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Review

# Abiotic and biotic constituents of oil sands process-affected waters

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ABSTRACT

The oil sands in Northern Alberta are the largest oil sands in the world, providing an important economic resource for the Canadian energy industry. The extraction of petroleum in the oil sands begins with the addition of hot water to the bituminous sediment, generating oil sands process-affected water (OSPW), which is acutely toxic to organisms. Trillions of litres of OSPW are stored on oil sands mining leased sites in man-made reservoirs called tailings ponds. As the volume of OSPW increases, concerns arise regarding the reclamation and eventual release of this water back into the environment. OSPW is composed of a complex and heterogeneous mix of components that vary based on factors such as company extraction techniques, age of the water, location, and bitumen ore quality. Therefore, the effective remediation of OSPW requires the consideration of abiotic and biotic constituents within it to understand short and long term effects of treatments used. This review summarizes selected chemicals and organisms in these waters and their interactions to provide a holistic perspective on the physiochemical and microbial dynamics underpinning OSPW .



## Introduction

The Albertan oil sands represent one of the largest global reservoirs of bitumen (i.e., a viscous mixture of hydrocarbons), with an estimated 174 billion barrels of recoverable oil sands ore (Allen, 2008a). The bitumen in these oil sands sediments is referred to as crude bitumen, which is a highly dense and viscous unrefined petroleum source that is enriched in heavy metals and has a low hydrogen-to-carbon ratio compared to conventional crude oil sources (Masliyah et al., 2011). The oil sands region in Northern Alberta is the largest in the world, and the third largest petroleum reserve in existence (Hewitt et al., 2020; Xue et al., 2018). Over $100 billion has been invested in the Canadian oil and gas industry since the early 2000s, with the oil sands contributing approximately $82.6 billion in profits in 2016 (Doluweera et al., 2017; Poveda and Lipsett, 2013). During the eleven-year period spanning 2017 to 2027, the Canadian oil and gas industry is expected to produce $2.7 trillion in revenue (Doluweera et al., 2017), and create and preserve nearly 10 million Canadian jobs between 2010–2035 (Honavar et al., 2011). The economic impact of the oil sands exceed natural gas and conventional oil (i.e., crude oil that is pumped from underground deposits) in Canada, as the oil sands industry generates higher profit than both of the aforementioned industries (Doluweera et al., 2017). Due to the large proven reserves of bitumen and the encouragement of investment into the oil sands industry, Canada is projected to be-

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come the fourth largest oil manufacturer by 2035 (Poveda and Lipsett, 2013).

Unlike most conventional methods of bitumen recovery, oil sands sediment is collected from the surface soil of mine pits instead of subterranean extraction (Allen, 2008a). Industry operators extract the bitumen through the Clark Hot Water Process, where caustic hot water is mixed in with the mined ore to create a slurry where the bitumen can be skimmed off for downstream refinement (Allen, 2008a). After being used in the extraction process, the water is referred to as oil sands process-affected water, or OSPW, which is comprised of 70%–80% by weight (wt%) water, 20–30 wt% solids (i.e., sand, silt, and/or clay), and 1–3 wt% residual bitumen (Allen, 2008a). In 1993, a zero-discharge policy was enacted by the Albertan Government to prevent the release of OSPW directly into environmental ecosystems and encourage reuse of OSPW in further bitumen extractions (Giesy et al., 2010; Hazewinkel and Westcott, 2015). To meet these mandates, industry operators store OSPW in man-made reservoirs called tailings ponds (Giesy et al., 2010). Although the OSPW in these tailings ponds is subsequently reused in further extractions, the water begins to accumulate salts from the mined ore and decrease in bitumen extraction efficiency (Hazewinkel and Westcott, 2015). As a result, of the 7.5-10 total barrels of water needed to extract 1 barrel of bitumen (Poveda and Lipsett, 2013), approximately 0.5-2.5 barrels are contributed by freshwater sources in order to maintain bitumen extraction efficiency (Quinlan and Tam, 2015). This results in the continuous expansion of tailing ponds and OSPW, with over one trillion litres of OSPW stored in open ponds across Northern Alberta at present (McNeill and Lothian, 2017). While these tailings ponds can undergo remediation of contaminants through passive processes such as oxidation and photodegradation facilitated by sunlight (Leshuk et al., 2016a; Qin et al., 2019a), currently no standardized, economically feasible, and/or high throughput OSPW remediation technique exists to allow its release back into environmental water systems (van den Heuvel, 2015).

There are multiple stakeholders involved in the remediation of OSPW. In the context of Albertan oil sands affected sites such as tailings ponds and mines, the definition of reclamation specifies that self-sustaining ecosystems must be created with a land use capacity that is comparable to the previously displaced area (Allen, 2008b). Ultimately, the Albertan government has indicated that the OSPW housed in tailings ponds be in a “ready-to-reclaim state” by 10 years post-mine closure (Martin, 2015). Businesses in the oil sands industry employ different proprietary methods to extract bitumen based on the quality of the ore collected at the mine site, leading to OSPW having different company-dependent constituents (Allen, 2008b; Mahaffey and Dubé, 2017). Characterizing the components in OSPW is normally performed using solid phase or liquid-liquid extraction and downstream gas chromatography and mass spectrometry analyses to identify compounds in these complex mixtures (Huang et al., 2021; Jiang et al., 2021). The heterogeneity in OSPW is further exacerbated as the composition also varies based on factors such as the geographic location, effluent age, and whether the water is being actively used in extractions or is stored in settling basins to initiate sedimentation (Bauer et al., 2019; Dube et al., 2021; Frank et al., 2016; Hewitt et al., 2020). This has led to industrial estimates of 15–70 years regarding OSPW reclamation (McNeill and Lothian, 2017), although a five-year remediation period has been proposed as the technology to treat these effluents currently exist and thus the shortest suggested estimate is contingent on the pooling of resources and collaborations between oil sands corporations (van den Heuvel, 2015). Potential remediation techniques that are currently being explored include the construction of artificial wetlands to treat both OSPW and sediment from the oil sands (Toor et al., 2013; Vander Meulen et al., 2021a), dewatering tailings to harden the sediment to make it usable for dry landscape reclamation approaches (Kalantari et al., 2012), capping dewatered sediment with soil or water-capping OSPW with freshwater to dilute the oil sands solid or fluid material (Richardson et al., 2020), applying advanced oxidation processes (AOPs) such as electro-oxidation or solar photocatalysis that oxidize contaminants in OSPW such as naphthenic acids (NAs) (Abdalrhman and Gamal El-Din, 2020; Meng et al., 2020), using microbial communities to degrade or modify constituents of OSPW (Demeter et al., 2014; Mahdavi et al., 2015; Xue et al., 2018), or flocculation to aggregate particulate matter in tailings that can then be physically removed (Wang et al., 2015a). Although these techniques require different resources, they can be integrated at various stages in the life cycle of OSPW – from raw OSPW to post-treatment – to initiate various remediation phases. To address reclamation on a provincial scale, the Mine Financial Security Program (MFSP) was established in 2011 by the Albertan government to collect funding from oil sands operators towards reclamation of surface mined landscapes (Cook, 2018). Currently, the total finances secured by industrial contributions ($1.4 billion) is lower than the estimates of reclamation, which range between $28-$260 billion (Corkal et al., 2020; Gosset and McNeill, 2020; Wheatley and Westman, 2019). While the oil and gas industry currently remain an integral part of the Albertan economy, considerations into how to remediate oil sands-affected materials (both fluids and solids) is of importance to multiple stakeholders.

Exposure routes of OSPW has been associated with ingestion, inhalation, bioaccumulation through food webs, and direct physical contact (Kindzierski et al., 2012), in addition to leaching of OSPW from tailings ponds into groundwater and subsequently the Athabasca River in Alberta (Frank et al., 2014). However, the low water-to-air transfer properties and octanol water partition values suggest that the inhalation and biomagnification routes contribute nominally to human exposure of OSPW, and ingestion or skin contact are considered the main exposure methods (Kindzierski et al., 2012). While heavy metals enriched in OSPW have been associated with several health problems, no studies of OSPW-derived heavy metals on human health have been performed (Ore and Adeola, 2020). Bioaccumulation of heavy metals in tissue has been shown in field studies with mussels downstream of oil sands developments, which may pose a risk to aquatic ecosystems (Pilote et al., 2018). Correlating toxicants of interest collected from wildlife sampling in the Athabasca oil sands region has demonstrated spatial patterns associated with elevated mercury levels in animals near bitumen upgrading facilities and open pit mining sites (Eccles et al., 2019). Analysis of surface water chemistry in Base Mine Lake (BML) in Alberta (i.e., a man made lake filled with remediated OSPW) has shown that most metal concentrations fall below aquatic toxicological guidelines, although this is mainly due to the dilution of OSPW through freshwater supplementation and the removal of water in BML for subsequent bitumen extractions (White and Liber, 2018). Due to the brackish nature of OSPW, salinity is expected to pose an issue in reclaimed OSPW reservoirs, with the long-term development of BML ultimately expecting to resemble an estuary rather than a freshwater lake without further intervention (White and Liber, 2020).

OSPW causes both acute and chronic toxicity under *in vivo* and *in vitro* conditions. *In vivo*-focused OSPW experiments have generally utilized fish, crustaceans, algae, amphibians, or insects as these organism are expected to populate reclaimed landscapes in the future, and also serve as bioindicators of toxicity for current OSPW remediation approaches or general constituents of concern (Bauer et al., 2019; Bilodeau et al., 2019; Colavecchia et al., 2004; Hagen et al., 2012; Kinley et al., 2016b; MacDonald et al., 2013; Mahdavi et al., 2015; Marentette et al., 2015; McQueen et al., 2017; Pomfret et al., 2021; Scarlett et al., 2013; Toor et al., 2013). Additionally, mice have also been used to gauge the toxicity of OSPW (Li et al., 2019; Rogers et al., 2002). *In vivo* toxicity is assessed via the generation of a species sensitivity distribution (SSD), where either: i) the proportion of a population of a species exposed to a specific chemical concentration, or ii) the concentration of a compound hazardous to a specific percentage of a species, is estimated (COSIA, 2021; Fox et al., 2021; Redman et al., 2018). *In vitro* toxicity of OSPW has been assessed using microbial organisms, cell lines, or primary cell cultures harvested from organisms (Frank et al., 2008; Garcia-Garcia et al., 2011; Hagen et al., 2012; Miles et al., 2019; Phillips et al., 2020; Qin et al., 2019b; Sansom et al., 2013). Cell-based data has demonstrated a loss of cell viability and a modification of antimicrobial responses, although *in vitro* outcomes may not accurately reflect *in vivo* results (and vice versa) due to the differences in complexity of models (Saeidnia et al., 2015). Altogether, OSPW has been shown to cause mortality and affect various biological responses such as immunological functioning, reproduction, and early stage development (Colavecchia et al., 2004; Hagen et al., 2012; Heuvel et al., 2012). Although OSPW exerts toxicity across multiple organisms, the effects are dependent on both species and the type of OSPW used. As a result, it is important to understand the sensitivity restrictions of models in addition to the composition and history of the OSPW used in these different assays.

To treat OSPW, understanding its major constituents, including both biotic (live organisms), and abiotic (non-living chemical and physical components) factors should be considered to help interpret specific modes of toxicity and future effects of various remediation techniques. Improved understanding of these elements will also help shed light on interactions between and within various categories of toxicants, as well as passive remediation processes that are underpinned by the biotic fauna. While the complete and specific identification of all the components of OSPW is challenging, there are key factors of OSPW that are generally recognized as contributors to its toxicity, in addition to emerging constituents of interest. This review will outline selected toxicants of concern in OSPW.

## 1. Abiotic constituents

### 1.1. *Naphthenic acids*

Naphthenic acids (NAs) are a group of polar carboxylic acids arranged in rings (although some naphthenic acids can be acyclic), which are generally described through the chemical formula $C_nH_{2n+z}O_2$, where $n$ is the number of carbons, and z is a negative even integer that indicates the loss of hydrogen atoms and cyclisation of the NA species (Brown and Ulrich, 2015). NAs can exist outside of the chemical formula identified above that delineates classical NAs to encompass atypical NA structures using the formula $C_nH_{2n+z}O_x$, where $x$ is between 2–5 and is indicative of NAs in aged tailings ponds (Hindle et al., 2013), or $C_nH_{2n+z}O_xN_\alpha S_\beta$, which includes NAs containing nitrogen or sulphur species (Vander Meulen et al., 2021b). Classical and atypical NAs are summarized as the naphthenic acid fraction compounds (NAFCs) associated in the organic fraction of OSPW, which can encompass thousands of NA species (Vander Meulen et al., 2021b). These compounds can range from 2.9 to 100 mg/L in tailings ponds (depending on the sample and analytical technique used), and have been reported in the Athabasca basin in concentrations of up to 10 mg/L (Ross et al., 2012). Monitoring of NAFC concentration has generally been completed using acidification, liquid-liquid extraction, and Fourier-transform infared (FT-IR) spectroscopy (Jivraj et al., 1995), although newer methods of mass spectrometry techniques have also been utilized for greater resolution such as gas chromatography-mass spectrometry (GC-MS), time-of-flight mass spectrometry (TOF-MS), and high performance liquid chromatography-mass spectrometry (HPLC-MS) (Ripmeester and Duford, 2019). NAs are soluble in alkaline conditions such as tailings ponds or rivers, but are insoluble at neutral pH (Brown and Ulrich, 2015). While NAFCs are naturally found in bitumen deposits, some compounds can serve as markers associated with oil sands developments, leading to attempts to distinguish between sources of NAFCs in the environment that are anthropogenic in origin versus ones that occur due the natural weathering of the oil sands deposits (Ahad et al., 2020; Frank et al., 2014; Headley et al., 2013; Hewitt et al., 2020; Vander Meulen et al., 2021b).

NAFCs have been the subject of multiple toxicity studies using either commercially obtained NAs collected from petroleum or extracts from OSPW (Brown and Ulrich, 2015). The mode of toxicity of NAFCs is suggested to be the nonspecific disruption of cellular membranes, a process termed narcosis (Li et al., 2017; Quinlan and Tam, 2015). NAFCs derived from OSPW are inherently more complex and may contain hundreds of thousands of NA species in a single sample compared to simple-structured commercial NAs (Brown and Ulrich, 2015; Xue et al., 2018). Furthermore, mixtures available for purchase may include additional toxicants such as alkylphenols that confound interpretations of NA-specific toxicity (West et al., 2011). As a result, model NAs have been suggested to serve as poor surrogates for extrapolating OSPW NAFC-dependent toxicity as the NA constituents in mixtures should be identified to understand toxicity in real-world, industrially sourced samples versus NA mixtures that can be bought (Bartlett et al., 2017; Garcia-Garcia et al., 2011; Marentette et al., 2015; Scott et al., 2005). However, commercial NAs are helpful in examining the mechanisms of toxicity in different biological models as well as potential biodegradation pathways of NAs in more defined conditions (Xue et al., 2018). Low molecular weight (LMW) NAs which are common in commercially available NA mixtures, are normally structurally simpler and more readily biodegradable than high molecular weight (HMW) NAs which are more complex, recalcitrant, and form a large proportion of the NAFCs in aged OSPW samples (Bartlett et al., 2017; Scott et al., 2005; Xue et al., 2018). Smaller NAs have been suggested to be more toxic compared to their larger counterparts due to HMW NAs possessing more hydrophilic carboxylic acid groups, reducing hydrophobic (and therefore toxic) effects on organisms (Frank et al., 2008; Holowenko et al., 2002). Due to the complexity of the NAFCs present in OSPW, target lipid models (TLMs) have been employed to infer the sensitivity and modes of toxicity that NAFCs exert on organisms through narcosis to generate model-informed SSDs (Redman et al., 2018).

It was shown during early OSPW remediation research that chemical and biological treatments of fluid tailings reduced acute toxicity in organisms, with the authors positing that the main toxicant in OSPW were NAs (MacKinnon and Boerger, 1986). As the NAFC forms a major component of OSPW toxicity, *in vitro* and *in vivo* biological assays have been used to examine their mechanisms of toxicity. For alternatives to animal testing, macrophage cell lines (Garcia-Garcia et al., 2011; Qin et al., 2019b), fish cell lines (Lee et al., 2008; Sansom et al., 2013; Trento et al., 2018), placental trophoblast cells (Raez-Villanueva et al., 2019), and the commonly applied Microtox® assay, which uses the bacterium *Aliivibrio fischeri* (Bartlett et al., 2017; Frank et al., 2008; Martin et al., 2010; Toor et al., 2013), have all been applied *in vitro* to examine the effects of NAFCs. In regards to animals, fish have been widely used as models for NAFC exposures due to aquatic species being at risk for OSPW-derived toxicants, using laboratory zebrafish (Reinardy et al., 2013; Scarlett et al., 2013; Wang et al., 2015b), goldfish (Hagen et al., 2012) and Japanese medaka (Bauer et al., 2019; Sun et al., 2017; Zhang et al., 2016), along with more ecologically relevant species such as trout (Lacaze et al., 2014; Leclair et al., 2013; MacDonald et al., 2013; Toor et al., 2013; Young et al., 2011) and walleye (Marentette et al., 2017). Other organisms used in NAFC toxicity studies include freshwater microcrustaceans in the *Daphnia* and *Ceriodaphnia* genuses (Kinley et al., 2016b; McQueen et al., 2017; Zubot et al., 2012), amphibians (Gutierrez-Villagomez et al., 2019; Melvin et al., 2013; Melvin and Trudeau, 2012), and semi-field work studies with tree swallow nestlings (Gentes et al., 2007), insects such as midges in the *Chironomus* genus (Anderson et al., 2012) or the mayfly *Hexagenia* species (Pomfret et al., 2021). Survival of target species is generally inversely correlated with NA concentrations (Anderson et al., 2012), although aged OSPW contain more recalcitrant NAs that seem to have less toxic effects on organisms as previously discussed (Bauer et al., 2019; Holowenko et al., 2002). However, this trend is not consistent amongst all OSPW samples, as in some studies aged and fresh OSPW exert similar toxicity effects (Marentette et al., 2015), or fresh OSPW had less toxic effects than aged OSPW (Bartlett et al., 2017). In addition to mortality, pupation and emergence of insect larvae (Anderson et al., 2012), developmental impediments at early life stages (Gutierrez-Villagomez et al., 2019; Melvin et al., 2013), immunosuppression (Garcia-Garcia et al., 2011), opportunistic disease infections (such as fin erosion and lymphocystis in fish) (Hogan et al., 2018; McNeill et al., 2012), reproductive impairments (Kavanagh et al., 2011; Raez-Villanueva et al., 2019), and liver damage (Rogers et al., 2002) have all been observed post-naphthenic acid treatment in experiments across multiple species. Interestingly, tree swallow nestlings exposed to NAs at 10-fold higher concentration than the “worst case scenario” of environmental NAs over the period of a week did not have any adverse outcomes related to growth, organ weights, liver metabolism, or blood biochemistry excluding an increased amount of blood cell production in the liver (termed extramedullary erythropoiesis) (Gentes et al., 2007). The differences of biological responses highlight the influence of diverse species used in OSPW exposure assays, and care must be taken when inter-species extrapolation is performed.

Despite studies supporting the toxicity of the NAFC, it must be stated that NAs and other ionisable dissolved organics derived from OSPW have little bioaccumulation potential based on their generally low hydrophobic properties at environmentally relevant pH values (Scott et al., 2020). This suggests that although NAFCs exhibit detrimental effects *in vivo* and *in vitro*, the risk of accumulating these components at higher trophic levels due to biomagnification is likely limited. Additional organism-driven processes such as metabolism and elimination processes are also expected to affect the bioaccumulation potential of NAFCs, although these phenomena can be more difficult to isolate and study *in vivo* (Scott et al., 2020). Although the current literature has shifted from NAs being the sole toxic contributor in OSPW to the entire organic fraction instead, it is evident that the NAFC does exert toxicity in laboratory experiments (Marentette et al., 2015; Marentette et al., 2017)

### 1.2. *Polycyclic aromatic compounds (PACs)*

Polycyclic aromatic compounds (PACs) cover thousands of constituents, including the well-studied polycyclic aromatic hydrocarbons (PAHs) that have only carbon and hydrogen elements, alkylated PAHs, dibenzothiothenes, and heterocyclic compounds consisting of nitrogen, oxygen, or sulphur atoms in their aromatic ring structure (Andersson, 2009; Cheng et al., 2018). The sources of PACs can be biogenic (synthesized by organisms such as microbes and plants during biotransformation of organic materials), petrogenic (associated with petroleum and its by-products), or pyrogenic (due to the incomplete combustion of organic materials) (Harner et al., 2018). Generally, LMW PACs (2–3 aromatic rings) are petrogenic and can be emitted into the air before being deposited onto the surface of the earth through precipitation events or gravity (a process termed atmospheric deposition), whereas pyrogenic PACs are primarily HMW components ($\geq$4 aromatic rings) and exist as particulate matter, such as soot

(Patel et al., 2020). HMW PACs are more recalcitrant, less soluble, and generally sediment-bound compared to their more volatile LMW counterparts that can be re-released into air and water (Wallace et al., 2020). To measure concentrations of dissolved or particulate PACs, the compounds are collected on a filter or sorbent and extracted with solvents or ultrasonification, and examined through GC-MS (Abdel-Shafy and Mansour, 2016). In environmental assessments of PAC concentrations in the Athabasca region from 2013–2015, alkylated-PACs predominated the PAC compositions with an average of 6.5 ng/L (Marvin et al., 2021). Selected PACs in the environmental surface water surrounding the Albertan oil sands in the monitoring (i.e., benzo[a]anthracene and pyrene) were found to exceed the Canadian Council of Ministers of the Environment (CCME) guidelines associated with each compound. PACs can be identified as anthropogenic or naturally occurring using carbon isotope signatures or molecular diagnostic ratios (Berthiaume et al., 2021; Jautzy et al., 2013; Kelly et al., 2009). These compounds are prominent contributors to oil sands toxicity and have been detected in sediment and water due to atmospheric deposition of aerosolized PACs (Bari et al., 2014; Kurek et al., 2013; Wnorowski et al., 2021), lichens (Studabaker et al., 2012), and snowpack (Kelly et al., 2009, 2010; Manzano et al., 2016) surrounding the Albertan oil sands region. Distance from an industrial oil sands site is generally inversely correlated with PAC concentrations in the environment (Cho et al., 2014; Kelly et al., 2009), although PACs have been found as far as ~90 km away from refineries in concentrations ~2.5–23-fold higher than before surface mining began in the region (Kurek et al., 2013). Although airborne PACs are of concern, an estimated 3400 tonnes of PACs have been disposed of in tailings ponds between 1994-2018, far outweighing PAC release in air (Berthiaume et al., 2021).

Due to the chemical heterogeneity of PACs, different physiological effects are observed on biological systems. Routes of exposure to organisms are associated with inhalation, ingestion, contact with skin, or in the case of plants, taken up through root systems (Patel et al., 2020). Exposure to PACs can occur simultaneously through more than one route, such as plants absorbing solubilized PACs in the soil in addition to receiving atmospheric deposits on their shoot systems (i.e., the aboveground parts of a plant). Like NAFCs, narcosis has been implicated as a mode of toxicity of PACs in addition to endocrine disruption, carcinogenicity, immunotoxicity, reproductive defects, oxidative stress, impaired survival, cardiotoxicity, and teratogenicity (Carls and Meador, 2009; Patel et al., 2020). While the majority of PACs exist in concentrations that exert acute toxicity in most settings, some compounds can increase in toxicity due to solar radiation interacting with PACs to generate reactive species (Marzooghi and Di Toro, 2017), or from by-products (i.e., phenols, catechols, and quinones) created by the metabolic breakdown of PAHs in organisms, which bind to DNA segments and lead to an increased risk of cancer (Moorthy et al., 2015).

Due to their persistence in ecosystems, PACs have been studied in OSPW where leaching of OSPW into the surrounding soil and groundwater is a concern. Laboratory assessments of Japanese medaka fish embryos exposed to the model PAC retene were shown to have higher incidences of pericardial edema and upregulation of *cyp1a*, a gene that is associated with the blue sac disease in fish fry, an effect that was not observed following OSPW-only exposures (Alharbi et al., 2016). Several field studies have also compared petrogenic PACs to NAFCs due to their ability to be aerosolized and deposited in ecosystems. PACs induce acute toxicity on aquatic fauna and birds (Abdel-Shafy and Mansour, 2016), species that would be present in proximity to oil sands mine sites. For example, nestling tree swallows within 5 km of oil sands mining areas were shown to have a complex mixture of 41 different PACs accumulated in muscle and feces, with a higher concentration of sequestered PAC in these biological samples compared to nestlings reared at reference sites over 100 km away from oil sands activities (Fernie et al., 2018). This phenomenon is also observed in the 425 fish collected between 2011 and 2012 around the Athabasca/Slave River systems (Ohiozebau et al., 2017). The Athabasca River site closest to the oil sands development had significantly higher PAC concentrations (a mean of 48 ng/g, wet mass) in the edible flesh compared to fish caught at the farthest site sampled from the oil sands in the Slave River region, averaging 13 ng/g wet mass of PACs in the muscle tissue. Passive ecological monitoring has shown that wolf and moose scat samples collected near oil sands developments have petrogenic PAC signatures, which may have been introduced via diet, atmospheric deposition, or social behaviours such as grooming in wolf packs (Lundin et al., 2015). Despite evidence of PACs in species surveyed near the oil sands, most PACs are metabolised by organisms after exposure, preventing most bioaccumulation and biomagnification events at increasing trophic levels (Abdel-Shafy and Mansour, 2016; Bilodeau et al., 2019). This has led to suggestions to re-evaluate the ecological risk of OSPW for some wildlife species such as birds (Beck et al., 2015).

Due to the omnipresence of PACs, exposure is inevitable for organisms. Monitoring the type and concentrations of PACs in the environment and in OSPW is critical to understand and prevent adverse health outcomes in humans and wildlife. While effects on biological systems are dependent on the composition of the PAC in question, monitoring water soluble and particulate PACs near oil sands mining sites is vital for developing appropriate risk assessments and effective treatment options.

### 1.3. *Other toxicants of interest in OSPW*

#### 1.3.1. *Volatile organic compounds (VOCs)*

Volatile organic compounds (VOCs) are emitted into the atmosphere due to ongoing mining activities in the oil sands, which can form secondary pollutants after interactions with ultraviolet radiation (UV) radiation such as ozone (Bari and Kindzierski, 2018; Ling et al., 2022). VOCs such as benzene, toluene, ethylbenzene, and xylene (BTEX) are added during bitumen extraction to reduce its viscosity during refinement processes (Siddique et al., 2007). VOC quantification is usually performed using proton-transfer-reaction mass spectrometry (PTR-MS) or proton-transfer-reaction ion-trap-mass-spectrometry (Warneke et al., 2011). Historically, these VOCs have existed in OSPW in concentrations exceeding the CCME guidelines for the protection of aquatic life (up to 3 mg/L in tailings ponds) (Allen, 2008a). There is little information about VOCs in the literature regarding OSPW

(Mahaffey and Dubé, 2017), however in a study using tailings as a substrate under anaerobic conditions, BTEX compounds were degraded by the microbial methanogen community in the order of toluene, xylene, ethylbenzene, and benzene, preferentially (Siddique et al., 2007).

#### *1.3.2. Inorganic ions*

The type and presence of inorganic ions affects the slightly alkaline and brackish properties of OSPW: total dissolved solids (TDS) and ions in OSPW such as sodium (99–608 mg/L), chloride (18–540 mg/L), sulphate (50–513 mg/L), bicarbonate (219–950 mg/L), and ammonia (0.03–14 mg/L) exceed the levels found in the Athabasca River by 8- to 200-fold, depending on the ion (Allen, 2008a). Ion chromatography (ICS) is used to quantify anions, while inductively coupled plasma mass spectrometry (ICP-MS) for cations is generally used (Qin et al., 2019b). Bicarbonate and sulphate ions have been shown to enhance or ameliorate the toxicity of metals present in OSPW based on studies using the freshwater invertebrate *Ceriodaphnia dubia* (Puttaswamy and Liber, 2012). Specifically, increased toxicity was observed when *C. dubia* was co-incubated with nickel and bicarbonate, whereas sulphate decreased the vanadium-associated toxicity effects, and a range of chloride concentrations demonstrated no effects on the fecundity of *C. dubia*. The same study also reported that ions can influence the type of metals released from the solid component of OSPW, where sulphate increased the concentration of the cationic metals nickel, manganese, and zinc into the water, and bicarbonate increased the mobilization of metals that can form oxyanions (aluminum, arsenic, molybdenum, and vanadium) into the surrounding water. Additionally, tailings (which consists of water, sand, clay, and residual bitumen) can slowly release more OSPW (i.e., expressed water) out as the sediment portion settles (Mahaffey and Dubé, 2017). This effect was seen in an experimental pond capped with tailings, which had an increase in salinity, pH, and naphthenic acids over a 13-year period despite the lack of further OSPW addition to the pond (Heuvel et al., 2012). Salinity has also been suggested to enhance cytotoxic effects observed in the inorganic fraction of OSPW (Miles et al., 2019; Sansom et al., 2013).

#### *1.3.3. Trace elements and metals*

Trace elements and metals enriched in bitumen can serve as indicators of oil sands related toxicants in the environment. Snowpack, sediment, plants, and water samples have been collected near the Athabasca oil sands region to examine the particulate and dissolved presence of these chemicals using various mass spectrometry approaches (Boutin and Carpenter, 2017; Gueguen et al., 2011, 2016; Kelly et al., 2010; Shotyk et al., 2017). Vanadium, nickel, rhenium, and molybdenum, metals that are enriched in bitumen, are increased downstream of oil sands mine-related activities compared to upstream sites sampled on the Athabasca River, where concentrations of rhenium averaged 2.4 ng/L upstream and 6.5 ng/L downstream of oil sands mining sites, for example (Shotyk et al., 2017). Snowpack analyses of trace elements have supported that vanadium and molybdenum positively correlate with proximity to an oil sands site, indicating anthropogenic sources facilitating atmospheric deposition (Gueguen et al., 2016). Silicon, titanium, potassium, iron, calcium, and aluminum elements smaller than 2.5 μm in diameter in the particulate matter phase ($PM_{2.5}$) were enhanced near the oil sands sites and exceeded levels of these elements found in the atmosphere of major Canadian cities (Phillips-Smith et al., 2017). However, there is conflicting research on the extent of trace elements and metals contributed by anthropogenic activities in the oil sands region. Previous work has identified elevated concentrations of silver, cadmium, antimony, and thallium over 200 km away from oil sands operations (Kelly et al., 2010), however these conclusions have been contested by a counter study that did not observe an enrichment of these elements downstream of the oil sands industry compared to upstream sites on the Athabasca River (Shotyk et al., 2017). Additionally, potentially toxic trace elements in the Albertan oil sands region have been shown to be generally lower in concentration than the rocks forming the upper continental crust of the Earth (Shotyk et al., 2021). With regards to toxicity, nickel and vanadium have been shown to act synergistically and enhance the toxicity observed against the survival and reproduction of *C. dubia* (Puttaswamy and Liber, 2012), with vanadium concentrations being inversely correlated with *C. dubia* survival (Puttaswamy et al., 2010).

#### *1.3.4. Bitumen*

Bituminous residue forms 1%-3% of the OSPW in tailings ponds, and is usually measured as oil and grease (Allen, 2008a; Dube et al., 2021). Previous oil and grease concentrations have been reported as ranging from 9 – 92 mg/L in tailings ponds from different oil sands operators (Allen, 2008a). Bitumen composition is largely dependent on ore quality at a mining site, and the residual bitumen concentration is difficult to quantify as its distribution changes in both breadth and depth in tailings ponds, although high-resolution microscopy has shown that residual bitumen is predominantly bound to particulate matter in tailings (Beck et al., 2015; Shende et al., 2016). As residual bitumen ranges from 0.6%-3% on the surface of these tailings ponds, this may not be at high enough concentration to cause biofouling of feathers in avian species that might interact with OSPW (Beck et al., 2015). Ultimately, it is unclear how much residual bitumen in OSPW specifically contributes to toxicity.

## 2. Biotic constituents

The microbial communities form an incredibly important component of OSPW, as most reservoirs with OSPW such as tailings ponds lack macro-scale animal inhabitants outside of reclaimed wetlands or terrestrial landscapes (Hawkes and Gerwing, 2019). Aside from bioengineering processes to directly treat abiotic factors such as the NAFC in OSPW, the microbial community actively degrades hydrocarbons and recalcitrant elements over a long-term period via a passive remediation process (Harner et al., 2011). The degradation of hydrocarbons requires two of the three domains of life (the prokaryotes Archaea and Bacteria), the involvement of geochemical cycles such as sulphur, carbon, nitrogen, and iron, and lastly, the generation of gas (i.e., methane, carbon dioxide and hydrogen sulphide) produced by the microbial communities in OSPW (Foght, 2015; Mori et al., 2019). While the indigenous community of microbes can degrade components such as NAFCs and reduce the overall toxicity of OSPW, it occurs at a very slow rate (Chegounian et al., 2020). Therefore, there are multiple studies aimed at selecting and enriching microbial consortia that are able to metabolize various abiotic constituents in OSPW (Chegounian et al., 2020; Demeter et al., 2014; Folwell et al., 2016; Kinley et al., 2016a; Mahdavi et al., 2015; Stasik et al., 2015), or accelerating the detoxification process by providing structurally simpler substrates with reduced recalcitrant components (such as treated OSPW) for microbial bioremediation (Martin et al., 2010).

There is an estimated $1 \times 10^6$ microbial cells per millilitre of OSPW (Foght, 2015), forming a diverse community that includes prokaryotic sulphate- and nitrate- reducing bacteria (SRB and NRB, respectively), nitrogen fixing bacteria, iron reducing bacteria (IRB), fermenters, methanotrophs, acetogens, aerobic species, methanogens, and photosynthetic and heterotrophic eukaryotes (Albakistani et al., 2021; Harner et al., 2011; Liu et al., 2016a; Richardson and Dacks, 2019; Ridley and Voordouw, 2018; Yergeau et al., 2012). While there is considerable diversity in OSPW microbial communities (An et al., 2013; Penner and Foght, 2010; Rochman et al., 2017), these environments are generally dominated by a small group of microbial taxa that are normally identified through 16S rRNA sequencing for prokaryotes, or 18S rRNA sequences for eukaryotes (Aguilar et al., 2016; Wilson et al., 2016). Like the abiotic constituents, the microbial composition of OSPW varies due to proprietary methods to extract bitumen that is dependent on the oil sands operator practices of OSPW management (Wilson et al., 2016; Yergeau et al., 2012). In comparison, the Athabasca River is estimated to have $1 \times 10^5$ - $2 \times 10^6$ cells per millilitre (Costerton and Geesy, 1979), with more types of available carbon sources reflecting more microbial diversity in the environmental samples as opposed to OSPW communities (Yergeau et al., 2012).

### 2.1. *Bacteria*

The bacterial community in OSPW is responsible for the degradation of hydrocarbons, and likely originate from the surface level bitumen ores (Foght et al., 2017). Additional microbial species are derived from the tailings effluent and freshwater incorporated into OSPW. Anaerobic processes such as sulphate reduction (the reduction of $SO_4^{2-}$ to $S^{2-}$) and methanogenesis (the generation of methane gas, $CH_4$) predominate most of the metabolic processes in OSPW, although Archaea, not Bacteria, exclusively perform the latter process. Some genera of bacteria that make up OSPW communities include *Pseudomonas, Thauera, Acidovorax, Desulfurivibrio, Thiobacillus, Brachymonas, Methylobacter*, *Methylovulum, Methylocaldum, Schumannella, Hydrogenophaga, Azonexus, Salinimicrobium, Achromobacter, Geobacter,* and *Thiocapsa* (Albakistani et al., 2021; Bordenave et al., 2010; Golby et al., 2012; Siddique et al., 2019; Yergeau et al., 2012). Overall, tailing pond sediments contain less diverse microbial species than environmental reference sites outside of oil sands mining sites due to the restricted carbon sources available (Yergeau et al., 2012).

Tailings ponds are stratified due to the particulate tailings found in OSPW, leading to various chemical gradients and subsequent niche differentiation of microbial residents in these reservoirs. Sulphate, for example, has been shown to be at the highest concentration at the surface of a Suncor tailings pond, yet the authors observed the highest sulphate reduction rate mediated by SRB over 12 meters below the surface water (Ramos-Padron et al., 2011). Methanogenesis rates fluctuated in the same tailings pond and did not correlate with depth range (Ramos-Padron et al., 2011), which is supported by similar observations from a Syncrude tailings dam (Holowenko et al., 2000). IRB in tailings ponds are also correlated with sulphur-associated bacteria, incorporating hydrogen sulphide ($H_2S$) into sulphide minerals such as pyrite (Stasik et al., 2014). Methanotrophic bacteria (use methane as a carbon source) reside in the oxic stratum (termed the oxycline) of OSPW reservoirs (Albakistani et al., 2021). NRB, which reduce nitrate ($NO_3^-$) ultimately to nitrogen gas ($N_2$) in a process called denitrification, have been recovered in OSPW reservoirs previously (Risacher et al., 2018). Nitrifying bacteria capable of oxidizing ammonia ($NH_3$) to nitrite ($NO_2^-$) or $NO_3^-$ are also present in the hypoxic zone in OSPW (Mori et al., 2019). Psychotropic bacteria have been identified in OSPW that are able to degrade hydrocarbons and methane at low temperature (4–15°C) conditions which may be influenced by Canadian seasonal variations (Albakistani et al., 2021; Kato et al., 2001).

Microbial community composition is also influenced by the presence or absence of NAs, although metabolic functions in these consortia have been shown to vary based on the time of year (Hadwin et al., 2006). Bacteria isolated from OSPW that can degrade or biomineralize recalcitrant components such as metals, BTEX, NAFCs, and PACs have been identified and enriched in *in situ* and *ex situ* conditions (Demeter et al., 2014; Golby et al., 2014; Mahdavi et al., 2015; Stasik et al., 2015). Bacteria tend to preferentially metabolize structurally simpler NAs over complex ones through aerobic biodegradation, which may be linked to acute toxicity exhibited by the LMW NAs (Frank et al., 2008; Smith et al., 2008). However, OSPW-derived consortia have been demonstrated to degrade more recalcitrant toxicants and are mainly responsible for the NAFC profile shift and reduced toxicity in aged OSPW observed in some studies using passively remediated water (Bauer et al., 2019; Demeter et al., 2014; Han et al., 2009; MacDonald et al., 2013; Toor et al., 2013; Whitby, 2010).

### 2.2. *Archaea*

The majority of Archaea in OSPW are methanogens that metabolize hydrogen gas ($H_2$), carbon dioxide ($CO_2$), acetate, methylamine, and dimethylsulphides to generate $CH_4$ in an anoxic setting (Harner et al., 2011). Methanogens are exclusive to the domain Archaea, and methanogenesis is performed in anaerobic conditions. Archaea reside in tailings ponds that are associated with methanogenesis, although not all tailings ponds are inherently methanogenic (Fedorak et al., 2002). Methanogen presence is dependent on whether oil sands operators supplement diluent to reduce the viscosity of bitumen for downstream refinement, whereby the diluent provides the substrates for methanogenesis in OSPW (Foght et al., 2017; Siddique et al., 2019). While the diluent is a propri-

**Table 1 – A summary of highlighted abiotic components and their role in oil sands process-affected water (OSPW).**

| Constituent | Role in OSPW | Examples | References |
|---|---|---|---|
| Naphthenic acid fraction compounds (NAFCs) | NAFCs are major contributors to OSPW toxicity via nonspecific membrane disruption (narcosis). Naphthenic acids (NAs) are diverse in composition and larger NAs are generally more recalcitrant. Impairment to reproduction, immune system functioning, and developmental progress can occur post NAFC exposures. | Classical NAs ($C_nH_{2n+z}O_2$), aged NAs ($C_nH_{2n+z}O_x$), and ones that include nitrogen or sulphur ($C_nH_{2n+z}O_xN_\alpha S_\beta$). | (Brown and Ulrich, 2015; Headley et al., 2013; Hindle et al., 2013; Li et al., 2017; Vander Meulen et al., 2021a) |
| Polycyclic aromatic compounds (PACs) | PACs are emitted into the atmosphere through oil sands mining activities and deposited into OSPW, as well as being present naturally in petroleum and its by-products. These compounds exist in various compositions, are generally recalcitrant and toxic. | Polycyclic aromatic hydrocarbons (PACs), alkylated PAHs, dibenzothiothenes, or heterocyclic compounds with nitrogen, oxygen, or sulphur elements. | (Andersson, 2009; Cheng et al., 2018; Harner et al., 2018; Wallace et al., 2020) |
| Volatile organic compounds (VOCs) | VOCs are introduced via diluent addition to reduce bitumen viscosity, and serve as substrates for methanogens. VOCs are known toxicants that can interact with solar radiation to form secondary pollutants while aerosolized. | Benzene, toluene, ethylbenzene, and xylene, known as the BTEX group. | (Siddique et al., 2007) |
| Inorganic ions | Inorganic ions are enriched in OSPW and change in composition over time. Ions may have additive or protective effects on toxicity *in vivo*. Sediment-bound metals can be mobilized to the aqueous phase based on ion presence. Ions that are associated with salinity also contribute to cytotoxic effects. | Sulphate and bicarbonate ions. | (Heuvel et al., 2012; Mahaffey and Dubé, 2017; Miles et al., 2019; Puttaswamy and Liber, 2012; Sansom et al., 2013) |
| Trace elements and metals | Specific metals and trace elements are enriched in bitumen deposits and can serve as indicators of oil sands mining. Some metals have been shown to negatively impact survival and reproduction *in vivo*. | Vanadium, nickel, rhenium, molybdenum, titanium, potassium, iron, calcium, and aluminum. | (Puttaswamy and Liber, 2012; Puttaswamy et al., 2010; Shotyk et al., 2017) |
| Bitumen | Residual bitumen is distributed heterogeneously in OSPW, and its effect on biological organisms in small concentrations is poorly understood. | Oil and grease. | (Allen, 2008a; Beck et al., 2015; Dube et al., 2021) |

etary mixture of specific compounds that vary based on company, it generally includes naptha (a light hydrocarbon), and members of the BTEX group (Cossey et al., 2021). Members of the archaeal methanogens in OSPW are associated with the acetoclastic *Methanosaetaceae* family, which exclusively use acetate as its carbon source, the hydrogenotrophic *Methanomicrobiales*, which produces methane using $H_2$ and $CO_2$ as substrates, and methylotrophic *Methanosarcinaceae*, which use methanol, methylamines, and methylated sulphides as intermediates in their production of $CH_4$ (Siddique et al., 2019; Sollinger and Urich, 2019; Wagner et al., 2018). Hydrocarbons that are generally considered to be recalcitrant have recently been shown to be metabolized under methanogenic conditions (Mohamad Shahimin et al., 2021), and their degradation can be further accelerated through supplying additional indigenous OSPW microbiota (Siddique et al., 2020). Bioreactor experiments using OSPW collected from a Syncrude tailings pond suggest that early stage tailings pond ecosystems are predominately bacteria inhabited, while archaeal abundance increases as the tailings age (Chi Fru et al., 2013). This shift is likely also dependent on the physiochemical parameters of each OSPW sample, as methanogenesis requires additional carbon substrates added post-bitumen extraction (Foght et al., 2017).

### 2.3. *Eukaryotes*

Currently, microeukaryotes in OSPW are poorly elucidated (Richardson and Dacks, 2019). Microeukaryotes refer to the microscopic, generally single-celled eukaryotic organisms that form part of the microbiome and span multiple different trophic levels such as some algae species, parasites, saprotrophs, or predators (Campo et al., 2019). Photosynthetic algae have been proposed as potential partners to bioremediate OSPW by supplying oxygen to facilitate aerobic degradation of NAs by prokaryotes (Whitby, 2010). Co-incubations with OSPW-derived bacteria and algae have shown the reduction of NAs and toxicity in lab scale experiments, however the bacterial biomass was responsible for most of the detoxification effort, and the bacteria alone treatment had the highest NA degradation rate (Mahdavi et al., 2015). Axenic algal cultures have also been used to examine their biodegradation potential for the acid extractable organics (AEOs) of OSPW, which mainly comprise of NAs (Ruffell et al., 2016). In the aforementioned study, a green algal *Stichococcus sp.* was effective at reducing AEO marker ions after being exposed in up to 100 mg/L concentrations of AEOs (Ruffell et al., 2016). NAs and PACs have been biodegraded by algae in previous studies (Beddow et al., 2016; Chan et al., 2006; Demeter et al., 2014; Folwell et al., 2016; Headley et al., 2008), and the NA tolerance of photosynthetic eukaryotes has also been examined (Leung et al., 2003; Woodworth et al., 2012). Regarding field surveys, microeukaryotes have been recently characterized through next-generation sequencing of two sources of OSPW from Syncrude oil sand sites (Aguilar et al., 2016). 18S rRNA libraries generated from anoxic sediments as well as the surface oxygenated waters identified 169 different operational taxonomic units (OTUs, a proxy for species) that were mainly associated with heterotrophic Fungi, Rhizaria, Amoebozoa, Chromalveolata, and phototrophic Chloroplastida higher taxonomic groups. Metagenomic analyses on the same samples were only able to identify a total of 75 eukaryotic SSU rDNA sequences (22 in the sediment, 55 for the surface water), which is likely an underestimation due to the dominance of prokaryotic signatures. Overall, the presence and role of eukaryotes within OSPW reservoirs is the focus of ongoing studies.

### 2.4. Viruses

Viruses are biological entities consisting of nucleic acids (either DNA or RNA) surrounded by a protein shell (Taylor, 2014). Depending on the virus and cell infected, a single virus can result in tens of thousands of progenitors within days (Chen et al., 2007; Chinchar, 1999). Although viruses are restricted to parasitizing cellular machinery of their hosts to reproduce, their range of infection as a whole encompasses all domains of life (Koonin et al., 2015). There are approximately $10^{31}$ viruses on earth, outnumbering prokaryotes by 10-fold in the environment (Hendrix et al., 1999). Bacteria represent one of largest biomass groups on earth, and as such, viruses that infect bacteria (termed bacteriophages, or phages) are expected to be the most abundant viruses in the biosphere (Bar-On et al., 2018; Breitbart and Rohwer, 2005). Once a phage has entered a cell, it can undergo the lytic lifecycle by hijacking the host cellular machinery to form viral factories and produce virions (i.e., complete virus particles), causing the eventual lysis of the bacterium (Howard-Varona et al., 2017; Li et al., 2020). Alternatively, a phage may initiate a lysogenic lifestyle by integrating its genetic material into the bacterial genome (where it is then termed a prophage) and replicating alongside the host without producing virions (Howard-Varona et al., 2017). Phages can also encode auxiliary metabolic genes (AMGs) scavenged from hosts during ancestral infections (Warwick-Dugdale et al., 2019). AMGs evolve independently from host homologs and increase phage fitness by altering host metabolic processes during viral infection, and are conserved across multiple phage lineages. Due to their inability to independently reproduce, viruses are not unanimously considered living or nonliving (Lopez-Garcia, 2012). The inclusion of phages in the biotic section of this review is meant to highlight their interactions with bacterial hosts that have been previously discussed above.

Phages have been shown to mediate the aggregation of suspended clay particles that are present in OSPW (Curtis et al., 2011). This form of phage-facilitated aggregation of particulate matter in tailings appears to be dependent on the presence of positively charged amino acid residues on the phage as well as hydrogen bonds (Curtis et al., 2013). Recently, metagenomic analyses have identified phages with large genomes in BML (Al-Shayeb et al., 2020). BML was commissioned by Syncrude as the first full-scale end-pit lake in Alberta that was filled with OSPW and capped with freshwater in 2013 (Richardson et al., 2020). After restricting searches in metagenomic datasets to select for phages with genomes greater than 200 kilobase pairs (kbp), the authors identified phages in BML with 206-395 kbp genomes (Al-Shayeb et al., 2020). In contrast, the most common phage genome ranges from only 40-45 kbp (Zrelovs et al., 2020). Further analyses of these phage genomes from BML (in addition to other Albertan tailings ponds and natural freshwater lakes containing methane sediments) identified the presence of *pmoC* (Chen et al., 2020). This gene is associated with the bacterial *pmoCAB* operon, which encodes particulate methane monooyxgenases (pMMOs), a metalloenzyme that is responsible for the conversion of methane to methanol in methanotrophs. Interestingly, the authors failed to identify phages that encoded either *pmoA* or *pmoB* genes but noted that the fastest growing methanotroph in a freshwater lake was infected by three *pmoC* containing phages, suggesting that pmoC-phage infection enhances bacteria methane oxidation.

## 3. Future perspectives

### 3.1. Abiotic constituents in OSPW

The abiotic components of OSPW are mainly generated via the bitumen extraction process, although they can also be associated with natural bitumen deposits (Wu et al., 2019). Table 1 summarizes the abiotic components highlighted in this review. The main toxicant of interest in OSPW is associated with the NAFC due to its acute toxicity effects observed in several biological models (Bartlett et al., 2017; Marentette et al., 2015; Morandi et al., 2015; Vander Meulen et al., 2021a). While smaller NAs have been suggested to be more toxic to microbes and metabolized preferentially compared to larger NAs (Whitby, 2010), the composition of the NA mixture itself, and whether it is commercially or oil sands-sourced affects the extrapolation between experimental results and true environmental outcomes (Bartlett et al., 2017; West et al., 2011). PACs can be emitted into the atmosphere and deposited into terrestrial and aquatic systems surrounding the oil sands region, and are also present in OSPW with recalcitrant components (Bari et al., 2014; Li et al., 2017). The addition of diluent to bitumen introduces VOCs such as the BTEX group into OSPW, which can serve as a substrate for microbial degradation (Siddique et al., 2007). Finally, trace elements, metals, and ions can be enriched in bitumen deposits as well as produced during the Clark Hot Water Process extraction (Allen, 2008a; Shotyk et al., 2017). In OSPW reservoirs, these chemicals may serve as co-stressors and affect *in vivo* toxicity effects of the NAFC, such as salt being demonstrated to affect the toxicity of NAs in yellow perch and mussels (Bartlett et al., 2017; Nero et al., 2006). Altogether, these components have been regarded as inducers of the majority of the detrimental effects seen in experiments using OSPW, although specific toxicity mechanisms are still not elucidated. Metabolic pathways affected in OSPW-exposed bacteria include oxidative stress, DNA damage, and protein stress (Morandi et al., 2017). For higher organisms, biomimetic extractions (BE) (i.e., quantifying the concentration of hydrocarbon mixtures that adhere to polymer fibres during solid phase microextraction through gas chromatography) can be used to predict toxicity of a complex mixture such as OSPW towards organisms through the generation of SSDs informed through BE (Letinski et al., 2014; Redman et al., 2018). Ultimately, more research is required to understand the toxicokinetics (i.e., how a toxicant enters an organism and reaches its target) and toxicodynamics (i.e., the symptoms that ensure after an effective dose of a toxicant is reached) (Croom, 2016) of OSPW.

### 3.2. *Biotic interactions in OSPW*

The microbiome of OSPW consists of eukaryotic, prokaryotic, and viral biological entities that are intimately involved with the biotransformation of abiotic constituents (Table 2). In OSPW, microbial communities are present planktonically (i.e., free-floating) and in biofilms, which are aggregates of multispecies cells encased in an extracellular matrix attached to a substrate (Golby et al., 2012). Around 40%-80% of prokaryotes are posited to exist as biofilms (Flemming and Wuertz, 2019), which may help reduce exposure to various toxicants such as OSPW constituents (Golby et al., 2012). While the microbial community in OSPW is composed of a core microbiome that can degrade limited substrates (i.e., hydrocarbons and petroleum related by-products) with a supportive accessory set of microbes that can metabolize side products, its constituents are dependent on the available carbon and energy sources in the local environment (Wilson et al., 2016). While most interactions are synergistic between members in these microbiomes, there may still be antagonistic interactions that develop due to competition for available substrates. Methanogenesis for example, is inhibited if sulphate is added to tailings sediment in laboratory assays to stimulate the growth of the SRB population (Fedorak et al., 2002; Holowenko et al., 2000). Sulphate is present in OSPW in the form of gypsum ($CaSO_4 \cdot 2H_2O$), which is added to OSPW by some oil sands companies to accelerate the sedimentation process of the particulates in OSPW (Fedorak et al., 2002). The rate of sulphate reduction is generally inversely correlated with methanogenesis, which is due to the competition for common substrates such as $H_2$ and acetate. Overall, SRB are stronger competitors if sulphate is not a limiting factor, as they gain more energy from their thermodynamically favourable metabolic pathways (Dar et al., 2008; Holowenko et al., 2000). Bacterial competition has been demonstrated to establish hierarchical dynamics in a single aquatic reservoir (Hussain et al., 2021), and successional microbial communities in water bodies show dynamic fluctuations at fine resolutions (Boucher et al., 2006), with distinct particle and free-living bacterial communities (Allgaier and Grossart, 2006). Future work examining the microbial succession in OSPW communities in individual tailings ponds would be valuable to understand the physiochemical differences that are potentially induced by microbes. In addition to interspecies competition, bacteriophages directly influence the microbial community composition by lysing cells and affecting population density of prevalent bacterial species (a Kill-the-Winner strategy), or by adopting lysogenic lifestyles to exploit hosts in low or high densities (a Piggyback-the-Winner model) (Knowles et al., 2016; Pannekens et al., 2019). Additionally, while multiple phages can infect a host cell at the same time (a process called superinfection), some prophages have developed mechanisms to prevent coinfection and increase host fitness, resulting in competition between closely related viruses (Kirchberger et al., 2021).

Distinguishing which members of microbial taxa are especially important in supporting these passive remediation processes will help shed light onthe detoxification progress of OSPW and other oil contaminated environments. Identifying hydrocarbon degrading microbial communities has been applied to multiple environments outside of the oil sands themselves, such as deepsea cold seeps (Pop Ristova et al., 2015; Van Landuyt et al., 2020), sediments contaminated by oil (Akbari et al., 2014), and high salinity coastal environments (Fathepure, 2014). Culturing OSPW communities to simulate endogenous conditions through biofilm-mediated techniques may offer better understanding of the interactions that occur on a microbial scale between members (Golby et al., 2012).

### 3.3. *Interfaces of interactions for abiotic and biotic constituents in OSPW*

Ultimately, both the biotic and abiotic constituents should be considered as interconnected components (Fig. 1). For example, biogeochemical cycles mediated by microbes have been shown to alter oxygen concentrations in the freshwater cap of OSPW reservoirs (Risacher et al., 2018). Additionally, the selection of the microbial community in OSPW is strongly influenced by the chemicals present in the water, serving as an energy sources for some species and inhibiting others that are unable to grow using these substrates. AOPs such as ozonation has been shown to remove up to 61% of NAs in one study (Perez-Estrada et al., 2011), while photocatalysis has been applied to remove 90% of commercially-sourced NAs (Liu et al., 2016b). However, the efficacy of photocatalysis is highly dependent on the source used, as Leshuk and colleagues demonstrated some OSPW samples have NA degradation occur 8.3x slower than samples obtained from a different company due to side reactions (Leshuk et al., 2016a). Additionally, recalcitrant NA species require substantially longer periods of treatment to ensure a complete mineralization of the NAFC (80% degradation at 6 hr of solar photocatalysis versus 14 hr for 100% removal), which can be an expensive and energy intensive investment when considering the sheer volume of OSPW that must eventually be treated (Leshuk et al., 2016b). AOPs have been shown to effectively degrade more complex NAs while microbes preferentially degrade LMW NAs (Leshuk et al., 2016b; Quinlan and Tam, 2015). This facilitates interdisci-

**Table 2 – Biotic constituents and their role in oil sands process-affected water (OSPW).**

| Constituent | Role in OSPW | Examples | References |
|---|---|---|---|
| Bacteria | Bacteria are important players in the OSPW biogeochemical cycling. The hydrocarbon degrading microbes are sourced from bituminous surfaces and the extraction process. These bacteria are also able to degrade recalcitrant components, contributing to the bioremediation of OSPW. These multispecies communities engage with other members synergistically or antagonistically. | *Pseudomonas, Thauera, Acidovorax, Desulfurivibrio, Thiobacillus, Brachymonas, Methylobacter, Methylovulum, Methylocaldum*, and *Schumannella* genera. | (Albakistani et al., 2021; Bordenave et al., 2010; Demeter et al., 2014; Golby et al., 2012; Siddique et al., 2019; Yergeau et al., 2012) |
| Archaea | Methanogenesis is performed exclusively by Archaea under anaerobic settings. Methanogen presence in OSPW is dependent on if diluent is used in the bitumen extraction. Methanogens utilize different carbon sources based on their physiology, and the community of OSPW shifts to a higher abundance of Archaea as tailings age. | *Methanosaetaceae, Methanomicrobiales*, and *Methanosarcinaceae* families. | (Chi Fru et al., 2013; Foght et al., 2017; Harner et al., 2011) |
| Eukaryotes | While there is limited research on eukaryotes in OSPW, laboratory studies have been performed with algae to degrade naphthenic acids (NAs) or acid extractable organics (AEOs). A mixture of heterotrophic and photosynthetic eukaryotes have been found in OSPW and oil sands sediments. Eukaryotic predation from heterotrophs also may affect the microbial community. | Fungi, Rhizaria, Amoebozoa, Chromalveolata, and Chloroplastida clades. | (Aguilar et al., 2016; Mahdavi et al., 2015; Richardson et al., 2020; Richardson and Dacks, 2019; Whitby, 2010) |
| Viruses | Viruses in OSPW are a new and developing field. Bacteriophages have been shown to aggregate clay particles in tailings. Recent metagenomic analyses have identified bacteriophages with large (>200 kbp) genomes in Albertan OSPW, with some encoding a methanotroph sourced gene. Viruses are also responsible for microbial turnover in environments, modifying the OSPW community composition. | pmoC-phages | (Al-Shayeb et al., 2020; Chen et al., 2020) |

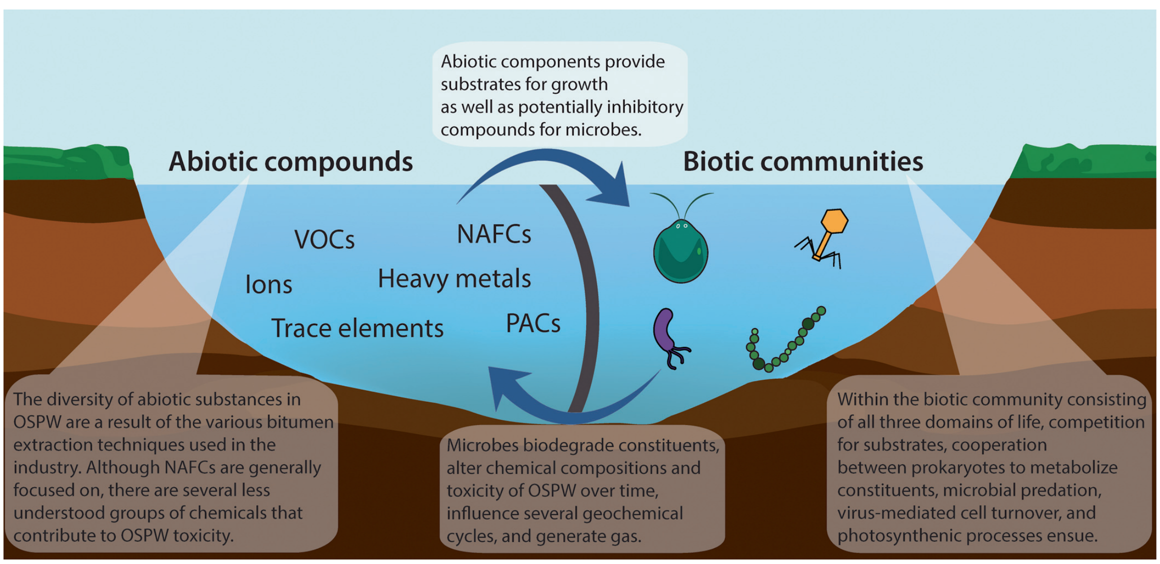


**Fig. 1 – A holistic perspective of the abiotic and biotic components in oil sands process-affected water (OSPW), and potential interactions within and between groups. Representative members in the biotic community are illustrated by a eukaryote (top left), bacteriophage (top right), and bacteria (bottom left and right).**

plinary approaches integrating an initial AOP treatment and subsequent bioremediation to more comprehensively reclaim OSPW in a fiscally responsible manner. For example, over 87.2% of total AEOs were removed in co-remediation treatments (ozonation and microbial degradation) compared to 13% AEO removal from endogenous microbes in non-AOP treated water (Hwang et al., 2013).

It may be infeasible to suggest a consolidated criterion that specifically stipulates constituents of OSPW. However, as several major constituents contributing to toxicity have been identified in OSPW, it is important to gain a holistic understanding of how potential interactions between toxicants of concern may occur in these dynamically complex waters. Future insight will better elucidate biological effects across species with the impacts of co-stressors incorporated, as well as more effective reclamation methods to reduce the toxicity of OSPW.

## 4. Conclusions

Due to the complexity of non-conventional bitumen extractions in Alberta, OSPWs contain multiple toxicants of concern that vary based on company techniques, locations of reservoirs, and age (Mahaffey and Dubé, 2017). The toxicants of interest established in previous literature such as NAFCs should be routinely monitored to record profile shifts in their composition that may be associated with toxicity as the OSPW ages over time (Marentette et al., 2015). The dynamics of hydrocarbon degraders in the prokaryotic community should be examined further to correlate changes in chemical constituents with the potential functional output of the microbial community. Altogether, the abiotic and biotic factors form unique mixtures that interact within and between themselves, and each must be considered individually and holistically to understand toxicity differences and remediation outcomes of OSPW in future reclamation projects.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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