TOXICITY OF SURFACE WATER AND PORE WATER FROM AN OIL SANDS PIT LAKE (LAKE MIWASIN) TO *Daphnia*

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ABSTRACT

Canada holds approximately one-third of the world's confirmed crude oil reserves, primarily concentrated in the Alberta Oil Sands (AOS) region, in the form of bitumen. However, the extraction process of oil sands generates significant volumes of tailings and process water, which raises concerns about their potential effects on aquatic life and the need for remediation. Lake Miwasin, constructed in 2018, serves as a pilot-scale pit lake and a model for this context. It features treated fluid tailings overlaid by a mixture of oil sands process-affected water (OSPW) and freshwater, exhibiting seasonal stratification. Since its establishment, Lake Miwasin has been colonized by various organisms, including the Crustacean, *Daphnia pulex*. Monitoring data from Lake Miwasin has shown the presence of compounds in both water and sediment that could be detrimental to aquatic organisms. Therefore, the primary goals of this research were to assess surface water and pore water toxicity, including an evaluation of selenium (Se) bioaccumulation in *Daphnia* species exposed to the conditions representative of Lake Miwasin at its early development stages and possibly other future pit lakes in the AOS region.

This study examined the toxicity of Lake Miwasin surface water (LMW) and pore water (LMP) using lab-cultured and native *Daphnia* species. Interestingly, LMW exhibited no acute or chronic toxicity towards *D. magna* and *D. pulex* (lab strains) and the native *Daphnia sp.* (collected from Humboldt Lake, SK, Canada). However, LMP demonstrated acute toxicity to both lab strains and the native *D. pulex* (collected from Lake Miwasin, AB, Canada). Lake Miwasin pore water also negatively impacted lab *D. pulex* reproduction, leading to a reduced number of offspring. Salinity emerged as a significant stressor in LMP, and a Toxicity Identification Evaluation (TIE) phase I suggested ammonia and metals in LMP as potential contributors to the observed toxicity in the tested organisms. On a related point, results showed that concentrations of dissolved Se in LMW from 2019 to 2021 exceeded the Canadian Council of Ministers of Environment (CCME) water quality guidelines for long-term aquatic life protection (1 μg/L), and the British Columbia Ministry of Environment (BC MoE) water guideline alert concentration for the protection

of aquatic life (1 μg/L). The latter water guideline was also adopted by the Government of Alberta. Further, another experiment assessed Se bioaccumulation in *D. pulex* through dissolved and dietary exposure routes, drawing comparisons to native specimens collected from Lake Miwasin. In semi-static tests (12 days), lab strain *D. pulex* exposed to selenate [Se(VI)] showed a Se concentration-dependent increase from days 5 to 12 for most treatments, whereas a lower bioaccumulation occurred at higher Se concentrations, which suggests a potentially internal regulatory mechanism. Native *D. pulex* exposed to LMW in laboratory conditions showed that Se bioaccumulation levels were similar to those of *D. pulex* collected directly from Lake Miwasin. Despite these findings, the Se concentrations in *D. pulex* from both lab exposures and Lake Miwasin collections remained below available regulatory guidelines for invertebrate tissue (4 μg/g), suggesting that *D. pulex* appears to pose minimal risk as a food source in the Lake Miwasin ecosystem.

These findings provide useful insights into the toxicity of Lake Miwasin water to aquatic invertebrates, the potential for Se bioaccumulation in daphnids in Lake Miwasin, the potential enhancement of pit lake monitoring programs, and the decision-making regarding the use of end pit lakes in the reclamation of OSPW and oil sands tailings.

Key words: oil sands process-affected water (OSPW), toxicity, *Daphnia*, selenium, bioaccumulation

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Para ti mama Carmen con todo mi amor.

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LIST OF ABBREVIATIONS

AOS	Alberta Oil Sands
OSPW	Oil Sands Process-Affected Water
Se	Selenium
LMW	Lake Miwasin surface water
LMP	Lake Miwasin pore water
TIE	Toxicity Identification Evaluation
CCME	Canadian Council of Ministers of Environment
MMB/D	Million Barrels Per Day
EPLs	End Pit Lakes
BML	Base Mine Lake
NAs	naphthenic acids
CPCs	compounds of potential concern
PAHs	polycyclic aromatic hydrocarbons
PASS	Permanent Aquatic Storage Structure
VOCs	Volatile Organic Compounds
BC MoE	British Columbia Ministry of Environment
ISQG	Interim Sediment Quality Guideline
PEL	Probable Effect Level
HL	Humboldt Lake
LM	Lake Miwasin
USask	University of Saskatchewan
HDPE	High-Density Polyethylene
DCW	Carbon-Filtered Municipal Water
RSW	Reconstituted Saline Water
RSP	Reconstituted Saline pore water
NaCl	Sodium Chloride
EDTA	Ethylenediaminetetra-Acetic Acid
SPE	Solid-Phase Extraction
DO	Dissolved Oxygen
ANOVA	Analysis of Variance
48-h LC_{50}	Medium Lethal Concentrations

<LOD	Under Limit of Detection
na	Not Analyzed
BBM	Bold’s Basal Medium
CRM	Certified Reference Materials
CW	Control Water
CA	Algae grown in BBM
ALM	Algae grown in Lake Miwasin

LIST OF APPENDICES

PREFACE

This thesis was prepared in a manuscript-style format following the University of Saskatchewan College of Graduate and Postdoctoral Studies guidelines. Accordingly, Chapter 1 serves as a general introduction to the work conducted while Chapters 2 and 3 have been prepared for peer-reviewed scientific journals. Chapter 4 is a general discussion that summarizes the research findings, addresses the study gaps identified by this research, and highlights areas for further study. As a result, some background information may be repetitive. All references are compiled at the end of the thesis and supporting information for the two research chapters is found in the appendices.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Oil sands

1.1.1 Overview

The Alberta Oil Sands (AOS) region is a place of significant industrial development and a major contributor to the country's economy. Canada has one-third of the proven global crude oil deposits (AER, 2015; Government of Canada, 2019) with the total reserves estimated at 171 billion barrels, representing 10% of the world's proven oil reserves (Government of Canada, 2019). Canadian crude oil production has shown a consistent increase over the years. According to AER (2022b), bitumen production grew from 1.3 million barrels per day (MMB/D) in January 2008 to 3.5 MMB/D in October 2021. It is projected that global energy demand will lead to a 27% rise by 2035 (CAPP, 2022a). Consequently, this trajectory is expected to lead to a crude oil production of 4.25 MMB/D by 2035 (CAPP, 2019), which will drive more intensive exploitation of crude oil from oil sands. During oil sands mining, the processes of removing ground material and extracting bitumen for recovery may have a significant impact on the surrounding land and water resources (Jordaan, 2012).

1.1.2 Tailings

Oil sands mining requires large volumes of freshwater, particularly in the process of separating bitumen from sand and clay referred to as extraction (AER, 2023b; Allen, 2008).). As a result, oil sands mining generates substantial quantities of tailings, and these tailings are subsequently pumped into tailing ponds (AER, 2023b; CAPP, 2022b). A "zero" discharge policy in Alberta, Canada required oil sands operators to confine all processed water on-site (Allen, 2008) to prevent its release into the surrounding environment. In fact, a holding of approximately 1360 Mm^3 of fluid tailings was calculated in 2020 (AER, 2021). Given the potential hazards linked with the accumulated tailings in the AOS, the current regulatory framework

promotes active treatment and progressive reclamation of tailings throughout the oil operation. This approach enables the disposal of tailings for reclamation purposes within a few years after the mine closure

The terms “tailing pond water” (Allen, 2008; MacKinnon & Boerger, 1986), “process-affected water” (Brown & Ulrich, 2015), and “oil sands processed-affected water” (Li et al., 2017; Mahaffey & Dubé, 2016; McQueen et al., 2017) have been used across studies to refer to oil sands industrial wastewater (the water sitting above mudline in tailings ponds). In a broader sense, the term “oil sands processed-affected water (OSPW)” encompasses designations such as fresh or aged tailings, raw or treated tailings, and seepage (Li et al., 2017). Furthermore, Allen (2008) stated that the composition of tailings is not consistent and depends on several factors, including ore quality, source, extraction processes, and age. Therefore, information regarding the composition or toxicity of oil sands tailings or OSPW collected from different sources needs to be used carefully for comparisons and assumptions.

1.1.3 Oil sands reclamation

Oil sands reclamation approaches strive to return the land to its original functions while adhering to government regulations (AER, 2023a). Under current legislation, operators are required to conduct tailings management to reduce the volume of stored fluid tailings (AER, 2021). Generally, reclamation plans include dry and wet landscape reclamations. For dry reclamation, it is crucial to stabilize the terrain by dewatering tailings, adding amendments to enhance soil quality, designing the topography, and establishing vegetation. In contrast, wet reclamation involves containing tailings in ponds and submerging them under water to create artificial lakes (BGC Engineering Inc., 2010).

Wet reclamation aims to create productive and sustainable landscape features. End pit lakes (EPLs) are constructed lakes that serve to treat tailings through bioremediation and salt dilution with the addition of freshwater (BGC Engineering Inc., 2010). EPLs offer additional potential advantages, such as controlling the flow of surface water and fluid tailings captured at the lake bottom (COSIA, 2021). The application of amendments to EPLs can accelerate the settling of tailings and the stabilization of organic substances. EPLs

are also a cost-effective method as they do not require additional space or heavy equipment (BGC Engineering Inc., 2010). However, concerns arise regarding the persistence of compounds within EPLs, particularly for salinity ions and dissolved organic contaminants that degrade over extended periods (Allen, 2008; BGC Engineering Inc., 2010; CEMA, 2012; COSIA, 2021). Additionally, there is a potential for the occurrence of meromixis and changes in water quality over time (CEMA, 2012), needing the implementation of a monitoring to evaluate the evolution of the EPLs. See CEMA (2012) for further background regarding EPLs.

Base Mine Lake (BML), located in the former Base Mine of the Syncrude Mildred Lake operation (AB, Canada) is the first full-scale EPL in the AOS region. Its primary objective is to demonstrate the efficacy of the water-capped tailing method in gradually improving water quality since 2012 by isolating fluid tailing beneath the water cap. Monitoring results from BML have revealed that fluid tailings were settling as predicted, accompanied by a progressive reduction in suspended solids over time (COSIA, 2021). However, some parameters (e.g., ammonia, nitrate, chloride, total boron, total phenolics, and hydrocarbons) have consistently or occasionally exceeded chronic or long-term guidelines for the protection of aquatic life (COSIA, 2021).

1.1.4 Compounds of potential concern

While there is an improvement in the tailings quality following treatment, their composition can still exhibit significant variability due to differences in oil sands ore, extraction methods, aging, and the treatments applied (Allen, 2008). Consequently, certain compounds may persist in treated tailings. Allen (2008) identified specific target contaminants that exceeded environmental water quality benchmarks, including naphthenic acids (NAs), volatile organic compounds, total dissolved solids, trace metals, bitumen, and ammonia. Afterward, Li et al. (2017) pointed out that the compounds of potential concern (CPCs) in OSPW and OSPW-derived fractions included NAs, polycyclic aromatic hydrocarbons (PAHs), metals, and salts.

Among organic CPCs found in OSPW, NAs are of the most concern since they have been postulated as the main source of toxicity to aquatic organisms (Brown & Ulrich, 2015; Clemente & Fedorak, 2005; Frank et al., 2009; MacKinnon & Boerger, 1986; Morandi et al., 2015). High-molecular-weight NAs, in particular, exhibit resistance to biodegradation and can persist in treated tailings (Allen, 2008). Further research needs to determine the limiting factors of NAs biodegradability, including molecular structure, abiotic factors, and their metabolic pathways by native microorganisms (Brown & Ulrich, 2015). In addition to NAs, PAHs represent another group of organic CPCs in OSPW capable of causing toxicity, whereas their impacts can be significantly mitigated by volatilization, dilution, and biodegradation in wet reclamation processes (Allen, 2008).

Among inorganic CPCs present in OSPW, metals, and salts hold particular significance. Metals are primarily derived from the bitumen deposits. Concentrations of aluminum, arsenic, chromium, copper, iron, lead, nickel, and zinc found in tailing water and reclamation ponds have exceeded water quality benchmarks (Allen, 2008), Moreover, operational bitumen upgrading methods can introduce additional metals into OSPW. For instance, vanadium, manganese, nickel, and molybdenum can originate from petroleum coke upon contact with OSPW (Squires, 2005). Elevated salinity levels within OSPW can potentially cause chronic toxicity in aquatic organisms. This is because high concentrations of salts can create an ion imbalance resulting in chronic stress to impair normal metabolic functions (Allen, 2008). Salinity can also impact biological productivity and community composition, or act as a stressor that increases the toxicity of other components (e.g., organic compounds and metals) to aquatic biota. Few studies have recognized or addressed salinity as a potential stressor in OSPW (Kavanagh, 2012; Nero et al., 2006).

Oil sands processed-affected water can be acutely and chronically toxic to a variety of organisms, including microorganisms, invertebrates, fish, birds, amphibians, and mammalian cell lines (Brown and Ulrich, 2015; Mahaffey and Dubé, 2016). The toxicity of OSPW can manifest through multiple modes of action such as narcosis, endocrine disruption, immunotoxicity, carcinogenicity, oxidative stress, DNA damage, pathological changes, delayed growth, and disruption of normal metabolic function (Brown and

Ulrich, 2015; Li et al., 2017; Morandi et al., 2017). It is important to note that due to the persistence of certain CPCs in OSPW, chronic toxicity might still be a concern in tailing management.

1.2 Pilot-scale end pit lake "Lake Miwasin"

1.2.1 Site description

Lake Miwasin, formerly known as the Suncor Demonstration Pit Lake, is situated to the south of the Suncor Millennium Mine, approximately 18 km north of Fort McMurray, AB, Canada (56° 53' N, 111° 23' W, Figure 1.1). The wet landscape reclamation approach has been used by Suncor. To ensure the feasibility of the reclamation plan, a pilot-scale project was executed, involving the implementation of Permanent Aquatic Storage Structure (PASS) treatment and watershed management (Suncor's closure plan) in the area. The PASS technology included the addition of a polymer flocculant and alum coagulant to the fluid tailings aiming for expediting the consolidation of fluid tailings.

Treated tailings have been sequestered in the lake bottom and capped with a mixture of OSPW and freshwater, aiming for a 50:50 ratio (COSIA, 2021; Suncor, 2023). This small lake covers an area of 1.2 hectares and has a maximum depth of around 10 m at its center. Within the first year, the treated tailings consolidated from an initial tailings depth of 9 m in 2017, to a tailings depth of ~6.3 m in 2018 after water cap placement. By 2019, there were 5.5 m of treated tailings overlaid by roughly 4.5 m of water. The eastern side of the pond features a littoral zone, accounting for 20% of the water's surface area, with a slope ranging from 0.2 to 5%. Lake Miwasin follows the seasonal cycle of layer mixing typically of a small boreal lake (CAPP, 2021).

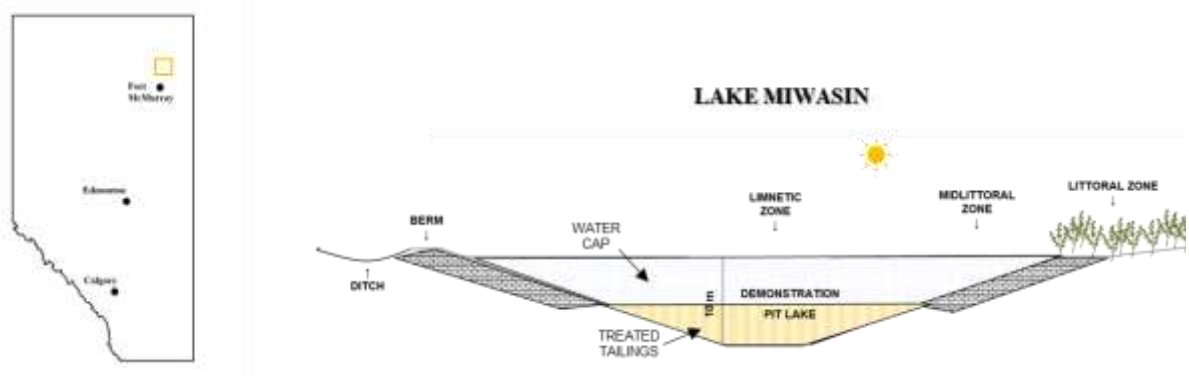

Figure 1. 1. Location and cross-section diagram of Lake Miwasin, AB, Canada.

The goal of Lake Miwasin is to establish a fully functional lake that reduces the mobility of CPCs in the treated tailings and facilitates the habitation of aquatic organisms, thereby promoting the development of a self-sustaining boreal ecosystem. In this case, the PASS process led to the formation of flocs, which then settle to the bottom sediments and reduce the concentrations of CPCs. However, some compounds in sediment may either persist at their initial levels or release from the pore water affecting the lake water chemistry (COSIA, 2021).

Lake Miwasin was constructed between 2017 and 2018 and its *in-situ* environmental monitoring is projected to continue for 15 years . The outcomes of the pilot project will provide valuable insights into the comprehensive closure plan for the entire mining operation on a large scale. The project is intended to verify the design assumptions for the lake and incorporates various monitoring approaches to evaluate its effectiveness related to pore water quality, treated tailings settlement rates, interactions with groundwater, and ecological development (CAPP, 2021).

1.2.2 Monitoring background information

Monitoring data collected by Suncor Energy Inc. between 2019 and 2021 (unpublished data) indicated a general improvement in water quality over time. This improvement can be attributed to the dilution effect

of freshwater inflows to the lake or the sequestering of tailings beneath the lake (COSIA, 2021), by the evidence of the decreasing concentrations of chloride, sodium, sulfate, total dissolved solids, conductivity, and ammonia. Additionally, pH levels have remained slightly alkaline over the years. Ammonia concentrations have shown a consistent decline, with occasional peaks observed between May and July each year (Figure 1.2).

Water monitoring data (2019-2021) also revealed a decrease in dissolved metal concentrations, e.g., boron, molybdenum, arsenic, uranium, cobalt, vanadium, antimony, chromium, beryllium, and nickel. In contrast, concentrations of dissolved strontium, barium, and aluminum remained stable over time. Additionally, fluctuations in dissolved manganese, zinc, copper, selenium, tin, and iron were observed across the monitoring years. On the other hand, sediment monitoring data collected between 2019 and 2021 indicates that concentrations of total metals such as iron, aluminum, manganese, barium, zinc, strontium, nickel, copper, cobalt, lead, uranium, and arsenic remained unchanged over the evaluation period.

Water monitoring data (2019-2021) have shown a decreasing trend in concentrations of hydrocarbons and NAs over time (average NAs concentrations were 10.2, 8.4, and 2.8 mg/L in 2019, 2020, and 2021 respectively). Volatile organic compounds (VOCs) such as benzene, toluene, ethylbenzene, and xylene have been reported below the analytical detection limits in the water since 2019. While for sediments, VOCs remained relatively the same concentrations across the monitoring years. In general, sediment monitoring results (2019-2021) showed that larger molecular weight hydrocarbons have experienced a slight decline, while smaller molecular weights have remained relatively constant. For example, smaller molecular weight PAHs (e.g., fluoranthene and naphthalene) had been consistently detected despite their declining concentrations (Figure 1.4). During 2019-2021, hydrocarbon fractions F2 F3 F4 were still persistent in sediment while fractions F2 and F3 remained in water.

1.2.3 CPCs in Lake Miwasin

The water and sediment quality data collected by Suncor Energy Inc. between 2019 and 2021 were screened against environmental guidelines established by the Canadian Council of Ministers of the Environment (CCME), the British Columbia Ministry of Environment (BCMOE) to identify the CPCs present in Lake Miwasin, and the Alberta Environmental Quality Guidelines (GoA) for cobalt and selenium.

Historical data collected by Suncor Energy Inc. in water showed that chloride and dissolved Se levels consistently exceeded guideline values during the 2019-2021 period. Specifically, all chloride concentrations were above the CCME long-term guideline of 120 mg/L but remained under the CCME short-term guideline of 640 mg/L (Figure 1.2). Similarly, all dissolved Se concentrations were above the CCME long-term guideline and the BC MoE alert concentration of 1 μg/L, with only 33% of cases above the BC MoE and GoA guidelines set at 2 μg/L (Figure 1.3).

Dissolved boron concentrations consistently exceeded the guideline in 78% of cases (Figure 1.3), mostly during 2019-2020. Dissolved cobalt concentrations exceeded the long-term BC MoE guideline of 4 μg/L in 13% of cases and the GoA guideline of 1.27 μg/L in 20% of cases in 2019 (Figure 1.3). Additionally, sulfate levels exceeded the BC MoE guideline of 309 mg/L in 16% of cases during 2019-2020, particularly in May and June (Figure 1.2). Furthermore, none of the PAH compounds exceeded the levels established in the CCME guidelines.

Regarding sediment data, the historical dataset showed that 12% of cases exhibited arsenic concentrations above the Interim Sediment Quality Guideline (ISQG) but not exceeding the Probable Effect Level (PEL) from the CCME guidelines during the period of 2020-2021 (Figure 1.4). Furthermore, the levels of various molecular weights of PAHs consistently exceeded either the ISQG or PEL guidelines (Figure 1.4).

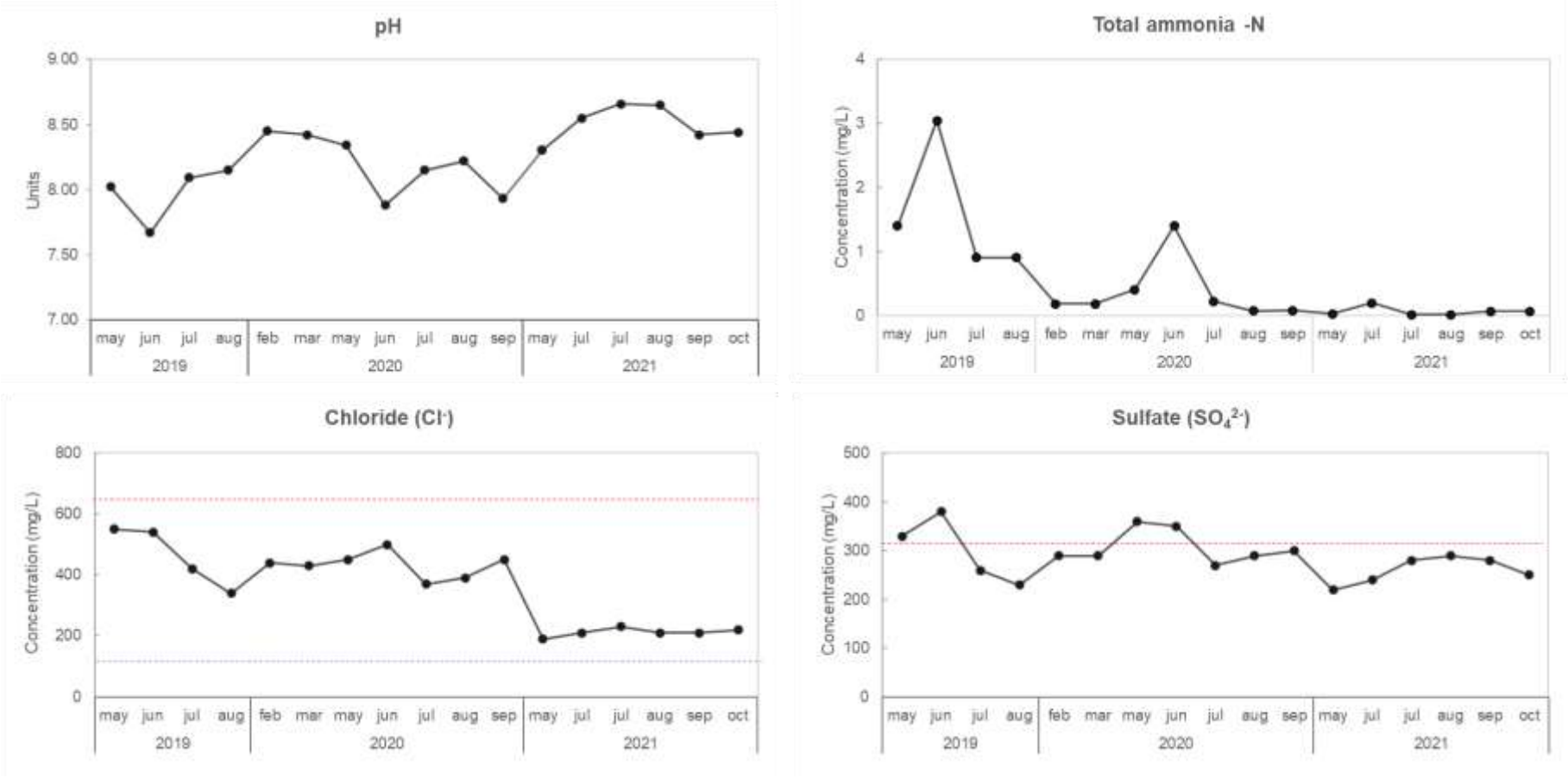

Figure 1. 2. Concentrations of pH, chloride, and sulfate in water collected in Lake Miwasin during 2019-2021. The red and blue dotted lines represent short and long-term guidelines, respectively.

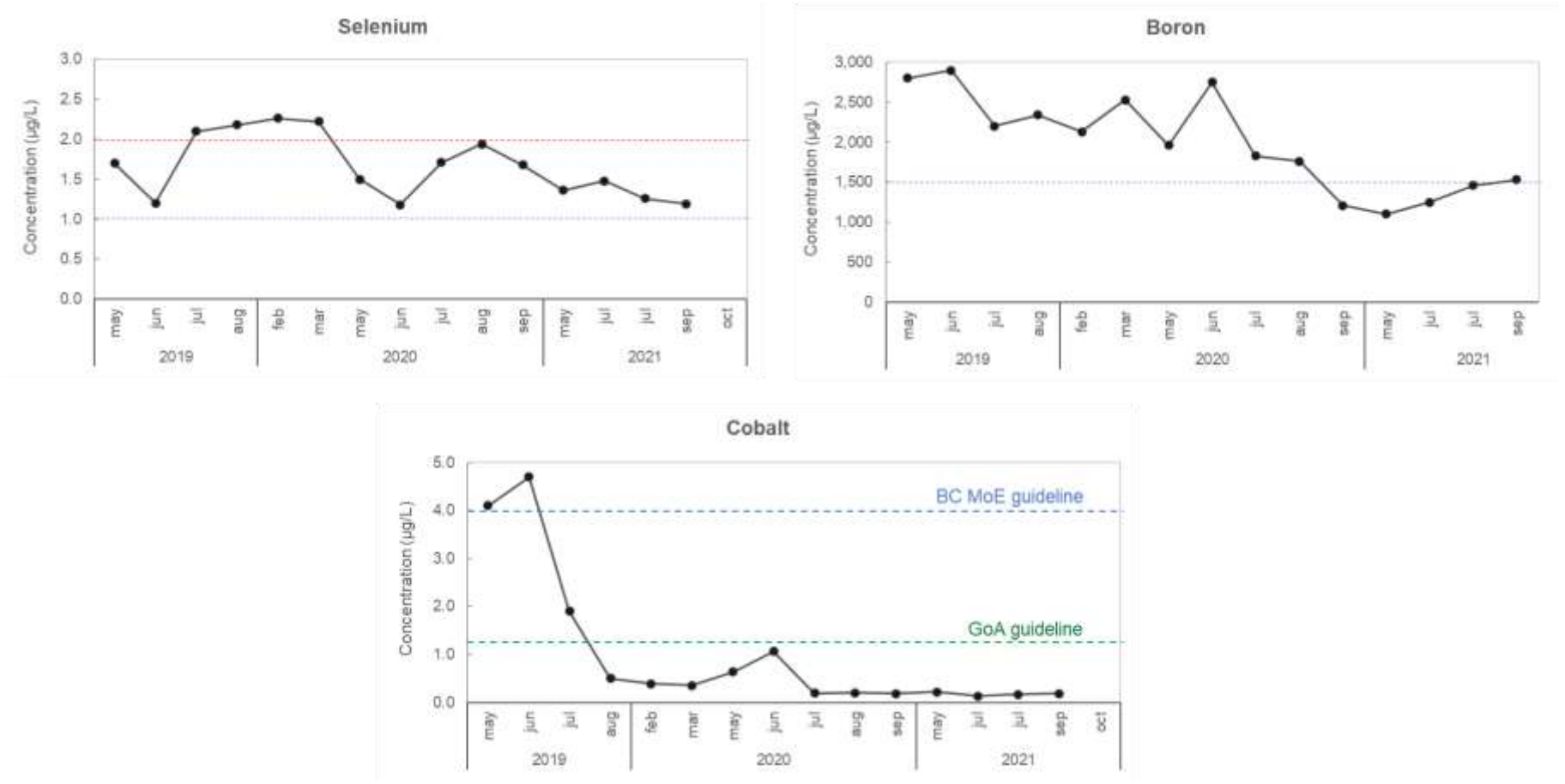

Figure 1. 3. Concentrations of dissolved metals in water collected in Lake Miwasin during 2019-2021. The red and blue dotted lines represent short and long-term guidelines, respectively.

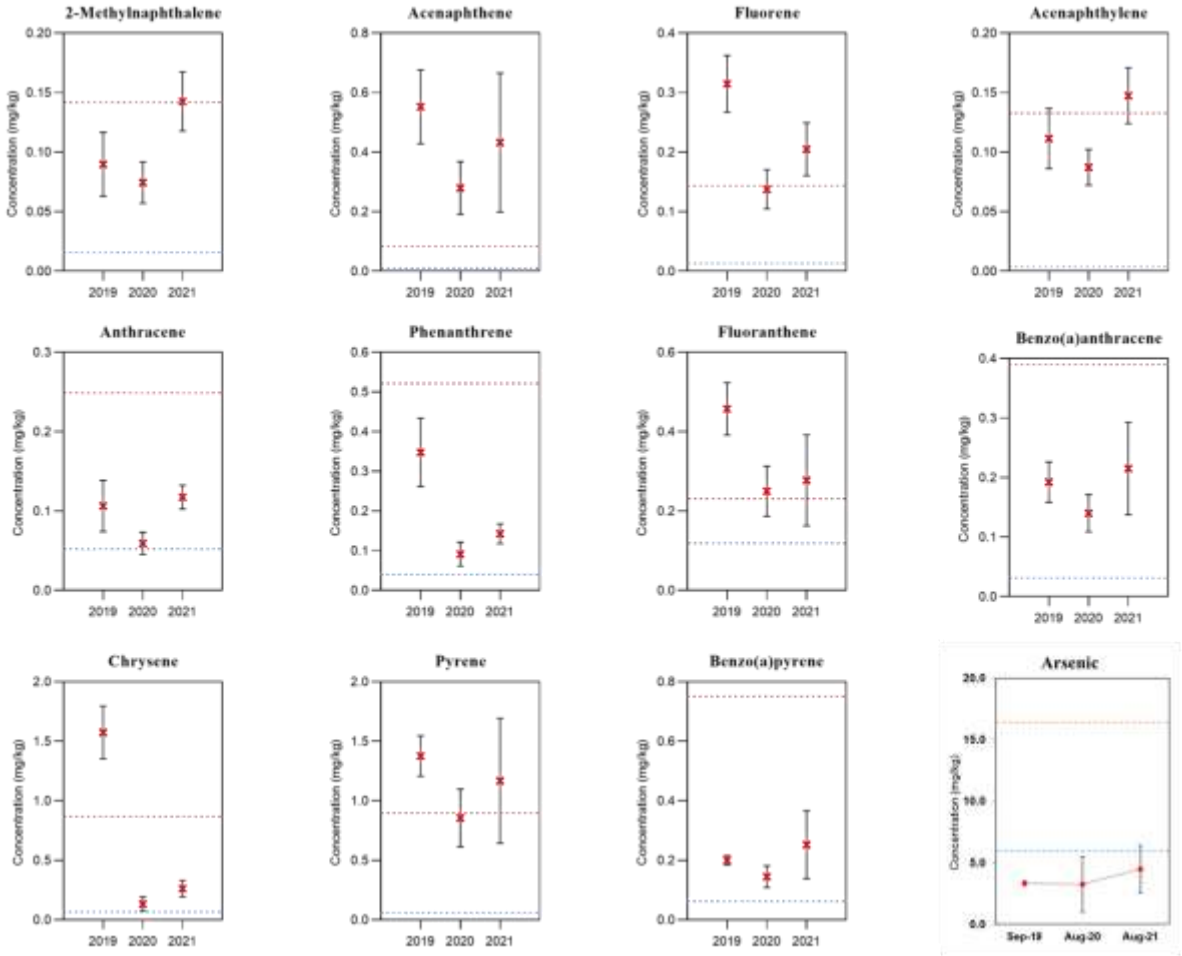

Figure 1. 4. Concentrations of parent PAH and arsenic in sediment collected in Lake Miwasin during 2019-2021. The red and blue dotted lines represent ISQG and PEL guidelines, respectively.

1.2.4 Selenium

Selenium (Se) plays a crucial role in aquatic ecosystems as it is an essential component of several enzymes with antioxidant and metal-binding functions (Janz et al., 2010; Young et al., 2010). Both nutrient deficiency and toxicity can result in reduced growth or survival (US EPA, 2016; Young et al., 2010). Selenium exists in aquatic environments in dissolved and suspended forms, transforming through biologically or chemically mediated reactions (Maher et al., 2010; Zhang & Moore, 1997). The bioavailability and toxicity of Se depend not only on its concentration but also on its speciation and interactions with other compounds (Hansen et al., 1993; Janz et al., 2010; Lo, 2014; Ogle & Knight, 1996). Furthermore, at the ecological level, the potential for Se to bioaccumulate is significant because it can persist in organisms and transfer through the food web from invertebrates to fish and birds (Janz et al., 2010).

Selenium uptake in aquatic food webs is primarily through diet (Presser & Luoma, 2010). Inorganic Se can be biotransformed by microorganisms and plants into selenomethionine, which can be assimilated by primary consumers and further converted to selenocysteine or transferred within food webs (Barwick & Maher, 2003). Selenium bioavailability depends on both selenium in water and food, as well as the assimilation efficiency of an individual consumer (Presser & Luoma, 2010). Given the different diets, assimilation, and elimination efficiencies among species, the degree of Se transfer can vary.

Previous studies have reported Se contamination related to coal mine operations had adverse effects on resident biota in the ecosystem of the Elk River watershed, BC, Canada (Miller et al., 2013; Orr et al., 2006; Young et al., 2010). For example, Miller et al. (2013) found that

invertebrates and fish in ponds exceeded the regulation criteria values with Se levels ranging from 16 to 50 μg/L. Similarly, Orr et al. (2006) observed higher Se concentrations in benthic invertebrates and fish in settling ponds and marshes receiving effluents from coal mines with Se concentrations ranging from 50 to 90 μg/L, compared to the reference sites.

Selenium can also be released from oil refining and mining activities (Cutter & San Diego-McGlone, 1990; Maher et al., 2010; Young et al., 2010), such as in the AOS region. This is particularly important in EPLs used for wet reclamation, where selenium can persist and potentially pose threats to higher trophic levels. Residual Se can potentially cause toxic effects on the aquatic organisms inhabiting Lake Miwasin, including phytoplankton, zooplankton, and benthic invertebrates (Hatfield, 2020). The data collected by Suncor Energy Inc. between 2019 to 2021 in Lake Miwasin water showed that in 77% of all samples, the consistent levels of dissolved selenium exceeded the long-term CCME and alert level BC MoE guidelines for water, which is set at 1 μg/L (Figure 1.3). It is important to note that Se concentrations alone in Lake Miwasin water do not provide sufficient insight into the prediction of Se bioaccumulation or trophic transfer (Stewart et al., 2010). Consequently, there remains a significant knowledge gap concerning the selenium bioaccumulation for the aquatic organisms within EPLs in the AOS.

1.3 *Daphnia* species

1.3.1 *Daphnia* as a model test organism

Daphnia are small freshwater crustaceans (0.3 to 6 mm) belonging to the Order Cladocera, commonly found in North American freshwater ponds and lakes, including western Canada

(ECCC, 2000). It is also found in saltwater lakes or estuaries (Ebert, 2022; Liao et al., 2015; Teschner, 1995). *Daphnia* are important representatives of the zooplankton, mainly because they are key primary consumers and prey of many planktivorous fishes (Ebert, 2022). In shallow and fishless ponds, large-bodied *Daphnia* are particularly important because they can temporarily dominate the community structure (Steiner, 2004). In open water, daphnids filter small suspended particles such as algae, bacteria, or detritus (Smirnov, 2014a).

Daphnia primarily reproduce asexually through parthenogenesis, when they inhabit stable environments, leading to the production of diploid females capable of indefinite reproduction (Ebert, 2022). This mode of reproduction maintains high levels of genetic homogeneity within the population. However, under unfavorable conditions (e.g., predation, crowding, shortage of food, temperature changes, and chemical exposure), *Daphnia* can switch to sexual reproduction, resulting in the production of resting eggs known as ephippia (Smirnov, 2014c).

Daphnia exhibits a broad sensitivity to various chemicals and displays a well-understood life history and ecology. Consequently, they find extensive use in reference methods for toxicity testing on survival and reproduction. These reference methods involve standard exposure experiments using *D. magna* and other *Daphnia* species (ECCC, 1990, 2000; OECD, 2004; US EPA, 1996). These methods involve various aspects of daphnid stocks maintained under laboratory conditions, including facilities, culture media, feeding, loading, as well as health criteria (ECCC, 2000; US EPA, 1996). Furthermore, *Daphnia* is easy to handle and care for, making them an ideal choice for assessing a wide range of contaminants (ECCC, 2000).

1.3.2 Response of *Daphnia* to saline environments

Generally, *Daphnia* populations can tolerate low to moderate levels of salinity, but higher salinity can result in toxic effects. Daphnids use hyperosmotic regulation as a mechanism to sustain the hemolymph hyperosmotic to the surrounding environment (Smirnov, 2014b). Their response to saline environments relies on several factors, including the level of salinity, duration of exposure, nutrient availability, predation pressure, genetic variability, and acclimation. The variability in salinity tolerance among *Daphnia* species is linked to their life history. Phenotypic or genetic adaptations to local salt conditions would determine the ability of populations to respond to salt stress (Ebert, 2022; Latta et al., 2012).

Different studies have reported lethal concentrations (LC_{50}) for sodium chloride (NaCl) in *D. magna*, ranging from 5.5 to 6.6 g/L (Martínez-Jerónimo and Martínez-Jerónimo, 2007; Schuytema et al., 1997) and from 2 to 3.3 g/L for *D. pulex* (Bezirci et al., 2012 Liu and Steiner, 2017). Nevertheless, concentrations above 5 g/L seem to be beyond the osmoregulation ability of *Daphnia* species leading to chronic toxicity (Arnér & Koivisto, 1993; Martínez-Jerónimo & Martínez-Jerónimo, 2007; Teschner, 1995). Additionally, the ionic composition significantly affects salinity tolerance CCME (2011). For instance, it was observed that the toxicity of cations in chloride salt modified its toxicity, with potassium showing greater toxicity compared to magnesium, calcium, and sodium salts during acute exposure to *D. magna* (Mount et al., 1997).

Salinity in aquatic ecosystems persists over extended periods, making long-term exposures more representative of realistic responses to salinity stress (Brown & Yan, 2015; Sarma et al.,

2006). Chronic exposure to salinity affects *Daphnia* in various ways, including decreased survival, alterations in life history traits (e.g., rate of population increase, time to reproduction, and the number of offspring) (Brown & Yan, 2015; Gonçalves et al., 2007; Huang et al., 2022; Martínez-Jerónimo & Martínez-Jerónimo, 2007; Sarma et al., 2006; Teschner, 1995), smaller body size (Huang et al., 2022), escaping behavior (Liu & Steiner, 2017), and decreased respiration (Arnér & Koivisto, 1993). Exposure to salinity has also affected the egg and embryonic development of *Daphnia*, resulting in a decreased population rate and significant ecological consequences at the population level (Brown & Yan, 2015).

Daphnia seem to increase their salt tolerance, with populations residing in high-salinity environments demonstrating greater resilience compared to those from low-salinity habitats. For instance, Arnér and Koivisto (1993) found that *D. magna* collected from a rockpool, that had acclimated to saline conditions, grew and reproduced better at salinity levels of 4 g/L in comparison to that of 0 and 8 g/L. Similarly, Liao et al. (2015) found that *D. magna* and *D. longispina* collected from saline rock pools exhibited higher salt tolerance (up to 3 g/L) as a result of local salinity adaptation. Coldsnow et al. (2017) demonstrated that *Daphnia* pre-acclimated for 2.5 months displayed enhanced tolerance to NaCl up to a certain threshold (1.5 g/L), beyond which survival rates decreased. This suggests that there may be limits to the extent of enhanced tolerance. The mechanism behind these differences in salt tolerance could be attributed to various factors, such as alterations in gene expression for osmoregulation, changes in ATP activity, shifts in allele frequency, or long-lasting epigenetic changes such as DNA

methylation (Coldsnow et al., 2017). To date, the mechanism responsible for salt tolerance in *Daphnia* is not yet fully understood.

1.3.3 Toxicity of OSPW on *Daphnia*

The effects of OSPW have been studied on many different aquatic organisms (Brown & Ulrich, 2015; Li et al., 2017), but studies with *Daphnia* species are limited. While some studies have suggested acute toxic effects of tailing pond water on *D. magna* (MacKinnon & Boerger, 1986), others showed no OSPW toxicity (Lari et al., 2017). This difference in results could be attributed to the specific features of OSPW, including its origin from ore mines, treatment, and residence time in ponds.

Considering that NAs have been postulated as significant contributors to toxicity in aquatic organisms exposed to OSPW, a study with monocarboxylic and dicarboxylic NAs-like surrogates spiked in OSPW resulted in acute toxicity to *D. magna*. Notably, the degree of toxicity increased with increasing NAs molecular weight (Frank et al., 2009). Lari et al. (2017) found only chronic effects (impaired reproduction and growth) in *D. magna* exposed to OSPW ranging from 1 to 10% (v/v).

Previous studies identified narcosis as a mechanism of toxicity for NAs (Frank et al., 2009; Morandi et al., 2015; Swigert et al., 2015). Narcosis is a nonspecific mode of action in which a hydrophobic compound disrupts the cellular membrane. The degree of narcosis depends on the size and lipophilicity of the molecule (Swigert et al., 2015). In contrast to NAs, the mode of action for whole OSPW is more complex due to the presence of many components (i.e., NAs,

PAHs, residual bitumen, metals, and salts), which can induce both single effects and potential interactions among different compounds. Furthermore, other mechanisms of toxicity in OSPW have been observed in fish and benthic invertebrates, such as oxidative stress and apoptosis (He et al., 2012; Wiseman et al., 2013), and the expression of genes related to endocrine disruption (Wiseman et al., 2013).

1.3.4 Selenium bioaccumulation by *Daphnia*

Selenium is an essential element for *Daphnia.* The lack of Se causes poor health in *D. magna* (ECCC, 1990; Elendt, 1990; Keating and Dagbusan, 1984). Conversely, higher Se levels (25-250 μg/L as selenate) can adversely affect their growth and reproduction (Johnston, 1987). Consequently, Se tends to accumulate in *Daphnia* tissue, leading to significant Se concentrations being loaded into *Daphnia* bodies. This, in turn, can potentially be transferred to higher-level vertebrates, increasing the risk of bioaccumulation (Boyum & Brooks, 1988).

Selenium assimilation by *D. magna* is highly influenced by its chemical form. Notably, *D. magna* preferentially uptakes Se from selenium-laden algae or selenomethionine in water rather than from inorganic forms of Se (Boyum & Brooks, 1988). However, when exposed to higher concentrations of Se, regulatory mechanisms come into play (Guan & Wang, 2004; Xu et al., 2001; Yu & Wang, 2002). Schultz et al. (1980) indicated that slow rates of Se elimination in *Daphnia* can lead to accumulation in food chains. Additionally, Guan and Wang (2004) and Yu and Wang (2002) have observed that Se elimination in *D. magna* is predominantly influenced by the body burden of the element rather than the Se concentration in their food. Excretion and

reproduction represent important routes of Se elimination in *D. magna* as well, which may be attributed to the essentiality of Se (Guan & Wang, 2004; Yu & Wang, 2002).

Several factors can affect Se uptake in *Daphnia*, such as elevated levels of sulfate, which reduce the accumulation of Se (as selenate) in *D. magna.* This effect is attributed to the utilization of specific Se proteins and permeases for sulfate adsorption (Hansen et al., 1993; Ogle & Knight, 1996).

1.4 Problem formulation

1.4.1 Rationale

The extraction of oil sands in Canada is a major contributor to the nation's economy. Due to the process of crude oil production generating larger quantities of tailings, which have been stored in tailings ponds throughout the AOS region for several decades. In consequence, concerns have arisen over the potential negative impacts of these tailing ponds on the surrounding environment. Specifically, the storage of tailings in these ponds can potentially cause land disruption, the release of volatile compounds and greenhouse gases, the infiltration of pollutants into groundwater, and wildlife exposure to those contaminants. To address these concerns, tailing management in oil sand projects continuously incorporates advances in treatment technologies and progressive reclamation. Reclamation plans involve progressive treatment of target compounds, ensuring that the long-term impacts of oil sand operations on the environment are minimized. The results of this research on Lake Miwasin are crucial due to its impact on future boreal oil sand development.

Lake Miwasin serves as a pilot project for the reclamation plan on the Base Plant Operation (Suncor Energy Inc.). To ensure the success of the project, a continuous monitoring program was implemented to track Lake Miwasin's evolution. Historical data collected by Suncor Energy Inc. between 2019 to 2021 revealed the presence of some CPCs in the overlying water and sediment. Therefore, further investigation was required to determine the effectiveness of the treatment approach and the self-sustainability of the lake.

Lake Miwasin is already a dynamic ecosystem and has shown the development of a simple aquatic community involving producers (e.g., algae) and primary consumers (e.g., *Daphnia*). To assess the potential toxicity of Lake Miwasin water, specific aquatic toxicity tests need to be conducted using sensitive and representative organisms. *Daphnia* species, known for their extensive use in toxicity testing and the presence of a similar organism in Lake Miwasin, were chosen as test organisms. Since Lake Miwasin salinity levels may affect the performance during exposures, *Daphnia* species will need to be pre-acclimated to the saline levels of the test waters before the assessment of other potential stressors can be performed. Both acute and chronic endpoints should be evaluated to determine the short-term and long-term effects. Moreover, a Toxicity Identification Evaluation (TIE) approach is needed to identify the class of compounds responsible for the observed toxicity. By considering these factors, the aquatic toxicity tests for Lake Miwasin water and pore water can help develop a comprehensive understanding of the effectiveness of the reclamation strategy.

Additionally, historical water quality data collected by Suncor Energy Inc. between 2019 to 2021 have consistently shown elevated Se levels above established water guidelines by CCME

(1987) and BCMOE (2014). To comprehensively investigate the potential impact of elevated Se levels on bioaccumulation in *Daphnia*, bioassays using *D. pulex,* a species resident in Lake Miwasin, are necessary. The results will help elucidate the potential negative impacts of Se on higher trophic level organisms. This study is the first investigation of Se bioaccumulation in EPLs located in the AOS region, and it provides critical information on the potential risks posed by present levels of Se.

1.4.2 Objectives and hypotheses

The specific research objectives and their null hypotheses were as follows:

1. Assess the potential toxicity of Lake Miwasin water and pore water to *Daphnia* and determine the major classes of CPCs using a TIE Phase I investigation.

   H_0: Acute or chronic toxicity of Lake Miwasin water and pore water to daphnids are not significantly different from their respective controls.

2. Assess the bioaccumulation of Se in *D. pulex* via dissolved and dietary exposure routes under conditions similar to those of Lake Miwasin and at dissolved concentrations of Se bracketing those possibly observed in future AOS pit lakes.

   H_0: There is no difference in Se bioaccumulation by lab D. pulex and native D. pulex exposed to either selenate spiked sources or Lake Miwasin water.

CHAPTER 2: TOXICITY EVALUATION OF WATER AND PORE WATER FROM A PILOT-SCALE PIT LAKE, ALBERTA OIL SANDS REGION, TO *Daphnia* SPECIES

Overview

The research in this chapter was designed to assess the toxicity of Lake Miwasin water and pore water to lab strains of *D. magna* and *D. pulex*, as well as to native *Daphnia* spp. This chapter will be submitted to the journal Archives of Environmental Contamination and Toxicology. The anticipated citation is Davila-Arenas, C., Doig L., Ji X., Panigrahi B., Ezugba I., Liber K. 2024. Toxicity evaluation of water and porewater from a pilot-scale oil sands pit lake to *Daphnia* species (in preparation).

Author contributions

Catherine Davila-Arenas (University of Saskatchewan): designed the study, collected, processed, and prepared samples used in the experiments, performed all statistical analyses, and drafted the manuscript.

Xiaowen Ji (University of Saskatchewan): assisted in the chemical analysis, reviewed, and revised the manuscript, and provided comments and corrections.

Banamali Panigrahi (University of Saskatchewan): reviewed and revised the manuscript and provided comments and corrections.

Immanuela Ezugba (University of Saskatchewan): reviewed and revised the manuscript and provided comments and corrections.

Lorne Doig (University of Saskatchewan): helped design the study, provided scientific input and guidance, revised the manuscript, and provided comments and corrections.

Karsten Liber (University of Saskatchewan): developed the project, assisted with study design, secured and oversaw funding, provided scientific input and guidance, revised the manuscript, and provided comments and corrections.

Abstract

Significant amounts of tailings and oil sands process-affected water (OSPW) are generated by bitumen extraction in the Alberta Oil Sands (AOS) region. These by-products are potentially toxic to aquatic organisms and require remediation. Lake Miwasin is a pilot-scale pit lake consisting of treated tailings overlayed with blended OSPW and fresh water. This study assessed the potential toxicity of Lake Miwasin surface water (LMW) and pore water (LMP) using saline-acclimated Cladocera, consisting of lab strains of *Daphnia magna* and *D. pulex*, and native *Daphnia* species collected in brackish Humboldt Lake (HL) and Lake Miwasin (LM) itself. LMW did not display acute or chronic toxicity to lab species and native *Daphnia sp* (HL). Conversely, LMP was acutely toxic to both lab species and native *D. pulex* (LM). In chronic tests (12 d exposure), LMP negatively affected reproduction in *D. pulex* (lab), with reductions in the number of offspring. In addition to salinity being identified as a stressor in LMP, toxicity identification evaluation (TIE) phase I findings demonstrated that the observed toxicity for *D. magna* (lab) and *D. pulex* (LM, native) may be attributed to ammonia and metals in LMP. Further investigations are required to confirm the contributions of these stressors to LMP toxicity.

Keywords: oil sands process affected water (OSPW), reclamation, pore water, toxicity, *Daphnia*

2.1 Introduction

Canada has one-third of the proven global crude oil reserves (AER, 2015; Government of Canada, 2019), with the greatest part occurring in Alberta's oil sands region (AOS) as bitumen (GoA, 2022; Zhou et al., 2008). The conversion of bitumen into synthetic crude oil requires significant amounts of freshwater, with every barrel of oil produced requiring two to four barrels of freshwater (GoA, 2022). As a result, oil sand mining generates substantial quantities of fluid tailings that are currently stored onsite in ponds. As of 2020, there was approximately 1360 Mm^3 of fluid tailings stored in the Alberta oil sands region (AER, 2021). Water, sand, silt, clay, and residual bitumen are usual components of tailings (AER, 2023b). As per the Tailings *Management Framework Objective of the Lower Athabasca Region Tailings Management Framework for the Mineable Athabasca Oil Sands* (2015), this material, both legacy and from ongoing production, is to be progressively reclaimed into the surrounding landscape. One option for tailings reclamation is its use in end pit lakes. However, there is limited historical information for oil sands-based end-pit lakes and therefore there are uncertainties regarding the efficacy of this approach.

The term oil sands process-affected water (OSPW) is broadly applied to different waters related to oil sands extraction processes (Mahaffey and Dubé, 2016), including tailings. Tailing pond water chemistry indicates that OSPW is moderately hard and slightly brackish (Allen, 2008) and contains various substances of concern including dissolved solids, ammonia, metals, bitumen, other hydrocarbons such polycyclic aromatic hydrocarbons (PAHs), benzene, toluene, ethylbenzene, and xylene (Allen, 2008; Li et al., 2017; Mahaffey & Dubé, 2016), and

naphthenic acids (NAs). OSPW composition is highly variable depending on ore quality, treatment method, and duration of aging in tailings ponds (Mahaffey & Dubé, 2016) and can be acutely and chronically toxic to a variety of organisms including microorganisms, invertebrates, fish, birds, amphibians, and mammalian cell (Brown & Ulrich, 2015; McQueen et al., 2017). OSPW may induce toxic effects via multiple modes of action including narcosis, endocrine disruption, immunotoxicity, carcinogenicity, oxidative stress, DNA damage, pathological changes, delayed growth, and disruption of normal metabolic function (Brown & Ulrich, 2015; Li et al., 2017; Morandi et al., 2017).

Lake Miwasin is a pilot-scale pit lake located in the AOS (Figure 1.1) consisting of a shallow excavated basin into which treated (consolidated) tailings were added as bottom substrate and then capped with a blend of OSPW and freshwater. Given the known presence of various contaminants of potential concern (CPCs), the objective of this research was to assess the potential acute and chronic toxicity of Lake Miwasin surface and pore water to *Daphnia*, a ubiquitous water-column invertebrate. Pore water, isolated from bottom sediment, was assessed for toxicity in addition to surface water, as this simulated a worst-case exposure scenario that could potentially occur during an extended period of pond stratification combined with pore water expression from consolidating bottom substrate (coagulated and flocculated fine tailings). For those samples demonstrating toxicity, the major classes of contaminants potentially causing the observed toxicity were assessed using a Toxicity Identification and Evaluation (TIE) Phase I consisting of physical and chemical manipulations (US EPA, 1991).

Few studies have recognized or addressed salinity as a potential stressor in OSPW (Kavanagh, 2012; Nero et al., 2006). Even with the addition of freshwater, Lake Miwasin displayed a brackish condition, particularly during the early stages, with the major ions consisting of sodium, chloride, and sulfate. Because salinity itself can be a potential stressor and influence toxicity test outcomes (CCME, 2011; ECCC, 2000), the present study assessed saline-acclimated test organisms to minimize the influence of osmoregulatory stress and determine whether other stressors might be contributing to the observed toxicity. This has been demonstrated to improve daphnid performance under brackish testing conditions (Arnér & Koivisto, 1993; Martínez-Jerónimo & Martínez-Jerónimo, 2007). Moreover, to better represent possible site-specific adaptations and local sensitivity of regional organisms to OSPW, testing was conducted using two laboratory species/strains of *Daphnia* and two field-collected species of *Daphnia*, one species collected directly from Lake Miwasin and the other from a similarly brackish lake in the broader region.

2.2 Materials and methods

2.2.1 Study site

Lake Miwasin (56° 53'N; 111° 23'W) is situated immediately south of the Suncor Millennium Mine, approximately 18 km north of Fort McMurray, AB, Canada (Figure 1.1). Lake Miwasin is a constructed end pit lake, part of a pilot-scale reclamation project (~15 years in duration) intended to create a functional, self-sustaining boreal lake ecosystem incorporating mine tailings, OSPW, and local surface water. Lake Miwasin was excavated in 2017 and the bottom was filled with treated tailings. This was capped in 2018 with a combination of treated

OSPW, consisting of coagulated tailings pond water and water expressed from the treated tailings deposit, and freshwater from the Lake Miwasin upland watershed and a nearby industrial pond, with a target ratio of approximately 50:50 (COSIA, 2021).

2.2.2 Sample collection and preparation

Lake Miwasin water (LMW) samples were collected in October 2021 by Hatfield Consultants at approx. 0.3 m above the sediment-water interface from five limnetic stations in Lake Miwasin using a Van-Dorn water sampler. Sediment samples were collected at the same time and locations using an Ekman grab sampler. Samples were immediately refrigerated in the dark and shipped at 4°C to the Toxicology Centre, University of Saskatchewan (Usask), SK, Canada. Lake Miwasin pore water (LMP) was isolated no longer than one week before testing by centrifuging sediment for 20 min × 1800 *g* twice in 50-mL conical high-density polyethylene (HDPE) tubes. A dark film of hydrocarbons was observed on the walls of the centrifugation tubes after pore water extraction. Aliquots of pore water were collected for analysis of water chemistry.

2.2.3 Toxicity experimental design

2.2.3.1 Test species

Lab strains of *D. magna* and *D. pulex* were used in the testing of LMW and LMP toxicity. These animals were cultured in-house at the Toxicology Centre, Usask. Culturing procedures followed general guidelines described elsewhere (ECCC 1990, 2000). Groups of 30 to 40 daphnid adults were maintained in 2-L glass jars in an environmental chamber at 23 ± 1°C

under a 16:8 h light:dark photoperiod. Aerated, carbon-filtered municipal water (DCW, City of Saskatoon) was used to renew water three times weekly. Daphnids were fed with a 1:1 ratio of algae (*Raphidocelis subcapitata* and *Chlamydomonas reinhardtii*) three times weekly. Based on the ECCC (1990) protocol, 2 μg/L of vitamin B_{12} and selenium were added at each water renewal as supplements. All tests were similarly conducted in an environmental chamber at 23 ± 1°C under a 16:8 h light: dark photoperiod.

Native *Daphnia* and associated water samples were collected in August 2021 from Humboldt Lake (HL), SK, a saline lake located in the boreal parkland ecoregion (52°08'N; 105°08'W) 105 km east of Saskatoon. In addition, a native strain of *D. pulex* was collected in Lake Miwasin in July 2021. Adult specimens were cultured in 2-L glass jars using aerated lake waters in an environmental chamber and maintained as described above for the lab strains. *Daphnia sp.* (HL) collected in Humboldt Lake and *D. pulex* (LM) collected in Lake Miwasin were cultured successfully under the same lab conditions.

Test organisms were acclimated gradually in control waters before testing. Two control waters were made, reconstituted saline water (RSW) and saline pore water (RSP). These were synthetic waters created to mimic Lake Miwasin surface water (near-bottom) and sediment pore water in terms of major ion composition by dissolving salts in DCW (Appendix, Table S 2). Acclimation to RSW and RSP used three gradual dilutions for at least 12-d (or until the third brood) per dilution. Because of constraints in saline acclimation, not all test organisms were used for all toxicity tests (Table S 1).

2.2.3.2 Salinity tolerance tests

Both the LMW and LMP have elevated salinity. Salinity tolerance testing was therefore performed to determine the suitability and relative tolerance of the test daphnid species for toxicity testing in these slightly brackish waters.

Salinity tolerance was evaluated for the lab strains of *D. magna* and *D. pulex* cultured in DCW (500 μS/cm, 0.265 psu; SO_4^{2-} 66 mg/L, Ca^{2+} 36 mg/L, Na^{+} 24 mg/L, Mg^{2+} 17 mg/L), as well as native daphnid strains *Daphnia sp* (HL) and *D. pulex* (LM) collected from Lake Humboldt (1500 μS/cm, 0.715 psu; Na^{+} 300 mg/L, SO_4^{2-} 260 mg/L) and Lake Miwasin (2010 μS/cm, 0.634 psu; Na^{+} 403 mg/L, Cl^{-} 308 mg/L), respectively. Ten neonates (< 24-h-old) were exposed to 200 mL of experimental solution in three replicates. Experimental solutions were prepared using DCW amended with sodium chloride (NaCl) up to a concentration of 8 g/L. Surviving individuals in each treatment were counted after 48-h of exposure. All salinity tolerance tests were conducted under static conditions.

2.2.3.3 Water exposures

LMW toxicity testing was conducted using both lab strains (*D. magna* and *D. pulex*), and the native *Daphnia sp.* (HL). Both RSW-acclimated and unacclimated daphnids were exposed simultaneously to LMW, except for *Daphnia sp* (HL), for which only RSW-acclimated animals were tested.

Acute toxicity of LMW was determined in 48-h static non-renewal lethality tests, following ECCC (2000) and OECD (2004) procedures. Briefly, three replicates of 10 neonates (< 24-h-

old) per beaker were placed in 200 mL of either control water (RSW) or undiluted LMW. The number of surviving individuals was recorded at 48-h, with immobilization as the indicator of mortality.

Chronic toxicity of LMW was performed in static renewal tests according to OECD (2012) and US EPA (1996). Lab strains were exposed for 21-d as per guidance, while native *Daphnia sp.* (HL) were exposed for only 12-d due to mortalities among the control adults. Briefly, ten replicates of individual neonates (< 24-h old) were placed in 40 mL for *D. pulex* and 50 mL for *D. magna* of control water (RSW) or undiluted LMW. Complete water renewals were performed every 48-h. At each water renewal, daphnids were fed with algae (*R. subcapitata and C. reinhardtii*) at a concentration of approx. 1×10^6 cells/mL, and trace amounts of vitamin B_{12} and selenium (2 μg/L). Each day, daphnids were checked for mortality and reproductive output (presence of offspring). Test endpoints included (*i*) survival of adults, (*ii*) time to produce first brood, (*iii*) number of living offspring produced in each brood, and (*iv*) total number of living offspring. Tests were considered acceptable if the mortality of the parent animals in the controls did not exceed 20% by the end of the test.

2.2.3.4 Porewater exposures

LMP toxicity testing was conducted using both lab strains (*D. magna* and *D. pulex*), as well as one field strain, *D. pulex* (LM). Both RSP-acclimated and unacclimated test organisms were exposed simultaneously to LMP.

Acute toxicity of LMP was determined in 48-h static lethality tests, following ECCC (2000) and OECD (2004) procedures. Briefly, three replicates of five neonates (< 24-h old) per beaker

were placed in 100 mL of either control water (RSP), 100% LMP or LMP diluted with RSP to achieve 50% (v/v), 25% (v/v), and 12.5% (v/v) LMP. The number of surviving individuals was recorded at 48 h, with immobilization as the indicator of mortality.

Chronic toxicity of LMP was performed in 12-d exposures (static renewal tests) following OECD (2012) and US EPA (1996) procedures. Briefly, three replicates of individual neonates (< 24-h old) were placed in 25 mL of control water (RSP), 100% LMP, or LMP diluted with RSP to achieve 50% (v/v), 25% (v/v), and 12.5% (v/v) LMP. Complete water renewals were performed every 48 h. At each water renewal, daphnids were fed and supplemented as indicated above. Each day, daphnids were checked for mortality and reproductive output (presence of offspring) as described above.

2.2.4 Toxicity identification evaluation

The toxicity identification evaluation (TIE) Phase I tests (US EPA, 1991) were conducted with undiluted LMP (initial pH 7.95) using *D. magna* (lab) and *D. pulex* (lab and native strains) in acute tests (48-h). The manipulations applied to LMP samples were as follows: (*i*) filtration through 0.7-μm pore size glass microfiber filter, (*ii*) aeration for 60 min, (*iii*) addition of zeolite SIR-600 (Resin Tech Inc) and filtration, (*iv*-*v*) additions of ethylenediaminetetra-acetic acid (EDTA) at 40 µM and 80 µM concentrations, and (*vi*) solid-phase extraction (SPE) through Thermo Scientific HyperSep C18 cartridges.

Briefly, filtration provides an indication of toxicity related to the particulate fraction. Loss of toxicity after aeration implies the presence of volatile constituents, oxidizable materials, or

surfactants. Elimination of toxicity through the addition of zeolite indicates toxicity due to ammonia. Removal of toxicity following the addition of EDTA suggests toxicity attributable to metals. C18 SPE columns remove non-polar to medium-polar organic compounds from solution (US EPA, 1991, 2007). Baseline toxicity was assessed using whole LMP without manipulation (positive control).

Three replicates of five neonates (< 24-h old) per beaker were placed in 25 mL of control and manipulated samples. The number of surviving individuals was recorded at 48 h, with immobilization as the indicator of mortality.

2.2.5 Chemical analysis

Routine water quality of test solutions was performed in-house at the Toxicology Centre, USask at the beginning of each test period from at least two randomly chosen beakers per treatment. Variables included pH, dissolved oxygen (DO) concentration, and conductivity (Thermo Orion Star A321 pH Portable Meter, Chelmsford, MA USA), alkalinity and total hardness (Hach, Titration cartridge, Romeoville, IL, USA), dissolved organic carbon (Shimadzu TOC-V CPN), and ammonia (Thermo Scientific™ Orion™ AQUAfast™ AQ4000 Colorimeter, Beverly, MA USA).

Dissolved ions (Na^+, Cl^-, and SO_4^{2-}) were measured by ion chromatography using an IonPac AS18 column (Thermo Dionex™ ICS 3000, Sunnyvale, CA, USA) in the Department of Soil Science at the University of Saskatchewan. Dissolved metals (24 elements) were analyzed at the Toxicology Centre by ICP-MS using an Agilent 8800 ICP-MS QQQ Triple Quadrupole

spectrometer, equipped with an ASX-500 autosampler and Masshunter software for instrument operation (Agilent Technologies, Santa Clara, CA, USA). Samples for dissolved metals analysis were collected in 8-mL HDPE bottles using syringe filters (0.45-μm pore size, polyethersulfone membrane, VWR International) and acidified (pH > 2) with high-purity nitric acid (HNO_3) (Optima Grade Nitric Acid, Fisher Scientific, Fair Lawn, NJ, USA).

The analysis data for naphthenic acids (NAs) and PAHs (acridine, benzo(a)anthracene, benzo(a)pyrene, benzo(b,j)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, dibenz(a,h)anthracene, indeno(1,2,3-cd) pyrene, quinolone) in LMW was provided by Hatfield Consultants. Additionally, analysis of PAHs (naphthalene, acenaphthylene, acenaphthene, phenanthrene, fluorene, anthracene, fluoranthene, pyrene, and chrysene), organochlorine compounds (1,3-dichlorobenzene, 1,4-dichlorobenzene, 1,2-dichlorobenzene, hexachloroethane, 1,2,4-trichlorobenzene, hexachlorobutadiene, 2-chloronaphthalene, 4-chlorodiphenyl ether, and bis(2-chloroethoxy)methane), phthalate esters (dimethyl phthalate, diethyl phthalate, dibutyl phthalate, and benzyl butyl phthalate), PAH derivative (carbazole), nitroaromatics (nitrobenzene, 2,6-dinitrotoluene, and 2,4-dinitrotoluene) and other hydrocarbon compounds (azobenzene and 4-bromodiphenyl ether), was performed in-house at the Toxicology Centre, USask using headspace-solid-phase microextraction (HS-SPME) combined with gas chromatography-Orbitrap high-resolution mass spectrometry (GC-Orbitrap-HRMS, Thermo Scientific, Mississauga, ON, CA).

Samples were placed on a TriPlus RSH autosampler and remained at room temperature (20 ± 0.5°C) until extraction. The RSH system was equilibrated to 65°C for 10 min with stirring.

Immediately prior to extraction, the SPME Arrow fiber was conditioned at 260°C for 1 min. The SPME Arrow was then inserted into the water sample and extraction was carried out at 65°C for 15 min. After extraction, compounds were desorbed from the arrow in the GC injection port for 1 min at 260°C. The GC oven profile was 35°C held for 10 min, the temperature was then ramped at 5°C/min to 200°C, this temperature was held for 8 min and then ramped at 15°C/min 300°C before a final hold of 5 min. The Orbitrap-MS was operated at 240,000 nominal resolution over the range of 50-550 m/z. After desorption, the SPME Arrow was cleaned in a dedicated cleaning port at 250°C for 15 minutes.

2.2.6 Statistical analysis

All data were assessed for normality and homogeneity by the Shapiro-Wilk and Levene's tests, respectively. In the salinity tolerance tests, 48-h LC_{50} concentrations and their corresponding 95% confidence intervals for each test species were calculated by Probit analysis. In toxicity testing, statistical differences between treatment groups were determined using a student's *t*-test or one-way analysis of variance (ANOVA) followed by a Turkey post-hoc test to analyze the interaction between treatments. In the case of non-normally distributed data, a Kruskal-Wallis ANOVA followed by Dunn's post-hoc test was performed. All statistical analyses were conducted using the IBM SPSS statistical package with $\alpha = 0.05$ (ver. 28, SPSS Inc., IL, USA).

2.3 Results

2.3.1 Salinity tolerance tests

Dose-response curves for the lab strains of *D. magna* and *D. pulex,* and native strains *Daphnia sp.* (HL) and *D. pulex* (LM), exposed to NaCl (0 to 8 g/L nominal concentrations) are shown in Figure S 1. Estimated toxicity values for NaCl concentrations are summarized in Table 2.1. Based on the 48-h LC_{50} values for NaCl, the most sensitive species was *Daphnia sp.* (HL), with an LC_{50} of 1.81 g/L NaCl. The rest of the species showed greater salt tolerance, with *D. magna* (lab) having the highest LC_{50} (6.04 g/L NaCl).

Table 2. 1. Medium lethal concentrations (48-h LC_{50}) for salinity tolerance based on nominal sodium chloride (NaCl) concentrations and corresponding mean conductivity values. Upper and lower 95% confidence intervals are shown in parentheses.

Species	48-h LC_{50} values in g/L	Conductivity (mS/cm)
Daphnia magna	6.0 (5.7–6.5)	11.1
Daphnia pulex	2.3 (2.1–2.5)	4.1
Daphnia sp. (HL)	1.8 (1.6–2.0)	3.3
Daphnia pulex (LM)	3.6 (3.2–4.1)	8.5

2.3.2 Water exposures

The acute toxicity of LMW to acclimated and unacclimated lab strains (*D. magna* and *D. pulex*) and native *Daphnia sp.* (HL) was assessed using 48-h exposures. Neither species

displayed significant mortality (p>0.05) between the control and the LMW treatment (data not shown).

Similarly, there were no significant differences (p>0.05) in chronic endpoints between the control and the LMW treatments over a 21-d exposure period (Figure 2.1). The mean survival in LMW was ≥ 80% for *D. magna* and *Daphnia sp.*; *D. pulex* (lab) showed <60% survival for unacclimated organisms (Figure 2.1). Whether acclimated or not, test organisms did not show significant differences (p>0.05) for reproduction in LMW compared to control water. The mean time to produce the first brood was ≤ 7.8 days for *D. magna* and *D. pulex* (lab) for all treatments, whereas *Daphnia sp.* (HL) showed a shorter time (≤ 6.3 days). For the production of offspring, *D. magna* reached greater values (15.9–17.3 neonates per brood and 93.3–100.4 total live neonates) compared to *D. pulex* and *Daphnia sp.* (HL) (

Table ***S 3***).

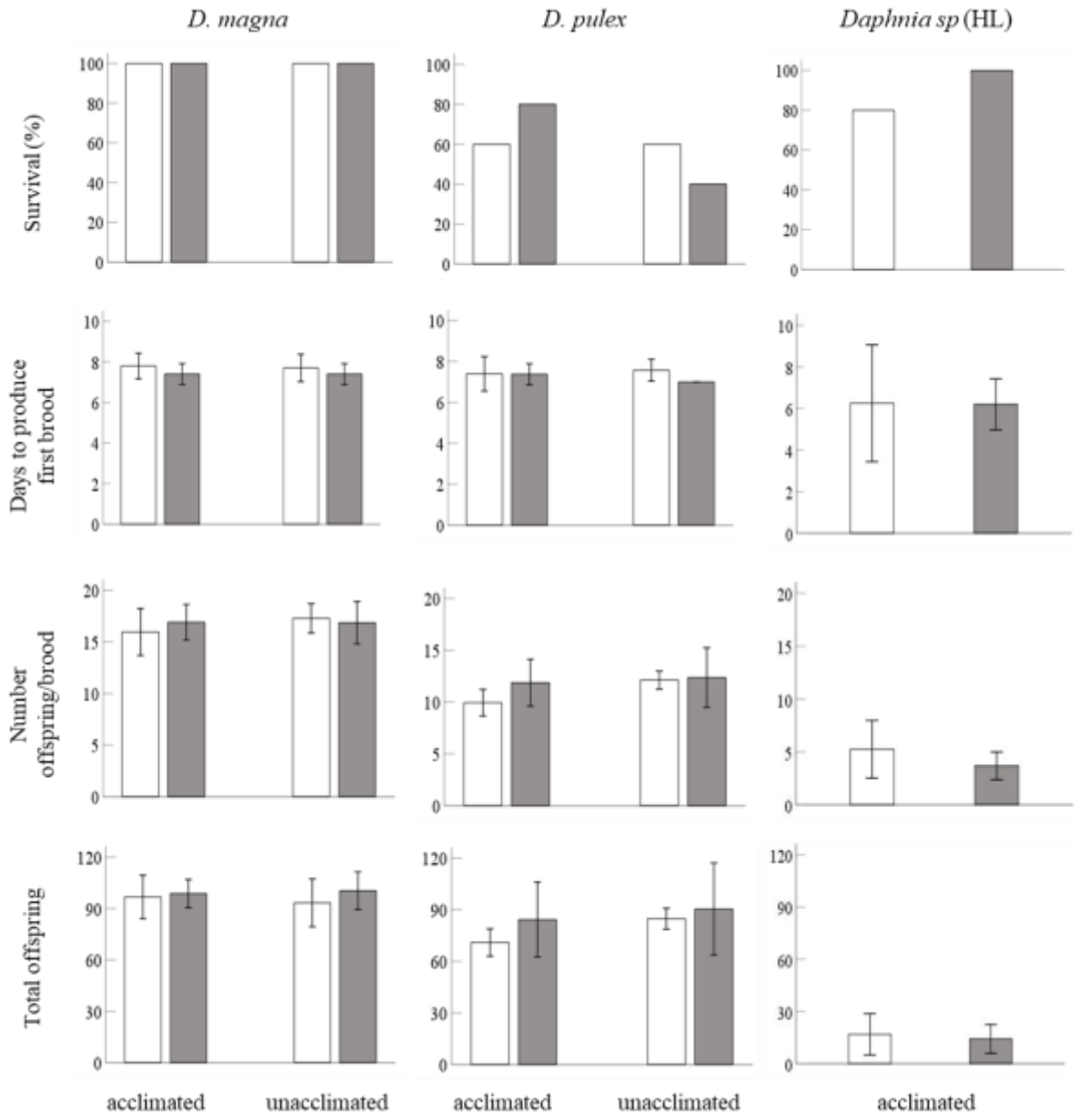

Figure 2. 1. Chronic toxicity for acclimated and unacclimated lab strains *D. magna* and *D. pulex* (21 d exposure) and a native strain *Daphnia sp.* (HL) collected in Lake Humboldt (12 d exposure) exposed to Lake Miwasin water (LMW) and control water (mean ± 1 SD; n = 10). White bars: control; grey bars: LMW.

2.3.3 Pore water exposures

The acute toxicity of LMP to acclimated and unacclimated *D. magna* (lab), *D. pulex* (lab), and *D. pulex* (LM) was assessed using 48-h tests. Results showed that LMP was acutely toxic to all test organisms (Figure 2.2). Of the three organisms assessed, *D. magna* was the most tolerant, showing significant differences in 48-h mortality compared to the controls only at 50% (p=0.019) and 100% (p<0.001) LMP (Figure 2.2). *D. pulex* (lab) and *D. pulex* (LM) were more sensitive, showing significant differences compared to the controls in 48-h mortality at ≥12.5% LMP (p<0.001) (Figure 2.2). No significant differences were observed between acclimated and non-acclimated organisms for any of the LMP treatments.

Chronic toxicity tests of LMP showed that survival of *D. magna* parents declined with increasing concentrations of LMP for both acclimated and unacclimated organisms, with a reduction in survival observed at 50% LMP (Figure 2.3). Both *D. pulex* (lab) and *D. pulex* (LM) showed a similar decline, with less survival of parent daphnids at 25% LMP (Figure 2.3). No reproduction by *D. pulex* (lab) was observed in ≥25% LMP.

Reproduction effects of LMP varied slightly depending on each treatment and species, but in general, it caused adverse effects whereas LMW did not (

Table ***S 3***). The mean time to produce the first brood for the lab strain of *D. magna* was delayed from 7.2 days (RSP, control) to ⩾8 days (exposed to 50% (v/v) of LMP). In addition, the mean number of offspring produced per brood for the unacclimated lab strain *D. magna* was reduced by ≤ 62% and ≤ 81% in treatments of 25 and 50% LMP, respectively, while that for the lab strain *D. pulex* was reduced by ≤ 75% in 12.5% LMP (

Table S ***3***). Significant differences between controls and the 12.5% LMP exposure were observed for both acclimated and unacclimated *D. pulex* (lab) for the following endpoints: time to produce first brood ($p<0.001$), number of living offspring produced in each brood ($p<0.05$), and total number of living offspring ($p<0.05$).

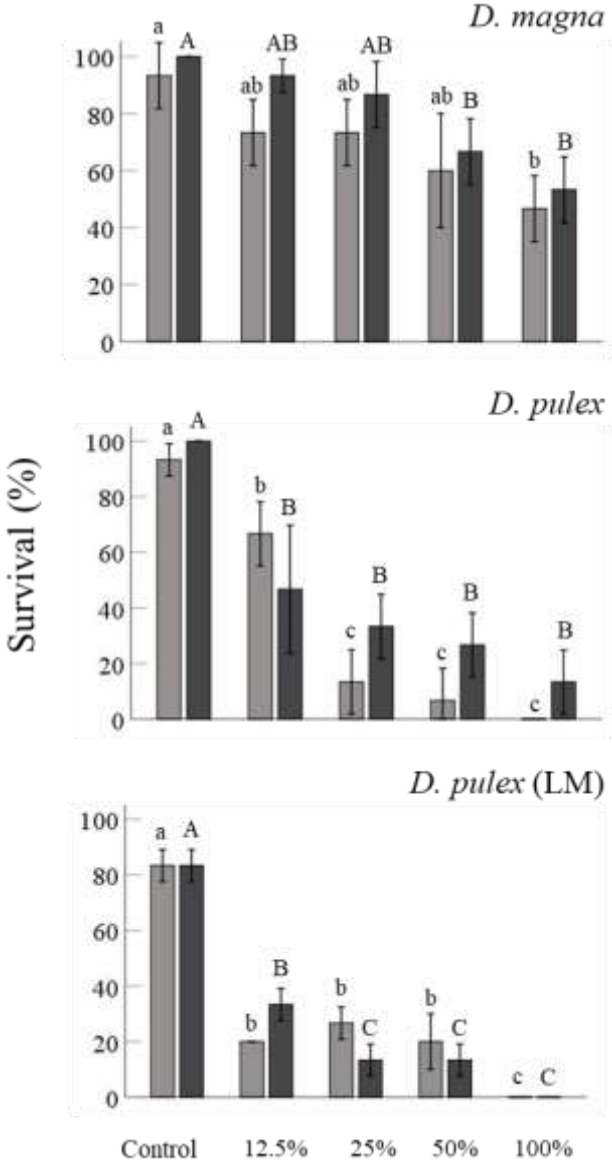

Figure 2. 2. Acute toxicity for acclimated and unacclimated lab strains of *D. magna* and *D. pulex*, and a native strain *D. pulex* (LM) collected in Lake Miwasin (48 h exposure) exposed to 12.5, 25, 50, and 100% Lake Miwasin pore water (LMP) and control water (mean ± 1 SD; n = 10). Lighter bars: acclimated; darker bars: unacclimated. Significant differences ($p \leq 0.05$) among treatments are shown in lower-case letters for acclimated organisms and capital letters for unacclimated organisms.

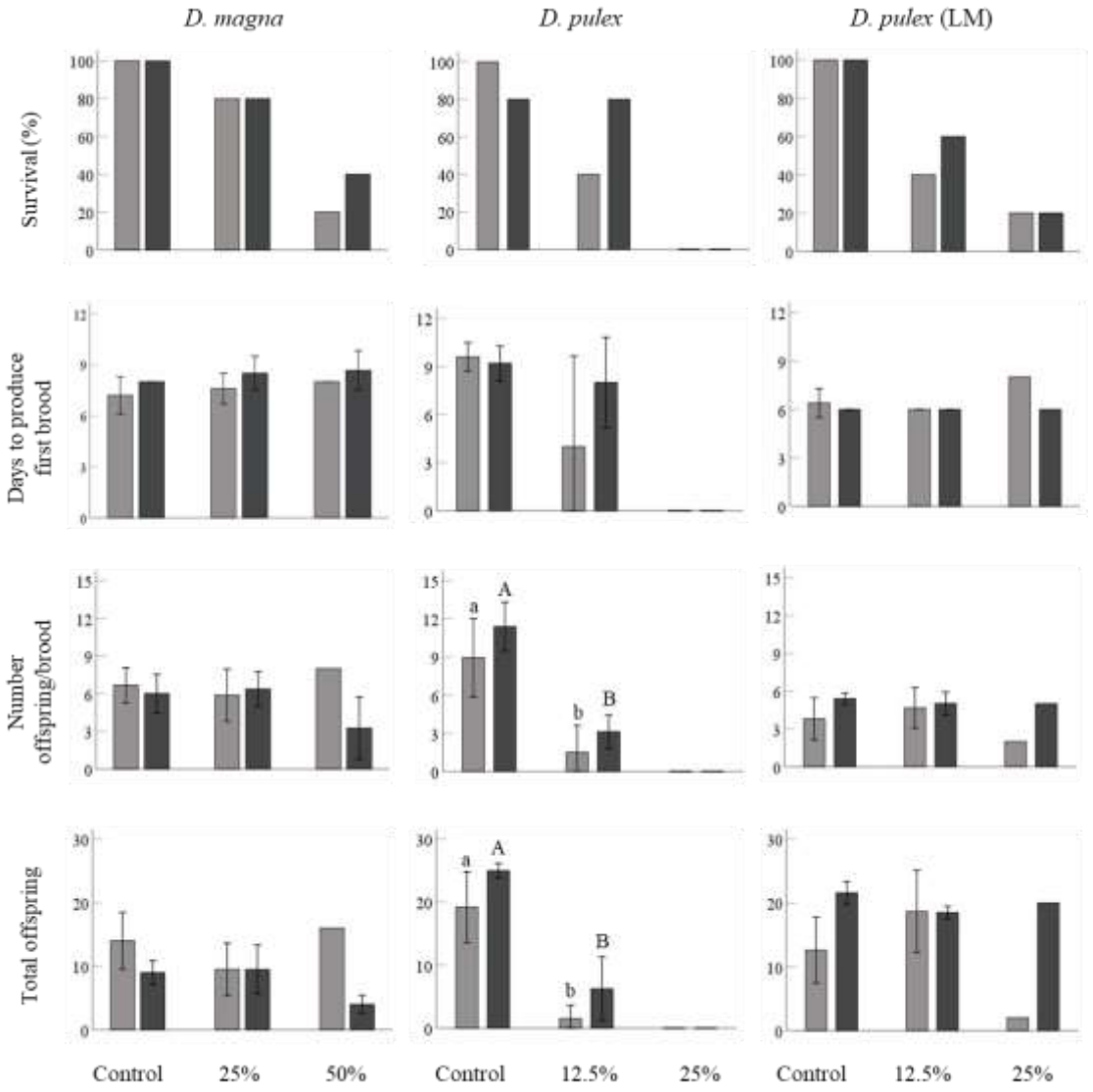

Figure 2. 3. Chronic toxicity for acclimated and unacclimated lab strains of *D. magna* and *D. pulex* and a native strain *D. pulex* (LM) collected in Lake Miwasin (12 d exposure) exposed to dilutions of Lake Miwasin pore water (LMP) and control water (mean ± 1 SD; $n = 5$). Lighter bars: acclimated; darker bars: unacclimated. Significant differences ($p \leq 0.05$) among treatments are shown in lower-case letters for acclimated organisms and capital letters for unacclimated organisms.

2.3.4 Toxicity identification evaluation – pore water

The survival of *D. magna* in LMP exhibited significant differences ($p<0.05$) across some TIE manipulations. Manipulations demonstrating statistical differences compared to baseline toxicity included filtration ($p=0.003$), C18-SPE ($p=0.044$), zeolite ($p=0.044$), and EDTA 40 μM ($p=0.044$). The survival of *D. pulex* (LM) revealed significant differences ($p<0.05$) for manipulations with zeolite ($p=0.022$) and EDTA 40 μM ($p=0.008$) compared to the baseline toxicity of LMP (Figure 8).

For *D. magna* in LMP, the mean survival in baseline, unmanipulated pore water was 53%, with four manipulations increasing survival, to 100% with filtration and 87% with C18-SPE, zeolite, and EDTA 40 μM. For *D. pulex* (LM) in LMP, the mean survival in baseline unmanipulated pore water was 33% with zeolite increasing survival to 80% and EDTA (40 μM) addition to 87%.

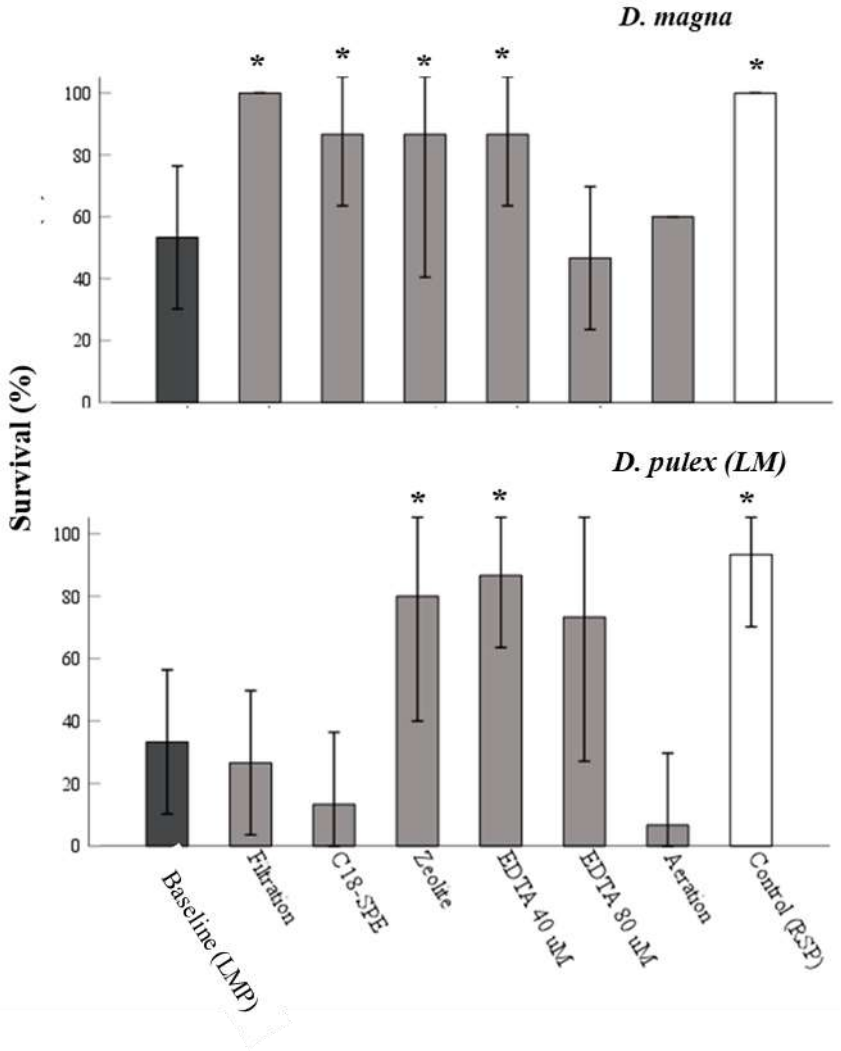

Figure 2. 4. Acute toxicity (48 h exposure) for acclimated lab strain of *D. magna* and a native strain *D. pulex* (LM) exposed to Lake Miwasin pore water (LMP) after TIE phase I manipulations displaying percent survival (mean ± 1 SD; $n = 5$). Darker grey bars = baseline (LMP); grey bars = TIE manipulations; and white bars = control (RSP). Significant differences ($p \leq 0.05$) are shown with an asterix between the treatment and the baseline (LMP).

2.3.5 Chemical analysis

The results of water chemistry analyses are shown in Tables 2.2 and 2.3. In general, LMP showed higher concentrations of analytes than LMW. Chloride concentrations in LMW and LMP were 156% and 323% above the long-term CCME guideline (120 mg/L), respectively. LMP was 284% over the CCME guideline for ammonia (0.173 mg/L) (Table 2.3). Only concentrations of fluoranthene (0.43 μg/L) and pyrene (1.21 μg/L) in LMP were above the CCME guidelines (Table 2.3). Dissolved Cd, Mo, and Se in LMW were over the long-term CCME guidelines while dissolved As, B, Cd, Mo, Se, and Zn in LMP exceeded either short- or long-term CCME guidelines (Table 2.3).

Table 2. 2. Summary of general water chemistry for Lake Miwasin near-bottom surface water (LMW) and sediment pore water (LMP) collected in October 2021, including their synthetic counterparts reconstituted saline water (RSW), and reconstituted pore water (RSP), and Lake Humboldt surface water.

	pH	Conductivity (μS/cm)	Na^+ (mg/L)	Cl^- (mg/L)	SO_4^{2-} (mg/L)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Test waters - Lake Miwasin							
LMW	8.40	2010	403	308	300	292	170
LMP	7.95	3634	768	508	930	607	189
Reconstituted synthetic waters							
RSW	8.26	1880	346	287	290	279	160
RSP	8.74	3367	708	560	580	380	192
Saline lake							
Lake Humboldt	8.51	1461	300	9	260	180	645

Table 2. 3. Mean concentrations of additional water chemistry variables in Lake Miwasin surface water (LMW) and sediment pore water (LMP) collected in October 2021.

Parameter	Units	Mean concentrations		Guidelines		
		LMW	LMP	Short-term (acute)	Long-term (chronic)	Source
pH		8.40	7.95	--	6.5-9.0	USEPA (1996)
Ammonia	mg/L	0.08	**6.65**	--	0.173[b]	CCME (2010)
Chloride	mg/L	**308**	**508**	640	120	CCME (2011)
Sulfate	mg/L	300	**930**	--	309	BC (2013)
Dissolved organic carbon	mg/L	28.67	130.30	--	--	
Organics						
Naphthenic acids (NAs)	mg/L	2.75[a]	na	--	--	
Acenaphthene	µg/L	<LOD	<LOD	--	5.8	CCME (1999)
Acenaphthylene	µg/L	<LOD	<LOD	--	--	
Acridine	µg/L	<LOD[a]	na	--	4.4	CCME (1999)
Anthracene	µg/L	<LOD	<LOD	--	0.012	CCME (1999)
Benzo(a)anthracene	µg/L	<LOD[a]	na	--	0.018	CCME (1999)
Benzo(a)pyrene	µg/L	<LOD[a]	na	--	0.015	CCME (1999)
Benzo(b,j)fluoranthene	µg/L	<LOD[a]	na	--	--	
Benzo(g,h,i)perylene	µg/L	<LOD[a]	na	--	--	
Benzo(k)fluoranthene	µg/L	<LOD[a]	na	--	--	
Chrysene	µg/L	<LOD	6.31	--	--	
Dibenz(a,h)anthracene	µg/L	<LOD[a]	na	--	--	
Fluoranthene	µg/L	<LOD	**0.43**	--	0.04	CCME (1999)
Fluorene	µg/L	<LOD	<LOD	--	3	CCME (1999)
Indeno(1,2,3-cd) pyrene	µg/L	<LOD[a]	na	--	--	
Naphthalene	µg/L	<LOD	<LOD	--	1.1	CCME (1999)
Phenanthrene	µg/L	<LOD	<LOD	--	0.4	CCME (1999)
Pyrene	µg/L	<LOD	**1.21**	--	0.025	CCME (1999)
Quinolone	µg/L	<LOD[a]	na	--	3.4	CCME (1999)
Dissolved metal(oid)s						
Aluminium	µg/L	10.26	5.29	--	840	BC (2023)
Arsenic	µg/L	1.95	**9.71**	--	5 (total)	CCME (2001)
Barium	µg/L	49.16	145.4	--	--	
Boron	mg/L	0.99	**2.98**	29 (total)	1.5 (total)	CCME (2009)
Cadmium	µg/L	**0.14**	**0.10**	1 (total)	0.09 (total)	CCME (1996)

Parameter	Units	Mean concentrations		Guidelines		
		LMW	LMP	Short-term (acute)	Long-term (chronic)	Source
Cobalt	µg/L	0.18	0.66	110 (total)	4 (total)	BC (2004)
Copper	µg/L	1.67	1.04	46.9	8.1	BC (2019)
Iron	µg/L	6.67	6.69	--	350	BC (2008)
Lead	µg/L	0.49	0.06	197 (total)	11 (total)	BC (1987)
Manganese	µg/L	0.22	309.23	3600	430	CCME (2019)
Mercury	ng/L	<LOD	<LOD	--	26 (total)	CCME (2003)
Molybdenum	mg/L	**45.06**	**59.92**	46 (total)	7.6 (total)	BC (2021)
Nickel	µg/L	5.68	4.89	775 (total)	86 (total)	US EPA (1995)
Selenium	µg/L	**1.33**	**1.96**	--	1 (total)	CCME (1987) BC (2023)
Silver	µg/L	0.04	0.04	--	0.25 (total)	CCME (2015)
Thallium	µg/L	0.04	0.04	--	0.8 (total)	CCME (1999)
Uranium	µg/L	2.34	2.83	33 (total)	15 (total)	CCME (2011)
Zinc	µg/L	2.13	**25.59**	37	7	CCME (2018)
Strontium	mg/l	0.43	812.59	--	--	
Chromium	µg/L	0.39	0.53	--	1.0 (VI) 8.9 (III)	CCME (1999)
Antimony	µg/L	0.28	0.27	--	--	
Vanadium	µg/L	0.85	0.63	--	--	
Titanium	µg/L	0.23	0.18	--	--	

Numbers in bold indicate exceedances of guidelines.

[a] Provided by Suncor Energy Inc.

[b] Ammonia guideline is pH and temperature dependent. The ammonia guideline used herein was calculated at pH 8.40 and 20°C.

<LOD: under limit of detection.

na: not analyzed.

2.4 Discussion

Overall, results indicate that there are notable differences in toxicity to daphnids between surface water and pore water from Lake Miwasin, with only pore water (LMP) demonstrating adverse effects. Controlling for osmotic stress linked to salt content, multiple classes of contaminants likely contributed to the observed pore-water toxicity. With regard to salinity, both LMW and LMP were elevated in various major ions, with LMP having concentrations of Na^+, Cl^-, and SO_4^{2-} almost twice that of LMW (Table 2.2). As a result, salinity was shown to be an additional stressor during chronic LMP exposures, negatively affecting control organism survival past a 12-d duration and with adverse effects on reproduction. More detailed discussion and broader implications are found below.

2.4.1 Salinity tolerance tests and acclimation

The short-term (48-h) tolerance to NaCl for test organisms used here followed the order: *D. magna* (lab) > *D. pulex* (LM) > *D. pulex* (lab) > *Daphnia sp.* (HL). The difference in salinity tolerance between *D. magna* and *D. pulex* has been previously demonstrated, with NaCl LC_{50} values of 5.48 g/L (Martínez-Jerónimo & Martínez-Jerónimo, 2007) and 6.6 g/L (Schuytema et al., 1997) for *D. magna*, and values of 2 g/L (Liu & Steiner, 2017) and 3.32 g/L (Bezirci et al., 2012) for *D. pulex*. These values are similar to the NaCl 48-h LC_{50} values reported here. It should be noted that although NaCl LC_{50} concentrations are above the salinity levels of LMW (~0.71 g/L) and LMP (~1.28 g/L), these differences are not large from a toxicological perspective.

The higher salt tolerance of *D. pulex* (LM) (48-h NaCl LC_{50} = 3.59 g/L) compared to our laboratory-cultured strain of *D. pulex* (LC_{50} = 2.32 g/L) could be related to a clonal variation and/or to the long term acclimation to the saline condition of Lake Miwasin. Weider and Paul (1987) stated that *D. pulex* clones have diverged physiological strategies for osmoregulation. It is possible that the *D. pulex* clones used in this study (lab and LM) belong to different strains that are able to tolerate slightly different levels of salinity.

Of the daphnids tested, the field-collected *Daphnia sp.* (HL) proved to be the most sensitive to salinity (48-h LC_{50} 1.81 g/L NaCl) and difficult to acclimate to LMP conditions. Although Humboldt Lake is a slightly brackish environment (conductivity = 1461 μS/cm), it has a particular ionic composition (Table 2.2) that may also account for the difference in tolerance with the rest of the test organisms. Lake Miwasin is elevated in both Na^+, Cl^-, and SO_4^{2-} while Humboldt Lake is elevated in Na^+ and SO_4^{2-} (Rawson & Moore, 1944), but has relatively little Cl^-. It is possible that this difference could have affected the NaCl tolerance of *Daphnia sp.* (HL) and thus call into question its potential use in toxicity testing related to the oil sands end pit lakes.

Salinity tolerance in daphnids can be acquired through exposure to saline environments. Liao et al. (2015) found that *D. magna* isolated from rock pools with historically high salinity levels (1440 μS/cm) exhibited greater salt tolerance compared to those from lower salinities (316 μS/cm). Additionally, Martínez-Jerónimo and Martínez-Jerónimo (2007) reported that salinity acclimation of the *D. magna* strain used in their study induced tolerance mechanisms within its physiological range, suggesting its suitability for use in toxicity assays of slightly

saline testing conditions. Thus, to minimize the influence of osmoregulatory stress under the brackish conditions of LMW and LMP, we performed salinity acclimation for the test organisms.

2.4.2 Surface water and pore water tests

Exposure to surface water (LMW) or pore water (LMP) from Lake Miwasin yielded mixed toxicity results. Based on seasonal data collected from 2019 to 2021, near-bottom surface water was typically elevated in various major ions and constituents of potential concern (CPCs) and was therefore more likely to demonstrate toxicity compared to water collected from higher up in the water column. Nevertheless, Lake Miwasin surface water (LMW), collected near-bottom, was not toxic to the *Daphnia* species and strains tested in either acute or chronic exposures. In contrast, exposure to LMP caused acute toxicity to *D. magna*, *D. pulex* (lab), and *D. pulex* (LM) (Figure 2.2), with mortality generally increasing with increasing pore water concentration. There were no significant differences between acclimated and unacclimated organisms. Overall, *D. magna* was the least sensitive of the species tested.

In the chronic tests, adult survival decreased by 50% and 12.5% LMP for *D. magna* (lab) and *D. pulex* (lab and LM), respectively, compared to no effects in LMW for a much longer exposure duration. However, only *D. pulex* (lab) demonstrated a significant ($p<0.05$) decrease in reproduction (Figure 2.3). This was observed at 12.5% LMP, the lowest pore water concentration tested, and the only LMP exposure for this species with acceptable survival in the controls until test termination.

The daphnid species selected for testing in this study are known for their tolerance of elevated salinity, and they performed well in all 21-d surface LMW exposures. Nevertheless, salt levels present in LMP were apparently stressful to the test organisms, as demonstrated by the difficulty acclimating *Daphnia* to salinity similar to that of LMP. In preliminary testing (data not shown), the stress caused by elevated salinity levels had adverse effects on reproductive endpoints and led to reduced survival of the tested organisms. As a result, the duration of chronic LMP exposures had to be reduced to 12-d.

Although acclimation to salinity can improve daphnid performance under brackish testing conditions (Arnér & Koivisto, 1993; Martínez-Jerónimo & Martínez-Jerónimo, 2007), chronic toxicity results (Figure 2.3, Table S3) revealed that the performance of acclimated organisms showed slight improvements compared to the unacclimated ones in control waters (RSW and RSP). Specifically, the mean total live offspring for the acclimated lab strain *D. magna* exhibited a 4% and 36% increase in RSW and RSP, respectively, while the acclimated lab and native *D. pulex* displayed a different trend. The total live offspring of acclimated lab strain *D. pulex* decreased by 16% and 23% in RSW and RSP, respectively, and acclimated native *D. pulex* (LM) experienced a reduction of 42% in RSP. This suggests that salinity acclimation of *D. pulex* strains did not adequately reduce the physiological stress induced by the brackish conditions of LMW and LMP. Bezirci et al. (2012) similarly found that salinity acclimation of *D. pulex* did not cause differences in terms of survival, fecundity, body width/body length ratio in chronic toxicity tests of NaCl concentrations up to 0.8 g/L, suggesting that *D. pulex* lacks physiological mechanisms to manage the negative effects of salinity at that level (0.8 g/L).

The osmolality of *Daphnia* hemolymph is attributed mainly to Na^+ and Cl- (Morris et al., 2021). Morris et al. (2021) observed that when the environmental Na^+ concentration was 30 mM, the hemolymph Na^+ concentration in *D. magna* increased from 53 to 75 nM and did not return to control levels observed at a Na^+ concentration of 10 mM. Unlike in situations with lower Na^+ ambient concentration (Na^+ < 20 Mm), where *D. magna* could recover to their internal baseline levels. Consequently, high concentrations of Na^+ impair *Daphnia*'s ability to adapt to increased salinity, leading to the accumulation of these ions in the hemolymph and potentially resulting in toxicity.

Test organisms used in this study were freshwater *Daphnia* which are hyperosmotic regulators. They reduce the permeability of ion-regulating epithelia and increase the uptake of NaCl to counterbalance losses (Aladin & Potts, 1995). So, they tend to take up ions from the media up to certain levels. That could explain why unacclimated *Daphnia*, in the slightly brackish conditions of this study, exhibited better performance in terms of survival and reproduction compared to acclimated *Daphnia*.

Exposure to LMP tested a worst-case scenario for Lake Miwasin, one where an extended period of stratification of the water column, combined with expression of pore water from consolidating tailings, enriches compounds of potential concern (CPCs) and salinity in near-bottom water. Visual observations during 2020 and 2021 site visits indicated that *D. pulex* commonly congregates near the bottom (approximately 4.3 m depth) in LM during daylight hours. Cladocera is known to exhibit diel vertical migration to avoid predators in the upper water column (Smirnov, 2017). This avoidance behavior exposes them to near-bottom

conditions that are enriched with salts and CPCs due to pore water expression that may be most concerning during seasonal stratification. It is probable that near-bottom water chemistry is likely the most impacted by pore water in the early times after lake formation when the rate of bottom compaction and associated pore water expression are highest, but progressively attenuate as the tailings compaction rate declines.

2.4.3 Toxicity identification evaluation for pore water

A TIE phase I study was conducted to identify the broad class of toxicant(s) responsible for the observed toxicity to daphnids exposed to LMP. The TIE phase I testing revealed that manipulations with zeolite and EDTA 40 μM were the only treatments that removed toxicity for both test species. Zeolite and EDTA can remove ammonia and metals in aqueous samples, respectively, suggesting that the aqueous toxicity of LMP is at least partially attributable to these stressors.

Ammonia is acutely toxic to many aquatic invertebrates at concentrations between 1.1 to 22.8 mg/L (mean 48-h LC_{50} values) with factors such as temperature and pH affecting speciation and toxicity (see review by CCME (2010)). Regarding *Daphnia*, ammonia concentrations > 0.5 mg/L have been shown to dramatically affect the population dynamics of *Daphnia obtusa, D. similoides*, and *D. similis* (Lyu et al., 2013). In the study presented here, the ammonia concentration in unmanipulated pore water (LMP, bulk pore water) was 6.65 mg/L and <1.5 mg/L in samples after treatment with zeolite. Therefore, ammonia could be causing toxic effects in test organisms exposed to bulk pore water from bottom sediment collected from Lake Miwasin.

On the other hand, EDTA chelates cationic metals producing relatively non-toxic complexes (US EPA, 1991). Concentrations of As, Ba, Mo, Mn, Sr, and Zn were higher in LMP compared to LMW with values of As, B, Mo, Se, and Zn exceeding CCME guidance for long-term exposure. Therefore, these trace elements could also contribute individually or in combination to the toxic effects observed in LMP. Notably, increasing the EDTA concentration to 80 μM from 40 μM did not significantly reduce toxicity. It is possible that the higher EDTA concentration could have directly caused some toxicity, but more likely, since EDTA chelates not only toxic metals but also essential ones, the reduction or depletion of essential trace elements might then indirectly have affected the survival of test organisms.

For *D. magna*, TIE phase I manipulations also showed that C18-SPE and filtration reduced the toxicity of LMP. For *D. pulex* (LM), only EDTA addition reduced toxicity. Filtration and C18-SPE are nonspecific treatments, acting also as filters or binding mediums of cationic metals (US EPA, 2007). So, TIE phase I results for *D. magna* must be interpreted with caution. US EPA (1991, 2007) suggested that additional TIE testing of the SPE fractions can be performed to determine toxicity for slightly hydrophilic to strong hydrophobic organic compounds. However, toxicity testing of the elution fractions containing compounds retained on the C18-SPE column was not performed in this study. Only fluoranthene and pyrene were above the utilized guidelines in LMP (Table 2.3), thus they are unlikely to cause acute toxicity. Acute toxicity of fluoranthene and pyrene to *D. magna* has been reported at levels as low as 4 μg/L (see review by CCME, 1999), which is still 10- and 5- times higher than the concentrations of fluoranthene and pyrene in LMP, respectively (CCME, 2010).

Discrepancies between the responses of the two test species to the various TIE phase I manipulations could be partly due to size differences of the daphnids. Burns (1968) demonstrated a strong positive correlation between the size of ingested particles and *Daphnia's* body size. Considering that *D. magna* is larger than *D. pulex* (LM), it is plausible that *D. magna* might have ingested larger filterable particles suspended in LMP that could not be ingested by *D. pulex*, thereby contributing to the observed toxicity in LMP (Figure 2.4). It is inferred that toxicants adsorbed to suspended particles but bioavailable upon digestion were removed through filtration and C18-SPE manipulations, leading to a decrease in LMP toxicity. Nevertheless, this is speculative, and analysis of CPC content on/in filterable solids was not performed in this study.

2.5 Conclusions

This study revealed that LMW collected from near-bottom was not toxic to lab strains of *D. magna* and *D. pulex* (lab) and to native *Daphnia sp.* (HL). However, exposure to LMP caused acute toxicity to *D. magna, D. pulex* (lab), and *D. pulex* (LM) at concentrations as low as 25% and 12.5% and caused chronic reproductive effects to *D. pulex* (lab) at 12.5%. In general, *D. magna* was the least sensitive to both acute salt exposure and exposure to LMP.

The acclimation of test organisms to brackish water prior to toxicity testing did not result in a better performance of testing organisms for either the LMW or LMP tests. In fact, the level of salinity of LMP and its control (RSP) caused adverse reproductive effects for *Daphnia* species in chronic 12-d exposures. Therefore, levels of salinity should be considered when

selecting test organisms for toxicity testing of brackish waters formed in future engineered oil sands and end pit lakes, with emphasis on near-bottom water as a worst-case exposure scenario.

Phase I TIE results indicated that ammonia and metals in LMP were likely responsible for toxicity to both native strain *D. pulex* (LM) and lab strain *D. magna*. Further TIE phases (II and III) are required to confirm the implication of ammonia and metals in LMP toxicity.

Exposure to both surface water and sediment pore water from Lake Miwasin yielded mixed toxicity results, illuminating the importance of understanding engineered system structure (constituents and CPCs), trajectory of physical processes (e.g., substrate compaction and pore water extrusion), and animal ecology (e.g., habitat use and diet) to assess the toxicological risks of surface water in this and future end-pit lakes in the oil sands region.

CHAPTER 3: SELENIUM BIOACCUMULATION IN *Daphnia pulex* VIA AQUEOUS AND DIETARY EXPOSURE

Overview

The research in this chapter was designed to assess the bioaccumulation of selenium by *D. pulex* strains via aqueous and dietary exposure. This chapter will be submitted to the journal Archives of Environmental Contamination and Toxicology. The anticipated citation is Davila-Arenas C., Doig L., Ji X., Panigrahi B., Ezugba I., Liu X., Liber K. 2024. Selenium bioaccumulation in lab-cultured and native *D. pulex* via dissolved and dietary exposure routes. *Archives of Environmental Contamination and Toxicology* (in preparation).

Author contributions

Catherine Davila-Arenas (University of Saskatchewan): designed the study, collected, processed, and prepared samples used in the experiments, performed all statistical analyses, and drafted the manuscript.

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Lorne Doig (University of Saskatchewan): helped design the study, provided scientific input and guidance, assisted in sample collection, revised the manuscript, and provided comments and corrections.

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Abstract

Pit lakes are currently being investigated as a way to store and reclaim waste materials in the Alberta Oil Sands (AOS) region, Canada. Lake Miwasin is a pilot-scale pit lake consisting of treated fine tailings overlayed with oil sands processed affected water (OSPW) blended with fresh surface water. Of various trace elements, in October 2021 the surface water contained a mean concentration of 1.33 ± 0.04 μg/L dissolved selenium (Se), above the Canadian Council of Ministers of Environment water quality guideline for long-term protection of aquatic life (1 μg Se/L). This study assessed the bioaccumulation of Se by the cladoceran *Daphnia pulex* under laboratory conditions through both dissolved and dietary exposure routes for comparison to field-collected specimens. In 12-d semi-static tests, a lab strain of *D. pulex* was exposed to water and algae grown in media spiked with selenate (1.76 ± 0.05 μg/L to 5.41 ± 0.04 μg/L). Results showed that Se bioaccumulation by lab *D. pulex* increased in all exposure treatments from days 5 to 12, with maximum Se concentrations of 3.08-3.47 μg/g dry weight (dw) observed within the exposure range tested. Interestingly, lower Se bioaccumulation concentrations (1.26-1.58 μg/g dw) were observed in the highest dissolved Se and dietary Se treatments, suggesting potential internal regulatory mechanisms. In addition, native *D. pulex* collected from Lake Miwasin and cultured in-house were exposed in 8-d semi-static tests to Lake Miwasin surface water (1.33 ± 0.04 μg/L) and algae cultured in Lake Miwasin surface water. Selenium bioaccumulation in native *D. pulex* (LM) ranged from 2.00 to 2.04 μg/g dw at day 8 and was not significantly different (p >0.05) compared to Se concentrations in *D. pulex* collected from Lake Miwasin (2.15 ± 0.28 μg/g) in summer 2022. The average Se

concentrations in both laboratory exposures and field-collected *D. pulex* were below the interim guideline (4 μg/g Se dw) for invertebrates established by the British Columbia Ministry of Environment (2014). Based on this, *D. pulex* living in Lake Miwasin poses little risk as a food source to higher trophic level organisms living in the lake and potentially other systems similar in water chemistry and dissolved Se concentrations. Nevertheless, the possible regulation of Se uptake by *Daphnia* at environmentally relevant Se concentrations merits further investigation to better assess the potential risk of Se in pelagic food webs at the AOS.

Keywords: selenium, dissolved selenium, bioaccumulation, dietary exposure, daphnia

3.1 Introduction

Selenium (Se) is an essential element for aquatic organisms; however, slightly elevated concentrations of Se in freshwater ecosystems can pose a toxicological risk to higher-level organisms, such as egg-laying vertebrates (Stewart et al., 2010). Selenium is accumulated in lower trophic organisms, such as algae and invertebrates, and is then transferred to higher trophic levels through their diet (Lemly, 1993; Young et al., 2010). For example, Se can bioconcentrate in algae 10^2- to 10^6-fold above dissolved Se concentration in water (Stewart et al., 2010). Once transferred to higher trophic-level organisms such as fish, deformities and mortality can occur if tissue residues reach a certain threshold (Janz et al., 2010). Therefore, small increases in Se above background concentrations can pose significant risks to exposed fish populations.

Selenium is extensively distributed to and cycled within environmental systems via natural and anthropogenic processes (Young et al., 2010). For example, Se occurs naturally in rocks and soils (Presser et al., 1994), but can also be released from industrial processes, including oil refining, fossil fuel combustion, agriculture, and mining (Maher et al., 2010). One potential source of Se includes waste products of oil refining (Cutter & San Diego-McGlone, 1990; Young et al., 2010) such as in the Alberta Oil Sands (AOS). In particular, pit lakes constructed using AOS waste materials are currently being explored as a means of reclaiming OSPW and tailings. Selenium associated with these materials will likely be elevated in such ponds and could pose a potential risk to higher trophic levels such as birds and fish.

Guidelines for long-term exposure to aqueous Se concentrations have been established by various jurisdictions including: the US Environmental Protection Agency (1.5 μg/L for lentic aquatic systems) (US EPA, 2016); the Canadian Council of Ministers of Environment (1 μg/L) (CCME, 1987), and the British Columbia Ministry of Environment (BC MoE) (alert concentration 1 μg/L, guideline concentration of 2 μg/L) (BCMOE, 2014). Nevertheless, the impact of bioaccumulation within this concentration range remains poorly understood and uncertainty persists regarding whether Se at low concentrations could, under some circumstances, lead to toxicity in higher trophic levels. The BC MOE recommends 4 μg/g dw or less as a guideline for Se in dietary tissue (invertebrate) to protect higher-level vertebrates from food-borne toxicity (BCMOE, 2014).

This study focused on Lake Miwasin, a pilot-scale end pit lake located in the AOS region. The mean (± 1SD) dissolved Se concentration in Lake Miwasin surface water (LMW) was 1.33

± 0.04 μg/L in October 2021. This value is similar to or above US and Canadian long-term exposure guidelines for total aqueous Se and thus raised concern about the potential risk of Se to biota in this and similarly engineered systems. Uptake by pelagic algae could lead to the transfer of Se to *D. pulex*, an abundant primary consumer in Lake Miwasin. Growth and reproduction in *Daphnia* are only affected at relatively high levels of Se (25-250 μg/L as selenate) (Johnston, 1987). Therefore, Se can accumulate in *Daphnia* at the observed concentrations and be transferred to higher-level vertebrates without *Daphnia* experiencing toxicity. By examining the uptake of Se in daphnids at low, environmentally relevant concentrations, this research contributes to resolving uncertainties related to Se biodynamics in the context of both current and future interactions within pit lakes.

Using Lake Miwasin as a test case, our goal was to assess the bioaccumulation of Se in *D. pulex* via dissolved and dietary exposure routes under conditions similar to those of Lake Miwasin and at dissolved concentrations of Se bracketing those likely observed in future AOS pit lakes. Specifically, the objectives were to assess Se bioaccumulation in (i) lab-strain *D. pulex* exposed to spiked water and fed with algae grown in media spiked with selenate and (ii) native *D. pulex* (LM) exposed to water and algae grown in LMW under lab conditions. Accumulation results were then (iii) compared to *Daphnia* specimens collected directly from Lake Miwasin.

3.2 Materials and methods

3.2.1 Test organism culture

Two *D. pulex* strains were used as test organisms. A native strain of *D. pulex* was collected from Lake Miwasin in July 2021 and cultured in LMW under laboratory conditions at the Toxicology Centre. The lab strain of *D. pulex* was obtained from an in-house culture at the Toxicology Centre, Usask, SK, Canada. Culturing procedures followed general guidelines described elsewhere (ECCC 1990, 2000). Briefly, groups of 30 to 40 daphnid adults were maintained in 2-L glass jars in an environmental chamber at 23 ± 1°C under a 16:8 h light: dark photoperiod. Aerated reconstituted saline water (RSW) was used as culture water. RSW was prepared by addition of salts (NaCl: 400 mg/L; $NaHCO_3$: 280 mg/L; K_2SO_4: 100 mg/L; Na_2SO_4: 248 mg/L) to dechlorinated (via biofiltration), carbon-filtered municipal water (DCW, City of Saskatoon). Daphnids were fed a 1:1 ratio of the algae *Raphidocelis subcapitata* and *Chlamydomonas reinhardtii*. Water renewals and feeding were conducted three times weekly. Daphnid culture requires the addition of Se to promote animal growth and reproduction (ECCC, 2000). Our attempts to culture daphnids without the addition of Se, to prevent any potential interferences with the experiments, yielded poor results (unpublished data). Therefore, as a compromise, controls of experiments had a reduced concentration of Se (1.76 ± 0.05 µg/L) compared to guidance protocols.

3.2.2 Exposure waters

All exposure waters were aerated for 24 hours prior to use. Samples for dissolved Se analysis were collected in 8-Ml high-density polyethylene (HDPE) bottles using polyethersulfone filters (0.45 μm pore size, VWR International, Radnor, PA, USA) and acidified (pH< 2) with high-purity nitric acid (HNO_3) (Optima Grade, Fisher Scientific, Hampton, NH, USA) from all treatments in duplicate at the beginning of exposures.

3.2.2.1 Selenium-spiked water

The Se concentrations in these experiments were selected to bracket those concentrations found in Lake Miwasin and potentially other future, similarly constructed pit lakes. A selenate stock solution (150 μg/L) was prepared using analytical reagent grade Na_2SeO_4 (>99.8%, Sigma-Aldrich, Saint Louis, MO, USA) in Milli-Q water (Millipore, Bedford, MA, USA). Selenium-spiked solutions were prepared by appropriate dilution of the stock standard solution with culture water. Nominal aqueous concentrations were control, 1, 2, 4, and 8 μg/l Se, and the background Se concentration in LMW (see Table 3.2 for measured Se concentrations).

3.2.2.2 Lake Miwasin surface water

Lake Miwasin (56° 53'N; 111° 23'W) is situated immediately south of the Suncor Millennium Mine, ~18 km north of Fort McMurray, AB, Canada (Figure 1.1). Surface water for use in experiments I and II was collected from Lake Miwasin in early October 2021 at 0.3 m above the sediment-water interface. This water was brackish (2010 μS/cm) with the predominant ions consisting of Na^+, Cl^-, and SO_4^{2-} (Table 2.2). LMW used for renewals during

each experiment was not filtered to minimize sample alteration. The mean (± 1SD) dissolved Se concentration in Lake Miwasin surface water (LMW) was 1.33 ± 0.04 μg/L (consisting of 45% selenite and 55% selenate) in October 2021.

3.2.3 Algal culture and selenization

Two algal species were cultured in selenized media to serve as food in the *Daphnia* exposures. The chlorophytes selected for dietary Se were *R. subcapitata* (CPCC #37) and *C. reinhardtii* (CPCC #243), both obtained from the Canadian Phycological Culture Centre (CPCC) at the University of Waterloo, ON, Canada. Algae cultures were grown in Bold's Basal Medium (BBM) (Stein 1973) and LMW at 22 ± 2 °C under continual light. Selenate was added to BBM to achieve the desired aqueous Se concentration prior to algae inoculation. Treatments for dietary Se were the same as those selected for aqueous exposure of daphnids and ranged from control (~1 μg/l) to 8 μg/l Se (nominal aqueous concentrations). An extra dietary Se treatment consisted of both algae grown in LMW (ALM).

Algae cell densities were determined using a Coulter Counter (Beckman Coulter Inc., Mississauga, ON, Canada) following a 7-day incubation period. After culturing, algal cells were pelleted by centrifugation at 2000 × g for 15 minutes. Algal cells were then resuspended in ultrapure water and pipetted to ensure the removal of the selenized growth medium. Duplicate algal cell samples were collected from each dietary Se treatment to determine the total Se concentration. Samples were stored at -20 °C until lyophilization, digestion, and Se analysis (described below).

3.2.4 Experimental setup

Experiments simulated conditions in Lake Miwasin where animals are exposed to both aqueous and dietary Se (Figure 3.1). All exposures were conducted using pre-cleaned glassware in an environmental chamber at 23 ± 1°C under a 16:8 h light:dark photoperiod. The algal feed consisted of a 1:1 (v/v) ratio of *R. subcapitata* to *C. reinhardtii* at a cell density of approx. 2 x 10^5 cells/Ml. Both experiments were conducted in 1-L borosilicate glass beakers containing 500 mL of water and 5 mL of each alga.

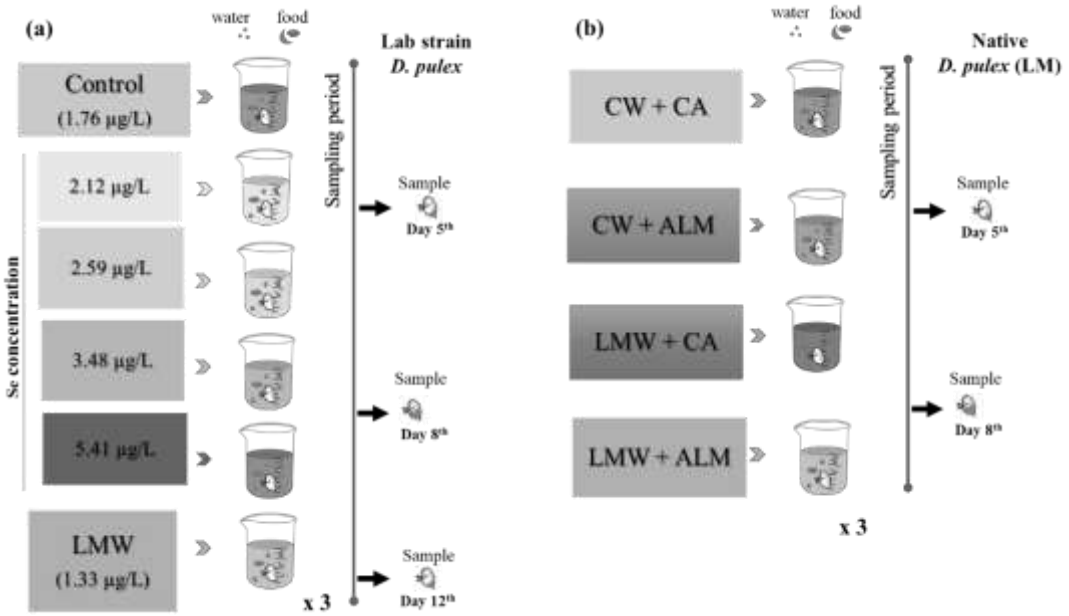

Figure 3. 1. Schematic of the experimental designs for Se bioaccumulation via dissolved and dietary exposure routes in (a) lab strain *D. pulex* exposed to selenate in spiked water and selenized algae and (b) native *D. pulex* (LM) exposed to control water (CW) or Lake Miwasin water (LMW) fed with either alga grown in BBM (CA) and LMW (ALM).

3.2.4.1 Experiment I

Lab strain *D. pulex* was exposed to Se-spiked water and selenized algae in six treatments (Figure 3.1a). Measured dissolved Se concentrations (Table 3.2) of water exposures ranged from 1.76 ± 0.05 μg/L (Se level in control water) to 5.41 ± 0.04 μg/L (highest treatment). Each treatment also received algae cultured in Se-spiked BBM. Selenized *R. subcapitata* had Se concentrations ranging from 0.03 ± 0.02 to 0.38 ± 0.00 μg/g dw. Selenized *C. reimhardtii* had Se concentrations ranging from 0.02 ± 0.01 to 0.56 ± 0.06 μg/g dw. The exposures were conducted for 12 days with three replicates per treatment. Fifty lab strain *D. pulex* neonates (< 24-h old) were transferred to each test vessel at day 0. Total water renewals, including exposure media and food, were conducted every two days. Daphnids were sampled for total Se tissue concentration analysis on days 5, 8, and 12.

3.2.4.2 Experiment II

In the second experiment, native *D. pulex* (LM) were exposed to four Se treatments under laboratory conditions (Figure 3.1b): control water supplemented with algae grown in either control media (CW+CA) or LMW (CW+ALM); and LMW supplemented with algae grown in either control media (LMW+CA) or LMW (LMW+ALM). LMW had a measured mean dissolved Se concentration of 1.33 ± 0.04 μg/L and *R. subcapitata* and *C. reimhardtii* grown in LMW had mean Se levels of 0.38 ± 0.00 and 0.56 ± 0.06 μg/g dw, respectively (Table 3.2). Fifty native strain *D. pulex* (LM) neonates (< 24-h-old) were transferred to each replicate on day 0. Tests were run for 8 days with 3 replicates per treatment. Water changes were as described above. Daphnids were sampled for total Se analysis on days 5 and 8.

Daphnids from the two experiments were not gut purged but placed in a 0.1 mM ethylenediaminetetraacetic acid (EDTA; Sigma Chemical Co., St. Louis, MO, USA) solution for 10 min to remove any surface-adsorbed Se (Vigneault & Campbell, 2005), and subsequently rinsed with ultrapure water.

Additional samples of daphnids were obtained directly from Lake Miwasin. Samples were collected in July 2022 by towing an 80-μm mesh plankton net through the water column at approximately 3-m depth. Once reaching the surface, samples were rinsed with lake water, placed into clear 50-mL plastic bottles, and stored on ice for transport to the Toxicology Centre, Usask. In the laboratory, samples were rinsed with ultrapure water and daphnids were sorted using a stereomicroscope. Daphnid specimens were then stored at -20 °C until freeze-dried prior to digestion and analysis of Se content.

3.2.5 Sample digestion

Freeze-dried tissue samples were digested for Se analysis using high purity, 69% HNO_3, and 30% H_2O_2 (Sigma Aldrich, St. Louis, MO, USA) in Teflon digestion vessels. Capped vessels were placed in a MARS-5 microwave digestion system (EM Corporation, Matthews, NC, USA) and held at 160°C for 20 min. Digests were then filtered and diluted to 2% HNO_3 before analysis.

Water, digestion blanks, and certified reference materials (CRM) were used to ensure the analytical accuracy of Se. The instrumental standard reference material, Natural Water 1643f (National Institute of Standards and Technology) was run with all samples with an analytical

accuracy of 93.7 ± 7.4 (n = 12). A CRM for trace metals, TORT-3 (lobster hepatopancreas, National Research Council Canada, Ottawa, ON, Canada), was digested with algae and daphnia samples with a percent recovery of 88.8 ± 13.4 (n = 12). Ultrapure water was also digested with samples as a method blank. Method blank Se concentrations averaged 0.03 ± 0.07 μg/L (n = 12).

3.2.6 Chemical analysis

Chemical characterization of test solutions was performed in-house at the Toxicology Centre, Usask at the beginning of each test period from at least two randomly chosen beakers per treatment. Additional test variables measured included pH, dissolved oxygen (DO) concentration, and conductivity (Thermo Orion Star A321 Ph Portable Meter, Chelmsford, MA USA), alkalinity and total hardness (Hach, Titration cartridge, Romeoville, IL, USA), and ammonia (Thermo Scientific™ Orion™ AQUAfast™ AQ4000 Colorimeter, Beverly, MA USA). Ions were measured using ion chromatography (Thermo Dionex™ ICS 3000, Sunnyvale, CA, USA).

Selenium concentrations were measured directly from filtered and acidified test waters. An aliquot of LMW was analyzed for Se speciation using liquid chromatography coupled to ICP-MS to separate inorganic Se species. Water samples, freeze-dried algae, and daphnia samples were analyzed for total Se content using inductively coupled plasma spectrometry (8800 ICP-MS Triple Quad, Agilent Technologies, Santa Clara, CA, USA). General water chemistry is summarized in Table 2.2 and Se speciation is in Table 3.1.

Table 3. 1. Mean (±1SD) concentrations of selenite and selenate in Lake Miwasin near-bottom surface water (LMW) collected in early October 2021.

Parameter	Unit	LMW
Total Se	μg/L	1.42 ± 0.02
Dissolved Se	μg/L	1.33 ± 0.04
Selenite	μg/L	0.61 ± 0.00
Selenate	μg/L	0.74 ± 0.05
Selenomethionine	μg/L	<LOQ
Seleno-L-cysteine	μg/L	<LOQ

<LOQ: under limit of quantification.

3.2.7 Statistical analysis

Daphnid Se concentrations were assessed for normality and homogeneity by the Shapiro-Wilk and Levene's tests, respectively. Statistical differences (p<0.05) among treatment groups were determined using a one-way analysis of variance (ANOVA) followed by a Turkey post hoc test to analyze for interaction among treatments. For non-normally distributed data, a Kruskal-Wallis ANOVA followed by Dunn's post hoc test was performed. All statistical analyses were conducted using the IBM SPSS statistical package (ver. 28, Chicago, IL, USA).

3.3 Results

3.3.1 Selenium in cultured algae

Selenium concentrations in cultured algae across all treatments are summarized in Table 3.2. Across selenized BBM treatments, *R. subcapitata* had Se concentrations spanning from 0.03 ± 0.01 to 0.26 ± 0.00 μg/g dw, and *C. reinhardtii* exhibited a range of 0.02 ± 0.01 to 0.22 ± 0.03

μg/g dw. Accumulation was relatively low, only elevated above the controls in the two highest treatments. Notably, algae cultured in LMW had the highest Se uptake for both *R. subcapitata* (0.38 ± 0.00 μg/g dw) and *C. reinhardtii* (0.56 ± 0.06 μg/g dw).

Table 3. 2. Mean (±1SD) Se in lab strain *D. pulex* (*µ*g/g dw) exposed to selenate-spiked water and selenized algae. LMW= Lake Miwasin water.

Treatment	Dissolved Se (*µ*g/L)	Se in algae (*µ*g/g dw)		Se in lab strain *D. pulex* (*µ*g/g dw)		
		R. subcapitata	*C. reimhardtii*	Day 5	Day 8	Day 12
Control	1.76 ± 0.05	0.06 ± 0.02	0.03 ± 0.00	1.28 ± 0.44	1.03 ± 0.35	1.62 ± 0.47
Spiked Se	2.12 ± 0.05	0.03 ± 0.02	0.02 ± 0.01	0.87 ± 0.38	1.85 ± 0.55	3.47 ± 0.13
	2.59 ± 0.05	0.03 ± 0.01	0.02 ± 0.01	1.06 ± 0.17	2.40 ± 0.58	3.08 ± 1.74
	3.48 ± 0.04	0.26 ± 0.00	0.11 ± 0.06	0.39 ± 0.13	1.77 ± 0.51	2.16 ± 0.15
	5.41 ± 0.04	0.14 ± 0.04	0.22 ± 0.03	1.14 ± 0.43	1.31 ± 1.53	1.26 ± 0.30
LMW	1.33 ± 0.04	0.38 ± 0.00	0.56 ± 0.06	0.99 ± 0.24	1.56 ± 0.75	1.58 ± 0.43

3.3.2 Selenium bioaccumulation by lab strain *D. pulex*

Selenium concentrations in lab strain *D. pulex* exposed via both dissolved and dietary exposure routes are shown in Figure 3.2 and Table 3.2. In general, increased Se concentrations were observed beyond day 5 in some treatments but not all. For example, statistically significant differences ($p < 0.05$) in daphnid Se concentration among sampling times were only observed in the 2.12 ± 0.05 μg Se/L treatment [F (17, 36) = 3.996, p<0.001]. In this treatment, Se increased 2.1-fold from day 5 to 8 (1.85 ± 0.55 μg/g dw) and 4.0-fold from day 5 to 12 (3.47 ± 0.13 μg/g dw). Lab strain *D.* pulex exposed to the highest Se treatment (5.41 ± 0.04 μg/L) or LMW treatment (1.33 ± 0.04 μg/L) did not show an increasing trend of Se bioaccumulation over time. Interestingly, increasing exposure concentration did not translate into a corresponding trend in tissue Se concentration.

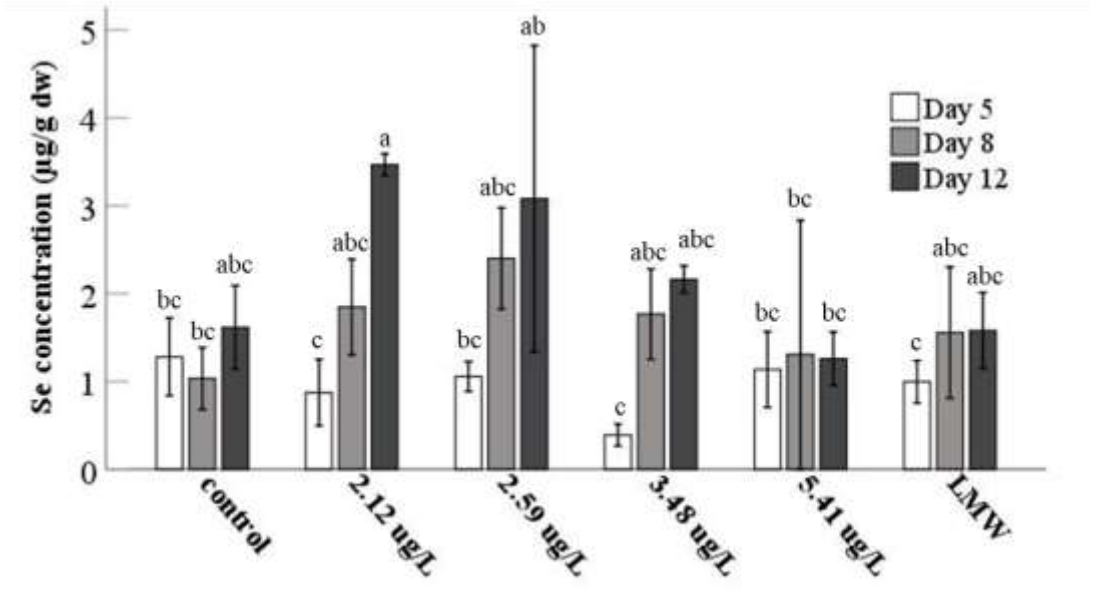

Figure 3. 2. Mean ± 1 SD whole-body Se concentration (μg/g dw) in lab strain *D. pulex* exposed to selenate-spiked water and selenized algae for three sampling periods (days 5, 8, and 12) (n=3). Different letters (a-c) represent a significant difference (p<0.05) in Se concentrations among treatment groups. It should be noted that sample biomass was low (<0.001 g) in treatment 3.48 μg/L. Therefore, caution should be used in interpreting those specific Se values. LMW = Lake Miwasin water.

3.3.3 Selenium bioaccumulation by native *D. pulex* (LM)

Se concentrations in native *D. pulex* (LM) exposed to combinations of waterborne Se and dietary treatments in CW and LMW are shown in Figure 3.3 and Table 3.3. There were no significant differences ($p > 0.05$) in Se concentration in *D. pulex* (LM) for either duration of exposure (day 5 versus day 8) or among treatments. Nevertheless, increased Se concentrations in *D. pulex* (LM) were observed from days 5 to 8, with the highest Se concentrations (2.00 ± 0.20 and 2.04 ± 0.48 μg/g) occurring on day 8 in treatments involving algae cultured in LMW (CW+ALM and LMW+ALM). In comparison, the mean concentration of Se in adult daphnids collected from LM (2.15 ± 0.28 μg/g dw) was similar to those of animals exposed in the lab to algae cultured in LMW (ALM), regardless of exposure to water source (Figure 3.3, Table 3.3).

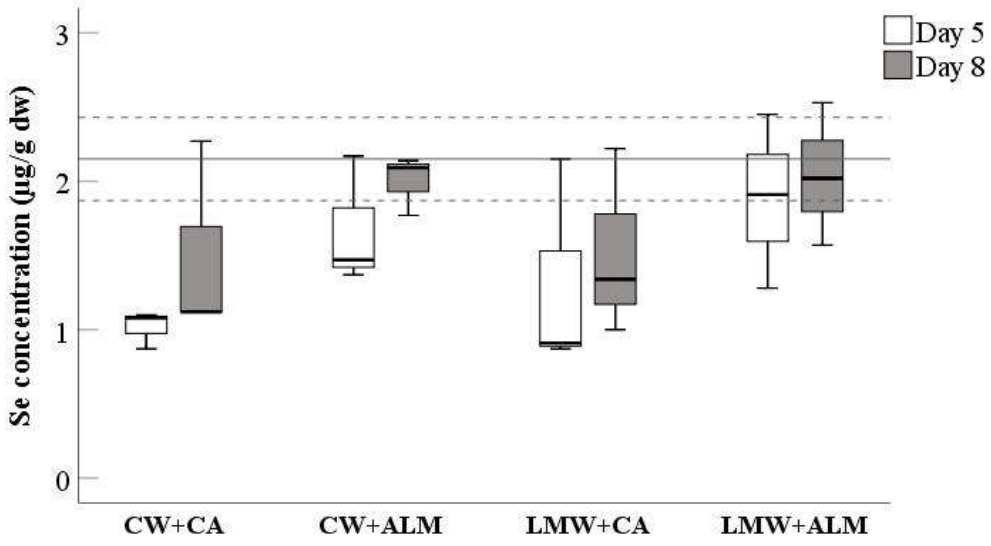

Figure 3. 3. Whole-body Se concentration (μg/g dw; mean ± 1 SD) in a native strain of *D. pulex* (LM) exposed to Control (CW) or Lake Miwasin water (LMW) and control algae (CA) or algae cultured in LMW (ALW) over two sampling times (days 5 and 8) (n=3). Horizontal solid and dotted lines represent mean ± 1 SD tissue Se concentration, respectively, in *D. pulex* collected directly from Lake Miwasin (n=7).

Table 3. 3. Mean (±1 SD) Se in native *D. pulex* (LM) (µg/g dw) exposed to control water (CW) or Lake Miwasin surface water (LMW) and algal cultured in control water (CA) or LMW (ALM).

Treatment	Se in native *D. pulex* (*µ*g/g dw)		Se in daphnid collected directly from Lake Miwasin (*µ*g/g dw)
	Day 5	Day 8	
CW + CA	1.02 ± 0.13	1.50 ± 0.66	--
CW + ALM	1.67 ± 0.44	2.00 ± 0.20	--
LMW + CA	1.31 ± 0.73	1.52 ± 0.63	--
LMW + ALM	1.88 ± 0.59	2.04 ± 1.09	--
Lake Miwasin	--	--	2.15 ± 0.28

3.4 Discussion

3.4.1 Algal selenization

Algae cultured in LMW had the highest uptake of Se despite the total dissolved Se concentration being similar to the control BBM. This suggests greater Se bioavailability in LMW, including the possible presence of more bioavailable Se species in LMW compared to selenized BBM. Although selenate is the predominant oxyanion in Lake Miwasin (Table 3.1), other forms of Se are present. In freshwater environments, green algae can accumulate different forms of Se, with organic Se (selenomethionine) and selenite being more bioavailable that selenate (Stewart et al., 2010). *C. reinhardtii* has shown a linear uptake rate of selenite compared to selenate, whose transport system would saturate at high selenate concentrations (Fournier et al., 2006). This might be one of the reasons why Se accumulation was greater in algae exposed to LMW compared to selenate-spiked media. Another potential explanation might be the sulfate content in BBM which may compete with selenate for uptake sites and thus affect the Se accumulation in the algal species.

A proportional increase in dissolved Se among algal batch cultures was expected to yield a similar trend in tissue Se concentrations in the two algal species cultured. However, the observed Se concentrations were low for the algae cultured in the control BBM and similar to

the two lowest treatments. This outcome could potentially be attributed to inherent variability at these low Se concentrations. Considering that the Se uptake by *R. subcapitata* did not display concentration-dependent uptake for the two highest selenized BBM treatments and the tested algae were grown in batch cultures, it might be also likely that differences in algal growth among the treatments influenced Se content per cell (i.e., growth dilution), leading to a deviation in the expected pattern of increased Se accumulation with increased dissolved Se exposure.

3.4.2 Selenium bioaccumulation by lab strain *D. pulex*

Selenium is largely accumulated in aquatic organisms through the diet (Lemly, 1993; Young et al., 2010) including *Daphnia* (Boyum & Brooks, 1988; Guan & Wang, 2004; Yu & Wang, 2002). However, against expectations, increased Se bioaccumulation was not observed with increased exposure to dietary Se (Table 3.2, Figure 3.2). The underlying cause for this observation remains unclear. Previous investigations on dietary assimilation of metals in *Daphnia* have demonstrated that the assimilation efficiency of Se decreased significantly with increasing Se concentrations in algal food items *C. reinhardtii* and *Scenedesmus obliquus* (Guan & Wang, 2004; Yu & Wang, 2002). Therefore, it is possible that at elevated Se concentrations, *Daphnia* uses regulatory mechanisms to reduce dietary Se assimilation.

These regulatory mechanisms could include saturation of binding sites available for Se in the daphnid digestive tract (Boyum & Brooks, 1988; Guan & Wang, 2004; Stewart et al., 2010), efficient excretion, lower ingestion/assimilation, and reproductive allocation (Yu & Wang, 2002). In other filter feeders such as marine Copepoda, increased levels of metal(loid)s in algae, such as selenium and cadmium, reduced metal assimilation efficiency due to efficient excretion or reduced ingestion of metal(loid)s (Xu et al., 2001).

Reproduction can also play a role in Se regulation (Yu & Wang, 2002), with Se allocation to offspring ranging between 20-40% of the total Se body burden (Guan & Wang, 2004; Lam

& Wang, 2006; Yu & Wang, 2002). In the present study, *D. pulex* began reproducing on day 6 and continued until the end of the experiment. Selenium transfer to juveniles was not measured in this study, either as a function of total reproductive output or percent transfer, so its influence on treatments is unknown. Although this and the above are possible mechanisms to explain the observed pattern of Se bioaccumulation, the exact cause(s) remain unknown.

Finally, it is likely that lab strain *D. pulex* started accumulating Se in the earliest life stage (<24-h old), even before exposure to the experimental Se treatments, which could have influenced the results. In our study, Se levels in control water and control algae contributed to Se accumulation in lab strain *D. pulex* (Table 3.2).

3.4.3 Selenium bioaccumulation by native *D. pulex* (LM)

Whether exposed to reconstituted control water or LMW, native strain *D. pulex* (LM) fed with algae grown in Lake Miwasin water (ALM) had higher levels of Se compared to daphnids grown in control media (CA) (Figure 3.3 and Table 3.3). Nevertheless, these differences were less than 1.3-fold and not statistically significant. The day-8 concentration of Se in *D. pulex* (LM) exposed to LMW and fed with ALM (2.04 ± 1.09 μg/g dw) (Experiment II) was slightly higher than that found in the lab strain *D. pulex* (1.56 ± 0.75 μg/g dw) exposed to the same conditions (Experiment I). Nonetheless, this difference was not statistically significant, indicating that both strains have a similar accumulation of Se under Lake Miwasin exposure conditions. Moreover, the Se concentration in daphnids collected directly from Lake Miwasin (2.15 ± 0.28 μg/g dw) fell within the range of Se in *D. pulex* (LM) exposed to LMW and fed with ALM under laboratory settings (Figure 3.3). This indicates that our lab strain of *D. pulex* is an appropriate model for native *D. pulex* (LM) and that our laboratory exposure conditions accurately simulated exposure conditions in Lake Miwasin.

Previous investigations have explored Se accumulation by *Daphnia* exposed to low, environmentally relevant levels of Se (<10 μg Se/L, as selenite or selenate) and found a wide

range of whole-body concentrations (0.15 to 6.04 µg Se/g dw) (Graves et al., 2019; Lo, 2014; Muscatello et al., 2008; Ogle & Knight, 1996). For example, Graves et al. (2019) found that Cladocera accumulated Se from 2.2 to 3.5 µg/g dw in boreal lake limnocorrals spiked with 0.12 µg/L selenite. Under laboratory conditions, *D. magna* exposed to lab media spiked with 5 µg/L Se (as selenate) accumulated 1.21 µg/g dw of Se (Ogle and Knight, 1996), and *D. magna* exposed to a lab media and food spiked with 10 µg Se/L (as selenate) accumulated 0.15 µg/g dw of Se (Lo, 2014). Collectively, these and other studies demonstrate that Se accumulation by *Daphnia* is influenced by Se speciation (selenate versus selenite), exposure duration, route of exposure (spiked water and/or food exposures), and the presence of modifying factors (e.g., sulfate).

In particular, exposure to low concentrations of selenate, the predominant species in Lake Miwasin, yields reduced accumulation of Se compared to selenite. Franz et al. (2011) found that *Chironomus dilutus* larvae significantly accumulated Se from the selenite and seleno-DL-methionine compared to the control and selenate treatments. Therefore, knowledge of Se speciation is very important in assessing Se biodynamics in freshwater aquatic ecosystems.

3.4.4 Selenium guideline for aquatic dietary tissue

In addition to water quality guidelines for the protection of aquatic life, the BCMOE (2014) proposed an interim Se guideline for invertebrate tissue (4 µg/g dw). This is considered a measure of dietary Se that links its transfer from primary consumers to higher trophic levels (e.g., fish). In this study, highest Se bioaccumulation from both dissolved and dietary exposure routes were 3.47 ± 0.13 µg/g dw in lab strain *D. pulex* (from selenate), 2.04 ± 0.48 µg/g dw in native *D. pulex* (LM) exposed to LMW under laboratory conditions, and 2.15 ± 0.28 µg/g dw in adult *D. pulex* collected directly from Lake Miwasin. These levels are below the BC MoE tissue guideline, suggesting limited risk to higher consumer species in Lake Miwasin.

3.6 Conclusions

This is the first study that investigates Se bioaccumulation in AOS end pit lakes in northern Alberta, Canada. It demonstrated that, when exposed to concentrations of Se relevant to Lake Miwasin, a pilot-scale end pit lake, both lab and native (LM) strains of *D. pulex*, do not bioaccumulate Se above the BCMOE interim guideline (4 μg/g dw) for invertebrate tissue. Therefore, the risk to higher trophic level organisms in LM, and potentially other systems with similar water chemistry and dissolved Se concentrations, appear limited. It is likely that *D. pulex* uses physiological mechanisms to regulate internal Se concentrations at the selenate exposure levels used in this study. However, this is speculative and warrants further investigation. Selenium bioaccumulation is influenced by various factors including Se speciation, route of exposure (water or food), the presence of exposure and toxicity modifying factors (e.g., sulfate), and duration of exposure. While beyond the scope of this study, further investigation into the interplay of these factors would benefit the predictability of Se uptake in *Daphnia* in future, larger AOS pit lakes.

CHAPTER 4: GENERAL DISCUSSION

4.1 Overall summary

The present study was undertaken as part of a larger research project that aims to develop novel and site-relevant tests to monitor environmental changes concerning the limnology and toxicity to aquatic organisms of water from a pilot scale pit lake, Lake Miwasin, in the Athabasca Oil Sands region. Specifically, the objectives of this thesis include (i) the assessment of the potential toxicity of Lake Miwasin water and sediment porewater to saline-acclimated and unacclimated *Daphnia* species, and (ii) the evaluation of the bioaccumulation of selenium via dissolved and dietary exposure routes by lab-cultured *D. pulex* exposed to spiked Se (as selenate) and native *D. pulex* exposed to Lake Miwasin surface water.

Overall, there was no observed acute or chronic toxicity of Lake Miwasin water to *Daphnia*. However, Lake Miwasin pore water was acutely toxic to lab-cultured *D. magna* and *D. pulex*, and to native *D. pulex* collected from Lake Miwasin. A toxicity identification evaluation (TIE) Phase I investigation revealed that metals and ammonia are the major classes of contaminants of potential concern with respect to the observed toxicity of Lake Miwasin pore water to daphnids. Further investigation is required to confirm these results. Secondly, Se bioaccumulation by both lab-cultured and native *D. pulex*, exposed to Se via dissolved and dietary sources, reached levels below the tissue Se guideline (4 μg/g dw) recommended by the British Columbia Ministry of Environment (BCMOE, 2014). These results suggest that, although measured dissolved Se concentrations are above the CCME guideline for long-term protection of aquatic life (1 μg/L), *D. pulex* may not transfer enough Se to higher trophic level organisms to cause Se toxicity, at least under the present conditions in Lake Miwasin.

4.2 Applicability

Lake Miwasin is a demonstration pit lake that is part of a pilot-scale reclamation project (~15 years duration) aimed at creating a functional lake using treated tailings overlain with a blend of treated OSPW, consisting of coagulated tailings pond water and water expressed from the treated tailings deposit, and freshwater from the Lake Miwasin upland watershed and a nearby industrial pond. The intent was to reclaim these materials while creating a self-sustaining boreal ecosystem. The configuration and materials used to create Lake Miwasin resulted, in the early years (2019-2021) in a seasonally stratified lake (dimictic) often with denser and saltier water near the bottom overlayed with less saline water. Because the treated tailings continued to consolidate and express pore water during the study period, near-bottom water typically had elevated concentrations of various dissolved constituents of concern and represented a worse-case exposure scenario for pond organisms. This study tested surface and pore water samples collected in 2021, the third year since the lake formation. Given that Lake Miwasin is experiencing temporal changes in water quality and has started to establish biological communities, our results reflect the conditions during the period of investigation (October 2021).

Daphnia, small freshwater crustaceans, are ideal test subjects for toxicity studies due to their ease of culturing and short life cycle. They are also sensitive to changes in water quality, offering valuable insights into the potential effects of contaminants on aquatic ecosystems. In this thesis, *Daphnia* species were used as test organisms to assess the toxicity of water and pore water from Lake Miwasin, as well as the potential for Se bioaccumulation. In 2019, *D. pulex* was discovered in Lake Miwasin, making this organism particularly relevant to this research. Additionally, in 2021, this specific strain was cultured under lab conditions and subsequently used in pore water toxicity testing and Se bioaccumulation exposures. Therefore, the results are not only comparable across various species/strains but also reflective of the particular sensitivities present in Lake Miwasin.

The findings presented here can assist environmental managers in enhancing the monitoring program in the pilot-scale Lake Miwasin, as well as in making decisions about the overall reclamation process for future pit lakes. From a toxicological perspective, more attention should be placed on sediment in the monitoring program since toxicity tests showed that pore water was acutely toxic to lab strains *D. magna*, *D. pulex,* and native *D. pulex*. Although these organisms are pelagic and largely remain in the water column, they may be affected by sediment pore water when treated tailings in sediments are consolidating and pore water is expressed (in the early years after lake formation) during water column stratification, or potentially during a resuspension event. Chronic exposure of saline-acclimated and unacclimated *Daphnia* species to pore water did not show differences when compared to the reconstituted saline water used as a control. This lack of distinction is likely due to the salinity levels in the test waters being above the physiological tolerance limits of the tested *Daphnia* species. Since salinity in Lake Miwasin could in itself be a stressor for salt-sensitive species, it is recommended that salinity be considered an important factor in the interpretation of all toxicity tests.

Based on Se bioaccumulation experiments, our results suggest that *D. pulex* may not transfer high enough levels of Se to higher trophic levels. However, changes in the water chemistry of Lake Miwasin could change Se speciation and/or sulfate levels, which appeared to be the main factors governing Se bioaccumulation by *D. pulex*. Even so, it is recommended to keep monitoring water chemistry for total Se and Se speciation and to consider the ameliorating effect of sulfate when evaluating potential Se toxicity in Lake Miwasin and other future pit lakes.

4.3 Limitations

4.3.1 Acclimation of *Daphnia* to saline conditions of pore water

Daphnia magna is a freshwater invertebrate frequently used in toxicity tests (ECCC, 1990, 2000; OECD, 2004, 2012; US EPA, 1996) and it has shown some tolerance to salinity in

previous studies (Arnér & Koivisto, 1993; Huang et al., 2022; Martínez-Jerónimo & Martínez-Jerónimo, 2007; Teschner, 1995). Reconstituted saline pore water (RSP) was prepared as a control solution to simulate the brackish conditions of Lake Miwasin pore water. For acclimation to saline control waters, daphnids were assumed to adjust their salinity tolerance to RSP in long-term exposures; however, they seemed to be flexible only within certain physiological limits.

The results of this thesis suggest that the tested *Daphnia* species could survive in the elevated levels of salinity in RSP, but their reproductive performance was influenced by the RSP controls regardless of whether organisms were acclimated to RSP or not. We observed reduced levels of offspring and delayed reproduction for RSP-acclimated *Daphnia* species when they were exposed to the RSP controls (3367 μS/cm) in 12-day exposures, resulting in no significant differences in comparison to the pore water treatments.

In general, aquatic animals can adapt to changes in salinity through two pathways: (i) they allow their internal conditions to adjust to the external environment through mechanisms of conformity, and (ii) they tend to make their internal conditions unaffected by the external environment via osmoregulation (Wilmer, 2005). Teschner (1995) and Arnér and Koivisto (1993) observed that *D. magna* could regulate its internal level of ions to a certain extent, beyond which it would switch to being an osmoconformer. The osmoconformer response in *Daphnia* species may explain why salinity acclimation did not seem to have a significant effect. For instance, Bezirci et al. (2012) found that daphnids could not mitigate the adverse effects induced by increasing salinity, leading to smaller body sizes and consequently fewer eggs produced per brood, ultimately leading to a population decline. Therefore, a limitation of this thesis is that the *Daphnia* species used for chronic toxicity testing lacked physiological tolerance to levels of salinity found in Lake Miwasin pore water. However, from a regional perspective, the fact that *D. pulex* is a natural resident acclimated to the current range of salinity

in Lake Miwasin implies that they adopt strategies to stay away from higher saline zones or that bottom water does not reach the salinity of pore water.

4.3.2 Porewater availability for TIE

Toxicity Identification Evaluation (TIE) Phase I results implicated ammonia and metals as the major classes of potential contaminants responsible for the toxicity observed in Lake Miwasin sediment porewater. However, further confirmation of these contaminants as the true causes of toxicity needs to be completed. A complete TIE investigation has three phases to focus on the particular class of chemicals and add other pieces of evidence to confirm the identity of the toxicants (US EPA, 2007). In this thesis, the extraction of porewater from sediments was the most time-consuming and labor-intensive work. Since the pore water: sediment recovery ratio was ~1:3, its extraction required larger volumes of sediment which was in limited supply. Although some adjustments were made to reduce the volumes of test waters in sediment porewater exposures, the three TIE phases involve several treatments and ultimately more volumes of sediment porewater. Therefore, the low availability of sediment samples prevented the pursuit of TIE Phase II and III tests.

4.3.3 Factors that modify selenium uptake by aquatic organisms

Different factors can combine to influence Se uptake on *Daphnia* (Janz et al., 2010). Among those factors, sulfate has shown decreased accumulation of selenate by *D. magna* due to a sulfate-selenate antagonism interaction (Hansen et al., 1993; Lo, 2014; Ogle & Knight, 1996). The mechanisms involved in such interaction can start at the absorption stage (Hansen et al., 1993), where sulfate would compete with selenate during the uptake processes (Ponton et al., 2020; Stewart et al., 2010). Furthermore, sulfate-selenium interaction may vary for different species. For example, *D. magna* and *Chironomus decorus* had different degrees of Se bioaccumulation under low sulfate concentrations (Hansen et al., 1993). This thesis involved

both dietary sources of Se and dissolved aqueous Se but has limitations in explaining more complex interactions.

Although sulfate may reduce the availability of selenate for aquatic invertebrates, no evidence exists that sulfate can completely block the absorption of selenate. Also, with the presence of other forms of Se (e.g., selenite or organic selenium species), the relationship between sulfate and selenate becomes complex and may result in a decrease in the impact of sulfate on the Se bioaccumulation in living organisms. Lake Miwasin is a pit lake where the aqueous constituents may experience seasonal and temporal variability that might affect Se bioavailability. However, this thesis did not evaluate the potential antagonistic or synergistic interactions of Se with other variables that might cause changes to its bioaccumulation and transfer to higher levels of the aquatic food chain.

4.3.4 Selenium speciation analysis

Given that the LMW treatment showed the highest Se uptake for both algae in comparison to the control and Se-spiked treatments (Se dietary source for experiments in Chapter 3), it is highly likely that LMW contains more bioavailable Se species, such as selenite. An analysis of Se speciation of LMW confirmed that organic Se (selenomethionine and seleno-L-cysteine) concentrations were negligible, while the oxyanions selenate and selenite displayed notable concentrations. A significant percentage of selenite (45%) (Table 3.1) was likely the explanation of why LMW dietary treatments showed higher Se bioaccumulation compared to the highest selenate treatments. Therefore, a better understanding of Se species, including both inorganic and organic Se forms, would help to better predict Se bioaccumulation patterns.

4.4 Future research opportunities

4.4.1 Selection of test organisms

McQueen et al. (2017) observed that aquatic organisms were more sensitive to OSPW in the following order: fish > aquatic invertebrates > macrophytes. These findings show the importance of the inclusion of different, yet representative, test species from different trophic levels to perform appropriate assessments. Lake Miwasin already has a freshwater community, including phytoplankton, zooplankton, and benthic macroinvertebrates (Hatfield, 2020). Given that *D. pulex* is an abundant primary consumer in Lake Miwasin, we thought that the inclusion of *Daphnia* spp., as test subjects in the experiments conducted in this study, could be valuable in terms of comparison. Several studies have demonstrated high variability in the toxicity of OSPW to *Daphnia* species (Lari, 2017; MacKinnon & Boerger, 1986; Zubot et al., 2012). This variability is attributed to the diverse contaminant profiles in OSPWs, which ultimately depend on several factors, including tailing's ore quality, its source, extraction processes, and age, making it challenging to compare toxicity data among OSPWs (Mahaffey & Dubé, 2016).

In the beginning, the Lake Miwasin water column did not show toxicity to *Daphnia* spp. Later, with the addition of pore water exposures, *Daphnia* spp. exhibited a poor performance at the salinity levels of the pore water. This made difficult the pore water assessment for reproductive endpoints. Nevertheless, future efforts to assess the toxicity of sediment and porewater from pit lakes could benefit from the inclusion of other suitable species such as *Chironomus* and *Hyalella*, which can tolerate higher salinities and may be more appropriate for this purpose. *C. dilutus* showed sensitivity to untreated OSPW through effects on pupation and adult emergence (Anderson et al., 2012). However, they also observed that salt control water did not cause any substantial effects on the behavior of *C. dilutus* compared to freshwater control organisms. Therefore, further research on reclamation ponds should involve testing with more species, possibly including species similar to those inhabiting brackish conditions, yet still sensitive to other contaminants.

4.4.2 TIE Phase II and III for Lake Miwasin pore water

The fundamental idea behind TIE is to utilize physical or chemical techniques to separate or alter the concentration or bioavailability of various toxic substances that may be present in a sample. Instead of relying on chemical analyses to detect changes in a matrix, a toxicity test is employed as an "indicator" to determine whether the manipulations have altered the sample's toxicity (US EPA, 2007). The TIE process can be divided into three distinct phases, Phase I (characterization), Phase II (identification), and Phase III (confirmation). In this study, we found that Lake Miwasin pore water resulted in acute toxicity to *D. magna* and *D. pulex*, and the subsequent TIE Phase I experiment identified the broad class of toxicants involved as ammonia and metals. Nevertheless, TIE Phases II and III investigations are needed to complete the assessment. During Phase II of the TIE process, more precise techniques can be employed to focus on the particular class of chemicals identified during Phase I. In TIE Phases II, the objective is to separate the toxicant(s) responsible for the toxicity from other chemicals present in the sample, thereby simplifying the sample for chemical analysis. In TIE Phase III, additional evidence is gathered to establish a convincing case that the suspected toxicant is indeed responsible for the observed toxicity. This is a crucial step before implementing any management strategies to address the problematic chemicals (US EPA, 2007).

There are research opportunities to further confirm whether ammonia and/or metals are responsible for the toxicity observed here. To verify that the toxicity of a sample of zeolite-treated porewater is caused by ammonia, one can add ammonia to the sample to restore the initial ammonia concentrations. It can demonstrate that the sample with added ammonia exhibits toxicity with the same magnitude as the original sample. In the case of metal toxicity, our study did not include adjusted pH tests, which may help confirm the toxicity of cationic metals. Other methods to investigate metal toxicity can include direct chemical analysis of metals in unfiltered pore water samples and solid phase of sediments, cation exchange resin, and sulfide addition.

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APPENDIX

Table S 1. Summary of test organisms used in experiments.

Test	Lab strains		Native strains	
	D. magna	*D. pulex*	*Daphnia sp.* (HL)	*D. pulex* (LM)
Salinity tolerance	✓	✓	✓	✓
Lake Miwasin water (LMW)	✓	✓	✓	
Lake Miwasin pore water (LMP)	✓	✓		✓
Toxicity identification evaluation (TIE)	✓			✓

Table S 2. Reconstituted saline water (RSW) and pore water (RSP) preparation.

Salt reagent	Grams in 1 L RSW	Grams in 1 L RSP
Sodium Chloride (NaCl)	0.4	0.8
Sodium Bicarbonate ($NaHCO_3$)	0.3	0.6
Potassium Sulfate (K_2SO_4)	0.1	0.2
Sodium Sulfate (Na_2SO_4)	0.3	0.5

Table S 3. Summary of the chronic test exposures to LMW and LMP for all test organisms, acclimated and unacclimated, lab strains *D. magna* and *D. pulex*, and native strains *Daphnia sp.* (HL) and *D. pulex* (ML). RSW = reconstituted saline water; LMW = Lake Miwasin water; RSP = reconstituted saline porewater; LMP = Lake Miwasin porewater; A = acclimated; NA = unacclimated.

	LMW exposure (21 days)				**LMP exposures (12 days)**							
	Control RSW		**LMW 100% (v/v)**		**Control RSP**		**LMP 12.5% (v/v)**		**LMP 25% (v/v)**		**LMP 50% (v/v)**	
	A	**NA**	**A**	**NA**	**A**	**NA**	**A**	**NA**	**A**	**NA**	**A**	**NA**
Parent survival at the end of the chronic test (%)												
D. magna	100	100	100	100	100	100	--	--	80	80	20	40
D. pulex	80	80	80	60	100	80	40	80	0	0	--	--
Daphnia sp (HL)	80	--	100	--	--	--	--	--	--	--	--	--
D. pulex (LM)	--	--	--	--	100	100	40	60	20	20	--	--
Mean time to produce first brood (days)												
D. magna	7.8 ± 0.6	7.7 ± 0.7	7.4 ± 0.5	7.4 ± 0.5	7.2 ± 1.1	8 ± 0	--	--	7.5 ± 1	8.5 ± 1	8	9 ± 1.4
D. pulex	7.4 ± 0.8	7.6 ± 0.5	7.3 ± 0.5	7.0 ± 0	9.6 ± 0.9	9.5 ± 1	4 ± 5.7	8 ± 2.8	--	--	--	--
Daphnia sp (HL)	6.3 ± 2.8	--	6.2 ± 1.2	--	--	--	--	--	--	--	--	--
D. pulex (LM)	--	--	--	--	6.4 ± 0.9	6 ± 0	6 ± 0	6 ± 0	8	6	--	--
Mean offspring produce per brood (number of neonates)												
D. magna	15.9 ± 2.3	17.3 ± 1.4	16.9 ± 1.7	16.9 ± 2.1	6.7 ± 1.4	6 ± 1.5	--	--	5.9 ± 2.1	6.4 ± 1.4	8	3.3 ± 2.5
D. pulex	9.9 ± 1.3	12.1 ± 0.9	11.9 ± 2.3	12.4 ± 2.9	8.9 ± 3.1	11.4 ± 1.9	1.5 ± 2.1	3.1 ± 1.3	--	--	--	--
Daphnia sp (HL)	5.2 ± 2.7	--	3.7 ± 1.3	--	--	--	--	--	--	--	--	--
D. pulex (LM)	--	--	--	--	3.8 ± 1.7	5.4 ± 0.5	4.7 ± 1.6	5 ± 0.9	2	5	--	--
Mean total live offspring (number of neonates)												
D. magna	97 ± 12.7	93.3 ± 13.9	98.7 ± 8.3	100.4 ± 11	14 ± 4.5	9 ± 1.9	--	--	9.5 ± 4.1	9.5 ± 3.9	16	4 ± 1.4
D. pulex	71 ± 8	84.8 ± 6.1	84.4 ± 22	90.5 ± 27	19.2 ± 5.6	25 ± 1.2	1.5 ± 2.1	6.3 ± 5.1	--	--	--	--
Daphnia sp (HL)	20 ± 10.5	--	14.2 ± 8.3	--	--	--	--	--	--	--	--	--
D. pulex (LM)	--	--	--	--	12.6 ± 5.2	21.6 ± 1.8	18.7 ± 6.4	18.5 ± 1	2	20	--	--

	LMW exposure (21 days)				LMP exposures (12 days)							
	Control RSW		**LMW 100% (v/v)**		**Control RSP**		**LMP 12.5% (v/v)**		**LMP 25% (v/v)**		**LMP 50% (v/v)**	
	A	**NA**	**A**	**NA**	**A**	**NA**	**A**	**NA**	**A**	**NA**	**A**	**NA**
Parent survival at the end of the chronic test (%)												
D. magna	100	100	100	100	100	100	--	--	80	80	20	40
D. pulex	80	80	80	60	100	80	40	80	0	0	--	--
Daphnia sp (HL)	80	--	100	--	--	--	--	--	--	--	--	--
D. pulex (LM)	--	--	--	--	100	100	40	60	20	20	--	--
Mean time to produce first brood (days)												
D. magna	7.8 ± 0.6	7.7 ± 0.7	7.4 ± 0.5	7.4 ± 0.5	7.2 ± 1.1	8 ± 0	--	--	7.5 ± 1	8.5 ± 1	8	9 ± 1.4
D. pulex	7.4 ± 0.8	7.6 ± 0.5	7.3 ± 0.5	7.0 ± 0	9.6 ± 0.9	9.5 ± 1	4 ± 5.7	8 ± 2.8	--	--	--	--
Daphnia sp (HL)	6.3 ± 2.8	--	6.2 ± 1.2	--	--	--	--	--	--	--	--	--
D. pulex (LM)	--	--	--	--	6.4 ± 0.9	6 ± 0	6 ± 0	6 ± 0	8	6	--	--
Mean offspring produce per brood (number of neonates)												
D. magna	15.9 ± 2.3	17.3 ± 1.4	16.9 ± 1.7	16.9 ± 2.1	6.7 ± 1.4	6 ± 1.5	--	--	5.9 ± 2.1	6.4 ± 1.4	8	3.3 ± 2.5
D. pulex	9.9 ± 1.3	12.1 ± 0.9	11.9 ± 2.3	12.4 ± 2.9	8.9 ± 3.1	11.4 ± 1.9	1.5 ± 2.1	3.1 ± 1.3	--	--	--	--
Daphnia sp (HL)	5.2 ± 2.7	--	3.7 ± 1.3	--	--	--	--	--	--	--	--	--
D. pulex (LM)	--	--	--	--	3.8 ± 1.7	5.4 ± 0.5	4.7 ± 1.6	5 ± 0.9	2	5	--	--
Mean total live offspring (number of neonates)												
D. magna	97 ± 12.7	93.3 ± 13.9	98.7 ± 8.3	100.4 ± 11	14 ± 4.5	9 ± 1.9	--	--	9.5 ± 4.1	9.5 ± 3.9	16	4 ± 1.4
D. pulex	71 ± 8	84.8 ± 6.1	84.4 ± 22	90.5 ± 27	19.2 ± 5.6	25 ± 1.2	1.5 ± 2.1	6.3 ± 5.1	--	--	--	--
Daphnia sp (HL)	20 ± 10.5	--	14.2 ± 8.3	--	--	--	--	--	--	--	--	--
D. pulex (LM)	--	--	--	--	12.6 ± 5.2	21.6 ± 1.8	18.7 ± 6.4	18.5 ± 1	2	20	--	--

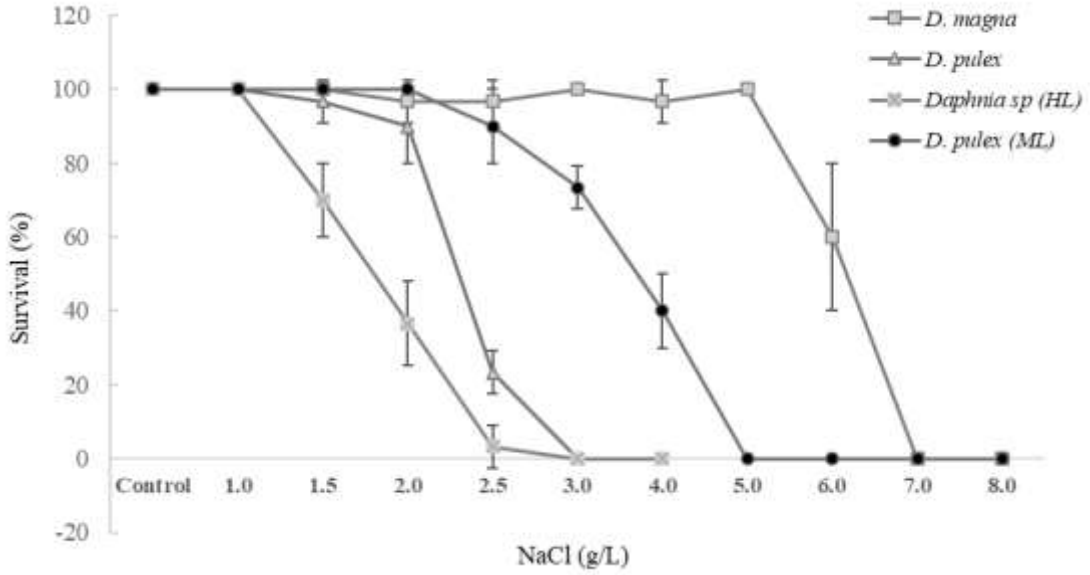

Figure S 1. Dose response curves for sodium chloride (NaCl) to the standard laboratory test species, *D. magna* and *D. pulex*, and two native zooplankton species, *Daphnia sp*. and *D. pulex*, collected in Humboldt Lake (HL) and Lake Miwasin (LM), respectively. Each data point represents the percent survival (mean ± SD; n = 10) at each NaCl concentration.