electrochemical biosensor as a dual platform for detecting both NA concentrations and their associated toxicity (2021, completed)
- Examining the long-term performance of the microbial electrochemical biosensor for detecting NA concentrations in real OSPW samples (2022-2023, in progress)

- Developing machine learning (ML) algorithms to analyze biosensor response under the interference of complex water matrix and environmental conditions (to be completed).
- Developing a miniaturized version of the microbial electrochemical biosensor to enable its use in the field (to be completed).

PROGRESS AND ACHIEVEMENTS

Investigation with real OSPW sample (2022)

A significant portion of our research focused on exploring the sensing capability of the biosensor applied for real OSPW samples for its practical application. Similar to biosensor calibration with model NA compounds (*reported in previous reports*), we first determined an adequate charging time for the charging-discharging (CD) operation by comparing multiple charging times (5 – 20 minutes) due to the complexity of OSPW samples (Fig. 1a-d). The optimal charging time was determined to be 15 minutes, which produced the highest transient peak currents with the highest sensitivity for the real OSPW sample (Fig. 1e). Further increasing the charging time to 20 minutes led to a slight decrease in the peak current and the use of a higher charging time will result in a longer detection time.

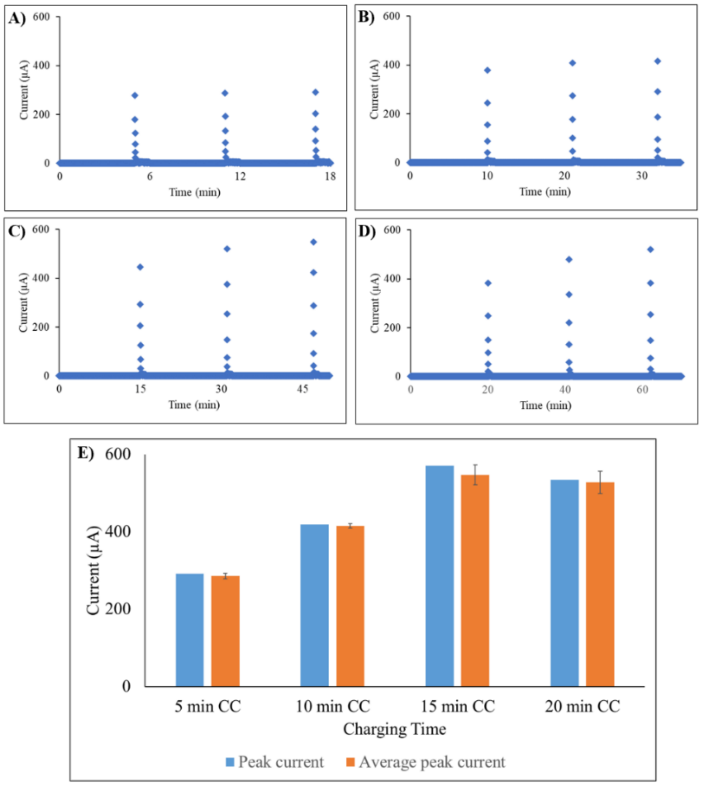

Fig. 1. *The performance of biosensor with real OSPW (without dilution, i.e., 100% OSPW) based on different charging times; a) 5 minutes; b) 10 minutes; c) 15 minutes; d) 20 minutes; e) a summary figure.*

Construction of calibration curve for OSPW biosensor (2022-Present). After determining the adequate charging time for CD operation, we constructed a calibration curve for our OSPW biosensor using 15-minutes charging time. In this phase, we recorded the peak currents produced from diluted OSPW samples (10-80%) (Fig. 2); the dilution of the OSPW sample would also dilute the initial concentration of NAs (24.9 mg/L). The results showed a linear relationship (R2=9202) between the % of OSPW and peak currents. Our results indicated our OSPW biosensor could be implemented to detect NAs in OSPW samples with a dilution of up to 10%.

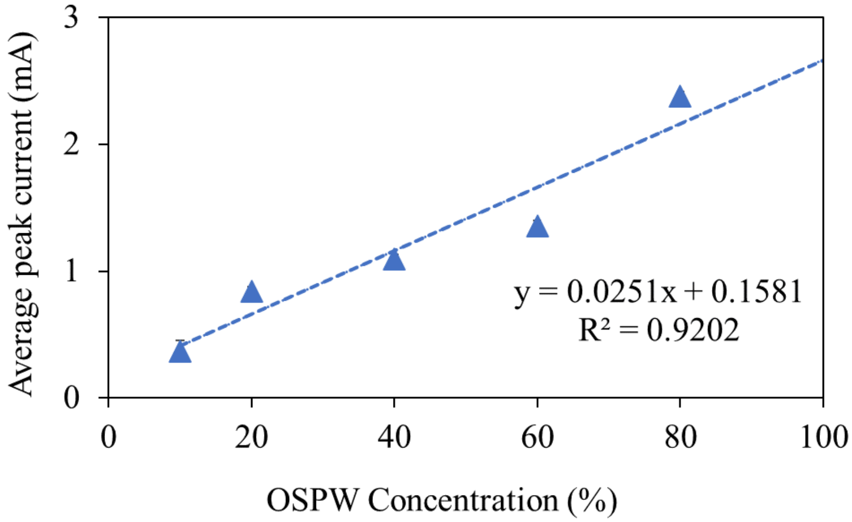

Fig. 2*. A calibration curve established based on transient peak currents from CD cycles operated with real OSPW samples. 100% indicates the real OSPW without any dilution.*

LESSONS LEARNED

Results with real OSPW samples have been promising and suggested our microbial electrochemical cell-based biosensors can be utilized as a simple bioanalytical tool for monitoring NA concentrations in OSPW samples. Our biosensor could also potentially be deployed in the field for on-site monitoring of NA concentrations in water streams around the oil sand tailings region. Additional research is required to overcome the current challenges of NA biosensors applied for real OSPW samples. The retardation of the enrichment process with OSPW as feedstock is a major hurdle considering a highly complex matrix of OSPW samples. The presence of more complex NA compounds compared to commercial and model ones makes it more difficult for the microbes to break down. The team is working to address these challenges.

PRESENTATIONS AND PUBLICATIONS

Journal Publications:

Published

Chung, T. H., Zakaria, B.S.; Meshref, M. N., & Dhar, B. R. (2021). Enhancing quorum sensing in biofilm anode to improve biosensing of naphthenic acids, emphasis on aromatic compounds. Biosensors and Bioelectronics, 210, 114275.

Chung, T. H., Meshref, M. N., & Dhar, B. R. (2021). A review and roadmap for developing microbial electrochemical cell-based biosensors for recalcitrant environmental contaminants, emphasis on aromatic compounds. Chemical Engineering Journal, 130245.

Chung, T. H., Meshref, M. N., & Dhar, B. R. (2020). Microbial electrochemical biosensor for rapid detection of naphthenic acid in aqueous solution. Journal of Electroanalytical Chemistry, 873, 114405.

Conferences, presentations, and workshops:

Presented

Chung, T.; Zakaria, B.S.; Dhar, B.R. (2019). Development of microbial electrochemical cell as a rapid biosensor for the detection of naphthenic acids, 11th Western Canadian Symposium on Water Quality Research, May 10, Edmonton, AB, Canada.

Chung, T.; Zakaria, B.S.; Dhar, B.R. (2019). Calibration of bio-electrochemical naphthenic acids sensor using electrical response from charging-discharging cycles, poster presented in Canada's Oil Sands Innovation Alliance (COSIA) Innovation Summit, June 7-8, Calgary, Alberta.

Barua, S.; Zakaria, B.S.; Dhar, B.R. (2018). Development of a self-powered biosensor for real-time monitoring of naphthenic acids, poster presented in Canada's Oil Sands Innovation Alliance (COSIA) Innovation Summit, June 7-8, Calgary, Alberta.

Barua, S.; Zakaria, B.S.; Dhar, B.R. (2018). Development of bioelectrochemical sensing device for naphthenic acids, poster presented in 53rd Central Canadian Symposium on Water Quality Research, February 22, Toronto, ON, Canada.

RESEARCH TEAM AND COLLABORATORS

Institution: University of Alberta

Principal Investigator: Dr. Bipro Dhar

Current list of trainees:

Name	Institution or Company	Degree or Job Title	Degree Start Date (For Students Only)	Expected Degree Completion Date or Year Completed (For Students Only)
Tae Chung	University of Alberta	PhD Student	2021	2025
Dr. Basem Zakaria	University of Alberta	Postdoctoral fellow	2022	2024

WJ0168 - Protecting the Athabasca River Basin: Bacterial Biosensors for Detection and Bioremediation of Oil Sands Process-Affected Water (OSPW)

COSIA Project Number: WJ0168

Research Provider: Athabasca University / Dr. Shawn Lewenza

Industry Champion: Canadian Natural (CNRL)

Industry Collaborators: None

Status: Year 5 of 5

PROJECT SUMMARY

The project has advanced a novel detection and quantification system for naphthenic acids (NAs). There is infrastructure in place around tailings ponds to collect seepage water and to prevent leakage of oil sands process water (OSPW) from the mine site. No untreated OSPW is released from the mine sites. Releasing OSPW from the mine sites in the future will require regulatory approval. It will be necessary to ensure all water quality criteria is met, including potential guidelines pertaining to NAs. Current analytical chemistry methods exist to determine the concentration of NAs in OSPW. These methods require complex sample extraction methods and either an FTIR analyzer or a sophisticated and expensive mass spectrometers to detect and characterize the compounds present in acid extracts. These methods are slow and not ideal for high-throughput sample measurement. Current methods are costly and the method proposed is expected to be far more cost effective. The previous *E. coli* biosensors can detect NA that are mostly commercially available acyclic compounds. There is a need for biosensors to detect a wide range of compounds and ideally those common in OSPW.

Biosensors are engineered bacteria that can be used as an alternative assay to indicate the presence of NA in water. A biosensor technology has been developed with applications in environmental monitoring and for rapid identification of novel bacterial strains that can degrade naphthenic acids. The researchers have established a process using bacterial genomics to identify how bacteria sense and respond to naphthenic acids, as well as a synthetic biology approach to rapidly construct naphthenic acid biosensor strains. The availability of these biosensors will enable rapid, cost-effective testing and monitoring of OSPW storage ponds and surrounding environments.

The biosensors permit the rapid identification of novel bacterial isolates that can degrade naphthenic acids. Novel strains and genes have been identified that degrade simple mixes of acyclic and single ringed NA compounds. The high throughput screening will be soon carried out to identify bacterial genes required to degrade NA extracted from OSPW. This novel strain and genetic information will increase our understanding of how bacteria degrade NA, which can be used to optimize bioremediation strategies.

PROGRESS AND ACHIEVEMENTS

Deliverables/Milestones

Year 1: Printing DNA promoters and molecular biology construction of naphthenic acid biosensors

A panel of 62 promoters (on/off switches) were shown to be induced by naphthenic acids in a Pseudomonas bacterial isolate recovered from OSPW. The promoters were printed using synthetic biology and cloned as transcriptional lux (bioluminescence) reporters on plasmids. All strains were tested for their ability to produce luminescence from these promoters in response to diverse naphthenic acid compounds and mixtures. From this panel, three promoters were chosen for further study (Steve Shideler).

Year 2: Validation of naphthenic acid biosensors: specificity and sensitivity.

This work was completed by Tyson Bookout, which formed the basis of a patent and is currently being prepared as a manuscript to be submitted for publication this summer. A summary of this work was presented to Canadian Natural in a recent progress presentation on March 8, 2022.

NA biosensor features:

- Detection of acyclic naphthenic acids compounds with the atuA-lux biosensor, with a lower limit of detection of ~15mg/L and an upper limit of >500 mg/L.
- Detection of naphthenic acid extracts from OSPW with the marR-lux biosensor, with a lower limit of detection of ~15 mg/L. This sensor detects complex naphthenic acid compounds, including those with rings containing nitrogen
- Detection of classic naphthenic acid compounds with the 3,680-lux biosensor, with a lower limit of detection of ~1 mg/L.
- All biosensors respond in a dose-dependent manner, with luminescence production linearly proportional to NA concentration. This supports the use of standard curves and quantitation of NA in a sample.
- All biosensors can detect NA in large panel of OSPW extracts and water samples.

The biosensors generated from this work have also been described in a patent (details below). The submission of the patent forced us to withhold our publication to avoid any disclosure. Now the patent is submitted, the work will be submitted for publication this summer/2023.

The overall goal of using this new biotechnology as an alternative approach to measure naphthenic acids has been successful and can be considered for use in environmental monitoring of OSPW/tailings pond water samples. These experiments were mostly performed with pure compounds and organic extracts of OSPW or other commercially available extracts of naphthenic acids.

Year 3: Genome mining to find candidate NA biodegradation genes.

Publications 1-3 described the genome sequencing of several bacterial isolates recovered from OSPW. Their genomes are currently under analysis to attempt to identify candidate genes for degradation of NA using bioinformatics and gene-prediction methods.

Year 3-5: Metagenomic screening to identify NA biodegradation genes.

This genetic screen to identify new strains capable of NA has been performed and is being expanded. The first approach was to screen E. coli isolates that express fragments of bacterial DNA cloned from oil sands soil samples. DNA from bacteria in soil is isolated, cloned and expressed in laboratory strains of bacteria and rapidly screened with our biosensors for the ability to degrade NA. In these experiments, we grow potential degraders in small-volume cultures with naphthenic acids. We remove the cells and measure the amount of NA remaining in the cell-free supernatant with our biosensors. All the methods used to date do not require organic extractions to detect NA. Samples can be tested directly. This allows us an alternative method of identifying the bacterial genes used by bacteria to degrade NA, without being able to grow the vast majority of microbes in the soil or water. These same soil microbes are likely those able to survive in tailings ponds. The methods to use our biosensors to measure degradation by bacterial strains are still under development. This novel application of the biosensors will greatly improve researchers' ability to identify bacterial strains that can degrade NA, which is normally a complex experiment, which is time consuming, costly and complex. This work is ongoing and planned to be completed within year 5, followed by another publication #5.

Year 4-5: Development of methods and testing of actual OSPW samples.

OSPW water samples are typically difficult to obtain. Canadian Natural has not provided any OSPW samples to date. My collaboration with Dr John Headley at Environment Canada has provided a small panel of ~20 OSPW water samples. Our biosensors have been used to detect NA within these samples. We are currently optimizing our standard curves in order to estimate the NA concentrations, and therefore to compare concentrations determined by analytical mass spectrometry methods. This data will be published in manuscript #4.

Access to additional OSPW and water treated in mesocosms and constructed wetlands has been provided due to our involvement in another collaborative project. The project and our role is briefly described below.

Preliminary evidence suggests our biosensor can detect naphthenic acids in OSPW in addition to detecting pure compounds or extracts that are diluted to the concentrations found in tailings ponds. However, due to the complex chemistry of an OSPW sample, there also appears inhibitory effects of OSPW on the growth of our bacterial biosensors. For these reasons, we are developing rapid extraction methods of OSPW with the hope of extracting the NA fraction and removing any potential inhibitors of bacterial growth, such as high salts or other organic compounds.

PRESENTATIONS AND PUBLICATIONS

Patent

Title: BACTERIAL BIOSENSORS FOR MONITORING AND DETECTION OF NAPHTHENIC ACIDS IN THE ENVIRONMENT

- Provision patent submitted Sept /21

- International PCT No. PCT/CA2022/051379 filed Sept /22

Journal Publications:

Published

Complete genome sequence of a *Pseudomonas* isolate species isolated from tailings pond water in Alberta, Canada. 2021 Microbiol Resour Announc. 2021 Mar 4;10(9):e01174-20. doi: 10.1128/MRA.01174-20.

Draft Genome Sequences of Six *Pseudomonas* spp. and One *Rheinheimera* sp. Isolated from Oil Sands Process-Affected Water from Alberta, Canada

Under review

Complete Genome Sequence of Pseudomonas veronii Isolated from Tailings Pond Water in Alberta, Canada

T Bookout, S Shideler, K Goff , E Cooper, J Headley, S Lewenza. Construction of whole cell bacterial biosensors as an alternative environmental monitoring technology to detect naphthenic acids in oil sands process-affected water.

Application of naphthenic acids biosensors to rapidly monitor and screen strains for bioremediation potential. To be completed in year 5 2023/24.

RESEARCH TEAM AND COLLABORATORS

Institution: Athabasca University, University of Calgary

Principal Investigator: Dr. Shawn Lewenza

Name	Institution or Company	Degree or Job Title	Degree Start Date (For Students Only)	Expected Degree Completion Date or Year Completed (For Students Only)
Dr. S. Lewenza	**Athabasca University**	**Associate Professor**		
Steve Shideler **Tyson Bookout** **Lauren Bowron** **Kira Goff**	**University of Calgary** **University of Calgary** **University of Calgary** **Athabasca University** **50% CNR/50% Mitacs Funding**	**M.Sc.** **M.Sc.** **Undergrad** **Post doc**	**Sept 2017** **July 2019** **Sept 2019** **Nov 2021**	**Sept 2019** **July 2022** **Dec 2019** **Oct 2024**

New Recent Collaboration

My lab is a co-investigator on a large, multidisciplinary, collaborative research project funded by a Genome Canada Large Scale Applied Research Project (LSARP). The title of the project is: Optimization Of Constructed Treatment Wetlands For Water Remediation.

Our contribution to the project is to develop potential new biosensors using bacterial gene expression data provided from the bacterial communities in sediment and rhizosphere of plants within a constructed wetland or mesocosm, that has been enriched with OSPW for the purpose of NA remediation. We are also testing our biosensor technology to monitor NA concentrations before and throughout the treatment period, when OSPW is filtered through greenhouse-scale mesocosms and the Kearl constructed wetlands (Imperial Oil) for water treatment. This will provide the opportunity for a head-to-head comparison of our NA biosensor technology with cutting-edge MS orbitrap analysis.

Project website: grow-genomics.ca

WE0085 - Petroleum Hydrocarbons Toxicity Limits for Oil Sands Mine Waters

COSIA Project Number: WE0085

Research Provider: Four Elements Consulting

Industry Champion: Syncrude Canada

Industry Collaborators: Canadian Natural (CNRL), Imperial Oil Ltd., Suncor Energy Inc.

Status: Year 1 of 2

PROJECT SUMMARY

Background:

Canada-wide Standards (CWS) for toxicity limits of Petroleum Hydrocarbons (PHCs) were initially developed by the Canadian Council of Ministers of the Environment (CCME) for soils, ostensibly to assess soils contaminated by leaking tanks at petroleum filling stations. PHCs are refined, non-polar hydrocarbons divided into four classes based on boiling point ranges for the corresponding n-alkanes– F1 (carbon numbers C6-C10), F2 (>C10 - C16), F3 (>C16 - C34) and F4 (>C34 - C50). Alberta Environment and Parks (now Alberta Environment and Protected Areas - AEPA) developed F1 and F2 hydrocarbon guidelines in water for protection of aquatic life, which were adapted from analysis completed in the CWS documentation. These have been subsequently used to derived end-of-pipe release limits for the release of surficial groundwater from the Aurora North site.

***Table 1**. CWS for toxicity limits of PHCs.*

CWS Fraction	Current AEPA EPEA Approval Release Limit	AEPA Acute Guideline	Notes
F1 Fraction	0.15 mg/L combined	150 ug/L	Interim. Based on toxicity reference value for gasoline and a safety factor of 10. Derived as whole product toxicity benchmarks for aquatic life (grass shrimp) in support CWS (CCME 2008).
F2 Fraction		110 ug/L	Interim. Based on toxicity reference value for fuel oil no. 2 and a safety factor of 10. Derived as whole product toxicity benchmarks for aquatic life (banded killifish/pumpkinseed) in support of CWS (CCME 2008).

This project considers the development of the AEPA guidelines for PHCs in water and the application of approved analytical methods to measure PHCs in oil sands mine waters.

Objectives:

1) Assess the process and assumptions used in the derivation of the AEPA guideline for protection of aquatic life for PHC in water and its applicability to oil sands mine waters.

2) Assess the appropriateness of approved analytical methods used to measure PHCs in oil sands mine waters. Specifically, assess the results of PHC analysis with removal of polar organic substances using the two approved CCME clean-up methods:

   a) in situ clean-up method, which is the current common clean-up method used by commercial labs

   b) column clean-up method with reverse surrogate, which is recommended for waters where the presence of polar organics is likely.

Polar organic compounds in test samples must be removed prior to PHC analysis and the column clean-up method with reverse surrogate is recommended if polar organic compounds are suspected in the sample. Polar organics are known to exist in oil sands mine waters, are commonly referred to as naphthenic acids. They are typically present in untreated mine process-affected waters at 20-70 ppm.

PROGRESS AND ACHIEVEMENTS

Assessment of the AEPA guideline for protection of aquatic life for PHC in water

The approach for this component was:

Review of literature on the technical approach and limitations behind the development of the F1/F2 toxicity thresholds.

Review of literature on toxicological characteristics of whole refined petroleum products with an objective of understanding compounds in petroleum hydrocarbon fuel mixtures that are direct markers of potential aquatic toxicity.

Presentation of characteristics (profiles) of hydrocarbon compounds in oil sands water to compare measured levels of F1/F2 subfractions to measured levels of hydrocarbon compounds considered direct markers of potential aquatic toxicity.

The analysis highlighted a number of deficiencies in the derivation of the guidelines, particularly for application to oil sands mine waters, which are fundamentally different from refined oil products.

This review identified a limitation in the aquatic organism data set used by CCME (2008) to derive the F2 fraction guideline. The guideline was based primarily on whole-effluent toxicity testing for using no. 2 fuel oil. The data set is from LC50 aquatic toxicity testing of fresh whole unrefined and/or refined oil products. However, the LC50 results for 16 species included the use of an oil dispersant linear alkylate sulfonate (LAS) with the F2 hydrocarbons, which had a threshold for mortality of more than an order of magnitude lower than for no. 2 fuel oil without LAS. (Rehwoldt et al. 1974). Comparing the results of the two tests (with and without LAS), it is readily apparent the presence of the oil dispersant poses significant acute toxicity to the six species as the LC50 results were six to 36 times higher for the tests without LAS. Rehwoldt et al. (1974) stated as much. “It is apparent that the toxicity of the dispersant far exceeds the toxicity of the oils for all species of fish tested.” The lowest LC50 was a grass shrimp, a species with no habitat in Alberta, where the test included use of LAS.

More recent studies (ECCC / Health Canada 2015) have been conducted for no. 2 fuel oil to compile acute toxicity endpoints for a variety of aquatic species. This study selected an acute-toxicity value to represent a critical ecotoxicological endpoint to aquatic organisms in their screening assessment – 1,900 μg/L. This value represented a 48-h EC50 endpoint for immobilization with the water flea (Daphnia magna). The results of the acute toxicity values, 1,900 ug/l and 2,000ug/l are similar.

Petroleum hydrocarbons such as no. 2 fuel oil are comprised of a mix of hydrocarbons. The substances thought to cause toxicity (i.e., toxicological drivers drivers) in no. 2 fuel oil include mono−aromatics (benzene, toluene, ethylbenzene, xylenes), di−aromatics (naphthalene, 2−methylnaphthalene) and n−hexane.

An analysis of PHC fraction in six oil sands mine water samples – four natural groundwater samples and two process-affected water samples – effectively illustrates the limitations of applying the F1 / F2 acute toxicity limits derived from refined products to naturally occurring aged (oxygenated) hydrocarbons. For all of these oil sands mine water samples, the F1 fraction was below detection limit. The F2 fraction for groundwaters was usually above the Alberta guideline of 150 ug/l , ranging from below detection to 1,800 ug/l. For process affected water, the F2 fraction for all the samples was above the Alberta guidelines, averaging 200 – 470 ug/l. However, when a detailed chemical analysis of more than sixty F2 compounds was conducted, virtually all results were below the low ng/l detection limit. The few that were above the detection limit had low (<100) ng/l values. Potential toxicological drivers i.e. hydrocarbon compounds considered direct markers of potential aquatic toxicity were measured at less than detection limits or only in minor (< 100 ng/L) amounts. They are present in much smaller quantities compared to F2 subfraction hydrocarbons. To a large extent, F2 subfraction hydrocarbons registering in these oil sands mine water samples are mostly unresolved (i.e., comprised of complex weathered hydrocarbons) and have little or no dependence on direct markers of potential aquatic toxicity.

Assessment of approved analytical methods used to measure PHCs

The measurement of F2 hydrocarbons in water can be confounded by high levels of polar organic substances called “biogenic” material or “unresolved complex mixtures” representing highly degraded organic material, including naphthenic acids, resulting in false positives. The standard method for analysis of CCME for hydrocarbon fractions allows for either in situ or ex situ (column) silica gel cleanup prior to chemical analysis to remove biogenic polar organics such as fats and humic acids. The column cleanup is considered more robust but is not routinely offered by commercial laboratories.

The hydrocarbon classes and standard analytical methods for British Columbia (BC) are very similar to the CCME Canada-Wide Standard. The BC method uses column cleanup only as studies completed during the development of the standard method showed in situ cleanups can be considerably less effective than column techniques for removing medium polarity biogenic material. The BC method also requires the use of a capric acid reverse surrogate added to all samples and quality control sample extracts immediately prior to silica gel cleanup. Capric acid is a fatty acid that should be 100% retained by the silica gel column with less than 2% breakthrough, thus ensuring all polar organics are removed prior to analysis for PHC.

Analysis of three oil sands mine water samples (one groundwater, two process-affected waters) and a natural spring in the mineable oil sands regions were completed by a commercial lab using both the in situ cleanup and the column cleanup. The results are summarized in the table below:

Table 2. *PHCs analysis of process-affected waters..*

		Petroleum hydrocarbon fraction (ug/l)			
Sample	**Cleanup**	**F1**	**F2**	**F3**	**F4**
Groundwater	In Situ	ND (<100)	600 – 700	1300 – 1400	ND (<200)
	Column	ND (<100)	<100	<200	ND (<200)
Process water 1	In Situ	ND (<100)	160	420	ND (<200)
	Column	ND (<100)	<100	340	ND (<200)
Process water 2	In Situ	ND (<100)	490	2000	ND (<200)
	Column	ND (<100)	<100	<200	ND (<200)
Natural spring water	In Situ	ND (<100)	430	1900	ND (<200)
	Column	ND (<100)	<100	<200	ND (<200)

In all cases, the F1 fraction was below detection limit regardless of the clean-up method. For the F2 fraction, all samples using the in situ cleanup method were above the Alberta guideline of 110 ug/l while all samples using the column cleanup method were below detection limit and below the guideline. It is interesting to note that the reverse surrogate results for the column cleanup were quite variable with recoveries ranging from 78 – 112% compared to the minimum of recovery of 98%. This may in part be because the column cleanup method is not commonly practiced by commercial labs at this time.

LESSONS LEARNED

The only way to ensure clean up of the high levels of polar organic substances found in both natural groundwaters in the oil sands region and process-affected waters is by using the column clean-up method with reverse surrogate to ensure removal prior to PHC analysis. When using this method, all samples tested to date meet the Alberta water quality guidelines for the F1 and F2 fraction.

PRESENTATIONS AND PUBLICATIONS

Future

RESEARCH TEAM AND COLLABORATORS

Institution: Four Elements Consulting

Principal Investigator: Tammy Rosner

Independent Consultant: Warren B. Kindzierski, PhD

WJ0182 – Using BE-SPME Passive Samplers for Ecological Hazard Assessment of Oil Sands Process-Affected Water

COSIA Project Number: WJ0182

Research Provider: Meta Analytical Inc.

Industry Champion: Imperial Oil Ltd.

Industry Collaborators: None

Status: ongoing

PROJECT SUMMARY

Oil sands process-affected water (OSPW) is typically comprised of a complex mixture of organic and inorganic constituents generated during the bitumen-extraction process used by the oil sands industry. Oil sands operations currently use large, on-site tailings ponds for storage of OSPW, with more than 92.7km2 being used to hold process water for recycling and reuse. Recent work by Redman et al. (2018) demonstrates bio-mimetic solid phase microextraction (BE-SPME) based passive samplers may be useful tools for hazard assessment of OSPW, as these devices can effectively provide a surrogate measure of dissolved organic contaminant residues that can cause adverse effects in aquatic organisms. Before this approach can be deployed under the Oil Sands Monitoring (OSM) program or for end-of-pipe testing of treated OSPW, additional laboratory and field-based studies are required to establish effectiveness of the passive samplers. Figure 1 shows a schematic illustration of scope of the current project. The current project is part of a larger overall research program involving development and testing of BE-SPME passive samplers for hazard assessment of petroleum-derived dissolved organic chemicals (phase I-III) reported in the 2020 Water Mining Research Summary. The overall goal of this research is to demonstrate the efficacy of SPME-based passive samplers for robust, cost-effective hazard assessment of OSPW. The primary objectives for the present work on BE-SPME passive samplers for hazard assessment of OSPW (phase IV) include:

1) utilize BE-SPME passive samplers to assess chronic effects of OSPW-derived dissolved organic chemicals in early life stage fish, including rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas).*

2) utilize BE-SPME passive samplers to assess chronic effects of OSPW-derived dissolved organic chemicals in early life stage amphibians, i.e., wood frog embryos (*Lithobates sylvaticus).*

3) compare observed concentration-response relationships for chronic exposure in early life stage fish (rainbow trout) and amphibians (wood frogs) to previous results and evaluate species-sensitivity distributions.

4) for comparison to BE-SPME data, utilize high-resolution mass spectrometry (Orbitrap-MS) to measure speciated and total naphthenic acid (NA) concentrations in OSPW, treated OSPW and surface water samples,

5) apply BE-SPME passive samplers for hazard assessment of different OSPW, treated OSPW and receiving waters within the Lower Athabasca River watershed.

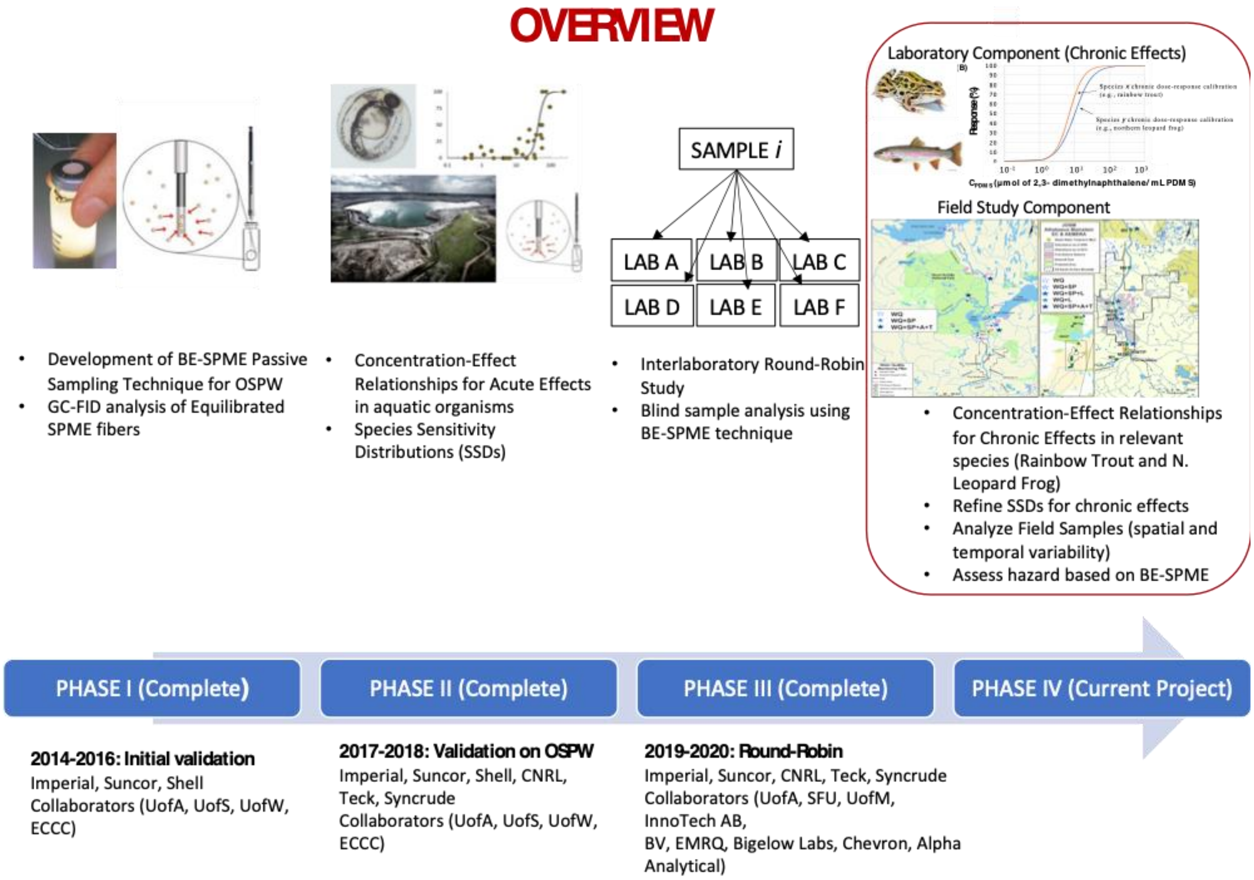

Figure 1. *Schematic illustration of the scope of the current project (phase IV), as well as phases related to BE-SPME development and testing and interlaboratory comparison studies (phase I-III).*

PROGRESS AND ACHIEVEMENTS

Sample Collections

In 2021, we collected three 1 m^3 totes of OSPW acquired from Imperial Oil (2021 OSPW), as well as 40 L samples of constructed wetland influent and effluent (Day 0 and Day 15 temporal sampling at Kearl Treatment Wetland). We also collected several samples of surface water from existing oil sands monitoring sites (Athabasca River and Muskeg River surface water). These field samples consisted of 2 L grab samples, collected during the months of June and October 2021. Samples were taken at three sites (corresponding to existing OSM sites), including one upstream of Fort McMurray and two sites near Fort Mckay. Triplicate samples were taken at each site.

In 2022, we collected four 1 m^3 totes of OSPW acquired from Imperial Oil (2022 OSPW), as well as 40 L samples of constructed wetland influent and effluent (Day 0 and Day 15 temporal sampling at Kearl Treatment Wetland). During this period, we also collected 300 L of “aged OSPW”, which consisted of Imperial Oil Ltd OSPW from Kearl aged in a 2-year old mesocosm experiment at the InnoTech Mesocosm facility, Vegreville, AB. Field surface water samples were collected from the Athabasca River and Muskeg

River sites, comprised of 2 L grab samples collected in June and October 2022. Also, we have submitted third party request for aged/treated OSPW from Syncrude (Base Mine Lake, Ponds).

Chemical Analyses

For the current project, OSPW, treated OSPW and surface water samples are analyzed for the total bioavailable dissolved organics using the developed BE-SPME method and speciated naphthenic acid (NA) concentrations using high-resolution mass spectrometry (Orbitrap-MS). Specifically, samples analyzed included Imperial OSPW (2021 and 2022 totes), treated wetland influent and effluent (Kearl Treatment Wetland in 2021 and 2022), and surface water samples collected from Athabasca and Muskeg River. Table 1 shows the observed BE-SPME and total NA concentrations measured in the various samples. Figure 2 shows the relationship between measured BE-SPME concentrations and total Naphthenic Acid concentrations measured via Orbitrap-MS for analyzed samples of OSPW and diluted OSPW used in exposure experiments.

Table 1. *Results of BE-SPME measurements, total naphthenic acid (NAs) concentrations, pH, total dissolved solids (TDS), and total hardness in 2021 and 2022 OSPW, closed-loop treatment wetland, aged OSPW (two-year phytoremediation trial) and Athabasca River watershed.*

	2021					2022				
	BE-SPME (mM)	Total NAs (µg/L)	pH	TDS (mg/L)	Hardness (mg/L CaCO3)	BE-SPME (mM)	Total NAs (µg/L)	pH	TDS (mg/L)	Hardness (mg/L CaCO3)
OSPW [a]										
1% OSPW	-	-	8 ± 0.02	-	-	0.6	249	7.2 ± 0.0	-	-
2.2% OSPW	4.7 ± 1	369	8 ± 0.02	-	-	2.3	468	7.2 ± 0.02	-	-
4.6% OSPW	8.9 ± 1.9	807	8 ± 0.03	-	-	3.8	976	7.2 ± 0.02	-	-
10% OSPW	20.6 ± 4.5	1,910 ± 55.1	8 ± 0.02	-	-	8 ± 0.7	1,820	7.4 ± 0.02	-	-
22% OSPW	42.9 ± 16.2	5,220 ± 318	8.2 ± 0.02	-	-	16.3	4,170	7.7 ± 0.03	-	-
46% OSPW	81.7 ± 8.6	11, 000 ± 603	8.4 ± 0.05	-	-	33.9 ± 3.7	7,510	8.1 ± 0.05	-	-
100% OSPW	112 ± 7.3	15,300 ± 1,000	8.6 ± 0.06	808	227	60.7 ± 11.3	11,500 ± 439	8.3 ± 0.06	902	204
Aged/Treated OSPW										
Aged OSPW (2-year Mesocosm) [b]	-	-	-	-	-	34.2	6,870	8.5 ± 0.07	736	126
Constructed Wetland (Day 0) [c]	47.2	19,600	8.5 ± 0.07	848	240	50	12,200	8.4 ± 0.08	954	227
Constructed Wetland (Day 15) [c]	42.8	9,100	8.6 ± 0.09	810	302	46.3	9,480	8.5 ± 0.08	1,020	333
Athabasca River Watershed (Surface Water)										
Athabasca River [d]	N.D	8.6 ± 0.4	7.9 ± 0.07	202	133	N.D	12.3 ± 6.3	-	-	-
Muskeg River [d]	N.D	9.5 ± 1.9	-	-	-	N.D	17.1 ± 1.9	-	-	-

[a] Raw OSPW (i.e., 100% OSPW) was obtained from Imperial Oil Ltd in 2021 and 2022. b Aged OSPW consisted of water from a small pond mesocosm involving OSPW at InnoTech Alberta. c Water was obtained in 2021 and 2022 from the Kearl closed-loop constructed wetland tests designed to study passive treatment of OSPW. d Field-collected surface water from existing monitoring sites. Coordinates for Athabasca R. and Muskeg R. sites were 57°08’09” N 111°36’ 32”W and 57°07’59” N 111°36’ 14”W, respectively.

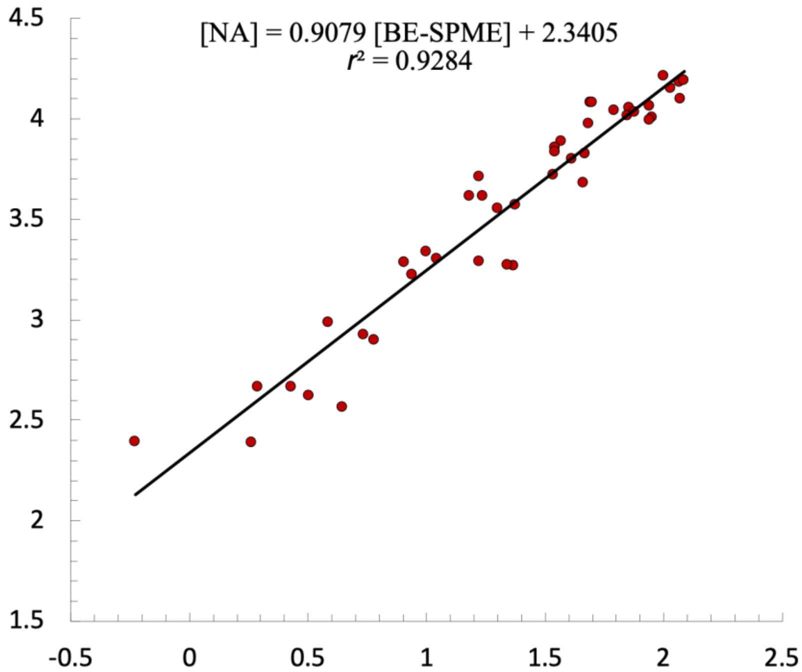

Figure 2: *Relationship between measured BE-SPME (mM 2,3 dimethylnaphthalene equivalent) via GC-FID and total Naphthenic acids (ug/L) via high resolution Orbitrap mass spectrometry. Results from all analyses of 2021 and 2022 OSPW samples (n=47).*

Chronic Toxicity Studies with Early Life Stage Fish

To date, we have completed 30 d chronic early life stage toxicity experiments with rainbow trout (Oncorhynchus mykiss) using with 2021 and 2022 Imperial Oil operational OSPW/diluted OSPW (100%, 46%, 22%, 10%, 4.6%, 2.2%, 1%, Controls), as well as exposure experiments involving treated and aged OSPW, including Kearl treatment wetland water and aged OSPW from a two-year mesocosm study.

For operational OSPW, the estimated LC50s for BE-SPME in 2021 and 2022 were 22.9 and 20.7 mM , respectively. The estimated LC50s for Total NA's in 2021 and 2022 were 2,290 and 3,390 µg/L, respectively. The lowest observable effect concentration (LOEC) related growth effects and deformities ranged between approximately 10 to 20 mM for BE-SPME and 1,000 to 2,000 µg/L total NA's. The observed dose-response relationship curves for rainbow trout chronic OSPW exposure is comparable to those previously reported for shorter-term/acute assays using different species. Significant total deformities observed from 10% OSPW in 2021 and from 22% OSPW in 2022, but not at lower concentrations.

OSPW treatment via a closed-loop constructed wetland system and via two-year phytoremediation mesocosm (aged OSPW) significantly reduced mortality and deformities. Compared to high mortality observed in constructed wetland influent (Day 0 water), no significant mortality was observed in treated constructed wetland effluent (Day 15 water) in 2021 and 2022. Constructed wetland effluent (Day 15 water) only exhibited significant difference in craniofacial deformities in 2021.

Chronic Toxicity Studies with Early Life Stage Amphibians

We collected egg masses of wild spawning wood frogs (Lithobates sylvaticus) during spring 2022 in Northern Alberta. Using the collected embryos, we conducted a 96-day chronic toxicity assay following

OECD Larval Amphibian Growth and Development Assay (LAGDA). Experiments involved exposure to Imperial Oil OSPW/diluted OSPW (80%, 46%, 22%, 10%, 4.6%, 2.2%, 1%, Controls).

Significant mortality of wood frogs that survived until metamorphosis was observed only in the 80% OSPW after 20-40 days (Figure 3a). Exposures of wood frogs to 40 and 80% OSPW treatments exhibited delayed development of wood frogs in time to metamorphosis with respect to its corresponding hard water control (Figure 3b). Time to hatch was unaffected by OSPW. Frogs at metamorphosis exhibited a higher incidence of severe scoliosis (skeletal deformities) at 20% compared to all other treatments and the control.

The LC50 for the tested operational OSPW in wood frogs was 96.2 BE units (2,3 mM dimethylnaphthalene equivalent), equivalent to a total Orbitrap-MS Total NA concentration of 10.2 mg/L. The apparent LC50 for wood frogs (96.2 BE mM) is higher than the LC50 for early life stage rainbow trout (20 mM). The lowest observable effect concentration (LOEC) related to skeletal deformities was 19.9 mM 2,3, dimethylnaphthalene, equivalent to a total Orbitrap-MS NA concentration of 3.6 mg/L. The apparent LOEC for chronic OSPW exposure in early life stage wood frogs is comparable to previously reported threshold effect levels in aquatic organisms (average EC50 values of~ 10 mM), Redman et al. (2018). The results provide important information regarding chronic effects of OSPW in early life stage wood frogs and application of BE-SPME for hazard assessment.

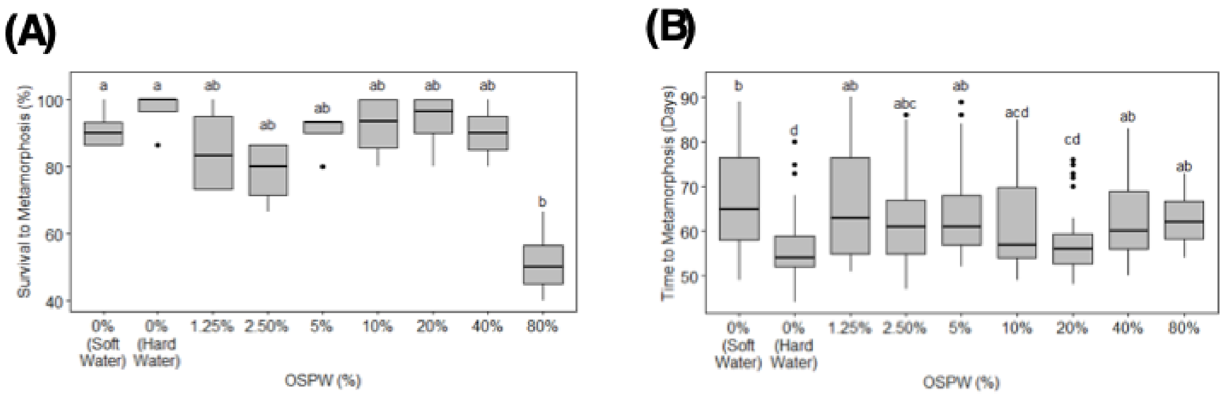

Figure 3: *(A) Percent of early life stage wood frogs that survived until metamorphosis (Kruskal-Wallis chi squared = 18.1, df = 8, p-value = 0.02, followed by a Dunn's Test for pairwise comparison). (B) Time to metamorphosis in days from the start of the exposure (Kruskal-Wallis chi squared = 37.4, df = 8, p < 0.01, followed by a Dunn's Test for pairwise comparison). Thick horizontal lines represent median values, and the surrounding box represent the lower and upper 25th and 75th percentiles and dotted lines depict the minimum and maximum values with the outliers as black circles. The replicates for each treatment were as follows: soft water control n=8; hard water and all OSPW treatments n=4. Different letters indicate significant differences between treatment groups.*

LESSONS LEARNED

There were several key findings and lessons learned from work conducted during this reporting period. One key finding was a strong positive correlation between measured BE-SPME concentrations via GC-FID and measured total naphthenic acid concentrations via high resolution Orbitrap-MS. This indicates the BE-SPME method is correctly measuring the target dissolved organic compounds in OSPW/treated OSPW samples. This is important, as a major goal of the developed BE-SPME approach is to provide a simple cost-effective surrogate measure of the potentially toxic dissolved organic substances (i.e., NAs) in OSPW and treated OSPW.

Another key finding from the work completed during this reporting period is the observed concentration-response relationship for rainbow trout chronic OSPW exposure was comparable to results previously reported for shorter-term/acute assays using different species. This indicates chronic effect levels are not substantially lower than previously believed, based on the available acute toxicity study results. For example, the BE-SPME based chronic LC50 value observed in the present 30-day exposure studies of rainbow trout (20 mM) is comparable to acute LC50 values observed for short-duration (4 d) OSPW exposure in early life stage zebrafish (Danio rerio), (45.7 mM) and fathead minnow (19.5 mM), (Redman et al. 2018).

Chemical analyses (BE-SPME and speciated NA's) and toxicity testing in treated OSPW (wetland effluent) and aged OSPW showed reduced contamination levels and lower chronic toxicity in early life stage fish. This is important information, as passive treatment of OSPW via constructed wetlands is viewed as a possible treatment technology for oil sands companies.

There is limited information regarding the toxicity of OSPW in amphibians. The present study provides novel results, which indicate a common amphibian species native to the oil sands region (early life stage wood frog) is apparently less sensitive to OSPW exposure than early life stage fish. However, while low mortality of wood frogs was observed, the data clearly shows exposure to raw OSPW does result in various sublethal effects, including higher incidence of deformities. It is important to note observations from the wood frog exposure experiments were highly variable, reducing the statistical power to detect significant differences between control and treatment groups. This is common for toxicity experiments using field-collected organisms (wood frog egg masses), due to higher natural variations compared to laboratory-raised test organisms.

Lastly, it is important to note observations of some inter-laboratory variability with the BE-SPME measurements. Results of an inter-laboratory comparison study for BE-SPME was recently published (Letinski et al., (2022), Environ. Toxicol. Chem. (2022).; https://setac.onlinelibrary.wiley.com/doi/10.1002/etc.5340). The study found relatively high variability between various laboratories analyzing a given OSPW sample. To provide an ongoing assessment of interlaboratory variability in BE-SPME results, we plan to conduct parallel split sample analyses at three laboratories, including InnoTech Alberta, Exxon Mobil and Simon Fraser University.

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Stenner, K; danis, b.; Bekele, A; Tanna, N.; Drozdowski, B.; Gobas,. Kelly, B.; Marlatt, V. 2022. Toxicity of Oil Sands Process-Affected Water in Early Life Stage Wood Frogs (Lithobates sylvaticus). Canadian Ecotoxicity Workshop. Winnipeg, MB, Canada.

Vega, V., Stenner, K; danis, b.; Bekele, A; Tanna, N.; Drozdowski, B.; Gobas,. Kelly, B.; Marlatt, V. 2022. Chronic Toxicity of Oil Sands Process-Affected Water in Early Life Stage Rainbow Trout (Oncorhynchus mykiss). SETAC North America Annual Meeting. Pittsburgh, PA., USA.

Stenner, K; danis, b.; Bekele, A; Tanna, N.; Drozdowski, B.; Gobas,. Kelly, B.; Marlatt, V. 2022. Chronic Toxicity of Oil Sands Process-Affected Water in Early Life Stage Wood Frogs (Lithobates sylvaticus). SETAC North America Annual Meeting. Pittsburgh, PA., USA.

RESEARCH TEAM AND COLLABORATORS

Institution: Meta Analytical Inc.

Principal Investigator: Barry C. Kelly

Name	Institution or Company	Degree or Job Title	Degree Start Date (For Students Only)	Expected Degree Completion Date or Year Completed (For Students Only)
Dr. Barry Kelly	Meta Analytical Inc.	Research Scientist	-	-
Dr. Frank Gobas	Simon Fraser University	Professor	-	-
Dr. Vicki Marlatt	Simon Fraser University	Professor	-	-
Katelyn Stenner	Simon Fraser University	Masters Student	Sep 2021	Dec 2023
Valeria Vega	Simon Fraser University	Masters Student	Sep 2021	Dec 2023

PIT LAKES

WJ0121 - Base Mine Lake

Research Provider: Multiple researchers and institutions

Industry Champion: Syncrude Canada Ltd.

Industry Collaborators: Imperial Oil Ltd., Suncor Energy Inc., Canadian Natural (CNRL)

Status: Multi-year project, ongoing

PROJECT SUMMARY

BML is the first, and currently the only full-scale commercial demonstration of the end pit lake technology in the oil sands industry. An oil sands end pit lake (EPL) is an area where overburden and oil sand has been removed and is then filled with fluids prior to closure. An EPL contains water (from the process of oil sands extraction or freshwater or both). It may or may not contain treated or untreated fluid tailings (FT), or other solids, such coarse tailings sand or overburden.

BML is located in the former West In-Pit (WIP) of the Syncrude Mildred Lake operation. It consists of a mined-out oil sands pit filled with untreated fluid fine tailings (FFT) comprised of silt, clay, process-affected water and residual bitumen. FFT are physically sequestered below a combination of oil sands process-affected water (OSPW) and fresh water. This pit lake configuration is often referred to as Water Capped Tailings Technology (WCTT). Based on previous research and modelling, WCTT is predicted to improve EPL water quality and sequester fluid tailings (or other tailings) below the water cap over time.

Freshwater is pumped in to Base Mine Lake from the Beaver Creek Reservoir. water is pumped out of BML to the tailings recycle water system, which uses it in the bitumen-extraction process. This flow through process dilutes the BML water cap over time and will be in place until a more substantial upstream surface watershed is reclaimed and connected to BML, and outflow is established into the Athabasca River. As the tailings continue to dewater over time, the lake water will get deeper.

Placement of fluid tailings began in 1995, was completed in late 2012, and BML was commissioned on December 2012. No tailings solids were added after this time. Fresh water and OSPW were added to the existing OSPW upper layer during 2013 to attain the final water elevation.

A key purpose of the BML Monitoring and Research Program (MRP) is to support an adaptive management framework. This program is designed to assess lake performance against key performance indicators and evaluate the need for management interventions. The initial focus of the research program is to support the demonstration of water-capped tailings technology and to provide a body of scientific evidence that demonstrates the lake is on a trajectory to become integrated into the reclaimed landscape. The outcomes from the program can be used to inform the design and management of future pit lakes, including those that may contain tailings materials, such as treated or untreated fluid tailings. At the same time, the program establishes a baseline of biophysical data to assess the changes in BML through time, and the state of the lake at certification, including water quality and other lake processes. The monitoring program is designed to track trends in the lake both seasonally and annually and measure these trends against some key performance metrics as outlined above. The research program focuses on key scientific questions designed to elucidate the mechanisms and processes that govern the current state of BML, and explain changes detected by the monitoring program. In other words, the monitoring program tracks the trends in the lake through time. The research program investigates why those changes are occurring.

The specific objective of the monitoring program is to provide information to support the validation of WCTT as a viable tailings management and reclamation option. In the early stages, the monitoring program will demonstrate fluid fine tailing are sequestered and the water quality in the lake is improving. The monitoring program is designed to do this by tracking the physical, chemical and biological changes in BML (Table 1). The program captures these changes both temporally and spatially, and eventually in the context of regional climate cycles. The monitoring program supports regulatory compliance, but also informs adaptive management of BML. The research program uses a multi-university, multi- and inter- disciplinary approach that focuses on the analysis and interpretation of monitoring data, hypothesis-driven research activities, and integration and collaboration among and between research programs. Research results are integrated with monitoring results on an ongoing basis, with the ultimate goal of identification and quantification of the processes and properties in BML that are responsible for the trends observed in the monitoring program. The various components comprising the monitoring and research programs are closely linked.

Table 1. *Base Mine Lake Monitoring Program Components*

Physical	Chemical	Biological
FFT Settlement	Water Balance Assessment	Aquatic Biology Assessment
FFT Geochemistry Assessment	Surface Water Quality Assessment	Surface Water Toxicity
Physical Limnology Assessment	Groundwater Assessment	Sediment Toxicity
Meteorological Monitoring	Chemical Mass Balance	
FFT Physical Assessment		

The current focus of the research program is to support the demonstration of the Water Capped Tailings Technology (WCTT). The program also provides supporting information about key processes fundamental to the progression of BML towards a functional component of the closure landscape. The current research programs were focused on key parameters influencing early BML development. The program focus is to validate WCTT. Several research programs will determine the potential fluxes from the FFT to the water column, including chemical, geochemical, mineral, gases and heat and bitumen. Physical, biological and chemical mechanisms are being investigated (Table 2).

Table 2. *Current Base Mine Lake Research Programs*

Research Component	Primary Objective	University	Researchers (PIs)
Physical limnology of BML and the potential for meromixis	To understand the circulation of BML and its potential for meromixis	University of British Columbia	Greg Lawrence / Ted Tedford / Roger Pieters
Characterization of controls on mass loading to an oil sands end pit lake	To define mass loading to Base Mine Lake by characterizing the mechanisms and distribution of heat and mass transfer from the tailings column to the overlying water column.	University of Saskatchewan	Lee Barbour / Matt Lindsay

Research Component	Primary Objective	University	Researchers (PIs)
Field investigation of BML water-cap oxygen concentrations, consumption rates and key BOD/COD constituents affecting oxic zone development.	To establish temporal and spatial variability in in situ BML water-cap oxygen concentrations, oxygen consumption rates and identify the biogeochemical processes linked to its consumption from the FFT-water interface to the BML water surface	University of Toronto / McMaster University	Lesley Warren / Greg Slater
Microbial communities and methane oxidation processes in Base Mine Lake	i) To study Biological Oxygen Demand (BOD) in the lake, ii) to examine a potential role of methanotrophs in the degradation of naphthenic acids (NAs), and iii) to examine the microbial community in BML, how the community changes over time with changes in lake chemistry, and the potential use of community analyses as an indicator of reclamation	University of Calgary	Peter Dunfield
Understanding Air-Water Exchanges and the long-term hydrological viability of Base Mine Lake	To measure and improve the understanding of the physical mechanisms controlling CH_4 and CO_2 fluxes across the air-water interface, to determine the factors that control evaporation from BML and to understand the long-term water balance of BML	McMaster University / Carleton University	Sean Carey / Elyn Humphreys
Characterization of Organic Compounds and Naphthenic Acids in Base Mine Lake: Implications for methane production, transport, oxygen consumption, and NA persistence	To understand methane production and release, the sources of naphthenic acids and petroleum hydrocarbons to the BML water cap, and the role of ebullition in transporting FFT constituents into the water cap	McMaster University	Greg Slater
Base Mine Lake Process Dynamics	To understand bitumen liberation to water surface and develop monitoring and mitigation tools for bitumen.	Syncrude	Barry Bara/Adedeji Oluwaseun

PROGRESS AND ACHIEVEMENTS

The two key outcomes for Base Mine Lake (BML) to valid the Water-Capped Tailings Technology (WCTT) are the physical sequestration of the fine tailings solids below the water cap and water quality improvements over time. Demonstrating the physical isolation of fines beneath BML's water cap is a key performance outcome related to the validation of WCTT. To date, the results from the monitoring and research program indicate the fine tailings are settling as forecasted by model predictions, the mudline is declining over time, the water cap is increasing in depth and there is generally a decrease in the suspended solids concentration over time, especially in the upper water layers, although the turbidity in the water cap fluctuates seasonally. The lake exhibits conventional boreal dimixis. These and other typical lake physical dynamics are important drivers of both the chemical and biological response in the lake. The water balance is dominated by pore water flux and fresh water import from Beaver Creek Reservoir. Bitumen sheen may have an effect on water evaporation from the lake surface. Surface water quality is also improving with time in Base Mine

Lake, as expected to demonstrate the viability of the WCTT. The lake water is not acutely toxic and has some residual chronic toxicity.

A key focus of the Base Mine Lake program is understanding the dynamics and mitigation of residual bitumen in the lake. Residual bitumen makes up a relatively small component of the fluid tailings. When FFT was placed in the mined-out pit, some of this residual bitumen separated from the FFT. This resulted in bitumen mats forming on the surface of the FFT, primarily focused in areas of the pit where tailings were discharged. As detected by the Base Mine Lake monitoring program, some residual bitumen is also present as a hydrocarbon sheen on the water surface, some of which has accumulated along the shoreline. This sheen is a result of methanogenic bacterial consumption of residual hydrocarbon in the bitumen mat producing methane bubbles with bitumen attached being released to the air-water interface. Observations and empirical data from both the research and monitoring program has led to the development of a conceptual model. This is being tested empirically through a number of research and monitoring programs and provides guidance for bitumen-mitigation activities.

LESSONS LEARNED

Lessons learned and key results are reported annually to the Alberta Energy Regulator and are publicly available here: Syncrude Canada Ltd. Reports

The most recent (202) report can be found here: Syncrude 2022 Pit Lake Monitoring and Research Report

PRESENTATIONS AND PUBLICATIONS

Journal Publications:

Published

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RESEARCH TEAM AND COLLABORATORS

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WJ0091 - Suncor Lake Miwasin

COSIA Project Number: WJ0091

Research Provider: Hatfield Consultants, AECOM, University of Alberta, University of Saskatchewan, Athabasca University, and University of Waterloo

Industry Champion: Suncor Energy Inc.

Industry Collaborators: Canadian Natural , Syncrude Canada Ltd., Alberta Innovates

Status: Year 5 of 6

PROJECT SUMMARY

Lake Miwasin Project Overview

Lake Miwasin is a scaled representation of Suncor's commercial scale pit lake at Dedicated Disposal Area 3 (DDA3, the future Upper Pit Lake (UPL)), which uses the Permanent Aquatic Storage Structure (PASS) process, an inline tailings treatment process of coagulant addition followed by flocculant addition. The PASS process enables:

- more rapid reclamation of the treated fluid tailings (FT) into a freshwater lake environment;
- integration of the lake into the surrounding watershed; and
- mitigation of potential adverse environmental effects.

The goal of the Lake Miwasin pilot study is to monitor and evaluate if the PASS process, when combined with the closure landform design, will accelerate the reclamation of a DDA to a self-sustaining boreal lake ecosystem. Specific objectives of the Research & Monitoring (R&M) Plan are to: (1) test assumptions in the pit lake design; and (2) address critical gaps in the pit lake design.

The Lake Miwasin pilot project is expected to have four operational and reclamation phases:

- Phase 1: Dewatering and treatment of FT (Q3 2017 to Q3 2018)
- Phase 2: Placement of the aquatic cover (Q3 2018)
- Phase 3: Controlled water flow through and release (~2019 to ~2023)
- Phase 4: Water release under natural flow (location and timing to be determined)

The Lake Miwasin project completed Phases 1 and 2 operations by the end of 2018 and is currently in Phase 3. Research and monitoring activities are planned to take place over a 15-year period (2018-2033) to meet the project goal and objectives.

Lake Miwasin Research and Monitoring Program

The Lake Miwasin Research and Monitoring (R&M) Plan adopts an Effectiveness Monitoring (EM) design within an adaptive management framework (CEMA 2013). EM is the process of identifying and monitoring key indicators of ecosystem response to evaluate the success of a reclamation initiative or goal. The EM framework is structured on a Goal – Objective – Assumption – Question – Indicator hierarchy. Following the EM design, measurable and obtainable assumptions were selected on the basis they are fundamental to achieving the Lake Miwasin project goal. Key test questions, hypotheses, and indicators were also identified. The R&M plan identifies three priority monitoring areas to monitor and assess the performance of the Lake Miwasin project: 1) treated tailings deposit; 2) aquatic cover and watershed; and 3) biodiversity. Research questions are identified and grouped into five research priority areas: 1) deposit characteristics; 2) water quality; 3) closure modelling; 4) landform design; and 5) performance trajectories.

The first five-year Lake Miwasin Research and Monitoring program was developed and implemented in 2019 following the overarching R&M Plan. The core monitoring program components currently include water quality, hydrology, hydrogeology (groundwater dynamics and quality), aquatic ecology and biodiversity (including aquatic, riparian and upland vegetation and amphibian communities), air quality, and tailings.

The current research programs comprise four studies led by multiple principal investigators and carried out by multiple universities, focusing on watershed hydrology, long-term fate and transport of COPCs (e.g. trace elements and organic compounds), and ecotoxicity.

1. Water fluxes and system evolution of Lake Miwasin (Athabasca University and University of Waterloo; PIs: Dr. Scott Ketcheson and Dr. Richard Petrone)

2. Investigation of trace element sources, transport and impacts at Lake Miwasin (SWAMP Lab, University of Alberta; leading PI: Dr. William Shotyk)

3. Experimental column, bioindicator and microbiology study (University of Alberta; PIs: Dr. Mohamed Gamel El-Din, Dr. James Stafford, and Dr. Patrick Hanington)

4. Advanced approaches to aqueous exposure and hazard characterization (University of Saskatchewan; PI: Dr. Karsten Liber)

Refer to the 2020 Water Mining Research Report (COSIA 2021) for a detailed description of each research program. An integrated Lake Miwasin modelling program was initiated end of 2019 and is currently ongoing.

PROGRESS AND ACHIEVEMENTS

The first five-year phase of the Lake Miwasin research and monitoring program started in March 2019 and continued through 2022.

Core Monitoring Program

Hatfield Consultants is retained to collect a full suite of physical, chemical and biological monitoring data from Lake Miwasin and its watershed. In 2022, 18 specialists and technicians worked on the Lake Miwasin monitoring program, conducting field sampling and maintaining the extensive array of instruments in the lake and throughout the watershed. Discrete and continuous data from 544 monitoring locations have been collected, which includes more than 50 continuous monitoring datasets, more than 700 environmental quality indicators, and more than 200 discrete sampling events. The monitoring team also collected samples and data for research teams from the University of Alberta, Athabasca University, and the University of Saskatchewan.

Analyses of the results of the 2022 program are underway, but key themes of findings from the monitoring program are the following:

- Lake Miwasin has been performing as expected both prior to and after the aquatic cover placement.

- Tailings and water quality conditions generally meet performance expectations with variation in sediment quality between the placed treated tailings and constructed littoral areas.

- No groundwater interaction with the Wood Creek Sand Channel (WCSC) aquifer has been observed.

- Our understanding of the water budget and physical dynamics of the lake continues to develop. The lake experiences typical patterns of stratification and mixing. Significant variation in climate from 2020 to 2022 is providing further insight into the hydrological network and water budget of the Lake Miwasin watershed.

- Vegetation continues to grow and expand across the upland, riparian and littoral areas. A mix of volunteer, including some exotics and planted species are successful. Trees, such as aspen and white spruce, are mixed with rough cinquefoil, raspberries, willows, and foxtail barley.

- Lake Miwasin water showed no toxicity to phytoplankton, invertebrates, or fish in laboratory tests. The lake has been colonized with native boreal aquatic organisms, including insects, plankton, and amphibians. Natural processes, such as primary production, nutrient and carbon cycling and community succession, are established in the lake and are supporting the establishment of the aquatic ecosystem.

Air Monitoring Program

AECOM is retained to monitor and assess air emissions from Lake Miwasin and the impacts of aerial deposition on the lake and upland ecosystems. The focus of air emissions monitoring in 2022 was on the use of a research-grade OP-FTIR operated over two two-week periods over the lake with two objectives: measure background concentrations expected when winds blow from emissions sources east and southeast of Lake Miwasin; and measure vertical profiles of concentrations above the lake to estimate emissions from the lake itself. About 30 chemicals were measured.

The background measurement program for east and southeast winds identified winds in summer come from this sector infrequently (less than 10% of the time). The contribution of other local emission sources to measured concentrations on the east side of Lake Miwasin was determined to be insignificant. It was inferred that measurements at Lake Miwasin are largely the result of non-local background emissions.

The vertical profile program continuously measured concentrations at three heights above ground – near ground level, and 5 m and 10 m above ground. The vertical radial plume mapping (VRPM) approach calculated emission rates from the surface of Lake Miwasin. Among the initial findings were:

- It appears the lake is not a measurable source of emissions of any of the target analytes.

- Many concentrations were not significantly different when comparing concentrations among the three measurement heights, suggesting the profiles were not from a ground-based (e.g., the lake) source.

- There was no indication that concentrations were different with winds blowing over the lake (background and lake sources) and winds blowing from the north (only background).

Overall, early results indicate emissions in Lake Miwasin are very low.

Watershed Hydrology Research Program

The Athabasca University and University of Waterloo research team continues to examine detailed field-scale hydrological processes and evolution that will be valuable to all partners when considering lake sustainability and design upscaling. Early (2020-2021) water table measurements of the "perched" groundwater system (<3m deep)) provided essential calibration values to the modelling team. Additional field measurements continue to be shared as requested to support other research groups.

Key findings of the watershed hydrology research program include:

- Spring snowmelt was identified as largest single contributing event to Lake Miwasin in 2021 and 2022. Mean peak 2022 SWE measurements across the upland (86 mm) were 16% higher overall compared to 2021 (74 mm). The flat areas and hummock features had significantly less snow ($p<0.05$) compared to the swales and opportunistic wetlands. Preliminary analyses indicate differing antecedent moisture conditions (dry 2020, wet 2021) led to different melt processes in 2021 and 2022 that are being investigated further.

- A comparison of the lake budget and mixing under dry (2020) versus wet (2021) conditions showed the importance of inflow from the upland to sustain lake levels: ~160 mm inflow was received by the lake from the upland during the 2020 open-water season when lake levels remained stable, while <10 mm was recorded in 2021 when lake levels dropped in response to high evaporation rates. Dry antecedent conditions created during the summer and fall of 2021 reduced surface runoff during the 2022 spring freshet. In spite of the reduced surface runoff, spring freshet was an important source of water for the lake, which contributed to the lake water level recovering to typical levels in spite of the dry preceding season.

- Distinct soil profile layers are observed, with higher saturated hydraulic conductivity and porosity and lower bulk density observed in the near-surface soil layer (~0 - 30 cm) than deeper in the profile (~30 – 125 cm).

- Preliminary water use efficiency results are beginning to highlight the critical and evolving role of the establishing vegetation and its water use on the trajectory of the upland, wetlands, and swales, especially with respect to water availability and runoff generation capacity from the uplands towards the lake, and the subsequent control on lake water levels. This represents a critical knowledge gap that needs to be addressed when scaling the Lake Miwasin design and modelling the system performance over future climate cycles.

- The role of the opportunistic wetlands in upland water budget is also being analyzed using the expanded suite of data variables collected during the 2022 summer. Understanding how the establishing vegetation water use influences overall upland water yield will be critical to being able to predict how sustainable water levels in the lake will be in the context of the region's climate cycle, while identifying any unique conditions under which they develop will help inform construction of future constructed site wetlands.

Since this is still a young system, new research questions continue to emerge as we learn more about it each year. Current research shows the swales are clearly evolving. As such, understanding how their changing hydrophysical properties and vegetation will influence their transmission role will elucidate information on stability of water levels as well. The upland soils are also evolving in response to vegetation growth. It will be important to address critical knowledge gaps by partitioning other components of the upland water budget and analyzing the evolution of the soil hydrophysical properties and how they affect water supply to the lake. Utilizing the reference lake dataset to inform key factors to consider when upscaling the design should also be prioritized.

Trace Elements Monitoring and Research Program

Early findings from the trace elements monitoring program suggest the trace element constituents of tailings are largely stabilized following the PASS treatment process. The concentrations of most trace elements have declined since the placement of aquatic cover. The lake serves as a sink for some trace elements contributed from the upland. Concentrations of dissolved molybdenum in Lake Miwasin surface waters have decreased from near 164 ppb to near 45 ppb between 2018 and 2022, and have stabilized. This suggests the flow-through design is effectively reducing the concentration of trace elements in Lake Miwasin over time.

Total arsenic concentrations in Lake Miwasin waters were consistently lower than the guideline value of 5 µg/L, and were overestimated when analyzed using ICP-MS. When measured using ICP-MS and HG-AFS, ICP-MS consistently overestimated the low arsenic concentrations in Lake Miwasin waters by 1-2 ppb. Selenium concentrations were similar using HG-AFS and ICP-MS.

Concentrations of trace elements in soils remained consistent at the same locations across several years. Spatial variation was observed, primarily associated with samples collected in swales compared to the hummocks and flat upland areas.

There were no acute toxic effects on *Daphnia magna* when exposed to Lake Miwasin waters and tailings. Limited toxic effects were observed during chronic exposures, including the uptake and accumulation of some trace elements. These outcomes were not altered by the addition of reference organic matter materials. Exposure to Lake Miwasin water significantly elevated the body burden of nickel in *Daphnia magna* while exposure to Lake Miwasin sediment significantly elevated the body burden of strontium. Significant accumulation of molybdenum and vanadium was also noted but this was rapidly depurated upon placement in clean water, indicating these elements are not bioaccumulated. Preventing access to sediments did not affect toxicity endpoints, suggesting a chemical rather than physical mode of toxicity. Additions of dissolved organic matter (DOM) did not significantly affect chronic exposure impacts on *Daphnia Magna*.

The pot experiment on uptake of Ni, V and Mo by foxtail barley (*Hordeum jubatum* L.) was completed. Samples of soils, plant biomass, and soil solutions were prepared and analyzed. Data analysis and interpretation were partially completed. Preparation and analysis of plant tissue samples are underway. The addition of fertilizer with Ni, Mo and V increased mean plant biomass. The addition of fertilizer alone did not increase mean plant biomass. The addition of Mo, Ni and V to soils without fertilizer also did not significantly impact plant biomass.

Bench-Scale Experimental Columns, Microbiology, and Bioactivity Study

Twelve experimental columns (height: 2.4 m, diameter: 200 mm), each with 0.72m of tailings and 1.44m of water cap, have been established at the University of Alberta under both oxic and anoxic conditions and results of the experimental columns are compared with the results obtained from the field to investigate the long-term impacts of treated tailings characteristics on the development of the lake ecosystem. Key results and findings are summarized below:

- Tailings consolidation has not reached stability; hence, further release of pore water and organics is expected in later years but at a lower magnitude. The mudline appeared within two hours of the column’s startup. Many physicochemical parameters, which showed fluctuations initially, displayed stability at the end of the fourth week in the capping water. Accordingly, the consolidation of tailings was prominent in this period and >14 cm of settling has been recorded at the end of two years. The settling rate was slightly higher in anoxic columns. There was no statistically significant difference in tailings consolidation between the anoxic and oxic columns. Dean-Stark analysis showed the bitumen content, water and solids were relatively stable at the top of tailings deposits in both oxic and anoxic columns. However, at the bottom, the water content was decreased and solids were increased. This observation clearly depicts the consolidation of sediments and release of tailings' porewater to the capping water. X-ray diffraction demonstrated the majority of the minerals in sediments were quartz, followed by kaolinite BISH 1989, and Muscovite 2M1. These minerals are typically found in oil sands tailings (Farkish and Fall, 2013). There were minimal differences between oxic column and anoxic column, as well as between top layer and bottom layer within each column, indicating oxygen condition and consolidation did not change the composition of mineral contents in sediments in the studied period.

- Physical parameters have shown stability; however, chemical parameters display slow release of organics. Alkalinity and salinity are increasing. The concentrations of dissolved organics (e.g., dissolved organic carbon, total organic carbon, naphthenic acids, acid extractable fraction) and conductivity gradually increased in the capping water of experimental columns with higher concentrations in anoxic columns. The slow increase in concentration could be attributed to the release of tailings' porewater in the capping water due to settling and consolidation. Further, slower biodegradation under anaerobic conditions could have resulted in the accumulation of naphthenic acids in anoxic conditions.

- The mobility of several metals/ions has been reduced or stayed unchanged, which signifies the efficacy of PASS treatment. The concentration of Mg, Sr, B, and Si in water have not changed over time in both oxic and anoxic conditions. An increase in Na and Cl concentrations was observed that increased the salinity and conductivity of the water cap. The sulfate concentration did not change over time significantly. On one hand, it shows alum was not dissociated upon PASS treatment of tailings. On the other hand, it also confirmed oxidation reduction potentials was not favorable for sulfate reduction. It is well-known that sulfate is highly water soluble and is extremely difficult to remove biologically. A sharp decrease in phosphate was recorded during the first few months of system's operation, indicating the main removal was due to physical processes, e.g. precipitation. The concentration of phosphate was nearly zero in field samples. This also reflected the initial increase in phosphate was probably due to disturbed conditions in the column's start-up phase. Accordingly, Ca concentration was also decreased, which may have been co-precipitated with phosphate. The concentration of other tested ions were relatively unchanged.

- The concentration of Total Kjeldahl Nitrogen (TKN) along with polyacrylamide (PAM) and acrylamide (AMD) has been decreasing over time. This is likely due to biodegradation of nitrogenous compounds as observed in lab-based studies. Acrylic acid is not detected throughout the study period. Similar concentrations of AMD were recorded between experimental columns and field samples, suggesting that experimental column may be used to understand PAM transformation processes in the field. The concentrations of ammonia, nitrite, and nitrate shows insignificant trends indicating active transformation of nitrogenous compounds. The field samples also depicted similar concentrations of TKN, nitrite, and nitrate as observed for DPL columns except for ammonia, which is not detected in the field samples.

- Different results from oxic and anoxic suggests unique mechanisms (bioactivity) for each system. However, as the study is still ongoing, it is difficult to make conclusive statements. This will also require attention to the chemical compositional changes at each level.

- Oxidation-reduction potential in capping water for both oxic and anoxic columns is not favorable for sulfate reduction and methanogenesis.

- Preliminary analyses on microbial autotrophy displayed presence of CO_2 fixation in the water column. Therefore, it can be deduced the system is able to support photosynthetic organisms with reduced toxicity.

The microbial communities of Lake Miwasin alongside the experimental columns are characterized using environmental DNA (eDNA) analysis to provide a comprehensive functional and taxonomic profile of bacteria and single-celled eukaryotes that provide essential ecosystem services in the boreal forest, including photosynthesis, carbon fixation, and nutrient cycling. By determining the microbiome of Lake Miwasin and associated sites over time, we can determine the extent to which biogeochemical processes necessary for a successful pit lake are consistently possible in the reclamation environment.

The research team has successfully developed a protocol for reliably extracting DNA from hydrocarbon-associated sediments, a key technological innovation necessary for eDNA analysis to be implemented more widely in the AOSR, as well as a robust bioinformatic pipeline for downstream computational analysis of eDNA to determine the taxonomic and functional information for the bacterial communities in Lake Miwasin. These have been successfully used to profile bacteria and single-celled eukaryotes in the experimental columns. Preliminary results showed a relatively small "core microbiome" dominated by methane cycling and sulfur reduction in the sediment. The microbial community structure of the water column was heavily influenced by oxygen availability, consistent with lakes in the region that freeze over in winter.

The bioactivity of Lake Miwasin at specific points during its construction (before and after water capping) was examined using macrophage bioassay. Post-inflammatory genes assessed showed acute activation between 2-10 hours of exposure using whole or fractionated water in quantitative polymerase chain reaction (qPCR) experiments. The nitric oxide (NO) pathway was also shown to be initiated in cells exposed to 10, 30 or 50% of the whole or organic water fractions, with an inverse relationship on exposure concentration and nitric oxide generation. Ultimately, the research completed had identified the organic fractions and post-water-capping samples as possessing the highest bioactive responses through gene- and protein-level analyses in mouse cell-line macrophages. Continued biomonitoring efforts of Lake Miwasin waters during mid-spring to late summer in 2022 has identified the persistence of bioactivity with samples spanning two different transects at benthic and littoral regions exhibiting concentrations of NO that exceed controls with cell-culture media and municipal tap water.

Over a 21-month (and counting) analysis of the experimental columns enacted to reflect anoxic and oxic parts of Lake Miwasin, it has also been demonstrated macrophage viability remains high at all sampled time points and cell death has been minimal across all samples. Interestingly, the concentration of NO during the early stages of the column sampling period (Week 1 to Month 4) had been shown to be noticeably higher than the negative media control. Month 5 samples had identified a separation in the anoxic and oxic responses, with the oxic columns inducing lower NO secretion on average compared to their anoxic counterparts. This trend had maintained itself until approximately the 18-month sampling period, with a reduction of NO quantified for all columns comparable to media controls.

The development of an in vitro bioassay for screening constituents of interest may be employed in future preliminary toxicity testing of aqueous mixtures in a reliable, cost effective, and rapid manner. Using macrophages to examine components that initiate a bioactive response may be used to supplement in vivo assays for a holistic understanding on mechanistic, biologically-informed, adverse-outcome pathways. By pinpointing observed bioactivity to specific samples post treatment or fractions, we hope to identify specific constituents of interest that contribute to the in vitro outputs observed.

Autonomous Sensor Network and Toxicity Characterization

Autonomous sensors are being used to track spatial and temporal changes in water quality, assess contaminant release from treated tailings over time, and monitor resuspension events at the sediment-water interface. Novel, site-relevant aquatic toxicity tests are also being conducted to track changes in the toxicity of surface water and substrate to aquatic organisms over time.

Autonomous sensors were able to capture and reproduce hourly, daily and seasonal variability of water quality parameters at discrete depths of the Lake Miwasin water column. Parameter values measured at the same depths and sampling dates using both sensors and grab samples were in close agreement, validating the sensor readings. The sensor data suggested:

- Lake Miwasin thermally stratifies during the summer months. This has implications for water quality nearest the sediment-water interface.
- Concentrations of dissolved salts have been progressively decreasing in the surface water of Lake Miwasin over time.
- There was no indication of spatial differences in water quality within the depth profiles of shallow and mid-depth sensor stations, suggesting water quality monitoring does not need to be expanded in these areas of Lake Miwasin.
- Sediment is a source of salts and trace metals to overlying water, either from pore water expression or geochemical redox processes.
- Periodic spikes in water column turbidity was possibly due to sediment resuspension or inflow from the lake catchment during summer storm events.

Overall, the autonomous sensors have performed well to date, collecting high-frequency water quality data at multiple sites and water depths. However, issues linked to cellular connectivity at site need to be addressed for improved real-time viewing of data. Sensor cables also need to be better protected from muskrats and minks.

The investigation of the toxicity of Lake Miwasin surface water is nearing completion, both at the near surface and near the sediment-water interface, including sediment pore water. No acute or chronic toxicity was observed for *D. magna* exposed to Lake Miwasin near-surface or near-bottom water. The acute and chronic toxicity of Lake Miwasin surface water (collected near-bottom, a worst-case exposure scenario) was also assessed using acclimated and unacclimated lab strains (*D. magna* and *D. pulex*) and native Daphnia sp. (from a natural lake). The results indicated Lake Miwasin surface water (collected from near-bottom) was not toxic under short- and long-term exposure conditions. Pore water isolated from bulk limnetic sediment was toxic to daphnid species, with more than one class of contaminant likely contributing to toxicity.

Sediment toxicity test results showed both limnetic and littoral (along the side slope) bulk sediment in Lake Miwasin (collected in 2020) were acutely toxic to midge larvae, with littoral sediment demonstrating comparatively less toxicity. However, limnetic sediment toxicity appears to have diminished with time. Interestingly, the top two cm of the limnetic sediment profile (collected in fall 2022) was not toxic to *C. dilutus* larvae in 10-d exposures. The agent(s) causing the observed toxicity in bulk sediment were not identified in whole sediment Toxicity Identification Evaluation (TIE) testing, with more than one class of contaminant likely contributing to toxicity.

LESSONS LEARNED

Lake Miwasin is still in an early stage for pit lake development. Preliminary results from the research and monitoring program indicate Lake Miwasin has been performing as expected since the commencement of the project. Some important findings have already been obtained (e.g., contribution of snow and spring freshet to the water budget and hydrological sustainability of the lake; influence of the upland on the hydrological and physical processes of the lake) from the early work. More insights and lessons learned will be gained with the progress of lake development and the completion of the first 5-year R&M program. Lessons learned across all components of the Lake Miwasin program will be discussed in detail at the end of the five-year monitoring program.

PRESENTATIONS AND PUBLICATIONS

Journal Publications:

Published

Choi S., Cuss C.W., Nagel A.N., Shotyk W., Goss G., Glover C. 2021. An Investigation of Chronic Trace Element Toxicity of Lake Miwasin Water and Sediment to Daphnia magna. 42nd annual meeting, SETAC North America. November 14–18, 2021 (Oral presentation)

Hussain N., Lillico D.M.E., Richardson E., Choo-Yin Y., Hanington P., Stafford J.L. 2021. An immune cell-based assay for detecting and monitoring bioactive constituents within oils sands process-affected waters (OSPW). Presented at Society of Environmental Toxicology and Chemistry (SETAC) North America 42nd Annual Meeting, Portland OR, USA. November 14-18, 2021 (Oral presentation).

Choi S., Cuss C.W., Shotyk W., Glover C.N. 2022. Chronic trace element toxicity of Lake Miwasin water and sediment to *Daphnia magna*. *Faculty of Agriculture, Life, and Environmental Sciences Graduate Student Symposium*, Edmonton, Alberta.

Choi S., Cuss C.W., Glover C.N., Goss G., Shotyk W. 2022. Chronic toxicity and bioaccumulation in daphnids exposed to water and sediment from an oil-sands tailings pit lake. *Canadian Ecotoxicology Workshop*, Winnipeg, Manitoba.

Choi S., Cuss C.W., Glover C.N., Goss G., Shotyk W. 2022. Chronic toxicity and bioaccumulation of trace elements in daphnids exposed to water and sediment from an oil-sands tailings pit lake. *43rd Annual meeting, SETAC North America*, Pittsburgh, Pennsylvania.

Hansika D., Cuss C.W., Du L., Noernberg T., Shotyk W. 2022. Influence of Concentration and Speciation of Trace Elements in Soil Solution on Plant Uptake and Accumulation by Hordeum Jubatum L. *Canadian Society of Soil Science annual general meeting*, Edmonton, Alberta.

Hansika D., Cuss C.W., Du L., Noernberg T., Shotyk W. 2022. Influence of Concentration and Speciation of Trace Elements in Soil Solution on Plant Uptake and Accumulation by Hordeum Jubatum L. *Faculty of Agriculture, Life, and Environmental Sciences Graduate Student Symposium*, Edmonton, Alberta.

Hansika D., Cuss C.W., Du L., Dyck M., Shotyk W. 2022. Influence of concentration and speciation of V, Ni and Mo in soil solutions on plant uptake and accumulation by *Hordeum jubatum L. Soil Science Society of America*, Baltimore MD.

Nagel A., Cuss C.W., Shotyk W., Glover C.N. 2022. Understanding the uptake and bioaccumulation of trace metals following exposure of aquatic invertebrate *Lumbriculus variegatus* to pit lake sediments from the Alberta Oil Sands Region. 43rd Annual meeting, SETAC North America, Pittsburgh, Pennsylvania.

Richardson E., Arslan M., Gamal El-Din M., Hanington P. 2022. Microbiome analysis of tailings reclamation protocols in the Athabasca Oil Sands Region. BioNet Conference, Calgary Alberta.

Lillico D.M.E., Hussain N.A.S., Choo-Yin Y., Gamal El-Din M., Stafford J.L. 2023. Establishing oil sands process-affected water bioactivity profiles using immune cell-based bioassays. Journal of Environmental Sciences. DOI: 10.1016/j.jes.2022.07.018

Hussain N.A.S., Stafford J.L.. 2023. Abiotic and Biotic Constituents of Oil Sands Process-Affected Waters. Journal of Environmental Sciences. DOI: 10.1016/j.jes.2022.06.012

Hussain N.A.S., Lillico D.M.E., Choo-Yin Y., Qin R., Zuo Tong How, Paul S., Gamal El-Din M, Stafford J.L. 2022. Detection and Tracking of Inflammatory Constituents in Environmental Water Samples Using Immune Cell Lines. Oral presentation. Society of Environmental Toxicology and Chemistry. Pittsburgh, USA.

Panigrahi B., Doig L., Davila A.C., Ezugba I., Liber K. 2022. Application of Machine Learning Algorithms to Predict Key Water Quality Parameters in an Oil Sands Demonstration Pit Lake Based on Real Time Sensor Data. Canadian Ecotoxicity Workshop (CEW), Winnipeg, MB, Oct- 2-5, 2022

Panigrahi B., Doig L., Davila A.C., Ezugba I., Liber K. 2022. Use of Autonomous Sensors to Monitor Spatio-temporal Changes in Water Chemistry in an Oil Sands Demonstration Pit Lake, Alberta. 20th International Symposium on Toxicity Assessment (ISTA 20) Saskatoon, SK, August 15-18, 2022

Davila C.E., Doig L.E., Panigrahi B., Ezugba I., Liber K. 2022. Effects of water from Suncor's Demonstration Pit Lake, Lake Miwasin, on saline-tolerant zooplankton 20th International Symposium on Toxicity Assessment (ISTA 20) Saskatoon, SK, August 15-18, 2022

Ezugba I., Doig L., Panigrahi B., Davila C. E., Liber K. 2022. Toxicity Assessment of bottom-substrate from a Pilot-Scale Pit Lake containing Polymer-Treated Oilsands Tailings. SETAC North America 43rd Annual Meeting, Pittsburgh Convention Center, Pittsburgh, Pennsylvania, USA, November 13–17, 2022

RESEARCH TEAM AND COLLABORATORS

Institution: Multiple consultants and research institutions

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WATER TREATMENT

WJ0132 - Water Return Demonstration Project

COSIA Project Number: WJ0132

Research Provider: Syncrude Canada Ltd.

Industry Champion: Syncrude Canada Ltd.

Industry Collaborators: Canadian Natural, Imperial Oil Ltd., Suncor Energy Inc., Teck Resources Limited, University of Alberta, Alberta Innovates

Status: Complete

PROJECT SUMMARY

This report describes the results of the Water Return Demonstration Project (WRDP). This was a large-scale pilot program designed to treat oil sands process water (OSPW) with petroleum coke (PC), a byproduct of Syncrude's Fluid Coking™ process used to upgrade bitumen into synthetic crude oil. Previous studies have shown PC can be used as activated carbon to reduce concentrations of dissolved organic compounds present in OSPW. Syncrude produces about 20 kg of product PC per barrel of synthetic crude oil produced. Based on current production rates, the technology has the potential to treat at least eight million m3 of OSPW per year.

The WRDP was based on a three-stage treatment process and involved the hydraulic deposition of a freshly produced slurry of OSPW and PC (Stage 1) into a containment facility (Stage 2), approximately 150 m wide by 465 m in length. The rate of collection of treated OSPW is controlled with an under-drain system installed at the base of the containment facility within the formed PC deposit. The reaction kinetics of the adsorption process are such that the longer the water is retained within the voids of the coke deposit (porewater residence time), the further the reduction in component concentrations. For the field program completed in 2021, porewater residence times were between about two weeks and two months. The collected water was then directed to an aerated pond (Stage 3) with a residence time of about eight days to permit additional treatment to reduce ammonia concentrations and for final quality testing of the treated OSPW.

The water quality testing program included two elements: (a) an onsite testing program to quantify the chemistry and acute toxicity of OSPW from the source and throughout the treatment process and, (b) a detailed aquatic toxicity study designed to test sub-lethal effects using a broad suite of toxicity tests. These included fathead minnows (Pimephales promelas), green algae (Pseudokirchneriella), Ceriodaphnia dubia, Hyalella 1zteca, fingernail clams (Sphaerium sp.), freshwater mussels (Lampsilis siliquodia), and walleye (Sander vitreus).

Mesocosms experiments filled with WRDP treated water and inoculated with periphyton and benthic invertebrates sourced from the Athabasca River, were also used to assess the WRDP-treated OSPW toxicity.

Literature cited:

AEP (Alberta Environmental Protection). (1995). Water Quality Based Effluent Limits Procedures Manual. Environmental Protection. Edmonton. Available at: http://environment.alberta.ca/01216.html

Four Elements (2022), Four Elements Consulting Ltd. Athabasca River Model Documentation. Available online at https://fourelements.sharepoint.com/sites/AthabascaRiverModel

PROGRESS AND ACHIEVEMENTS

The treatment process reduced concentrations of dissolved organic compounds and produced treated OSPW that was not acutely toxic based on bacteria, zooplankton, and fish bioassays. Removal efficiencies for naphthenic acids (NAs) were about 85%. Removal of phenolic compounds and hydrocarbons exceeded 95%. Removal of polycyclic aromatic hydrocarbons (PAHs) exceeded 99%. Reductions in concentrations of total suspended solids (TSS) and ammonia were greater than 98%.

The most sensitive sub-lethal endpoint was found to be periphyton growth rates when treated OSPW was mixed with Athabasca River at concentrations of 3.2% and higher. At treated OSPW concentrations less than 3.2%, there were no effects to periphyton or any of the species tested. This result was used because it is the most sensitive and assuming a treated OSPW release of eight million m3/year into the Athabasca River flowing at the 1Q10 flow condition (~99m^3/s), modelling calculations indicate a 3.2% dilution would be achieved between about 100 and 200 m downstream of the release point (Four Elements, 2022). This is about an order of magnitude more conservative than the two-km length restriction as per chronic mixing zone guidance recommended for industrial and municipal discharges in Alberta (AEP, 1995).

PRESENTATIONS AND PUBLICATIONS

Journal Publications:

Published

Syncrude Research and Development. 2022. Water Return Demonstration Project. DOI: https://doi.org/10.7939/r3-qctf-dy81

RESEARCH TEAM AND COLLABORATORS

A project team of highly experienced individuals was assembled to advance the closed-circuit phase of the WRDP and collect a defendable body of scientific information to support potential release of treated water to the Athabasca River. Because Reactor 1 of the WRDP is fed by an operating coke sluicing line, which is an integral component of Syncrude's crude oil production process, the entire treatment facility was operated and maintained by Syncrude staff.

To support the toxicological and biological evaluation of the treated water, Syncrude retained Hatfield Consultants Inc. Hatfield is a leading provider of aquatic environmental monitoring in western Canada, with clients in various sectors including; pulp and paper, mining, oil and gas, and government.

Hatfield also retained the professional services of Limnotek Research and Development, Nautilus Environmental, and Environment Canada and Climate Change (ECCC) to provide additional scientific expertise to support the closed-circuit evaluation. Limnotek has been providing research and consulting services since 1984 and has extensive experience in the design, field implementation and interpretation of mesocosm studies. They have worked on numerous jointly implemented studies with ECCC and have a history of innovative custom equipment design for field-based studies. Nautilus Environmental operate toxicity testing laboratories in Calgary and Vancouver, which are accredited by the Canadian Association of Laboratory Analyses (CALA). They have extensive experience operating mobile testing laboratories and conducting long-term tests with species such as fathead minnows and other organisms. Modern approaches to the use of mesocosms to evaluate water quality were pioneered by ECCC in the 1980s and

ECCC scientists supported the mesocosm experiments with respect to design and construction. They will continue to provide a key role by informing data analyses and interpretation.

WJ0188 - Optimizing OSPW Treatment in Constructed Wetland Systems with Genomic-Based Tools

COSIA Project Number: J0188

Research Provider: University of Calgary and Natural Resources Canada

Industry Champion: Imperial Oil Ltd.

Industry Collaborators: None

Status: Year 2 of 4

PROJECT SUMMARY

A broad family of organic compounds called naphthenic acid fraction compounds (NAFCs) are constituents of oil sands process-affected water (OSPW) and are known to be major contributors to its toxicity. Constructed wetland treatment systems (CWTS) are a scalable and cost-effective method being considered for the treatment of OSPW. The optimal conditions required to establish specialized wetland biological communities able to degrade and detoxify NAFCs, as well as the specific degradation pathways involved in these processes, are currently not well understood. This research project aims to apply genomic tools to better understand NAFC-biodegradation processes in CWTS and provide insight on approaches to enhance the efficacy of CWTS for OSPW treatment. Using mesocosm-scale greenhouse experiments and a pilot-scale CWTS on Imperial's Kearl Oil Sands site as experimental systems, this project will:

(1) Assess the dynamics and efficacy of CWTS for the treatment of OSPW under varying conditions;

(2) Identify key biological components (microbes, plants, genes) that operate in reducing OSPW toxicity and NAFC biotransformation;

(3) Examine the fate of NAFCs from biodegradation;

(4) Advance the development of passive samplers and biosensors for NAFC toxicity and quantification;

(5) Develop contaminant-fate models for the optimization of CWTS design;

(6) Integrate emerging experimental findings with advanced social-science methods to provide technical recommendations and social perspectives with the potential to inform practices and policies related to the use of genomics for improving water treatment using CWTS.

This transdisciplinary project is funded by Genome Canada as part of the Large-Scale Applied Research Project (LSARP) Program. It involves 16 research groups from universities and federal institutions across Canada. While this project is focused on the remediation of NAFCs, the approaches and outcomes are expected to contribute to the know-how and application of CWTS treatment of other contaminants in various industrial and municipal wastewater around the world.

PROGRESS AND ACHIEVEMENTS

This research project consists of five integrated research activities that have all been progressing in parallel.

Activity 1 – Optimize the rate of NAFC remediation using CWTS mesocosm systems

In this activity, replicated surface-flow greenhouse mesocosm experiments are conducted to determine the impact of various parameters, such as plant species and temperature, on microbial community structure, and the remediation and toxicity reduction of OSPW. While providing valuable insight for the optimization of future CWTS, these experiments contribute rhizosphere/root samples for metagenomics analyses (Activity 2) as well as data to construct predictive models (Activity 4). Two experiments were completed using different plant species and temperature regimes. The plant species used in these experiments included *Carex aquatilis* in Experiment #1 and *Triglochin maritima* and *Scirpus microcarpus* in Experiment #2. The effect of temperature (i.e. 20°C day, 10°C night vs 10°C day, 5°C night) was assessed in Experiment #2. No-plant control mesocosms were analyzed alongside the planted mesocosms in both experiments. General substrate and OSPW quality and characteristics were collected and high-resolution Orbitrap MS was performed to characterize NAFC attenuation. As an example of NAFC reduction in these mesocosms, the total NAFC concentration decreased approximately 50% over a 4-week treatment period. Within the NAFCs, the O2 class gradually decreased in relative abundance (~10%) to other classes, while O3 and O4 NAFCs correspondingly increased in relative abundance.

Extensive analysis of the microbial communities present in rhizosphere (soil attached to roots), roots, and sediment samples from Experiment #1 was performed (analysis is ongoing for Experiment #2). Results demonstrated microbial communities within *Carex* roots (endophytes) are clearly distinct from those of the other compartments, but also indicated: 1) rhizosphere communities can be distinguished from those of the sediments; 2) sediments from unplanted mesocosms are different from those of planted mesocosms; and 3) communities significantly change over time in the mesocosms (Figure 1).

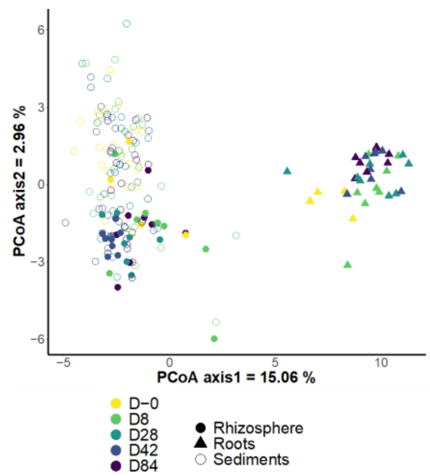

***Figure 1**. Principal coordinate analysis of microbial communities in various compartments (rhizosphere, roots, sediments) of the mesocosms at different time points based on Bray-Curtis dissimilarities of shotgun metagenomics data. D-0: samples collected before the addition of OSPW in the mesocosms; D8, D28, D42, D84: samples collected eight, 28, 42 or 84 days after the addition of OSPW in the mesocosms.*

Activity 2. Deepen the understanding of the mechanisms of NAFC metabolism and enrich for microorganisms with enhanced NAFC-degrading ability

Activity 2 aims to enhance our understanding of genes and pathways involved in NAFC degradation and toxicity reduction in plants and microorganisms through the metagenomics and metatranscriptomics analysis of samples from Activity 1, the enrichment and isolation of NA degrading bacteria and algae from samples collected in activity 3, and the implementation of plant laboratory experiments. An important first step for this activity has been to review the literature on microorganisms and genes currently known to be involved in NA degradation. This information has been summarized in a review paper that will be submitted for publication shortly. A list of genes generated from this work can now be used to screen metagenomic datasets. Based on this list, metagenomic data analysis of samples from mesocosm Experiment #1 identified several NA degradation genes whose relative abundance was influenced by *Carex* roots. A community shift over time was also identified, with the cluster of NA degradation genes at the end of the experiment returning to a structure similar to that observed at the beginning of the experiment, and concomitant with a reduction in NAFC in water through time. Further analysis will investigate the presence of other metabolic and stress-related genes in the metagenomes as well as the level of gene expression to complete the material for the first manuscript pertaining to metagenomics and metatranscriptomics results.

Assessment of the contribution of resident bacterial and algal communities and isolates to NAFC degradation and toxicity reduction is also ongoing. Numerous bacterial and algal isolates were enriched for their ability to grow on NAs as a sole carbon source and identified by sequencing. These isolates will be studied further to identify NA degradation genes and to test their potential as contributors to NAFC degradation in bioaugmentation studies.

Finally, experiments are ongoing to further characterize the uptake/degradation of NAs by plants and identify potential genes involved in this process. Experiments have been initiated to understand the fate of NAs taken up by wetland plant tissues using isotopically labelled model NAs and NAFCs from OSPW. This research builds on previous work of the team on upland plant species and will provide insight on cellular metabolism of NAs and the accumulation, tissue localization and composition of NA degradation products. A genetic screen using a model plant species is also progressing with the identification of several plant lines having growth tolerance to high NA treatments. This approach allows for the functional identification of NA-tolerant plant genes, as genetic selection is based on the ability of specific lines to successfully grow under high NA treatment conditions.

Activity 3. Analyze the biological dynamics and bioremediation ability of a pilot CWTS

The Kearl CWTS is a 1 ha recirculating, horizontal surface flow wetland that currently operates with a 14-day water retention time and 400 m^3 outflow per day. The wetland has operated since 2013. OSPW was first introduced into the system in 2021. Single-pass reduction in NAFCs (14 days) was observed from 21 mg/L to 14 mg/L (FTIR) in 2021. The Orbitrap NAFC data showed an attenuation of O2 components, increasing proportions of O3 and O4 components, and a decrease in lower molecular weight O4 components. Metagenomic analysis of samples collected in 2021 in the different cells of the pilot CWTS before the addition of OSPW (day -0) and on day 15 and 45, after the addition of OSPW, showed bacterial community structure is influenced by sample type (i.e., sediment, rhizosphere, roots), plant species (*Carex/*Cattail), and time (Figure 2). The increase in the relative abundance of the genus *Pseudomonas* after the addition of OSPW in the pilot wetland (mostly in the roots of Cattail) indicate its tolerance and/or capability to degrade toxic compounds found in OSPW. Indeed, some members of the *Pseudomonas* genus are known for their involvement in NA degradation and several of the NA tolerant isolates recovered from the pilot wetland (Activity 2) belong to this taxonomic group. Numerous genes involved in the degradation of xenobiotics were identified in the wetland bacterial communities, including several known NA-degradation genes.

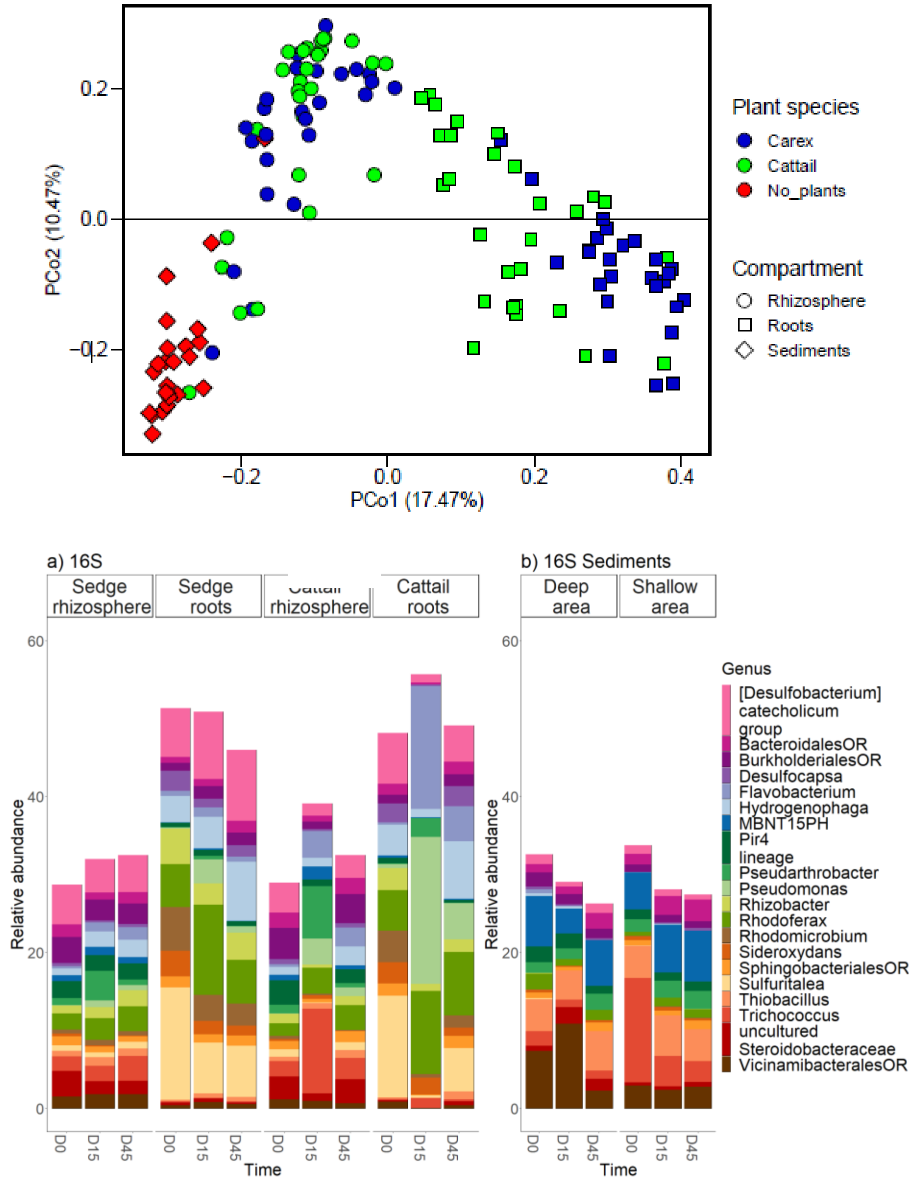

Figure 2. *Principal coordinate analysis (upper panel) and taxonomic profiles (lower panel) of microbial communities in various plant species (Carex (sedge) and Cattail) and compartments (rhizosphere, roots, sediments) of the Kearl CWTS (2021 samples). D0: samples collected before the addition of OSPW in the CWTS; D15 and D45: samples collected 15 or 45 days after the addition of OSPW in the CWTS.*

Year 2 sampling of the Kearl wetland during OSPW flow was comprehensive, with more than 1,000 samples collected throughout the field season (from May to Sept. 2022), including water samples, sediments, and plant tissues (*Carex* and cattail) for physico-chemical, microbiological, and genomics analyses. Similar NAFC attenuation and O2 class dynamics were observed in this season compared to 2021. Metagenomic and metatranscriptomic analyses are ongoing.

This project also aims to enhance and develop simple and efficient ways of testing for OSPW toxicity and NA remediation efficiency. These tools have the potential to reduce costs and effort, use small water volumes, and eliminate the use of animals for toxicity testing. Biomimetic Extraction - Solid Phase Micro Extraction (BE-SPME) has been shown to be an effective "passive sampler" of the bioavailable NAFCs in treated OSPW. This project will further calibrate the BE-SPME assay approach using rainbow trout and walleye embryo development. Results have successfully demonstrated consistency of rainbow trout toxicity testing with BE-SPME. As other research groups have observed, approaches that test walleye embryo development in response to OSPW treatment are challenging. However, experiments performed to date have shown some success in calibrating BE-SPME data to walleye embryo toxicity, and this work will be continuing.

Sensitive and specific biosensors relying on the diverse environmental and metabolite sensing mechanisms of bacteria are also being developed as a simple, rapid, inexpensive and high throughput technology to detect and quantify NAs. DNA sequences from gene promoters (switches) that activate genes found to be highly overexpressed in response to NAFC exposure (based on metatranscriptomics results from Activity 2) will be isolated and used to express bacterial reporter genes as a NAFC concentration indicator.

Activity 4 – Integration and synthesis of CWTS and genomics data using bioinformatics and mass balance modelling

The development of genomic-based and mass balance models in this project will (i) explore the role of genomic data in relation to other physical, chemical and ecological characteristics/data affecting wetland treatment; (ii) relate observations at the lab, mesocosm and field scales; (iii) extrapolate results from this study to alternative conditions in the wetland or other wetlands in Canada; and (iv) document and formalize the findings of this study for future studies.

A literature review was conducted to identify the best genomic modelling options for this project (see publication by Correa-Garcia et al.) and some statistical learning tools (Lasso, Ridge and Elastic Net regression) were tested, but the data is yet not sufficient to generate robust models. Once a substantial amount of data is generated, the selected tools will be applied to identify genetic markers that predict with a high accuracy the decrease in NAFC concentrations and in the toxicity of the outflow water.

Contaminant fate modelling is another modelling approach used in this study to integrate findings on plant uptake and metabolism, microbial degradation, wetland characteristics (e.g., dimensions, hydraulic retention), environmental conditions and toxicity. A previously developed model using the Kearl pilot wetland as study site was further developed to evaluate treatment efficiency under both study conditions (i.e., fully recycled) and commercial operation conditions (i.e., flow through). The modelling results illustrate the interplay between different contaminant removal processes that control the treatment of OSPW in the wetland throughout the course of the treatment process. For example, results indicated that initially microbial and plant-based breakdown play a minor role in the removal of NAs and other organics from OSPW, but the importance of microbial and plant degradation increases over time to become the dominant process controlling OSPW treatment. Data for model development, evaluation and testing continues to be generated in other activities and will be integrated in the model as it becomes available.

Activity 5 – Using advanced social science methods to provide technical recommendations and social perspectives related to the use of genomics for improving water treatment with CWTS

This activity examines social, cultural, economic, legal and artistic dimensions associated with genomic-informed bioremediation. It aims at better understanding public perceptions and preferences on the topic. The components of this activity are integrated throughout the project (activities 1 through 4) and will combine multiple advanced social science methods. This includes engaged research in the form of initial and progressively deepening experimental decision laboratories (EDL), focus groups, Q-sorting, a national survey, and arts-based research-creation. This research is progressing well. Details are not provided here as this research is outside of the scope of this report.

LESSONS LEARNED

This project is advancing knowledge and understanding of the mechanisms underlying NAFC degradation and OSPW toxicity reduction in CWTS by applying state-of-the-art genomic tools to the study of 1) meso-scale replicated experimental systems allowing to measure the effect of multiple treatments under controlled conditions and 2) a pilot-scale experimental system reflecting *in situ* conditions. Although the project is still in its early stages, this work has shown plant-associated microbial communities are dynamic in response to OSPW exposure and the shift in communities follows the NAFC degradation pattern. While further work is needed to clearly identify microorganisms, genes and pathways that drive NAFC biodegradation and develop strategies to enhance this process, the detection of several NA degradation genes in our samples, the isolation of multiple NA-tolerant bacterial and algal strains, and the identification of plant genetic variants that demonstrate growth tolerance on high levels of NAs are all important first steps towards achieving project goals.

PRESENTATIONS AND PUBLICATIONS

Journal Publications:

Published

Sara Correa-Garcia, Philippe Constant, Etienne Yergeau. 2022. The forecasting power of the microbiome. Trends in Microbiology. DOI: https://doi.org/10.1016/j.tim.2022.11.013

Submitted, under review

Kaitlyn E. Trepanier, Ian J. Vander Meulen, Jason M.E. Ahad, John V. Headley, and Dani Degenhardt. Evaluating attenuation of naphthenic acids in constructed wetland treatment systems planted with Carex aquatilis.

In preparation

Paula C.J. Reis, Julien Tremblay, Sara Correa-Garcia, Aurélie Beaulieu-Laliberté, Douglas Muench, Jason Ahad, Étienne Yergeau, Jérôme Comte, Christine Martineau. Microbial degradation of naphthenic acids: metabolic and genomic insights for improved bioremediation of affected water.

Conferences, presentations, and workshops:

Presented

Aurélie Beaulieu-Laliberté, Paula Reis, Christine Martineau, Jason Ahad, and Jérôme Comte. Oil sand process-affected water remediation through microbial biodegradation in a constructed wetland treatment system. Conference of the Society of Canadian Aquatic Sciences. Montreal, QC. February 22-25, 2023.

Annie Zymela, Lisa Gieg and Douglas Muench. Bioaugmentation of the Plant Rhizosphere to Support Constructed Wetland Treatment Systems for Oil Sands Process-Affected Water. Poster session at the 59th annual Alberta Soil Science Workshop, Calgary, AB. February 21-23, 2023. First prize, student poster session.

Mitchell Alberts, Christine Martineau and Douglas Muench. Application of Genomics to Enhance Constructed Wetland Treatment Systems for Remediation of Oil Sands Process-Affected Water. Oral presentation at the 59th annual Alberta Soil Science Workshop, Calgary, AB. Feb 21-23, 2023.

Aurélie Beaulieu-Laliberté, Paula Reis, Christine Martineau, Jason Ahad, and Jérôme Comte. Oil sand process-affected water remediation through microbial biodegradation in a constructed wetland treatment system. GRIL Annual Symposium. Montreal, QC. February 20-22, 2023.

Sara Correa-Garcia, Julien Tremblay, Marie-Josée Bergeron, Kaitlyn Trepanier, Dani Degenhardt, Christine Martineau, Etienne Yergeau. Metagenomics to enhance the remediation of oil sands process-affected water in northern environments. EcotoxicoMic 22. Montpellier, France. November 17, 2022.

Michelle Tilford-Shaw, Lori Bradford. Application of Genomics to Enhance Wetland Treatment Systems for Remediation of Processed Water in Northern Environments. COSIA Mining Subcommittee Social Science Discussion. Edmonton, AB. November 15, 2022.

Christine Martineau, and Douglas Muench. Genomics applications for optimization of constructed treatment wetlands for oil sands process-affected water. Canada's Oil Sand Innovation Alliance (COSIA) 2022 Mine Water Management Science Workshop. Edmonton, AB. October 18-19, 2022.

Sara Correa-Garcia, Julien Tremblay, Marie-Josée Bergeron, Kaitlyn Trepanier, Dani Degenhardt, Christine Martineau, Etienne Yergeau. Genomics and statistical learning to enhance the remediation of oil sands processed water. ISME-18. Lausanne, Switzerland, August 16-19, 2022.

Aurélie Beaulieu-Laliberté, Paula C.J. Reis, Jason Ahad, Christine Martineau, Jérôme Comte. Microbial degradation of naphthenic acids: metabolic and genomic insights for optimized bioremediation of oil sands process-affected water. GRIL Annual Symposium. Montreal, QC. March 15-16, 2022

Mitchell Alberts, Christine Martineau and Douglas Muench. Application of Genomics to Enhance Constructed Wetland Treatment Systems for Remediation of Oil Sands Process-Affected Water. Oral presentation at the International Phytotechnology Conference. May 23-26, 2023, Chicago.

RESEARCH TEAM AND COLLABORATORS

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WJ0174 - Development of a Passive, Plant-Based Naphthenic Acid Remediation Technology for Treatment of Oil Sands Process-Affected Water

COSIA Project Number: WJ0174

Research Provider: University of Calgary, Northern Alberta Institute of Technology (NAIT)

Industry Champion: Canadian Natural

Industry Collaborators: Imperial Oil Ltd.

Status: Year 3 of 3 (A no-cost one year project extension has been approved)

PROJECT SUMMARY

Surface mining of bitumen in the Canadian oil sands results in the accumulation of oil sands process-affected water (OSPW). This research project is aimed at determining the capacity of selected upland plant species and their associated microbes to remove organics from OSPW in an irrigated soil environment. This treatment approach builds on numerous examples of successful water treatment strategies that use an irrigated plantation approach but is tailored to OSPW. It takes advantage of the natural genetic diversity and extensive metabolic capacity of plants and microbes to remediate contaminated environments. This upland plant-based approach could be applied on a large scale using a gravity-based water distribution system capable of irrigating large volumes of OSPW through plant root systems on a sloped landscape lined with a clay or geotextile base.

The organic fraction of OSPW is comprised of thousands of different compounds that include recalcitrant forms of naphthenic acid fraction compounds (NAFCs). The benefit of using this approach is native plant species provide diverse genetic complexity with the potential to enhance NA remediation. We will screen for the most promising plant species and native genotypes for growth and remediation in an OSPW-irrigated environment.

The specific objectives of this research are to:

1) Screen natural genotypes of diverse, native plant species and genotypes to determine their suitability and capacity for growth in an OSPW irrigated environment and their ability to take up NAFCs. (Phase 1)

2) Perform greenhouse mesocosm experiments with the selected genotypes from Phase 1 to determine the effectiveness of communities of plants and their associated microbes to reduce toxicity and remove NAFCs from OSPW. (Phase 2)

PROGRESS AND ACHIEVEMENTS

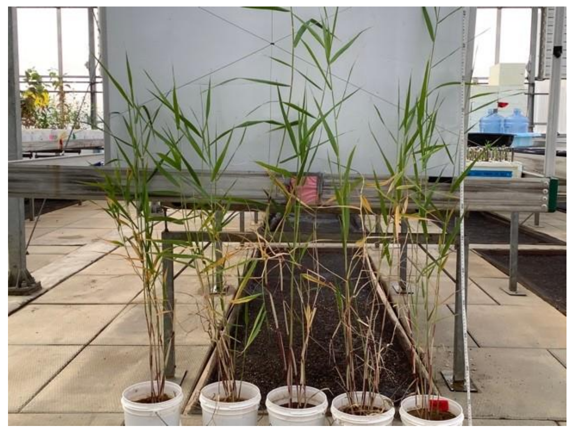
Phase 1 – Screen for species/genotypes suitable for growth under OSPW stress conditions and establishing assays for NAFC removal ability

Phase 1 was executed in three distinct components. In phase 1A, we optimized our plant genotype screening approach to integrate screening for OSPW toxicity as well as other key environmental extremes (flooding and drought). In phase 1B, we screened genotypes from three genera (*Calamagrostis canadensis*, *Solidago canadensis*, and *Salix* spp. including *Salix interior*). A total of 64 genotypes, with a short list of 14 genotypes, were selected for phase 1C (refer to Figure 1 for a visual example). Twenty genotypes from six additional species (*Populus balsamifera*, *Populus tremuloides*, *Cornus sericea*, *Carex aquatilis*, *Elymus trachycaulus* and *Phragmites australis*) were also included in phase 1C, though they were not pre-screened in phase 1B as there were too few individually viable genotypes for comparisons.

Phase 1C, which compared the ability of individual genotypes and species in their ability to treat OSPW, was completed in summer 2022. While many of these genotypes were selected from a broader pool of genotypes in phase 1B, we did still observe differences in growth and survival between genotypes and species (Figure 1). A major challenge of this phase was ensuring all species experienced similar volumes of exposure to OSPW. As these species grew at different rates and tended to consume water differentially, this necessitated that some species were allowed to grow for a greater length of time. To account for this experimental difference, we maintained a sample set of unplanted controls that were harvested periodically throughout the entire greenhouse phase from the time when the first species were harvested and for an additional three weeks beyond the final plant harvest. These controls allowed us to understand the soil-based influence/changes in NAFC degradation over time, which did tend to increase. While interpretation of the NAFC data set is ongoing, we have found good agreement between NAFC analysis conducted in our lab with BE-SPME (biomimetic extraction) results conducted on the same sample set.

***Figure 1**. Visual images of individual genotypes and plant species tested in phase 1C.*

Salix spp. genotype 12	*Salix* spp. genotype 11
	
Salix interior genotype 8	*Salix interior* genotype 8

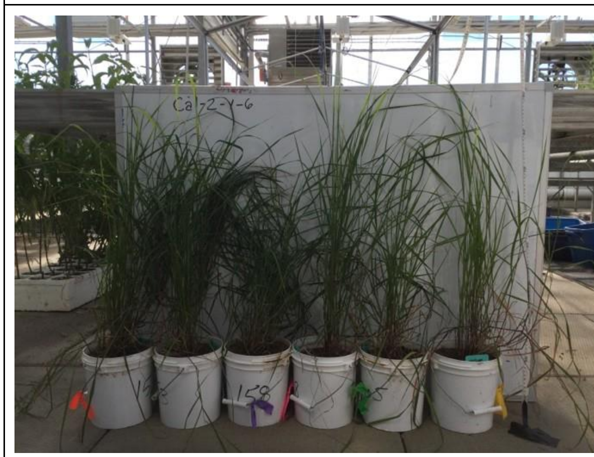
	
Populus balsamifera genotype 7	*Phragmites australis* subsp. *americanus* genotype 1
	
Cornus sericea genotype 6	*Cornus sericea* genotype 5
	
Populus tremuloides genotype 3	*Populus tremuloides* genotype 1

Solidago canadensis genotype 3

Solidago canadensis genotype 14

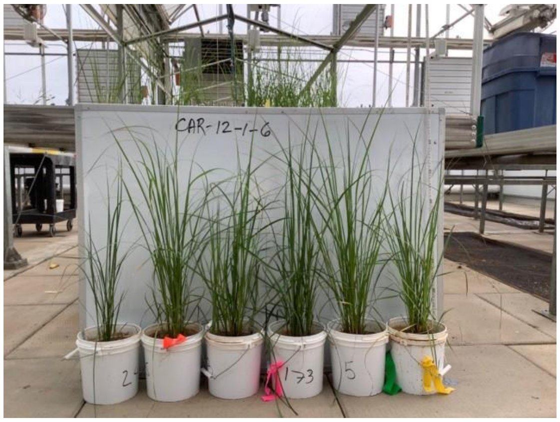
Calamagrostis canadensis genotype 2

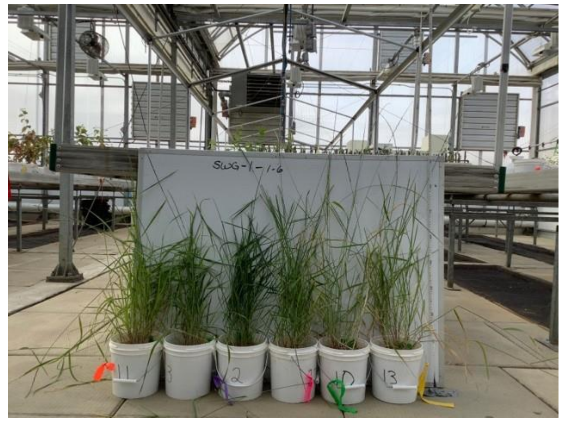
Calamagrostis canadensis genotype 6



Carex aquatilis genotype 12

Elymus trachycaulus genotype 1





Phase 2 – Greenhouse mesocosm studies using selected plant species/genotypes

Replicated greenhouse mesocosm systems have been developed, tested and implemented. These mesocosms are recirculating subsurface flow systems with 25 to 150 L of soil substrate. Experiments included planted and no-plant control mesocosms. Substrate, water flow, nutrient addition and sampling regimes are optimized. Temperature, evapotranspiration, plant biomass, redox, pH, toxicity (BE-SPME and aquatic organism), water quality, and high-resolution mass spectrometry were collected from each mesocosm throughout the study.

A replicated mesocosm experiment tested the ability of willow plants to reduce toxicity and NAFC content (Orbitrap and FTICR mass spectrometry) in consecutive cycles of fresh OSPW circulation to determine the OSPW treatment efficiency over extended periods of time. Eight one-week treatment cycles were performed and demonstrated a significant reduction in OSPW toxicity (as determined by BE-SPME) and >40% reduction in NAFC concentration in recirculating passes after one week, as determined by Orbitrap mass spectrometry. The NAFC reduction was more extensive in the latter cycles, suggesting the ability of this phytoremediation approach is sustained over longer treatment periods. Toxicity reduction was also maintained throughout the cycles.

Next, a mesocosm experiment incorporated two plant species selected in Phase 1 of this study for their high abiotic stress tolerance and NAFC-uptake ability. Single selected genotypes of *Calamagrostis canadensis* (Canada bluejoint) and *Solidago canadensis* (Canada goldenrod) were tested in single- and mixed-species mesocosms. Two 30-day cycles of OSPW flow through the mesocosms were performed, with water sampling performed throughout each cycle. BE-SPME assays indicated a decrease in toxicity that correlated to levels below chronic *Daphnia magna* EC 20 toxicity levels. Orbitrap and FTICR mass spectrometry analysis of the water samples from this mesocosm experiment are ongoing.

LESSONS LEARNED

Phase 1 experiments have demonstrated the selection of specific stress-tolerant genotypes will be critical for the development of effective OSPW phytoremediation technologies. Practical aspects, such as ease of propagation, will also be an important parameter for genotype selection, as field-scale implementation of this technology will require resilient, easy-to-grow genotypes with a high rate of survivability. Phase 2 experiments performed to date have demonstrated our closed-loop, subsurface flow greenhouse mesocosm setup is a tractable and effective approach to test the ability of upland plant species to reduce toxicity and remove organics from OSPW under variable treatment conditions. The experiments performed to date demonstrate a subsurface-flow phytoremediation system of this type, where OSPW moves directly through the root systems of selected upland plants, can maintain a high level of NAFC depletion and toxicity reduction in treated OSPW over extended periods. This research supports future efforts to implement upland plant pilot studies under field conditions.

PRESENTATIONS AND PUBLICATIONS

Future

RESEARCH TEAM AND COLLABORATORS

Institution: University of Calgary

Principal Investigator: Doug Muench

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WE0025 - Industrial Research Chair in Oil Sands Tailings Water Treatment – Second Term

COSIA Project Number: WE0025

Research Provider: University of Alberta

Industry Champion: Syncrude Canada Ltd.

Industry Collaborators: Canadian Natural, Imperial Oil Ltd., Suncor Energy Inc., Teck Resources Limited

Status: Year 6 of 6

PROJECT SUMMARY

Rationale

As one of COSIA's identified priority areas, water management is vital for the continuous development of the oil sands industry, managing current and future water allocations, and preserving healthy ecosystems and human well-being. The majority of water currently used for surface mining operations is recycled from settling basins while the remainder of the required water is withdrawn from the Athabasca River.

Oil sands process water (OSPW) is generated during the extraction process. No federal regulation is currently in place to allow the release of OSPW, treated or untreated, to the environment. Because continued water storage is unsustainable and inconsistent with the closure objectives, OSPW needs to be treated and released into the environment. There is a possibility for disposal of OSPW into deep geologic formations. In that case, minimal pre-treatment (if any) would be required to manage the OSPW. Therefore, water treatment/reclamation approaches are required to ensure OSPW quality is safe for its release.

Project Scope and Objectives

The NSERC IRC Program - Second Term (July 2017 to February 2024) focuses on developing and assessing innovative water treatment/reclamation technologies and strategies through a combination of passive (low-energy) and semi-passive treatment approaches to promote and protect the environment and public health.

The short-term objectives of the NSERC IRC Program - Second Term are:

- Understand the fundamentals of semi-passive and engineered passive treatment processes.
- Conduct life-cycle assessments and cost analyses of different treatment approaches.
- Assess the performance of selected treatment processes at the pilot-scale level.
- Assess the performance of four large field pilots on active mine sites.
- Develop a "toolbox" with the best available technologies for different water stream scenarios.

The long-term objectives, including those beyond the period covered by this program, are:

- Train highly qualified personnel with the skills necessary to promote and protect environmental and public health.
- Support current research programs focused on the treatment/reclamation of OSPW by facilitating the transfer of knowledge and new discoveries.
- Integrate the knowledge gained into actual water management options by the oil sands industry.

Methodology

To achieve the objectives of the NSERC IRC Program – Second Term, 29 projects were established. The projects were grouped into seven research areas: water and tailings quality, advanced oxidation processes, electro-oxidation processes, biological treatments, material development, piloting tests, and cost assessment (see Table 1).

Table 1. *Projects conducted during NSERC IRC Program – Second Term*

Project ID #	Research Area	Title	Status
1	Water and Tailings Quality	Long-Term Assessment of OSPW Quality Due to Self-Attenuation	Completed
2a	Advanced Oxidation Processes	Solar Photocatalytic Treatment of OSPW Using Bismuth Tungstate Based Photocatalysts	Completed
2b	Advanced Oxidation Processes	Assessing the Catalytic Potential of the OSPW Inorganic Matrix on Advanced Oxidation Process	Completed
2c	Advanced Oxidation Processes	Application of Persulfate-Based Advanced Oxidation Processes for OSPW Treatment	Completed
2d	Advanced Oxidation Processes	Comparison of Catalytic Ozone, UV/H_2O_2, UV/ Peroxymonosulfate and UV/Fenton in Degrading the NAs in OSPW	Completed
2e	Advanced Oxidation Processes	Treatment of OSPW by Ferric Citrate under Visible Light Irradiation	Completed
2f	Advanced Oxidation Processes	Solar-Based Advanced Oxidation Processes for OSPW Treatment Using Zinc Oxide as Catalyst	Ongoing
2g	Advanced Oxidation Processes	Treatment of NAs in OSPW Using Low-Dose CaO_2 as an Additive under Sunlight Irradiation	Completed
3	Advanced Oxidation Processes	In Situ Generation of Reactive Oxygen Species Using Sewage Sludge Biochar as a Catalyst	Ongoing
4	Biological Treatments	Understanding of Engineered Passive Processes for OSPW Treatment Using Mesocosms	Ongoing
5a	Electro-Oxidation Processes	Application of Electro-Oxidation for the Degradation of Organics in OSPW	Completed

5b	Electro-Oxidation Processes	Treatment of OSPW Using Electro-Oxidation and Electrochemically Activated Reactive Sulfate Species Using Boron Doped Diamond Electrode	Completed
5c	Electro-Oxidation Processes	Treatment of OSPW by Packed-Bed Electrode Reactor	Completed
5d	Electro-Oxidation Processes	Electro-Oxidation of NAs in OSPW by Polyaniline Modified Biochar/Graphene Electrode	Completed
6	Biological Treatments	Remediation of OSPW Using Deep Biofilters – From Bench to Scale-Up Tests	Completed
7	Electro-Oxidation Processes	Degradation of NAs and Real OSPW Using Combined Electro-Oxidation and Chemically Activated Peroxymonosulfate (PMS)	Completed
8a	Material Development	Adsorption Using Carbon Xerogel	Completed
8b	Material Development	Applications of Cellulose Nanofibres for Process Water Remediation	Completed
8c	Material Development	Application of Sludge-Based Materials for Adsorption Treatment	Completed
8d	Material Development	Preparation of Biochar-Chitosan Composite and its Application for Metals Removal from OSPW	Completed
8e	Material Development	Evaluation of Adsorption-Desorption of Contaminants of Potential Concern (COCs) in OSPW onto Different Types of Reclamation Materials	Completed
9	Piloting Tests	Coke-Treatment Piloting	Postponed
10	Piloting Tests	Wetland Piloting	Postponed
11	Piloting Tests	Vegreville Mesocosm Piloting	Completed
12	Piloting Tests	Suncor Demonstration Pit Lake (DPL) Piloting	Ongoing
13	Cost Assessment	Economic Analysis and Policy Options	Ongoing
14	Water and Tailings Quality	Application of Biomimetic Solid-Phase Micro-Extraction (BE-SPME) Method as a Screening Tool	Completed
15	Water and Tailings Quality	Assessing the Effects of Polymers and Polymer Degradation on Water Chemistry and the Quality of the Tailings	Completed
16	Water and Tailings Quality	Development of Mass Spectrometry Based Analytical Methods for the Detection of Multiple NAs and Identification of By-Products	Completed

Significance of the Research to the Industry

The NSERC IRC Program - Second Term aligns with ongoing efforts for the sustainable economic development of Alberta oil sands resources. Assessment of the various types of low-energy OSPW treatment processes (i.e., semi-passive and engineered passive approaches) will contribute to a better understanding of how treated/reclaimed OSPW could be safely discharged into the environment. The comprehensive characterization of OSPW before and after treatment, the dose-response analysis of toxic effects induced by different OSPW fractions, and the use of different treatment processes for OSPW will allow for the development of reclamation strategies for the safe release of OSPW into the environment and, ultimately, protection of public health.

PROGRESS AND ACHIEVEMENTS

The NSERC IRC Program - Second Term is achieving many short- and long-term objectives, including: training of highly qualified personnel with multidisciplinary expertise; researching technical issues of strategic importance to Canada, and promoting cooperation and knowledge exchange between academia, industry, and government.

In terms of water and tailings quality, we have assessed the OSPW quality due to self-attenuation (Project #1) and studying the stability and degradation of anionic polyacrylamides (PAM) in oil sands tailings (Project #15). We have also developed a method using atmospheric pressure gas chromatography time-of-flight mass spectrometry (APGC-TOF-MS) combined with solid-phase microextraction (SPME) for the simultaneous analysis of hydrocarbons and naphthenic acids (NAs) species. We have conducted Biomimetic Extraction (BE) using SPME and analyzed polydimethylsiloxane (PDMS)-coated fibres using gas chromatography with flame ionization detector (GC-FID) to quantify bioavailable organics (Project #14). The BE-SPME technique developed with Imperial could serve as a benchmark technology and as a quick assessment method to monitor the toxicity of dissolved organics in different treatment processes (e.g., wetland biodegradation) and in the natural environment. We have also developed analytical methods for the analysis of multiple NAs in a single run and methods for the identification of treatment by-products (Project #16). This has allowed a more accurate study of the degradation of NAs in real systems and to understand the removal mechanisms involved in different treatment processes.

Studies conducted under the NSERC IRC Program have shown advanced oxidation processes (Projects #2c, #2d, #2f) will play a key role in enhancing the remediation of OSPW by accelerating the degradation of NAs and other organics in OSPW. Our findings also suggest the photodegradation efficiency of OSPW treatment strategies can be enhanced in the presence of the inorganic photosensitizer in OSPW (Project #2b). Our results have shown nitrate could be used as a natural photosensitizer to induce photo-oxidation of organic compounds in OSPW and could potentially be utilized in a low-cost passive solar system for the remediation of OSPW with extended exposure time. Simulating the formation process of reactive oxygen species based on iron complexes in nature to degrade organic compounds would also be an environmentally friendly remediation process for OSPW (Project #2e). We have also demonstrated NAs in OSPW could be efficiently degraded by solar photocatalytic treatment using bismuth tungstate (Project #2a). Our findings suggest zinc oxide (ZnO)-based photocatalytic degradation of NAs and polyaromatic hydrocarbons in OSPW could be a significant treatment process aimed at detoxifying OSPW. We have demonstrated the immunotoxicity of OSPW was considerably reduced by the ZnO-based photocatalytic treatment (Project #2f). Our results have also shown photo-active materials on a floating support media can be used in the pit lakes and wetland systems to target recalcitrant organics (Project #2f).

Considering the nature and structure of NAs and the characteristics of OSPW, with its high electrical conductivity, electro-oxidation seems to be an effective and cost-efficient option for OSPW treatment (Projects #5a, #5b, and #5c). Applying anodic oxidation by using inexpensive electrodes materials, such as graphite, and under low-voltage conditions can preferentially degrade NAs of higher cyclicity and carbon number while decreasing the number of rings and molecular weight without resulting in complete mineralization. Therefore, the application of electro-oxidation could enhance OSPW biodegradability and reduce the toxicity. The lower voltages required for the treatment will result in a sustainable and environmentally friendly process that can be operated by solar energy, and the exclusion of the need for chemicals addition will prevent the production of any additional hazardous sludge. Electro-oxidation using boron doped diamond (BDD) electrode was also found to be an efficient treatment technique for the remediation of dissolved organic in OSPW as complete degradation of NAs and PAHs was obtained within 2 h of electrolysis at 5 mA cm^{-2} or above and complete dissolved organic carbon removal attained in 6 h at similar current densities (Project #5b). We have also developed new electrode materials (Project #5d).

In terms of material development, studies conducted under the NSERC IRC Program have reported, for the first time, the mechanism of adsorption of NAs onto carbon xerogels (Project #8a). Our results have demonstrated that mesoporous carbonaceous materials such as carbon xerogels can successfully be used to adsorb persistent and toxic organic contaminants from OSPW, resulting in a treated water that may be less acutely and chronically toxic to aquatic and mammalian life. We have also developed non-toxic and biodegradable cellulose nanofibres (CNFs) for adsorption treatment (Project #8b). The vast sources of raw material, only one step in aqueous modification, together with the semi-commercialized production allow the tailored CNF material to be applied in large scale in industrial process water treatment. We have also developed sludge-based biochar (Project #8c) and biochar-chitosan composite (Project 8d). Our results have shown sludge-based and hardwood-based biochars exhibit excellent adsorption performance due to their highly mesoporous character. They could be used as passive treatment method in tailing ponds for the removal of organic matter.

In terms of biological treatment, biofiltration and mild ozonation show complementary advantages for the degradation of NAs (Project #6). The biofiltration-ozonation-biofiltration process shows higher NA removal than the biofiltration of raw OSPW. The biofiltration pretreatment can benefit the ozonation of NAs while the post-biofiltration process shows its contribution to the improved removal of the oxidized NAs from OSPW. We have found bioaugmentation could be a useful strategy to improve the existing remediation potential of petroleum coke-based biofilters. Studies conducted using basalt fibres have indicated they can effectively emulate the functions of plant roots. Basalt fibres form a ball-like aggregate, recognized as a biological nest or “bio-nest” that allows the development of aerobic, anoxic, and anaerobic bacterial communities within the bio-nest. The presence of rich and diverse bacterial communities within the system actively drives a range of essential biogeochemical processes, such as organic transformation, nitrogen cycling, and methanogenesis.

LESSONS LEARNED

The following are the key outcomes achieved so far:

- The characterization and treatment of OSPW pose many challenges, including the presence of dissolved organic compounds such as NAs, other organic acids, total suspended solids, salinity, and other dissolved organic and inorganic compounds. The water characterization and toxicity assessment of the OSPW in both untreated and treated OSPW will help to achieve a much better understanding of the potential impacts of treated OSPW on the environment.

- The BE-SPME method presented in the NSERC IRC program could serve as a rapid and convenient analytical screening tool for estimating the toxicity of raw and treated OSPWs due to dissolved organics. The BE-SPME technique could serve as a benchmark technology to monitor the transformation of bioavailable NAs in treatment processes (e.g., wetland biodegradation), as well as to monitor the natural transfer and transformation of bioavailable NAs in the natural environment.

- Atmospheric pressure gas chromatography time-of-flight mass spectrometry (APGC-TOF-MS) combined with SPME will allow the simultaneous analysis of hydrocarbons and NAs species.

- Using sewage sludge as a precursor for the production of sewage sludge-based material (sludge-based biochar) has many advantages. It can be used to adsorb organic compounds from OSPW. The sludge-based biochar can also be used as catalysts for advanced oxidation processes.

- Low-current electro-oxidation is a promising pre-treatment option for OSPW while being routed to pit lakes and/or wetlands as it can lead to improved biodegradability and reduced toxicity of OSPW organics. The lower voltages required and low-cost graphite electrodes will result in a sustainable and environmentally-friendly process that can be operated by solar energy or can be used for in-pipe treatment.

- In situ catalytic oxidation may play a critical role in enhancing the remediation of OSPW when applied as a pre-treatment step to accelerate the degradation of NAs, among other organics, in OSPW.

- Solar-based advanced oxidation processes using photo-active materials either in suspension or on a floating support media can be used in the pit lakes and wetland systems to target recalcitrant organics.

- Biofiltration possesses remarkable advantages, such as low energy costs and low capital demand. The fixed-bed biofilm reactor shows high potential to be applied and scaled up for in situ treatment of OSPW.

Providing innovative multi-barrier treatment approaches and water-reuse/release scenarios will help promote and protect environmental and public health, enhance water quality, and support the ongoing efforts to assist the economical and sustainable development of Alberta oil sands resources.

PRESENTATIONS AND PUBLICATIONS

To date, there have been 76 publications in peer-reviewed journals and 111 presentations. Ten recent publications are listed below.

Journal Publications:

Published

Abdelrahman, A., S.O. Ganiyu, and M. Gamal El-Din. 2023. Degradation of surrogate and real naphthenic acids from simulated and real oil sand process water using electrochemically activated peroxymonosulfate (EO-PMS) process. Sep. Purif. Technol., 306, 122462.

Arslan, M., S.O. Ganiyu, D.M.E. Lillico, J.L. Stafford, and M. Gamal El-Din. 2023. Fate of dissolved organics and generated sulfate ions during biofiltration oil sands process water pretreated with sulfate radical advanced oxidation process. Chem. Eng. J., 458, 141390.

Ganiyu, S.O., M. Arslan, M. Gamal El-Din. 2022. Combined solar activated sulfate radical-based advanced oxidation processes (SR-AOPs) and biofiltration for the remediation of dissolved organics in oil sands produced water. Chem. Eng. J., 433, Part 1, 134579.

How, Z.T., Z. Fang, P. Chelme-Ayala, S.O. Ganiyu, X. Zhang, B. Xu, C. Chen, and M. Gamal El-Din. 2023. Ozone-activated peroxymonosulfate (O3/PMS) process for the removal of model naphthenic acids compounds: kinetics, reactivity, and contribution of oxidative species. J. Environ. Chem. Eng., 11(3) 109935.

Ju, Y., C. Liu, S.O. Ganiyu, Y. Zhao, and M. Gamal El-Din. 2023. Electrochemical degradation of dissolved organic matters in oil sands process water using continuous-flow packed bed electrode reactor. Sep. Purif. Technol., 320, 124135.

Li, J., Z.T. How, and M. Gamal El-Din. 2023. Aerobic degradation of anionic polyacrylamide in oil sands tailings: impact factor, degradation effect, and mechanism. Sci. Total Environ., 856, Part 1, 159079.

Lu, Q., L. Yang, P. Chelme-Ayala, Y. Li, X. Zhang, and M. Gamal El-Din. 2023. Enhanced photocatalytic degradation of organic contaminants in water by highly tunable surface microlenses. Chem. Eng. J., 463, 142345.

Medeiros, D., P. Chelme-Ayala, and M. Gamal El-Din. 2023. Sorption and desorption of naphthenic acids on reclamation materials: mechanisms and selectivity of naphthenic acids from oil sands process water. Chemosphere, 326, 138462.

Medeiros, D., P. Chelme-Ayala, and M. Gamal El-Din. 2023. Sludge-based activated biochar for adsorption treatment of real oil sands process water: selectivity of naphthenic acids, reusability of spent biochar, leaching potential, and acute toxicity removal. Chem. Eng. J., 463, 142329.

Meng, L., Z.T. How, P. Chelme-Ayala, C. Benally, and M. Gamal El-Din. 2023. Z-Scheme plasmonic Ag decorated Bi2WO6/NiO hybrids for enhanced photocatalytic treatment of naphthenic acids in real oil sands process water under simulated solar irradiation. J. Hazard. Mater., 454, 131441.

RESEARCH TEAM AND COLLABORATORS

Institution: University of Alberta

Principal Investigator: Mohamed Gamal El-Din (Professor, Department of Civil and Environmental Engineering)

Name	Institution or Company	Degree or Job Title	Degree Start Date (For Students Only)	Expected Degree Completion Date or Year Completed (For Students Only)
Dr. Mohamed Gamal El-Din	**University of Alberta**	**Professor**		
Rongfu Huang	**University of Alberta**	**Research Associate**		**2019 (completed)**

Pamela Chelme-Ayala	University of Alberta	Research Associate		
Lingling Yang	University of Alberta	Research Associate		
Grant Hauer	University of Alberta	Research Associate		
Selamawit Messele	University of Alberta	Postdoctoral Fellow		2020 (completed)
Mingyu Li	University of Alberta	Postdoctoral Fellow		2020 (completed)
Shailesh Sable	University of Alberta	Postdoctoral Fellow		2020 (completed)
Soliu Ganiyu	University of Alberta	Postdoctoral Fellow		
Zou Tong How	University of Alberta	Postdoctoral Fellow		2022 (completed)
Muhammad Arslan	University of Alberta	Postdoctoral Fellow		
Ming Zheng	University of Alberta	Postdoctoral Fellow		2023 (completed)
Muhammad Usman	University of Alberta	Postdoctoral Fellow		
Isaac Sanchez	University of Alberta	Postdoctoral Fellow		
Zhijun Luo	University of Alberta	Visiting Professor		2019 (completed)
Abdallatif Abdalrhman	University of Alberta	PhD Student	2014	2019 (completed)
Rui Qin	University of Alberta	PhD Student	2014	2019 (completed)
Lei Zhang	University of Alberta	PhD Student	2014	2018 (completed)
Lingjun Meng	University of Alberta	PhD Student	2017	2022 (completed)
Chelsea Benally	University of Alberta	PhD Student	2012	2018 (completed)
Jia Li	University of Alberta	PhD Student	2019	2023 (completed)
Monsuru Suara	University of Alberta	PhD Student	2019	2023
Zhexuan An	University of Alberta	PhD Student	2019	2023
Akeen Bello	University of Alberta	PhD Student	2019	2023
Foroogh Mehravaran	University of Alberta	PhD Student	2019	2023
Deborah Medeiros	University of Alberta	PhD Student	2019	2023 (completed)
Jerico Fiestas Flores	University of Alberta	PhD Student	2020	2024
Hadi Mokarizadeh	University of Alberta	PhD Student	2021	2025

Zhi Fang	**University of Alberta**	**Visiting PhD Student**	**2017**	**2017 (completed)**
Hande Demir	**University of Alberta**	**Visiting PhD Student**	**2019**	**2019 (completed)**
Junying Song	**University of Alberta**	**Visiting PhD Student**	**2019**	**2019 (completed)**
Yue Ju	**University of Alberta**	**Visiting PhD Student**	**2019**	**2021 (completed)**
Qi Feng	**University of Alberta**	**Visiting PhD Student**	**2021**	**2022 (completed)**
Adriana Vasquez	**University of Alberta**	**Master Student**	**2021**	**2023**
Ali Abdelrahman	**University of Alberta**	**Master Student**	**2019**	**2021 (completed)**
Jia Li	**University of Alberta**	**Master Student**	**2017**	**2018 (completed)**
Sanya Mehta	**University of Alberta**	**Research Assistant**	**2020**	**2021 (completed)**
Lekha Patil	**University of Alberta**	**Research Assistant**	**2019**	**2022 (completed)**
Ali Abdelrahman	**University of Alberta**	**Research Assistant**	**2021**	**2022 (completed)**
Alice Da Silva	**University of Alberta**	**Research Assistant**		**2018 (completed)**
Shimiao Dong	**University of Alberta**	**Research Assistant**		**2018 (completed)**
Yanlin Chen	**University of Alberta**	**Research Assistant**		**2019 (completed)**

Research Collaborators:

- Dr. James Stafford, Associate Professor, Department of Biological Sciences, University of Alberta.
- Dr. Patrick Hanington, Associate Professor, School of Public Health, University of Alberta.
- Dr. Yaman Boluk, Professor, Nanofibre Chair in Forest Products Engineering, Department of Civil and Environmental Engineering, University of Alberta.
- Dr. Dev Jennings, T.A. Graham Professor of Strategy and Organization and the Director of the Canadian Center for Corporate Social Responsibility (CCCSR), Alberta School of Business.
- Dr. M. Anne Naeth, Professor of Land Reclamation and Restoration Ecology, Department of Renewable Resources, Associate Dean Research and Graduate Studies in the Faculty of Agricultural, Life and Environmental Sciences, Director of the Land Reclamation International

Graduate School (LRIGS) and Director of the Future Energy Systems (FES), University of Alberta.

- Dr. Vic Adamowicz, Professor and Vice Dean, Faculty of Agricultural, Life and Environmental Science, University of Alberta.
- Dr. Xuehua Zhang, Professor, Department of Chemical & Materials Engineering, University of Alberta.
- Dr. Sandra Contreras Iglesias, Professor, Department of Chemical Engineering, Universitat Rovira, Spain.
- Dr. Bin Xu, Associate Professor, College of Environmental Science and Engineering, Tongji University, China.
- Dr. Chunmao Chen, Associate Professor, State Key Laboratory of Heavy Oil Processing, State Key Laboratory of Petroleum Pollution Control, China University of Petroleum-Beijing, China.
- Dr. Zhijun Luo and Dr. Zhiren Wu, Professor, School of the Environment and Safety Engineering, Jiangsu University, China.

Non-COSIA Collaborators:

- EPCOR Water Services
- Alberta Innovates
- Alberta Environment and Protected Areas

WJ0164 - SolarPass Demonstration for Treatment of Dissolved Organics in Water

COSIA Project Number: WJ0164

Research Provider: H2nanO Inc.

Industry Champion: Canadian Natural

Industry Collaborators: Imperial Oil Ltd.

Status: Complete

PROJECT SUMMARY

Background

Oil sands process-affected water (OSPW) may require treatment of dissolved organic compounds, such as naphthenic acid fraction components (NAFCs), prior to release to watersheds. H2nanO has developed a passive water treatment solution called SolarPass, which uses buoyant catalyst beads and sunlight to break down persistent organic compounds. SolarPass demonstrated promise for OSPW treatment in prior COSIA projects, although variations in OSPW composition may have affected treatment rates.

Scope

Evaluate SolarPass for OSPW treatment with expanded analytical scope and rigour to better understand impacts of OSPW composition on treatment outcomes.

Objectives

1. Evaluate OSPW composition and matrix effects on photocatalytic detoxification rate of two OSPW samples (from Canadian Natural Resources Ltd., and Imperial Oil Ltd.)

    1.1 Determine the influence of water matrix chemistry on SolarPass treatment performance

    1.2 Evaluate SolarPass treatment performance toward other water components of potential concern (PAHs, hydrocarbons/diluents, ammonia, sulfide)

2. Apply treatment monitoring tools for photocatalytic detoxification

Methods

H2nanO treated three OSPW samples (beyond the two originally planned) with its SolarPass process in batch recirculating raceway pools (800 L) indoors under simulated sunlight. The most comprehensive analytical test program H2nanO has deployed to date was implemented to rigorously monitor changes to water-quality parameters during treatment, and their correlation with fish aquatic toxicity.

Importance

Regulatory guidelines for treated OSPW release are expected as early as 2024. The Oil Sands Mine Water Science Team is considering the best available technology economically achievable (BATEA) toward technology-based effluent limits (TBELs). As SolarPass is a leading treatment solution under consideration, it was crucial to evaluate its simultaneous treatment performance toward multiple OSPW components of potential concern (COPCs).

PROGRESS AND ACHIEVEMENTS

SolarPass treatment successfully removed acute toxicity in all three OSPW samples in less than eight days, with significant removal of dissolved organics throughout the treatment period, replicating the key outcomes of prior SolarPass evaluations. In most cases, the levels of other OSPW COPCs in untreated water, including ammonia, polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs, and trace elements (Co, Cu, Mn, Ni, Sr, U, Zn) were below acute toxicity limits. Notwithstanding, SolarPass also achieved significant removal of these other COPCs demonstrating the technology's simultaneous multi-target treatment capabilities. The treatment mechanism was confirmed as photocatalysis by comparison to catalyst-free and dark experimental controls. Specific conclusions for each OSPW sample are summarized below:

CNRL:

- As-received OSPW sample had similar toxicity and water chemistry as in prior tests (the untreated sample was acutely toxic to fish, albeit with higher ammonia than previously)
- Acute toxicity to fathead minnow was eliminated within four days of treatment exposure. Rainbow trout acute toxicity was eliminated between four and eight days. Acute toxicity associated with dissolved organics was removed within the first four days, while acute toxicity from ammonia was fully eliminated between days four to eight.

IOL:

- As-received OSPW sample was acutely toxic to fish at start of treatment testing, with toxicity eliminated in less than one day of treatment (improving upon the two days required to remove acute toxicity in prior tests)
- SolarPass treatment kinetics of dissolved organics was highly reproducible between the two OSPW samples (received approximately three months apart), including virtually complete elimination of the Biomimetic Extraction – Solid Phase Micro-Extraction (BE-SPME) signal. BE-SPME is a fast test to assess the whole effluent toxicity (WET) of waters using relatively inexpensive, readily available analytical devices.

LESSONS LEARNED

A "light touch" treatment removes acute and chronic toxicity from OSPW

Results from testing in prior years demonstrate only a relatively mild treatment may be required to remove the acute and chronic fish toxicity of OSPW per standard regulatory whole effluent toxicity (WET) bioassays, without requiring significant reductions in the concentrations of NAFCs or acid extractable organics (AEO). Substantial changes to these conventional aggregate analytical chemistry measures of dissolved organics proved unnecessary for SolarPass to transform OSPWs from acutely lethal to completely non-toxic to both acute and chronic fish endpoints. This finding has potentially profound implications for treatment of OSPW for release.

OSPW toxicity is poorly correlated with standard water-quality metrics

Results from testing in prior years showed poor correlation between conventional measures of OSPW dissolved organics (naphthenic acids (NAs) using FTIR, Total Organic Carbon, dissolved organics using liquid chromatography coupled with ultra-high resolution mass spectrometry) and WET outcomes. This called into question the use of these conventional methods as toxicity surrogates. As some of these water chemistry metrics may be under consideration for monitoring OSPW treatment for release, results from this project suggest WET bioassays may be more protective of aquatic life. H2nanO's non-target petroleomic mass spectrometry analyses found organosulfur NAFCs to be significantly correlated with OSPW toxicity. This may have been a hidden factor underlying most of the OSPW toxicology literature to date. More work is needed characterize the toxicity of these organosulfur NAFCs.

SolarPass achieves multi-target treatment

A key lesson of this study was SolarPass simultaneous treated multiple organic and inorganic COPCs to achieve OSPW detoxification. These included NAFCs and dissolved organics, PAHs and alkyl-PAHs, and ammonia (NH_3). This was enabled by the most rigorous and comprehensive analytical chemistry program used to study SolarPass to date. Elimination of NAs and NAFCs was achieved simultaneously – in the same unit operation – with ≥90% elimination of PAHs and alkyl-PAHs (although most of these were already below toxicity limits in the untreated water)), as well as a number of trace elements of potential concern. Together, these results highlight the comprehensive water quality improvement SolarPass provides and helps situate the technology as a BATEA within a TBEL framework.

PRESENTATIONS AND PUBLICATIONS

Journal Publications:

In preparation

Timothy M. C. Leshuk, Zachary W. Young, Brad Wilson, Zi Qi Chen, Danielle Smith, Greg Lazaris, Mary Gopanchuk, Sean McLay, Corin Seelemann, Theo Paradis, Asfaw Bekele, Rodney Guest, Hafez Massara, Todd White, Warren Zubot, Daniel J. Letinski, Aaron D. Redman, Frank Gu. (2023). A light touch: solar photocatalysis detoxifies oil sands process-affected waters prior to significant treatment of naphthenic acids or acid extractable organics.

RESEARCH TEAM AND COLLABORATORS

Institution: H2nanO Inc.

Principal Investigator: Frank Gu, CEO

Name	Institution or Company	Degree or Job Title	Degree Start Date (For Students Only)	Expected Degree Completion Date or Year Completed (For Students Only)
Frank Gu	H2nanO	Chief Executive Officer		
Tim Leshuk	H2nanO	Chief Technology Officer		
Jeff Martin	H2nanO	Senior Scientist, Water Treatment		
Theo Paradis	CNRL	Technical Project Lead		
Asfaw Bekele	IOL	Environmental Research Advisor		

WJ0046 - Wetland Treatment of Oil Sands Process-Affected Water

COSIA Project Number: WJ0046

Research Provider: Simon Fraser University (Frank Gobas) with financial support from NSERC and Imperial

Industry Champion: Imperial Oil Ltd.

Industry Collaborators: None

Status: Ongoing

PROJECT SUMMARY

Large volumes of oil sands process-affected water (OSPW) have been generated through mining operations and bitumen extraction in the Canadian oil sands. Oil sands operators have successfully reduced fresh water requirements during bitumen extraction, To date, industry has adhered to a zero-release practice. Treatment technologies are being evaluated to reduce the ecological footprint and enable release of treated OSPW to the Athabasca River. Ongoing efforts to find feasible treatment solutions have identified several technologies with the potential to improve water quality of OSPW. Treatment wetlands have emerged as viable solutions to various wastewater challenges, including municipal and domestic wastewater, mine water, agricultural runoff, leachate, and industrial wastewaters. To investigate the application of treatment wetlands in the oil sands mining sector, a 1-ha surface-flow wetland was constructed on Imperial's Kearl Oil Sands site in 2013.

The study's overall goal is to improve the science of treatment wetland technology in Canada's oil sands. Using a combination of passive samplers and aqueous grab sampling, wetland treatment efficiency was evaluated at the Kearl Treatment Wetland for polycyclic aromatic hydrocarbons (PAHs) and naphthenic acids (O_2-NAs). This data was used to test and evaluate a contaminant-fate model for both neutral and polar organic contaminants to determine which contaminants can be removed via wetland treatment. Wetland treatment to remove toxicity was also evaluated using biomimetic extraction of hydrocarbons with solid-phase microextraction (SPME) fibres and through bulk OSPW toxicity testing. This project's specific objectives are to:

(i) investigate the ability of the Kearl Treatment Wetland to treat OSPW from Kearl Oil Sands site (Imperial Oil Resources Ltd.) in northern Alberta,

(ii) apply, test, and calibrate a contaminant-fate model of the Kearl treatment wetland, and

(iii) evaluate changes to OSPW toxicity as a result of wetland treatment

PROGRESS AND ACHIEVEMENTS

In previous years, the concentration-reduction and mass-removal of both PAHs and O_2-NAs in the Kearl Treatment Wetland was demonstrated by employing both passive sampling and aqueous grab-sampling techniques – see the 2021 Mine Water Research Summary. This data was used to test and evaluate a contaminant-fate model of the Kearl Treatment Wetland. While changes to the concentration and mass of constituents of concern are important to assess the feasibility of treatment wetlands, we must also consider the effects of wetland treatment on OSPW toxicity to fully demonstrate the application of treatment wetlands. Therefore, 30-day toxicity tests were performed on early-life stage rainbow trout (O*ncorhynchus* *mykiss)* at 1, 3.2, 10, 32, and 100% OSPW. Dose measurements were evaluated using biomimetic solid-phase microextraction (BE-SPME) to quantify the concentration of bioavailable organic compounds in each treatment group. BE-SPME was performed by autoinjection of polydimethylsiloxasne (30um) fibres (100 min) into acidified OSPW dilutions and analyzed by GC-FID. Endpoints evaluated were mortality, development (weight, length, and yolk sac absorption) and deformities.

Our measurements indicate strong linearity between BE-SPME concentrations and OSPW dilutions (R^2=0.96), suggesting that BE measurements adequately represent the amount of total bioavailable hydrocarbons being exposed to early-life stage rainbow trout during toxicity testing. Our results illustrates the OSPW used in this study exhibits concentration-dependent toxicity, with 100% mortality occurring in both the 32% and 100% OSPW treatment groups, corresponding with BE-SPME concentrations of 47.72 mM and 90.80 mM, respectively (mM = mmol of dimethylnaphthalene per L of polydimethylsiloxane). Our findings show a BE-derived LC50 (BE-SPME concentration at which 50% of the test species was affected) of 21.95 mM corresponds well with values reported in Redman et al. (2018). Toxicity thresholds for length and weight were 3.96 mM and 8.91 mM, respectively. The EC25 for deformities was 9.53 mM DMN, where deformities were indicative of cardiovascular toxicity and included pericardial and yolk sac edema and craniofacial malformations.

LESSONS LEARNED

Treatment wetlands have emerged as a viable option for OSPW remediation. Our findings reported in the 2021 Mine Water Research Summary have demonstrated the removal of both PAHs and O2-NAs from OSPW and our contaminant-fate model provides a valuable tool for industry operators to assess the feasibility of wetland designs, operation, and treatment objectives. An important part of this work is to evaluate how wetland treatment contributes to toxicity reduction of OSPW. The work presented here provides a basis for that evaluation since wetland treatment can now be assess using BE-SPME measurements correlated with this toxicity data for early-life stage rainbow trout. Given the complexity of OSPW and variable composition across the industry, these results help support the use of BE-SPME as a rapid, animal-free tool to directly assess the acute bioavailable toxicity of OSPW, changes in bioavailable hydrocarbon concentration, and the reduction in OSPW toxicity following wetland treatment without quantifying the concentration of individual organic constituents of concern.

PRESENTATIONS AND PUBLICATIONS

Journal Publications:

Published

Cancelli, A. M., Borkenhagen, A.K., Bekele, A. 2022. A Vegetation Assessment of the Kearl Treatment Wetland following Exposure to Oil Sands Process-Affected Water. Water,14, 3686.

Cancelli, A.M.; Gobas, F.A.P.C. 2022. Treatment of naphthenic acids in Oil Sands Process-Affected Waters with a Surface Flow Treatment Wetland. Environmental Research, 213.

Cancelli, A.M.; Gobas, F.A.P.C. 2020. Treatment of Polycyclic Aromatic Hydrocarbons in Oil Sands Process-Affected Water with a Surface Flow Treatment Wetland. Environments, 7, 64.

Conferences, presentations, and workshops:

Presented

Cancelli, A. M., Gobas, F.A.P.C. November 30-December 1, 2021. Treatment of OSPW in the Kearl Treatment Wetland. Canadian Oil Sands Innovation Alliance, Mine Water Management Workshop 2021, virtual.

Cancelli, A. M., Gobas, F.A.P.C. November 3-7, 2019. The treatment of oil sands process-affected water with the Kearl Treatment Wetland. Society of Environmental Toxicology and Chemistry, 40th North American Annual Meeting, Toronto, ON.

Cancelli, A. M., Gobas, F.A.P.C., Bekele, A. June 3-4, 2019. The removal of organic contaminants from OSPW in the Kearl Treatment Wetland. Canadian Oil Sands Innovation Alliance, 2019 Oil Sands Innovation Summit, Calgary, AB.

Cancelli, A. M., Gobas, F.A.P.C., Bekele, A. May 10, 2019. The removal of organic contaminants from OSPW in the Kearl Treatment Wetland. 11th Western Symposium on Water Quality Research, Edmonton, AB.

Cancelli, A. M., Gobas, F.A.P.C. November 4-8, 2018. A model of contaminant removal from oil sands process-affected waters in the Kearl Treatment Wetland. Society of Environmental Toxicology and Chemistry, 39th North American Annual Meeting, Sacramento, CA.

Cancelli, A. M., Gobas, F.A.P.C. June 7-8, 2018. Quantifying the removal of polycyclic aromatic hydrocarbons in the Kearl Treatment Wetland. Canadian Oil Sands Innovation Alliance, Oil Sands Innovation Summit, Calgary, AB.

Cancelli, A. M., Gobas, F.A.P.C. Sept 6-7, 2018. Model performance evaluation using passive samplers at the Kearl Treatment Wetland. Canadian Oil Sands Innovation Alliance, Science Workshop: Oil Sands Process Wastewater Characterization, Identification, and Treatment, Calgary, AB.

RESEARCH TEAM AND COLLABORATORS

Institution: Simon Fraser University

Principal Investigator: Frank A.P.C. Gobas

Name	Institution or Company	Degree or Job Title	Degree Start Date (For Students Only)	Expected Degree Completion Date or Year Completed (For Students Only)
Prof. Frank A.P.C. Gobas	Simon Fraser University	Professor		
Alexander M. Cancelli	Simon Fraser University	Postdoctoral Fellow		
Nina Pigott	Simon Fraser University	Master's student	2018	2022