

**DELINEATING EFFLUENT EXPOSURE AND ASSOCIATED RISK TO AQUATIC  
INVERTEBRATES DOWNSTREAM OF A NORTHERN SASKATCHEWAN  
URANIUM MINE**

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By

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## ABSTRACT

Treated effluent from the McClean Lake uranium milling operation in northern Saskatchewan is released into the east basin of McClean Lake, which could potentially cause a variety of both chemical and physical disturbances to the aquatic ecosystem. This study aimed to delineate diluted effluent exposure focusing on combined metals and major ions using autonomous sensor technology, identify the associated risk to aquatic invertebrates, and determine the potential effect of that risk on macroinvertebrate communities within McClean Lake. Autonomous sensor units were deployed at ten locations in and upstream of McClean Lake. Water, sediment and benthic macroinvertebrates were also collected at the same monitoring locations. Complementary surface water was collected from selected sites to perform bioassays with larvae of the midge *Chironomus dilutus*. Results indicated temporal and spatial variations in effluent exposure based on sensor electrical conductivity (EC) measurements in the McClean Lake east basin. Individual Hazard Quotients (HQs) for water ranged from ‘moderate’ (0.40 – 0.69) to ‘very high’ (>1) for silver, cadmium, arsenic, selenium, mercury, iron and thallium. At all sites, major ions risk was <1. Individual HQs for sediment registered ‘moderate’ (0.40 – 0.69), ‘high’ (0.7 – 0.99) and ‘very high’ (>1) values for vanadium and cadmium. The cumulative risk in water and sediment for all metals combined was >1 at some sites in Vulture Lake (discharging into McClean Lake) and McClean Lake. More detailed estimation of aqueous selenium and arsenic risk, the only two metals with good correlation with sensor EC data, indicated that their 90th percentile HQ values were <1 in McClean Lake, suggesting that these contaminants of potential concern do not represent a significant direct risk to aquatic invertebrates. The metrics of macroinvertebrate communities (total abundance and Margalef index (MI)) did not follow the diluted effluent pattern. The final model from a Generalized Additive Modelling (GAM) exercise confirmed that EC, selenium, and chloride in water, and total organic carbon and cadmium in sediment are key elements that collectively may have influenced macroinvertebrate community composition as measured by MI at the study sites. Finally, across all test endpoints in the bioassays, exposure to lake water from Vulture Lake and McClean Lake had no statistically significant effects on *C. dilutus*.

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## LIST OF ABBREVIATIONS

API	Application Programming Interface
CCA	Canonical-correlation analysis
CCME	Canadian Council of Ministers of the Environment
CCREM	Canadian Council of Resource and Environment Ministers
CWQG	Canadian Water Quality Guidelines
COPC	Constituents of potential concern
DO	Dissolved oxygen
DOC	Dissolved organic carbon
EC	Electrical conductivity
GAM	Generalized additive model
GC-MS	Gas chromatography-mass spectrometry
HPLC	High-performance liquid chromatography
HQ	Hazard quotients
ICP-MS	Inductively coupled plasma mass spectrometry
IDE	Integrated Development Environment
ISQG	Interim sediment quality value
LC-MS	Liquid chromatography mass spectrometry
MI	Margalef index
NTU	Nephelometric turbidity unit
ORP	Oxidation-reduction reactions
PEL	Probable effect level
RMSE	Root mean squared error
SD	Standard deviation
SE	Standard error
SQV	Sediment quality value
S/V TEMS	Sink/Vulture treated effluent management system

SWQG	Surface Water Quality Guidelines
SWQO	Surface Water Quality Objectives
TBC	Toxicological benchmark concentration
TDS	Total dissolved solids
TOC	Total organic carbon
US EPA	United States Environmental Protection Agency
UV-VIS	Ultraviolet–visible spectroscopy
WTP	Water treatment plant

## **PREFACE**

This thesis was prepared in a manuscript style format following the University of Saskatchewan College of Graduate Studies and Research guidelines. Accordingly, Chapter 1 of this thesis is a general introduction to the work conducted while Chapters 2 and 3 have been prepared for peer-reviewed scientific journals, and Chapter 4 is a general discussion pertaining to the above chapters. Therefore, there may be some overlap and redundancy of background information. All references are cited at the end of the thesis while supporting information for the above chapters are within the appendices.

## **CHAPTER 1: GENERAL INTRODUCTION**

### **1.1. The McClean Lake mine operation**

The McClean Lake uranium mining and milling operation is located in the Athabasca Basin Ecoregion in Northern Saskatchewan, Canada, approximately 350 km northeast of La Ronge near Wollaston Lake in the Boreal Shield Ecozone (Figure 1.1). Orano Canada Inc. (formerly AREVA Resources Canada) is the majority owner and operator of the McClean Lake Operation (70%), followed by Denison Mines Inc. (22.5%) and OURD Canada Co. Ltd. (7.5%). The McClean Lake mill processes undiluted, high-grade uranium ore, supplied by the Cigar Lake mine operated by Cameco Corporation (average grade 15%  $U_3O_8$ ). At the Cigar Lake operation, the ore is extracted from the orebody, mixed into a slurry, and trucked to the McClean Lake mill site. The McClean Lake mill can produce 10886.2 tons of yellowcake (uranium concentrate) annually, and in 2020 achieved total production of 10.1 million pounds  $U_3O_8$  (Orano, 2021).

The current configuration of the major infrastructure comprising the McClean Lake operation consists of three main areas (AREVA, 2016a):

- The JEB area includes the permanent camp, the McClean Lake mill, the JEB tailings management facility and the JEB water treatment plant (WTP).
- The Sue mining area includes the mined-out Sue A/C, Sue B, Sue E pits, and the Sue WTP.
- The Sink/Vulture Treated Effluent Management System (S/V TEMS) includes Sink Reservoir and its control structure, Vulture Lake and the effluent diffuser system that discharges effluent into the east basin of McClean Lake.

### **1.2. Uranium mining and milling processes**

The McClean Lake mill currently receives all the ore it processes from the Cigar Lake mine, which is an underground mine operated by Cameco Corporation. Ore is ground up and thickened in underground processing circuits and pumped to the surface in slurry form. At the surface, the ore is loaded into special containers and transported by truck to the McClean Lake mill for processing (AREVA, 2016a).



**Figure 1.1.** Location of the McClean Lake operation (red square).

(From, Canadian Nuclear Safety Commission – Comprehensive Study Report, 2012)

Milling processes (Figure 1.2) start by extracting the uranium from the ore (average grade of 18%) in a process called leaching, in which the ore is dissolved into a sulphuric acid solution containing ferric sulphate and hydrogen peroxide. This is a non-selective process, meaning that



other naturally occurring elements such as iron, arsenic and molybdenum are also dissolved. Then, counter-current decantation washes the uranium solution from the waste solids in the leached residue. The waste solids, containing a very small amount of soluble uranium, are sent to a Tailings Neutralization circuit. Clarification removes suspended solids from the uranium solution after decantation, then a process called solvent extraction creates a purified and more concentrated uranium solution. Subsequently, precipitation converts the uranium from a solution to 60% solid after going through a centrifuge wash. Ammonia is used to adjust the pH to bring the uranium out of the solution, producing ammonium diuranate solids. This form of uranium concentrate is yellow, giving it the term yellowcake ( $U_3O_8$ ). After that, calcining dries the yellowcake at a high temperature to a black powder called uranium oxide concentrate to be completely dried in a calciner. The final product contains about 85% uranium with less than 0.5% moisture (AREVA, 2016a).



**Figure 1.2.** Schematic of the uranium milling process.

### 1.3. Wastewater management and treatment

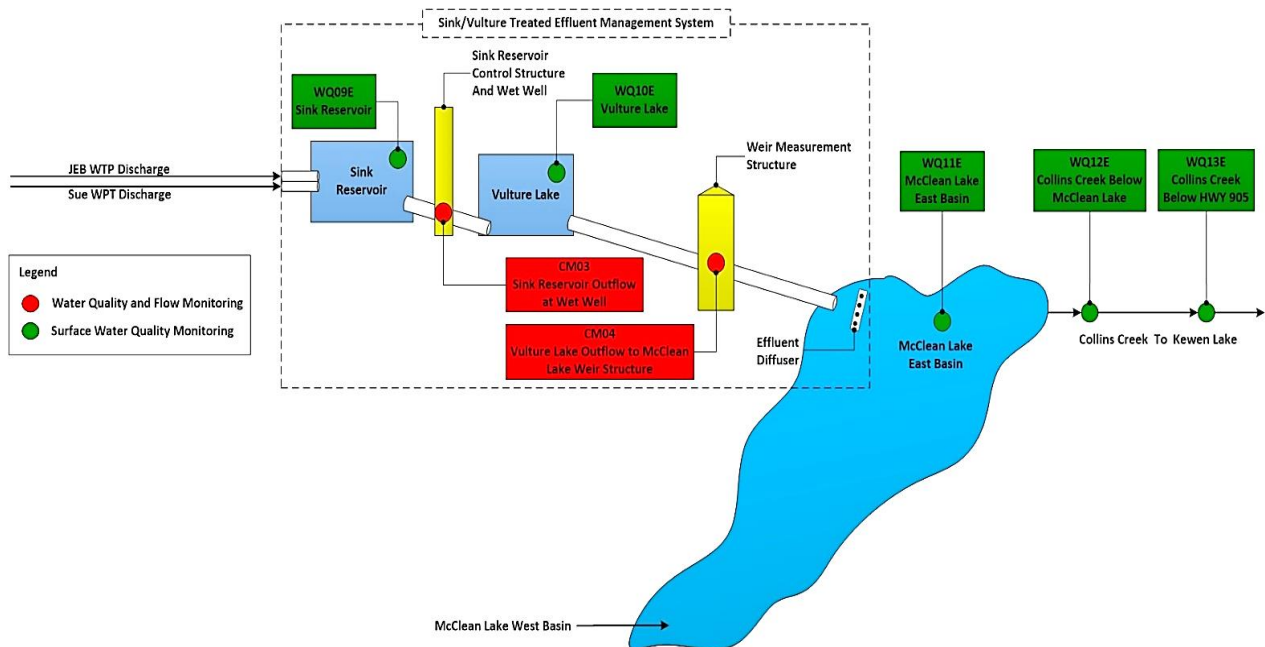
The wastewater treatment and management at the McClean Lake operation consists of the following facilities (AREVA, 2016a):

- **The JEB water treatment plant** receives contaminated water feeds mainly from the mill and JEB tailings management facility areas. The plant removes radionuclides, dissolved metals and suspended solids and then discharges treated effluent to the Sink/Vulture Treated Effluent Management System (S/V TEMS).
- **The Sue water treatment plant** receives contaminated water feeds from the Sue mine site. It removes radionuclides, dissolved metals and suspended solids and also discharges treated effluent to the S/V TEMS.
- **The Sink/Vulture Treated Effluent Management System (S/V TEMS)** is a central component of the McClean Lake operation wastewater management strategy. This system is

the final and common repository for all wastewater generated at the McClean Lake operation.

#### 1.4. Effluent discharge

Wastewater inputs to the S/V TEMS consist of treated effluents from the JEB WTP, the Sue WTP, and the JEB dewatering well system. The S/V TEMS consists of Sink Reservoir, Vulture Lake and all structures and connecting pipelines from Sink Reservoir to the east basin of McClean Lake (Figure 1.3). Water is discharged from Sink Reservoir through Vulture Lake and into McClean Lake, after which it enters Collins Creek (McClean Lake output) and eventually flows to Collins Bay in Wollaston Lake.



**Figure 1.3.** Sink/Vulture Treated Effluent Management System (from AREVA, 2016).

During the operational period, flows of treated water from the JEB and Sue WTPs and pit-perimeter dewatering wells are released to Sink Reservoir. Outflow from Sink Reservoir is controlled and subject to downstream flow conditions in Collins Creek. Releases from Sink Reservoir flow to Vulture Lake and then via a discharge pipeline to McClean Lake. Collins Creek flows from McClean Lake to Keweenaw Lake, continuing to Collins Bay of Wollaston Lake (AREVA, 2016a).

The discharge pipeline is equipped with a weir measurement structure to allow a flow diffuser to enhance the dispersion of treated effluent in the east basin of McClean Lake. The S/V TEMS provides a means of storing effluent, as required, to minimize effects to the downstream receiving environment while allowing water treatment to proceed (AREVA, 2016a).

## **1.5. McClean Lake background information**

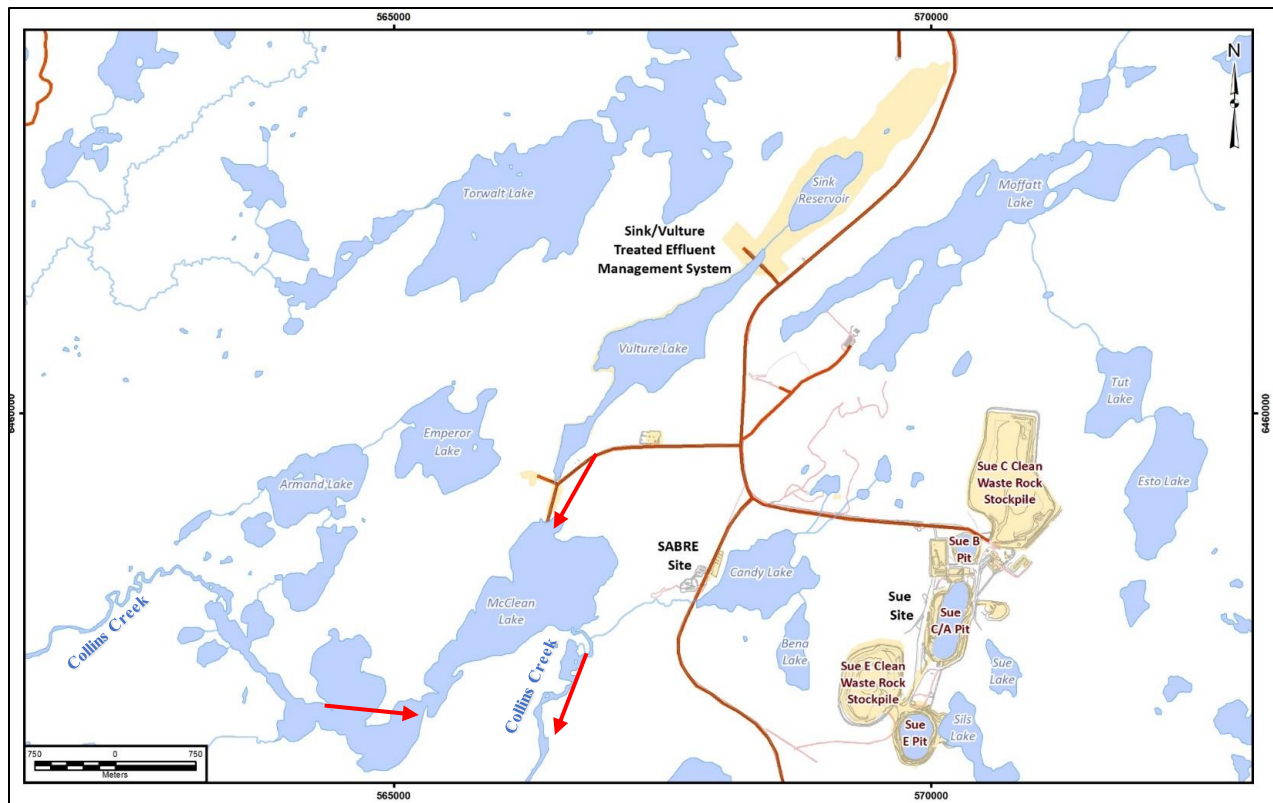
### **1.5.1. *Study sites characteristics***

McClean Lake is divided into two basins, an eastern and a western, with the division at the narrows, about midway along the long axis of the lake (Figure 1.4). The east basin of McClean Lake has a mean depth of 1.38 m, and the maximum measured depth is 3.65 m. Much of this basin is shallow, with about 53.7% of the total lake volume within the 0 to 1 m depth range. The total water volume in this basin is  $1.62 \times 10^6 \text{ m}^3$  and the surface area is 117.2 ha (AREVA, 2016a).

The West Basin of McClean Lake is similar to the east basin in volume ( $1.59 \times 10^6 \text{ m}^3$ ) and surface area (107.2 ha). The shoreline is much less regular, with several bays extending inland from the long axis of the basin. The western basin is quite narrow over much of its length and approximates a riverine system. Most of this basin is very shallow, with 48.8% of the lake volume occurring within the 0 to 1.0 m range. The maximum measured depth is 7.9 m (AREVA, 2016a). Finally, Vulture Lake is small and relatively shallow. It has a surface area of 73.5 ha and a volume of  $1.65 \times 10^6 \text{ m}^3$ . The mean and maximum depths of Vulture Lake are 2.25 m and 3.4 m, respectively. More than 40% of the total water volume is in the 0 to 1 m range (AREVA, 2016a).

### **1.5.2. *Surface water quality***

At the McClean Lake operation, the potential effects of effluent discharge associated with mining and milling activities depend primarily on the quantity and quality of discharge from the WTPs. The complete list of constituents of potential concern (COPC) at the McClean Lake operation includes (i) the following general water quality parameters: ammonia, chloride, fluoride, potassium, sulphate, and total dissolved solids (TDS); (ii) the metals/metalloids arsenic, cobalt, copper, lead, molybdenum, nickel, selenium, and zinc; and (iii) the radionuclides uranium, thorium-230, radium-226, lead-210, and polonium-210 (AREVA, 2016b).

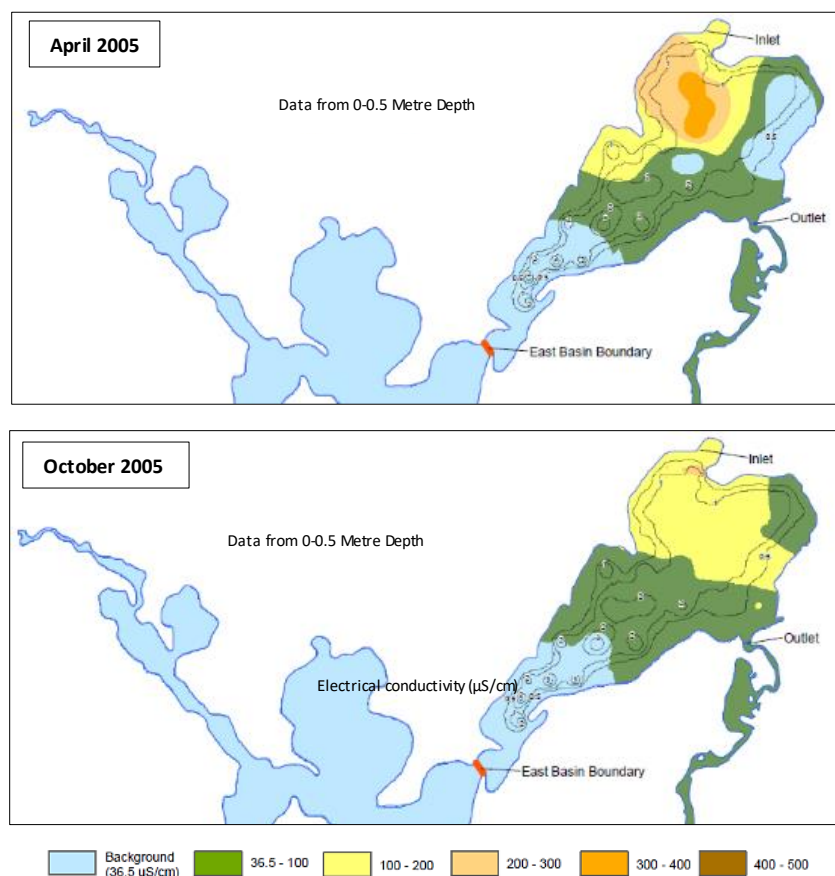


**Figure 1.4.** Location of Vulture Lake and McClean Lake within the mine lease. Red arrows show the flow direction of the water (from AREVA, 2016).

Between 2011 and 2015, there were some water quality monitoring programs implemented at the McClean Lake operation. The waterbodies sampled included McClean Lake west basin (reference site), Sink Reservoir outlet, Vulture Lake outlet, and McClean Lake east basin (primary exposure sites), and Collins Creek (downstream site). According to the monitoring programs, surface water quality within each primary exposure area generally showed stable or decreasing trends in COPC concentrations over time. Radionuclide levels were generally below or near the detection limits, and most parameters were below available guidelines. They concluded that the overall trend of decreasing COPC concentrations in the primary exposure area across the long-term dataset was a result of the status of the operation, which was in “care and maintenance” for most of the 2011 to 2015 assessment period, and was therefore releasing only smaller volumes of effluent with lower COPC concentrations to the S/V TEMS (AREVA, 2016a).

An additional study was conducted in 2005 to determine the extent and pattern of effluent distribution in the east basin of McClean Lake. The objective of this study was to delineate the

spatial extent of treated effluent in McClean Lake during the fall and winter seasons. Electrical conductivity was chosen as the parameter for mapping the effluent plume (Figure 1.5).



**Figure 1.5.** Plume delineation data for McClean Lake east basin in 2005 (from AREVA, 2016).

They concluded that seasonal effects were apparent in the spatial pattern of the effluent plume in the east basin of McClean Lake. Based on conductivity as an effluent tracer, under ice cover (April 2005), treated effluent concentrations appeared higher in the deeper areas and illustrated a distinct concentration gradient with depth. Complete water column mixing was evident during the open water season (October 2005), reflecting mixing through wind action and generally higher flows (AREVA, 2016a).

### **1.5.3. *Sediment quality***

The sediment chemistry monitoring program at the McClean Lake operation was developed and refined between 1999 and 2015. Sediments were collected from McClean Lake West Basin (reference site) and from Sink Reservoir, Vulture Lake, McClean Lake east basin, and Collins Creek as the primary exposure sites. The COPCs included calcium (major ion), metals and trace elements such as antimony, arsenic, barium, boron, cadmium, cobalt, copper, iron, lead, mercury, molybdenum, nickel, selenium, strontium, thallium, uranium, vanadium and zinc; and the radionuclides uranium, thorium-230, radium-226, lead-210, and polonium-210 (AREVA, 2016b).

The analysis of sediment chemistry data collected at the McClean Lake operation indicated that several COPCs had accumulated in the surficial sediment layer (0-2 cm) above their historical and reference values. The primary objective of the sediment sampling program was to evaluate changes in the sediment quality over time. They concluded that most COPCs were present at levels elevated above background conditions. They had either stabilized or displayed a slight decrease after higher levels were observed in 2005 or 2008 (AREVA, 2016b).

### **1.5.4. *Macroinvertebrate communities***

Operational monitoring of benthic invertebrate communities was performed at the McClean Lake operation in 1999, 2002, 2005, 2008, 2011, and 2015. The water bodies sampled included Sink Reservoir, Vulture Lake, McClean Lake east basin (exposure lakes) and McClean Lake West Basin (reference lake). In summary, the results indicated statistically detectable differences in macroinvertebrate abundance and richness (core values) in both Sink Reservoir and Vulture Lake compared to the West Basin of McClean Lake. However, the abundance and richness values in 2015 fell within the normal range of variation of values observed during the mine's baseline period and/or in reference waterbodies. Similarly, there was no evidence of changes in benthic invertebrate abundance or richness in the McClean Lake east basin. Benthic biomass was unusual in 2008, but returned to near normal values in 2011. In 2015, benthic biomass was slightly above the upper limit of normal in the McClean Lake east basin. However, there was no indication that the high biomass values in 2008 and 2015 reflect degraded conditions, given that the fauna remained diverse with a typical assortment of taxa.

## **1.6. Environmental concerns with uranium mining effluent**

Uranium mining and milling activities typically generate elevated concentrations of trace elements (e.g., arsenic [As], molybdenum [Mo], nickel [Ni], selenium [Se] and uranium [U]) and major ions (e.g., sulphate, ammonium) that can be released into the surrounding aquatic systems (Klaverkamp et al., 2002; Pyle et al., 2001; deRosemond et al., 2005; Muscatello et al., 2006; Muscatello & Janz, 2008) where they can affect the local biota. Especially, elevated concentrations of selenium (Se) have been reported in water, sediments and fish tissues downstream of uranium mining and milling operations in northern Saskatchewan, Canada (deRosemond et al., 2005; Muscatello et al., 2006). Once released into the aquatic environment, selenium can be accumulated through the food chain, reaching levels that can cause deleterious effects (e.g., impaired reproduction) in top predator fish species (Lemly, 1997).

From an ecotoxicological perspective, the cumulative effect of metals, metalloids and some none-metals (hereafter metals), as well as major ions in an effluent release could potentially represent a toxicological risk to aquatic organisms since cumulative toxicity has often been underemphasized. Thus, a major current interest of uranium mining companies is to understand the potential impacts of 'non-radioactive' components of their discharges. Generally, surface water and sediment are two main routes of contaminant exposure to biota, and hence possible causes of toxicity within effluent impacted areas. Surface waters within receiving environments are generally more representative of recent effluent releases than sediments. Similarly, surface waters typically act as the main source of contaminant exposure to pelagic organisms, but can also affect organisms that live at the water-sediment interface. On the other hand, the main compartment for the accumulation of metals within the environment is generally the sediments of receiving waters. Since metals do not degrade, their presence within receiving environments poses a long-term environmental risk because sediment contaminants could also be a source of metal redistribution after effluent discharges stop and surface water contamination drops (Robertson, 2006). Metal bioavailability is generally related to the composition of the sediment, the geochemical fraction of each metal in sediments at each site of interest, general water quality variables such as pH and redox status, and the feeding and digestive properties of the organisms of interest (Baumann & Fisher, 2011; Griscom et al., 2002; Tessier & Campbell, 1987).

Metals transported into an aquatic system are mainly incorporated into bottom sediments through adsorption, flocculation, and precipitation from the water column (Botte et al., 2007; Cheng et al., 2015). Metals that settle out of the water column are likely to be re-suspended and re-dissolved into pore water, from where sediment-associated metals can be released into the overlying water by diffusive fluxes (de Mora et al., 2004; Yu et al., 2012). Diffusive fluxes can result in a concentration gradient at the sediment-water interface, deteriorate the water's quality, and potentially cause secondary contamination to the water environment (Covelli et al., 1999; Li et al., 2015). During this process, pore water, either alone or in combination with sediment ingestion, plays the role of an intermediary pollutant exchange between sediment and overlying water. Some researchers have suggested that only free metal ions in interstitial water could directly produce biological effects (He et al., 1998; Blasco et al., 2000; Kalnejais et al., 2015).

Concerning macroinvertebrates, some studies have shown that the composition of some invertebrate communities changes due to exposure to uranium mining activities in Northern Saskatchewan (Kilgour et al., 2018). The number of benthic invertebrate taxa (richness) downstream of uranium mine/mill effluent releases points tends to be lower than the number of taxa observed in reference conditions. This means that benthic taxon richness is generally negatively correlated with measured sediment elements, especially when it comes to selenium and uranium concentrations (Kilgour et al., 2018). The general decreasing trend in taxon richness with increasing metal concentration or radionuclide activity is also observed in some reference lakes, implying that there also is a natural background influence of metal concentrations and radionuclide activity on benthic taxon richness at some locations (Kilgour et al., 2018). Occasionally, sediment metal concentrations at reference sites within northern Saskatchewan, which represent natural background conditions, exceed existing guidelines derived for use in Canada (e.g., CCME, 2011; Thompson et al., 2005).

Overall, uranium mining/milling effluent could theoretically cause toxicity to aquatic organisms through surface water and/or sediment environments. Surface waters are usually more representative of current or recent effluent releases, whereas sediments represent the accumulation of effluent-derived constituents over time.



### **1.7. Autonomous sensor systems for water quality monitoring**

As water quality and availability are emerging as a key challenge worldwide, there is a growing need for water quality monitoring across various areas, from drinking water, municipal and industrial wastewaters, to environmental waters (rivers, lakes, groundwater, and oceans). In this regard, the initial concept of an autonomous water quality monitoring system started with an online monitoring system including online automated equipment for data acquisition, networks for transmission, and software to analyze the data. Online monitoring aims to measure physicochemical parameters in waters (river, stream, lake, ocean, groundwater, industrial wastewater, urban drainage, etc.) in real-time (Dong et al., 2015). With a water quality monitoring system in place, there is a better chance to have a straightforward method to determine water quality status in real-time, quickly identify potential non-compliance issues in water quality, and improve system security (Papoutsas et al., 2010; Mutchek, 2013; Ramadan, 2012). Further, a remote water monitoring system would allow water managers to protect the integrity of water resources against any natural or anthropogenic threats, take immediate corrective and mitigation measures, and report the suitability of water for designated beneficial water uses. Such a real-time water monitoring network lays the foundation for greater environmental security and water resources management (Sallam & Elsayed, 2015).

Existing laboratory-based methods can be too slow to develop operational response and do not provide a level of protection in real-time. Besides, traditional spot/grab sampling is unlikely to give a good estimate of the true maximum and/or mean concentration for a particular physicochemical parameter in a water body, especially one with marked temporal variability in contaminant input. Therefore, when contaminant fluctuations occur, it is likely only to be detected through relatively high-frequency measurements, which would have the capability of detecting sporadic peaks of contaminant concentrations. In this context, autonomous sensors are capable of continuous measurements of parameters and thus would provide more frequent and up-to-date information and better coverage of long-term trends in fluctuations of contaminant concentrations (O'Flynn et al., 2010). The use of advanced monitoring sensor technologies and strategies means that proactive measures can be implemented to improve water quality (Greenwood et al., 2008).

A sensor node can be defined as a device containing the primary sensing element, a signal amplification unit, and a filtering system with dedicated software for data processing and compensation; wireless sensors are usually equipped with a wireless transmission element and a source of energy (e.g. batteries or solar panels). The primary sensing element is usually based on various materials' physical, chemical, and biological characteristics. Wireless sensors used for water quality monitoring are typically categorized as chemical, biological, and physical sensors (Dong et al., 2015). These include the J-Mar Biosentry™, a laser-based technology designed for the continuous online measurement of particles in water, the submersible UV-VIS scan measuring multiple water quality parameters. Gas chromatography-mass spectrometry (GC-MS), an automated system that can detect volatile trace organic micropollutants, liquid chromatography-MS (LC-MS), and high-performance liquid chromatography (HPLC) have also been used in an online capacity by water utilities. They can provide reliable information on micropollutants, particularly in water intake monitoring, near to real-time (Storey et al., 2011). Biological monitors, such as bacterial bioluminescence and fish monitors have been in use for many decades. TOXcontrol-(microLAN) is a real-time biological toxicity monitor used to measure toxicity in environmental samples and is based on the ability of *Vibrio fischeri*, a luminescent bacterium, to produce light as a by-product of its cellular respiration (Meighen, 1991). Bacteria react rapidly to toxicants changing their metabolism and therefore emitting a small amount of light.

Generally, autonomous sensor devices record physical or chemical parameters related to water quality, but they do not provide the source of contamination. Combining smart monitoring with a risk assessment approach for the early detection of water contamination may be helpful (Saab et al., 2019). Risk assessment provides an approach to determine the most probable causes of elevated risk related to toxicity or pollution. In the water industry, risk assessment can inform individuals and organizations about the nature and risk of their water and help inform how to mitigate those risks (Adimalla & Wu 2019; Li et al., 2016; Percival et al., 2000). The outcome of risk assessment helps in identifying priorities in corrective actions. A high-risk event requires an emergency response, while a low risk needs a lower priority of attention.

Several literature surveys on smart water quality monitoring systems have been done in recent years (Storey et al., 2011; Trescott & Park 2013; Nasirudin et al., 2011; Strobl & Robillard 2008; Gómez et al., 2011; Glasgow et al., 2004). For instance, in Lake Yahuarcocha in Ecuador,

key sensor locations were deployed to assess the influence of weather features, anthropogenic activities, and other non-point pollution sources (Jácome et al., 2018). In Lake Manzala, Egypt, real-time water quality monitoring was used to measure the regional and seasonal variations of some water quality parameters (Sallam & Elsayed, 2015). Huang and Chang (2003) provided an analysis of environmental informatics and systems analysis for autonomous water quality monitoring. Samsudin et al. (2018) developed a smart system in Malaysia to monitor water quality, measuring pH and turbidity to ensure clean water was continuously maintained. Saab et al. (2019) combined smart water monitoring with a risk assessment approach to ensure early detection of water contamination (turbidity and chlorine) in Strasbourg, France. Trescott and Park (2013) developed remote sensing models using Landsat satellite data to monitor algal blooms in Lake Champlain and across Canada and the USA. Similar technologies (smart grids) have also been used in smart water quality monitoring systems (Fang et al., 2012; Gao et al., 2012).

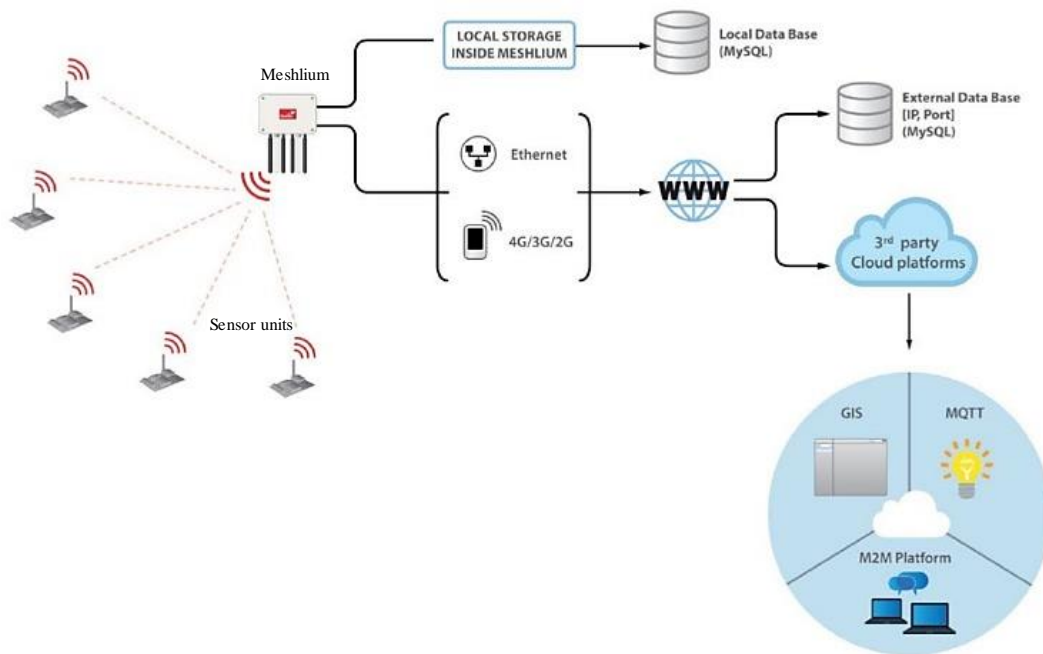
#### **1.7.1. *The Libelium Smart Water system***

The Libelium Smart Water® platform provides remote, real-time monitoring of various environmental compartments and parameters. The smart model for surface water quality monitoring (Smart Water units) works through the use of Waspote Plug & Sense sensor devices (Figure 1.6). The Smart Water units are autonomous, solar-powered, and programmed to take specific water quality measurements at specific times (Figure 1.7). They have been developed to facilitate remote environmental monitoring by deploying them in the field with attached probes (up to 6) that can measure pH, dissolved oxygen (DO), electrical conductivity (EC), oxidation reduction potential (ORP), turbidity and temperature (Libelium Technical guide, 2018).

Programming of the Smart Water units is performed within the Waspote Pro IDE (Integrated Development Environment) using the Waspote Pro API (Application Programming Interface) from Libelium. The Waspote IDE is the software development kit used to write and upload code (firmware) to the sensor units and monitor serial output (real-time measurements) and debugging. The Waspote API contains libraries necessary for compiling programs and is constantly updated whenever improvements are made. The software Waspote IDE v.38, provided by Libelium, is used as the template code for programming to measure ORP, EC, pH, DO, turbidity, temperature, and other variables.



**Figure 1.6.** Libelium Waspote Plug & Sense sensor device  
(from Libelium Technical guide, 2018).



**Figure 1.7.** Libelium IoT platform (from Libelium Technical guide, 2018).

Prior to deployment and use, probes are calibrated against standard solutions to ensure the accuracy and reliability of results. The Smart Water® units are connected to a computer via a USB cable, and the template code from the Waspote v.38 software is uploaded to each unit. According

to the Smart Water technical manual, the probes are individually connected to each unit and calibrated (Libelium, 2018). Values are measured in millivolts (mV) and then converted into the respective probe unit (i.e., C°, NTU, pH log scale, percent of dissolved oxygen,  $\mu\text{S}/\text{cm}$ ) by the instrument. After calibration, the values obtained are inserted into each node's programming code. Sensor probe measurements can subsequently be compared to parallel measures taken with calibrated hand-held meters allowing for further validation of reading from the sensor probes. The final code is based on the original template (Waspote v.38) with modifications to accommodate the specific research needs.

With respect to data collection, nodes communicate with a Libelium Meshlium® device, which acts as a Wi-Fi access point via a local cellular network (each unit requires a mobile SIM card and data plan). The Meshlium receives and parses the data frames from the sensors and data are uploaded to a Meshlium cloud connector (ThingSpeak™). The latter provides instant visualizations of data posted by sensors to the ThingSpeak cloud, which can be accessed from a computer or mobile device.

### **1.8. Importance of benthic macroinvertebrates**

Under the Metal and Diamond Mining Effluent Regulations, benthic macroinvertebrates are considered an indicator of fish habitat condition. Where indices of benthic community composition (e.g., abundance, richness, diversity) are shown to be impaired, fish habitat is considered impaired (Kilgour et al., 2005). Because most fish eat benthic organisms during at least part of their life cycle, benthic production influences fish production through bottom-up effects (Boisclair & Leggett 1989).

Generally, macroinvertebrate communities are monitored as they are an important part of the aquatic food chain and possess many characteristics of 'ideal' sentinel organisms. These characteristics include being largely sedentary in nature and sufficiently long-lived to allow simultaneous sampling of multiple age classes and developmental stages, thereby reflecting site-specific exposure to both water-borne and sediment-accumulated contaminants (Bonada et al., 2006). Additionally, benthic macroinvertebrate communities respond to multiple environmental factors, and there are many interactions among these factors. These include water quality (Clews et al., 2014; Miserendino & Masi 2010), substrate composition (Schröder et al., 2013), and the

distribution and abundance of macrophytes (Declerck et al., 2005). Water quality, hydrodynamic conditions, and substrate conditions are the main abiotic factors influencing benthic macroinvertebrate communities (Yi et al., 2018).

Invertebrate communities are useful for assessing ecosystem health, as they are common and widespread, with high species diversity and varying sensitivity to environmental disturbances (Rosenberg, 1993). The sensitivities of different macroinvertebrate taxa to pollution depend not only on their inherent physiological sensitivity, but also on their life history attributes and feeding behaviours (Giudici et al., 1988). Consequently, different species have considerably different pollution tolerances (Arimoro & Ikomi 2008). Thus, it is often possible to find some macroinvertebrate taxa-specific indicators for water quality assessment. Taxa-specific indicators refer to specific macroinvertebrate taxa, which tolerate a certain water quality condition, but do not exist in other water quality conditions (Xu et al., 2014). Some taxa-specific indicators have been reported. For instance, most species of Chironomidae are very tolerant to water pollution and can survive in very poor water quality (Langdon et al., 2006). Similarly, extremely high percentages of Oligochaeta, Arachnida, and Gastropoda are indicators of contamination from organic pollution (Nadushan & Ramezani 2011).

Fish are more commonly collected for bioaccumulation studies because they can provide an adequate tissue sample size for contaminant analysis. Also, they are often at the top of the aquatic food chain and therefore may contain the maximum contaminant concentrations in a specific aquatic system (humans and some terrestrial wildlife species may also eat them directly). However, the monitoring of resident fish may not be a suitable approach in many cases, and there are some distinct advantages to using benthic macroinvertebrates to monitor bioaccumulation (Cain et al., 1992; Poulton et al., 1995). Although there can be some problems when using invertebrates for bioaccumulation studies, including the necessity of using smaller tissue sample sizes and, in some cases, multiple species, invertebrates can be advantageous as a monitoring tool. First, they can accumulate high levels of metals and other elements such as selenium and arsenic. Second, they are relatively sedentary and represent exposure at the site of collection. Finally, as a food source, they can provide a means of transferring potentially toxic elements to higher trophic levels (Cain et al., 1992; Poulton et al., 1995).

Finally, there is a challenge to monitoring benthic invertebrates in lakes as it is often difficult to find suitable reference/unexposed lakes to mining operations that are good matches for lakes exposed to mining influences (Bonada et al., 2006; Mackey et al., 2013). Generally, the composition of benthic invertebrate communities among lakes changes predictably with average lake depth, depth of sample collection and substrate type, among a variety of other subtler influences (Brinkhurst, 1974; Saether, 1979). The results of monitoring programs that describe differences in fauna composition between reference and exposed lakes are often confounded with natural physical, chemical and biological characteristics. Regardless, monitoring of lakes is an important aspect of the regulatory compliance monitoring of active uranium mines and mills in Saskatchewan, Canada (Kilgour et al., 2018).

### **1.9. Rationale for using *Chironomus dilutus* in bioassays**

Chironomids are a group of freshwater non-biting flies, commonly referred to as midges, widespread throughout Canadian aquatic ecosystems. They have four life stages: egg, larval, and pupal stages (all aquatic), and then emerge into terrestrial adults. The entire lifecycle spans approximately 25 to 40 days, depending on species and environmental factors, although they spend most of their life as sediment-dwelling larvae, most of which are detritivores. The larvae of *Chironomus dilutus* comprise an important component of the diet of fish and waterfowl. Therefore, they have become a standardized test species in toxicity tests of contaminated sediment and water (Environment Canada, 1997).

Chironomids are an ecologically diverse family of Dipterans and probably the most ubiquitous of all aquatic insects due to their physiological tolerance to a wide range of environmental conditions, such as salinity or temperature, and reduced dissolved oxygen levels. Their increasing use in bioassays and toxicity testing is mainly due to this wide distribution and ecological importance, their short lifecycle, their ability to be reared in the laboratory, and the ease of identifying different stages (Anderson, 1977). In addition, they can be used to evaluate both water and sediment toxicity (Ibrahim et al., 1998).

Furthermore, Chironomidae is one invertebrate group that plays a vital role in the structure and function in the aquatic environment of the McClean Lake east basin. Results from the benthic community assessment of McClean Lake east basin during the baseline period (1993 and 1996)

and the operational period (1999, 2002, 2005, 2008, 2011, and 2015) indicated that the benthic community was numerically dominated by Chironomidae (~ 50% to 70%) and Pisidiidae clams ( $\geq 20\%$ ) (AREVA, 2016a).

## **1.10. Research goals and objectives**

### **1.10.1. *Scope of work***

This research was conducted at the McClean Lake uranium milling operation located in the Athabasca Basin ecoregion in northern Saskatchewan, Canada. The geology of the Athabasca Basin results in natural concentrations of some metals, trace elements and radionuclides in surface water and sediments that are higher than in other regions. Treated effluent from the operation is released into the east basin of McClean Lake. Thus, individual metals in the effluent, or the cumulative effect of these metals, could potentially represent a toxicological risk to benthic macroinvertebrates. Because effluent is typically a complex mixture of constantly changing constituents, persistent fluctuations and sporadic contaminant concentration peaks are likely to be detected only through relatively continuous measurements. Therefore, the application of autonomous sensor technology to monitor real-time water quality is an extraordinary tool to better describe temporal changes in contaminant exposure to aquatic organisms over longer periods of time.

### **1.10.2. *Experimental objectives***

The overall goal of this research project was to delineate effluent exposure at the McClean Lake uranium operation using autonomous, real-time water quality monitoring sensors (Libellium Smart Water® sensors). This would, in turn, help identify the associated risk from contaminant exposure (metals and major ions) to aquatic macroinvertebrates and better predict toxicological risk of metals to benthic macroinvertebrates in McClean Lake.

**Objective 1:** Describe temporal and spatial variations of effluent distribution and exposure of key contaminants in the McClean Lake east basin using remote sensor technology. Specific aims included:

- i. The use of EC as a surrogate for effluent tracing to determine the extent and variability of the effluent within the lake system.



- ii. Autonomous sensors programming and calibrations for sensors deployment and data collection.

*H<sub>01</sub>: There are no temporal and spatial variations of effluent distribution and exposure of key contaminants in McClean Lake east basin using remote sensor technology.*

**Objective 2:** Estimate the ecotoxicological risk from cumulative effluent exposure (metals and major ions) to aquatic invertebrates in McClean Lake east basin. Specifically, this objective includes:

- i. Characterization of surface water chemistry and the physicochemical characterization of sediment.
- ii. Calculation of individual and cumulative Hazard Quotients for the ecotoxicological risk assessment following a screening level approach.

*H<sub>02</sub>: There is no ecotoxicological risk from cumulative effluent exposure to aquatic invertebrates in McClean Lake.*

**Objective 3:** Predict the pattern of the toxicological risk of metals to aquatic invertebrates in McClean Lake east basin using remote sensor data. This objective included:

- i. The use of correlations between hand-held meter EC readings and specific metals to generate empirical relationships.
- ii. Estimation of the toxicological risk to aquatic invertebrates using sensor data and correlation equations; calculate the 90<sup>th</sup> percentile HQ for metals of concern.

*H<sub>03</sub>: There is no predictable pattern of toxicological risk from metals to aquatic invertebrates using autonomous sensors in McClean Lake.*

**Objective 4:** Determine whether there is an effluent effect on macroinvertebrate communities in McClean Lake east basin. Specifically, this objective includes:

- i. Determination of standard community composition metrics (e.g. total abundance and Margalef index)

- ii. Development of a generalized additive model (GAM) to determine the effect of contaminants and physicochemical parameters on aquatic macroinvertebrates.

*H<sub>04</sub>: There is no actual effluent effect on macroinvertebrate communities in McClean Lake east basin.*

**Objective 5:** Perform a bioassay to characterize the toxicity of surface water from effluent-exposure sites in McClean Lake to a representative freshwater species (*Chironomus dilutus*). This objective included:

- i. Calculation of percent larval survival and adult emergence of *C. dilutus* after exposure to McClean Lake surface water.

*H<sub>05</sub>: There is no toxicity from surface water collected from effluent-exposed sites in McClean Lake to a representative freshwater invertebrate species (Chironomus dilutus).*

**CHAPTER 2: DELINEATING EFFLUENT EXPOSURE AND CUMULATIVE  
ECOTOXICOLOGICAL RISK OF METALS DOWNSTREAM OF A SASKATCHEWAN  
URANIUM MILL USING AUTONOMOUS SENSORS**

## PREFACE

The main objective of Chapter 2 was to use autonomous sensors to monitor EC as a surrogate for metals and major ions to better understand temporal and spatial contaminant variability in McClean Lake east basin. The data generated from this study were used to identify elements of concern that may affect benthic macroinvertebrates in McClean Lake (Chapter 3). Chapter 2 has been prepared in a manuscript style and has been accepted for publication in the journal *Environmental Toxicology and Chemistry*.

Cupe-Flores, B., Mendes, M., Panigrahi, B., & Liber, K. (2022). Delineating effluent exposure and cumulative ecotoxicological risk of metals downstream of a Saskatchewan uranium mill using autonomous sensors. *Environmental Toxicology and Chemistry*

### Author contributions:

Cupe-Flores, B (University of Saskatchewan): Performed and helped design the field and laboratory work (sensor deployment and water and sediment collection). Conducted the data analysis and wrote the draft of the manuscript.

Mendes, M (University of Saskatchewan): Helped conduct the fieldwork and sample processing and analysis in the laboratory. Provided feedback and edits on the manuscript.

Panigrahi, B: Helped perform the statistical analysis (regression analysis) and provided feedback and edits on the manuscript.

Liber, Karsten (University of Saskatchewan): Helped design and implement the laboratory and fieldwork. Helped perform the fieldwork and provided feedback and editorial assistance on the manuscript.

## Abstract

There is increasing interest in using autonomous sensor technologies to monitor aquatic ecosystems in real-time and for using such monitoring data to perform better ecological risk assessments. Here, sensor units were deployed at seven locations in McClean Lake in northern Saskatchewan, receiving diluted uranium milling effluent to track effluent distribution and help predict potential biological effects on aquatic invertebrates. Water was also collected from each location on multiple occasions to measure major ions, dissolved metals, and routine water quality. In addition sediment was sampled to analyze total metals. The ecotoxicological risk to aquatic invertebrates was estimated using Hazard Quotients (HQs). The cumulative risk was estimated by summing the individual HQs, while the major ions risk was based on total osmolarity. Results indicated temporal and spatial variations in effluent exposure based on sensor electrical conductivity (EC) measurements in the McClean Lake east basin. Individual HQs for water ranged from ‘moderate’ (0.40 – 0.69) to ‘very high’ (>1) for silver, cadmium, arsenic, selenium, mercury, iron and thallium. At all sites, major ions risk was <1. Individual HQs for sediment registered ‘moderate’ (0.40 – 0.69), ‘high’ (0.7 – 0.99) and ‘very high’ (>1) values for vanadium and cadmium. The cumulative risk in water and sediment for all metals combined was >1 at some sites in Vulture Lake (discharging into McClean Lake) and McClean Lake. More detailed estimation of aqueous selenium and arsenic risk, the only two metals with good correlation with sensor EC data, indicated that their 90<sup>th</sup> percentile HQ values were <1 in McClean Lake, suggesting that these potential contaminants of concern do not represent a significant direct risk to aquatic invertebrate communities.

## 2.1 Introduction

Effluent is typically a complex mixture of constituents constantly changing at least somewhat with time, so fluctuations and sporadic peaks of contaminant concentration are likely only to be detected through relatively high-frequency monitoring or continuous measurements. Traditional spot/grab sampling is sometimes unable to provide a good estimate of the true maximum or mean concentration for a particular physicochemical parameter in a water body, especially one with marked temporal variability in input (O'Flynn et al., 2010). In this regard, autonomous sensors are capable of continuous measurements and can provide more frequent and up-to-date information, besides providing better delineation of long-term trends in fluctuations of contaminant concentrations (O'Flynn et al., 2010).

The use of advanced sensor technologies and strategies means that measures to improve water quality can be implemented more quickly (Greenwood et al., 2008). Several surveys on smart water quality monitoring systems have been performed. For instance, Samsudin et al. (2018) developed a smart system in Malaysia to monitor water quality, measuring pH and turbidity to ensure clean water was continuously maintained. Saab et al. (2019) combined smart water monitoring with a risk assessment approach to ensure early detection of water contamination (turbidity and chlorine) in Strasbourg, France. Trescott and Park (2013) developed remote sensing models using Landsat satellite data to monitor algal blooms in Lake Champlain and across Canada and the USA. Similar technologies (smart grids) have also been used in smart water quality monitoring systems (Fang et al., 2012; Gao et al., 2012).

Active Canadian uranium mines and mills are located in the Athabasca Basin ecoregion of Northern Saskatchewan. The geology of the Athabasca Basin results in natural concentrations of some metals, trace elements and radionuclides in surface water and sediments that are higher than in other regions (Kilgour et al., 2018). The McClean Lake milling operation (operated by Orano Canada Inc., previously AREVA Resources Canada Inc.), located in that ecoregion, discharges treated effluent to the aquatic environment via a treated effluent management system to control the effluent release. The system includes Sink Reservoir, Vulture Lake, the East Basin of McClean Lake, and all connecting structures located within the surface lease boundaries. Water is

discharged in a controlled manner from Sink Reservoir to Vulture Lake and subsequently into McClean Lake, after which it enters Collins Creek (McClean Lake outflow).

Typically, effluent discharges coming from uranium milling activities generate elevated concentrations of many different trace elements (e.g., arsenic (As), molybdenum (Mo), nickel (Ni), selenium (Se) and uranium (U)) and ions (e.g., sulphate ( $\text{SO}_4$ ) and ammonia ( $\text{NH}_3$ )) that can be released into the surrounding aquatic systems (Pyle et al., 2001; Klaverkamp et al., 2002; deRosemond et al., 2005; Muscatello et al., 2006; Muscatello & Janz 2008). The cumulative effect of these elements in an effluent release could potentially represent a toxicological risk to aquatic organisms since cumulative toxicity has often been underemphasized.

Surface waters within receiving environments are generally more representative of recent effluent releases than are sediments. They typically act as the main source of contaminant exposure to pelagic organisms and affect organisms living at the water-sediment interface or in the surficial sediment layer. On the other hand, the main compartment for metal accumulation in the environment is generally the sediments of receiving waters. Since metals do not degrade, the presence of bioavailable metal fractions within receiving environments can pose a long-term environmental risk through direct sediment and porewater exposure. Moreover, sediment contaminants could become a source of metal redistribution after effluent discharges stop and surface water contamination drops (Robertson, 2006). Metals transported into an aquatic system are mainly incorporated into bottom sediments through adsorption, flocculation and precipitation from the water column (Botte et al., 2007; Cheng et al., 2015), and they may be toxic to aquatic organisms when threshold concentrations are reached. Nevertheless, metals that settle out of the water column are more likely to be re-suspended and re-dissolved into pore water, from where sediment-associated metals can be released into the overlying water by diffusive fluxes (de Mora et al., 2004; Yu et al., 2012).

At the McClean Lake operation, the potential effects of effluent discharge associated with milling activities depend primarily on the quantity and quality of discharge from the water treatment plants. The complete list of constituents of potential concern (COPC) at the McClean Lake operation includes the following water quality ions; ammonium ( $\text{NH}_4^+$ ) chloride ( $\text{Cl}^-$ ), fluoride ( $\text{F}^-$ ), potassium ( $\text{K}^+$ ), sulphate ( $\text{SO}_4$ ); total dissolved solids (TDS); the following

metals/metalloids; arsenic (As), cobalt (Co), copper (Cu), lead (Pb), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn); and the following radionuclides; U, thorium-230, radium-226, lead-210, and polonium-210 (AREVA, 2016b).

At present, high-quality sensors for metals and other trace elements are not commercially available, especially for autonomous deployment. However, due to the potential toxicity of a few elements (incl. Se and As) in the McClean Lake Operation effluent release, it is important to monitor their concentrations at sites receiving continuous effluent input. The overall goal of this research was to use autonomous sensors to monitor, at high-frequency, electrical conductivity (EC) as a surrogate for elements of concern to better understand temporal and spatial contaminant variability and conduct a better ecological risk assessment for inorganic contaminants in the McClean Lake east basin. This goal was addressed through a series of research objectives, including efforts to (1) describe temporal and spatial variations in effluent exposure in McClean Lake east basin using autonomous, real-time water quality monitoring units, (2) estimate the associated risk from cumulative exposure to inorganic contaminants (metals and major ions) to aquatic invertebrates, and (3) predict the pattern and toxicological risk of metals to aquatic invertebrates using remote sensor technology.

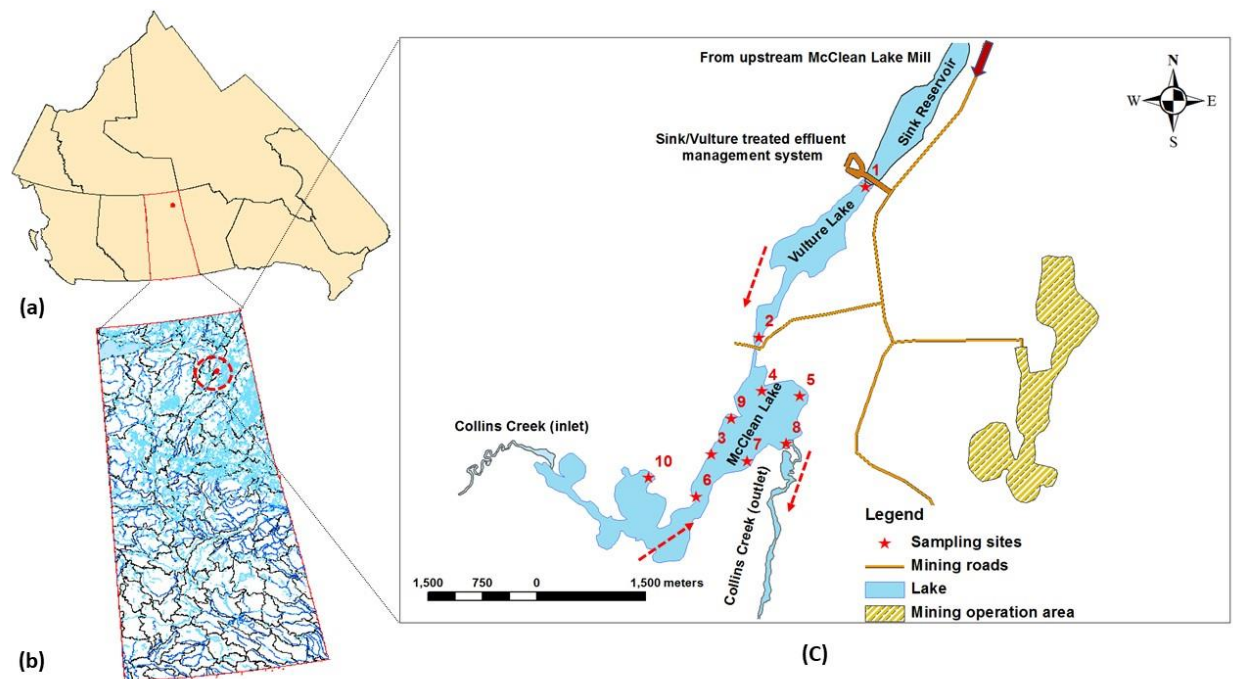
## **2.2 Materials and methods**

### **2.2.1 Study site**

The study sites for this project were located in the aquatic receiving environment downstream of the McClean Lake uranium milling operation in northern Saskatchewan, Canada (Figure 2.1). The Sink/Vulture Treated Effluent Management System (S/V TEMS) controls the effluent release and includes Sink Reservoir, its control structure, Vulture Lake, and the effluent diffuser system which discharges effluent into the East Basin of McClean Lake. The monitoring locations used for the present study were chosen to delineate effluent distribution and movement within the system and cover different areas within the downstream lakes, especially in the McClean Lake east basin. However, since the effluent mixing in McClean Lake east basin has been observed to be uneven and variable depending on the day and season, effluent concentrations in different parts of the lake were predicted to be highly variable. For this reason, one objective of this project was focused on understanding the effluent mixing pattern in McClean Lake east basin while also



considering data from Vulture Lake (McClean Lake inflow) and Collins Creek (McClean Lake outflow) as supplemental information. Thus, ten (10) monitoring locations were selected along Vulture Lake, McClean Lake east basin (exposure sites), Collins Creek (downstream site), and McClean Lake west basin (reference location) to delineate effluent distribution during late summer – early fall 2018 and summer – early fall 2019.



**Figure 2.1.** Map of the McClean Lake study site (c) showing the ten 2019 monitoring locations (red stars) along Vulture Lake, McClean Lake and Collins Creek. The other two maps show the location of the McClean Lake operation within Canada (a) and Saskatchewan (b). Dashed arrows in (c) indicate flow direction.

In 2019, the reference site (Site 10) was moved a few hundred meters to the north in the McClean Lake west basin, and Site 8 was repositioned from just inside the creek to immediately before the creek near the outlet of McClean Lake. Both sites were moved to obtain a better cellular signal and a more consistent benthic habitat. All monitoring sites were located at a water depth ranging from 0.6 to 1.2 m and in locations with some detritus/organic material covering the sediment surface. Both McClean Lake east and west basins are relatively shallow with surface areas of 117.2 ha and 107.2 ha, respectively. The basin volumes are  $1.62 \times 10^6 \text{ m}^3$  for the east basin

and  $1.59 \times 10^6 \text{ m}^3$  for the west basin. Approximately 50% of the lake areas are shallower than 1 m deep. Vulture Lake is also relatively shallow with mean and maximum depths of 2.2 m and 3.4 m, and it has a surface area of 73.5 ha and a volume of  $1.65 \times 10^6 \text{ m}^3$  (AREVA, 2016a).

### **2.2.2 *Sensors and sensor deployment***

The focus of the sensor component was solely on delineating contamination of surface water and relied on the use of Smart Water® sensors (Libelium Communications). These devices are part of an autonomous, solar-powered, real-time water quality monitoring system that can be programmed to take specific water quality measurements at specific times. The frequency of measurements was every 4 hours for 5 weeks in 2018 and every 12 hours for 7 weeks in 2019. Ten Smart Water® units were deployed in custom-built acrylic boxes (30 cm x 25 cm x 25 cm) designed to protect the sensors from field conditions and allow them to float on an anchored Styrofoam platform (Figure 2.2).



**Figure 2.2.** Sensor unit deployed at McClean Lake east basin in 2019.

Each sensor included four attached probes positioned at a depth of ~50 cm below the water surface to measure pH, EC, turbidity, DO, and temperature. In 2018, EC, pH, DO, temperature

and turbidity Smart Water® probes (Libelium Communications) were used in the deployment. However, in 2019, new EC, pH, and DO probes (Atlas Scientific) were purchased, which were compatible with the Libelium system while keeping the turbidity and temperature probes from Libelium. Solar panels were attached to the top of each box at a 45° angle and pointed south to allow the sensor battery to be charged during the day and used at night and maintain sensor autonomy in the field. Upon retrieving the sensors in 2018 after the five-week monitoring period, biofilm was observed growing on the probes. For that reason, sensor probe cleaning was performed after 18 days of deployment during the seven-week monitoring period in 2019 to ensure accurate and consistent readings from the sensors and thus to better understand effluent distribution.

Regarding data collection, nodes communicated with a Libelium Meshlium device, which acts as a Wi-Fi access point via the local 2G network (each unit required a mobile SIM card and data plan). The Meshlium received and parsed the data frames from the sensors in the field, and data were uploaded to a Meshlium cloud connector called ThingSpeak. The latter provided instant visualizations of data posted by sensors to the ThingSpeak cloud, which could be accessed from a computer or mobile device. Sensor data collected in 2018 were considered only preliminary work for sensors deployment due to a few technical issues that were then corrected in 2019. Details of sensors programming and calibration are described in appendix AA.

### ***2.2.1 Sample collection***

Water samples ( $n = 3$  per site) were collected twice (August and September) in 2018, and three times (July, August and September) in 2019 at a water depth of ~50 cm at all monitoring locations using a Wildco® 2.2-L acrylic Van Dorn horizontal beta water sampler (Wildlife Supply Company). Water from the sampler was placed into an acid-washed container (1 L approximately) to be transferred and filtered through 0.45- $\mu$ m polyethersulfone membrane to two sets of 30-mL HDPE Nalgene™ bottles using 5-mL syringes. One set of samples was only syringe-filtered for posterior major ions and physicochemical analysis, and the other set of samples was acidified to a pH of  $\leq 2$  with 192  $\mu$ L of 69% ultra-pure HNO<sub>3</sub> (Fisher Scientific) for subsequent metal and trace element analysis.

Sediment samples ( $n = 3$  per site) were collected in September 2018 and September 2019 at the same monitoring locations as water collection using a standard (15-cm x 15-cm) Ekman grab

sampler. Processing included removing the top ~2 cm layer of the sediment grabs with a stainless steel spoon and direct transfer of that layer into 60-ml plastic snap cap vials. Both sediment and water samples were refrigerated and transported to the University of Saskatchewan Toxicology Centre (Saskatoon, SK) in ice-packed coolers.

#### **2.2.4. Characterization of surface water chemistry**

Along with the collection of water samples, *in situ* parameters (pH, EC, temperature, and DO) were measured at a water depth of ~50 cm in 2018 using a Sevensgo duo pH/conductivity meter (Mettler Toledo) and an S4 dissolved oxygen meter (Mettler Toledo), and in 2019 using a Thermo Scientific™ Orion Star™ A329 pH/ISE/conductivity/dissolved oxygen portable multiparameter meter (Thermo Scientific). Dissolved metals and other trace elements analyses (B, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sn, Sb, Ba, Hg, Tl, Pb and U) were performed at the Toxicology Centre via inductively coupled plasma mass spectrometry (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). Instrumental quality assurance/quality control (QA/QC) values were always within  $\pm 20\%$  of certified values (e.g., reference material 1640a, trace elements in natural water, National Institute of Standards and Technology). An example of those data for two key elements, Se and As, is presented in Appendix O. Analysis for specific major ions ( $\text{Na}^+$ ,  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{+2}$ ,  $\text{Ca}^{+2}$ ,  $\text{F}^-$ ,  $\text{Cl}^-$  and  $\text{SO}_4^{-2}$ ) was performed via ion chromatography with a Dionex ICS 2000, using an IonPac AS18 column (Thermo Fischer Scientific) in the Department of Soil Science at the University of Saskatchewan. Conventional water quality analyses (Hardness, Alkalinity and Turbidity) were performed in-house at the University of Saskatchewan Toxicology Centre. Finally, dissolved organic carbon (DOC) content was analyzed using a Total Organic Carbon Analyzer TOC-VCPN (Shimadzu Corporation) in the Department of Soil Science.

#### **2.2.5. Physicochemical characterization of sediment**

Sediment samples were dried at 60° C for 72 h, sieved (<2 mm) subsequently ground, hydrofluoric acid was added to a subsample (47-51%), and the sample was digested in a MARS-5 Microwave Accelerated Reaction System with closed Teflon vessels (CEM Corporation) for four hours. The reference material used for quality assurance analysis was marine sediment PACS-3

(National Research Council). The elements of interest (B, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sn, Sb, Ba, Hg, Tl, Pb and U) were analyzed using an Agilent 8800 ICP-MS QQQ Triple Quadrupole spectrometer, equipped with an ASX-500 autosampler and Masshunter software for instrument operation (Agilent Technologies). Total organic carbon (TOC) analysis and particle size (% gravelly sand, % sand, % silt and % clay) determination were performed in the Department of Soil Science. Sediment subsamples for TOC determination were pre-treated with approximately 1 mL sulphurous acid (6%) to prevent inorganic carbon analysis. Subsequently, samples were analyzed for TOC in a LECO C632 TOC analyzer (LECO Corporation).

#### **2.2.6. *Electrical conductivity adjustment***

Electrical conductivity was used as a surrogate for effluent tracing to determine the extent and variability of the effluent plume within the lake system, especially in McClean Lake east basin. Electrical conductivity was chosen since it is commonly used to examine the mixing of freshwater with other water sources; for instance, seawater (Hiscock et al., 1996), precipitation and subsurface water (Kobayashi, 1986), as well as for the dilution gauging of stream discharge (Dingman, 2002). In addition to the amount and composition of ionic species, EC is strongly dependent on temperature. Consequently, to have meaningful data interpretation, water temperatures need to be corrected to values corresponding to a standard temperature (Hayashi, 2004). Thus, the EC handheld meter and EC sensor data were adjusted to a standard water temperature of 13°C, which was the median temperature from the sensor data collected in 2018 and 2019. Adjustments of EC values were performed using equations from Hayashi (2004).

$$EC_{t0} = EC_t / [1 - c(t - t_0)] \quad (1)$$

Where  $EC_{t0}$  is electrical conductivity at a standard temperature  $t_0$  and  $c$  is a constant given by:

$$c = a/[1 + a(t_0 - 25)] \quad (2)$$

For equation number 2,  $a$  (the temperature compensation factor) was set at 0.0191 as recommended by Clesceri et al., (1998).

### 2.2.7. Risk assessment procedure

The ecotoxicological risk assessment for surface water followed a screening level approach (Tier 1 assessment) using Hazard Quotients (HQs), which are the ratio between measured exposure and a corresponding effect-based threshold on aquatic biota. Hazard quotients were calculated according to the Canadian Ecological Risk Assessment Guidance for Federal Contamination Sites (FCSAP, 2012).

$$\text{Hazard Quotient (HQ)} = \frac{\text{Exposure Level}}{\text{Threshold Effect Level}} \quad (3)$$

The exposure terms for the HQs were derived from measured and estimated (via sensor data regressions) metal concentrations at the different study sites. The corresponding effect-based thresholds were mainly based on the Saskatchewan Surface Water Quality Objectives (SWQO), benchmarks that directly adopt the generic Canadian Council of Ministers of the Environment (CCME) Guidelines, and the Canadian Water Quality Guidelines (CWQG) for the protection of freshwater aquatic life. Hazard quotients were classified according to White and Liber (2018) as follows: Very high >1, High (0.7 – 0.99), Moderate (0.40 – 0.69), Low (0.10 – 0.39) and Very low (<0.1). The cumulative risk was estimated by summing only HQs > 0.4 (moderate risk) per site, making the still conservative assumption that the risk from key effluent contaminants is additive.

The cumulative risk for major ions (anions and cations) was calculated using equations derived by Mount et al., (2019). They developed concentration-based submodels for chronic toxicity to *Ceriodaphnia dubia* using adjustments of the acute models presented by Erickson et al., (2018) based on major ion solutions (Na, Na/Ca, Mg/Na, Ca and their mixtures) and nominal osmolarity (total ion concentration). Thus, the HQs were estimated based on total osmolarity (the sum of Na, K, Mg, Ca, Cl, Fl, and SO<sub>4</sub> molar concentrations) divided by an estimated threshold effect level (EC50) derived using the Mount et al., (2019) equation. This resulting equation for the chronic EC50 general ion toxicity submodel based on osmolarity was:

$$EC50 = \log\left(\frac{83.5}{2.14}\right) + \frac{0.91 \times \log\{Ca\}}{2.39 \times (\log\{Ca\} + 2.39)} \quad (4)$$

Where {Ca} is the calcium molar concentration (mM) in lake water.

When the cumulative HQs and HQs estimated from sensor data were  $<1$ , it was assumed that metal concentrations do not represent a likely risk and major ion concentrations only a minor risk of adverse effects to aquatic invertebrates. However, when cumulative HQs were  $>1$ , it represents a potential risk of adverse effects to aquatic invertebrates (FCSAP, 2012). Finally, risk assessment for sediment contaminants followed a similar approach using individual and cumulative HQs. However, it was based on available toxicity benchmarks for total metals and trace elements from the following sources: Canadian Council of Ministers of the Environment (CCME, 2021), Canadian Nuclear Safety Commission Working Benchmarks (Thompson et al., 2005), and No-effect and Reference-level sediment quality values (Burnett-Seidel & Liber 2013). HQs were calculated separately for sediment samples collected in September 2018 and 2019.

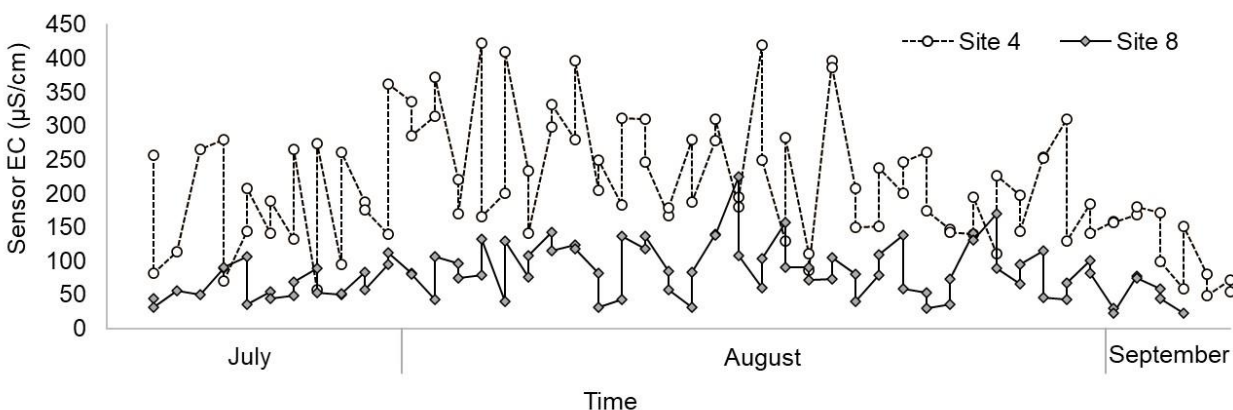
#### **2.2.8. Statistical analysis**

Statistical analyses were conducted in SPSS 20 (SPSS Inc., IBM), adopting a significance value of  $\alpha=0.05$  after checking for compliance with parametric assumptions of normal distribution (Shapiro-Wilk test) and homoscedasticity. Datasets from both monitoring years were combined and natural log-transformed to assure normality of residuals. Pearson correlation analysis ( $r$ ) was employed to describe the relationship between hand-held meter EC measurements and ICP-MS metal concentrations in McClean Lake. Once the correlation was established, Linear Models (LM) were used to perform linear regressions between adjusted EC and individual metal concentrations. The ultimate goal was to use EC to estimate the aqueous concentration of key contaminants of concern, specifically considering metals/elements with HQs higher than 0.4. Thus, the whole dataset was combined using 80% of it to generate a regression equation, and the remaining 20% was used to cross-validate the model. The model validation was based on calculating the root mean square error (RMSE) to measure the absolute errors between the model estimates and the observed values, which were considered acceptable when lower than 20%. After validation, EC regression equations were used to estimate concentrations of aqueous metals/elements not measured directly from the water sampling but derived from EC sensor data. Therefore, it was possible to use EC sensor data to estimate key contaminant concentrations and calculate HQs to determine the toxicological risk of aqueous contaminants in 2019.

## 2.3. Results

### 2.3.1. Sensor data

After applying the standard temperature adjustments of 13°C to the EC measurements from sensors deployed in Vulture Lake, McClean Lake, and Collins Creek, results indicated spatial and temporal differences in effluent exposure at all sites (July, August and September 2019). At the higher exposure site (Vulture Lake), sensor measurements showed that EC values started at approximately 1700  $\mu\text{S}/\text{cm}$  in July and decreased to 870  $\mu\text{S}/\text{cm}$  by September. At McClean Lake east basin, Site 4 (approx. 50 m from the diffuser inflow) registered EC values ranging from 48 to 420  $\mu\text{S}/\text{cm}$  (Figure 2.3). A significant aspect of this site is a high temporal variability with sensor EC data, showing variations with peaks of 280  $\mu\text{S}/\text{cm}$  and low values of 50  $\mu\text{S}/\text{cm}$  in July. Fluctuations continued with increasing EC values in August, reaching peaks of 420  $\mu\text{S}/\text{cm}$  and low values of 80  $\mu\text{S}/\text{cm}$ . The EC measurements decreased until reaching 48  $\mu\text{S}/\text{cm}$  in September. Moving downstream to the outflow of McClean Lake east basin, close to Collins Creek (Site 8), EC values ranged from 22 to 225  $\mu\text{S}/\text{cm}$  (Figure 2.3). In July, there were variations with peaks as high as 112  $\mu\text{S}/\text{cm}$  and low values of 30  $\mu\text{S}/\text{cm}$ , while in August, these variations reached highs and lows of 225  $\mu\text{S}/\text{cm}$  and 30  $\mu\text{S}/\text{cm}$ , respectively, dropping to 22  $\mu\text{S}/\text{cm}$  in September. The reference site in the west basin of McClean Lake (Site 10) displayed less variability in EC values which ranged between 12 and 24  $\mu\text{S}/\text{cm}$ . Additionally, variations were lower in McClean Lake west basin since it receives no effluent.



**Figure 2.3.** Electrical conductivity (EC) data measured with the Smart Water sensor units during 2019 for Site 4 at McClean Lake east basin (near diffuser) and Site 8 (at Collins Creek outflow).



### **2.3.2. Routine surface water chemistry**

After EC adjustments to 13°C, hand-held meter measurements indicated that mean  $\pm$  SD EC in Vulture Lake registered values (maximum of  $1550 \pm 3$   $\mu$ S/cm) higher than in the other locations. These values decreased at McClean Lake Site 4 (inflow) and continued decreasing at Site 8 (outflow), showing a lower mean  $\pm$  SD value of  $27 \pm 0.5$   $\mu$ S/cm. Mean  $\pm$  SD pH in Vulture Lake varied from  $7.02 \pm 0.1$  to  $7.98 \pm 0.2$ , while the values in McClean Lake and Collins Creek remained relatively unchanged, around 7.0. Dissolved oxygen concentrations showed mean  $\pm$  SD values as high as  $12.4 \pm 0.4$  mg O<sub>2</sub>/L in Vulture Lake and McClean Lake. Regarding DOC, Vulture Lake registered a mean  $\pm$  SD measurement of  $2.8 \pm 0.1$  mg/L, and Collins creek means as high as  $5.9 \pm 0.1$  mg/L. Concerning physiochemical parameters, hardness values decreased moving downstream and away from the effluent input source in Vulture Lake. Alkalinity values remained relatively constant in 2018 while decreasing in July 2019, and turbidity measurements did not have high variations within the study sites. Details of routine water quality are presented in Appendices A and B.

### **2.3.3. Metals and major ions**

Overall, dissolved metal concentrations of B, Ni, As, Se, Sr, Mo, Ba, and U in the surface water presented a good correlation with the diluted effluent pattern based on EC measurements ( $r > 0.5$ ). Higher concentrations were detected in Vulture Lake and decreased along McClean Lake east basin as they reached Collins Creek's outflow. However, other metals such as Al, V, Cr, Mn, Co, Zn, Ag, Cd, Sn, Sb, Hg, Tl and Pb did not correlate with the diluted effluent concentration and did not display any pattern, suggesting that metal values at all sites represented normal background concentrations with negligible effluent impact. Not surprisingly, major ions displayed a similar pattern to the EC measurements, with higher values in Vulture Lake, decreasing at the inflow to McClean Lake and dropping to even lower values near the outlet at Collins Creek. Cation concentrations (Ca<sup>+2</sup>, Mg<sup>+2</sup>, K<sup>+</sup> and Na<sup>+</sup>) remained constant among monitoring locations in 2018. Conversely, in 2019, except for Mg, cations displayed higher concentrations in Vulture Lake, decreasing significantly along McClean Lake and Collins Creek. Regarding anions (Cl<sup>-</sup>, SO<sub>4</sub><sup>-2</sup>, and F<sup>-</sup>), concentrations remained relatively similar in Vulture Lake. However, with the exception of F<sup>-</sup>, anion concentrations were higher in 2018 than in 2019. Details of major ions and metal concentrations are presented in Appendices C to I.

#### ***2.3.4. Ecotoxicological risk in surface water***

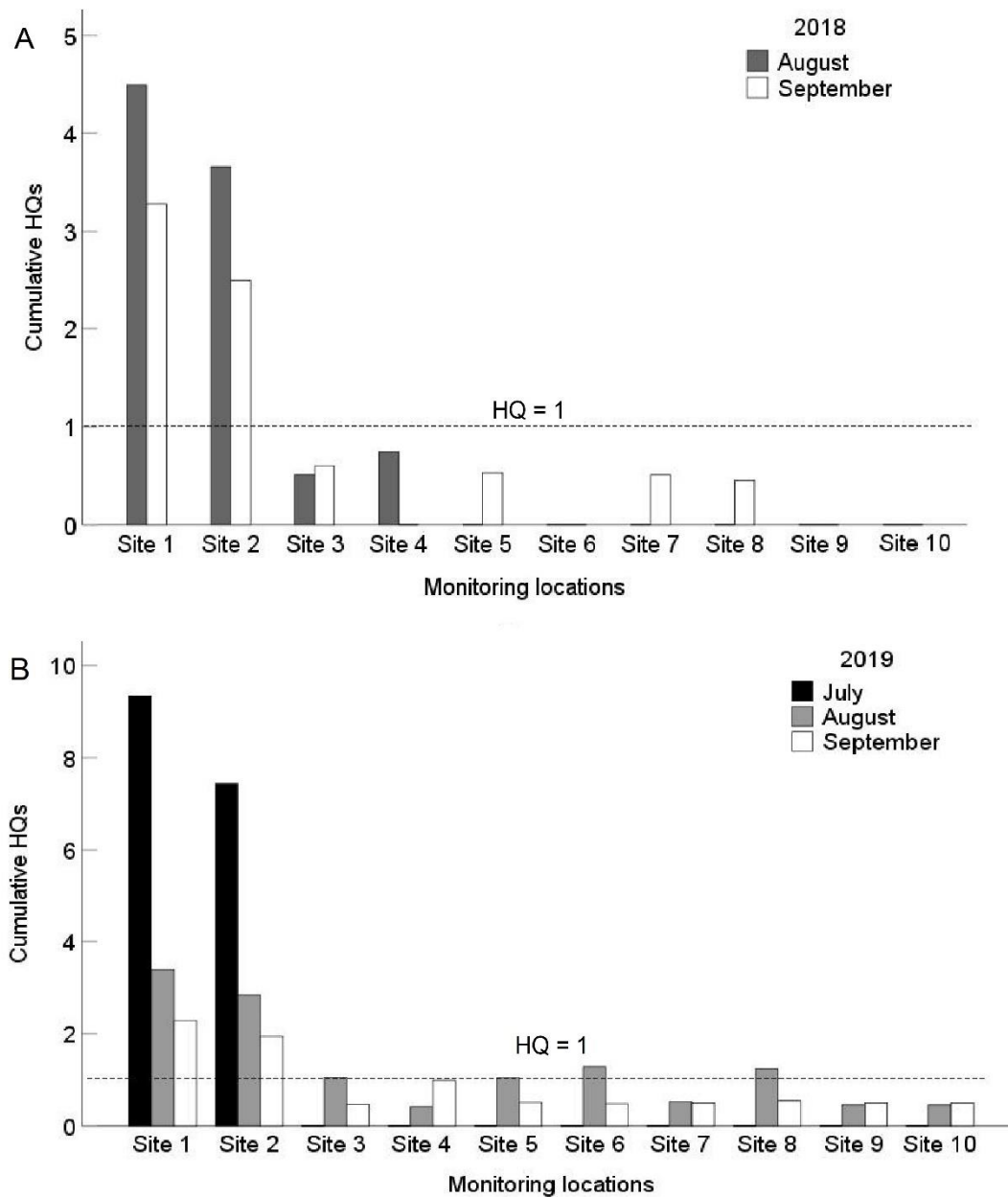
Due to the lack of available benchmarks (e.g., CWQG and US EPA) and adequate aquatic toxicity data for invertebrates for Sb, Sn, Ba, Mn and Sr, aqueous metal toxicological risk could not be assessed. For the 18 metals with benchmarks, individual risk estimates for 2018 exposure data indicated that silver (Ag), As, Se, and Hg had HQs with moderate ( $>0.4$ ) to very high ( $>1$ ) risk (Table 2.1). Overall, Se (2.61) and Hg (1.09) registered the highest HQs in Vulture Lake. Additionally, Se at Site 4 (inflow) and Ag at several locations (sites 3, 5 and 7) showed moderate to high risk in the McClean Lake east basin. For 2019, individual HQs showed that As, Se, Fe, Cd, Hg and Tl had moderate to very high HQs (Table 2.1). Similar to 2018, the higher HQs were associated with Se (4.14) and Hg (3.45) in Vulture Lake, while in McClean Lake east basin, Se registered moderate risk at Site 4 only. In addition, Cd and Fe had ‘moderate’ HQs at several monitoring locations in McClean Lake, including the reference site and Collins Creek. Except for As and Se, none of the assessed element concentrations (Ag, Hg, Fe, Cd and Tl) followed the diluted effluent pattern, based on EC measurements, with higher values in Vulture Lake decreasing by the outlet in Collins Creek and in the reference site (Table 2.1). The remaining dissolved metals (B, Al, Ti, V, Cr, Co, Ni, Cu, Mo, Pb and U) analyzed in both years were of negligible toxicological concern.

Regarding the cumulative risk for dissolved metals in 2018, HQs were  $>1$ , only at Sites 1 and 2, suggesting a potential risk of cumulative adverse effect to aquatic invertebrates in Vulture Lake. These cumulative HQs were higher in August (4.19) than in September (3.28) (Figure 2.4A). The remaining monitoring locations in McClean Lake east basin, Collins Creek and McClean Lake west basin all had cumulative HQs  $<1$ , suggesting that dissolved metal concentrations do not represent a likely risk of cumulative adverse effects to aquatic invertebrates. Cumulative HQs for dissolved metals in 2019 were  $>1$  for Vulture Lake during all three monitoring periods (July, August and September) and at some locations in McClean Lake east basin and Collins Creek in August 2019 only. The highest HQs observed in July (9.34 and 7.45) were recorded in Vulture Lake, while values were lower for August and September (Figure 2.4B). The remaining monitoring locations registered cumulative HQs  $<1$ .

**Table 2.1.** Hazard quotients (HQs)<sup>a</sup> for aquatic ecotoxicological risk of select individual metals of concern in water for 2018 (August and September) and 2019 (July, August and September).

Site	Month	Ag		As		Se		Hg		Fe		Cd		Tl	
		2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
1	Jul.		0.08		0.56		4.14		3.45		0.06		0.65		0.54
	Aug.	0.01	0.16	0.49	0.56	2.61	2.37	1.09	-	0.02	0.06	0.26	0.46	0.37	0.34
	Sept.	0.64	-	0.46	0.48	2.18	1.80	0.11	-	0.06	0.09	0.34	0.36	0.39	0.23
2	Jul.		0.07		0.53		2.92		2.88		0.07		0.69		0.42
	Aug.	0.01	0.08	0.49	0.52	2.40	1.87	0.77	-	0.10	0.09	0.27	0.45	0.27	0.29
	Sept.	0.27	-	0.42	0.46	2.08	1.48	0.04	-	0.06	0.14	0.30	0.37	0.32	0.21
3	Jul.		-		0.04		0.19		-		0.26		-		0.01
	Aug.	-	0.10	0.07	0.07	0.08	0.13	0.51	-	0.01	0.47	0.34	0.59	0.02	0.03
	Sept.	0.60	-	0.05	0.04	0.12	0.01	0.27	-	0.33	0.47	0.20	-	0.03	-
4	Jul.		-		0.07		0.16		-		0.23		-		0.03
	Aug.	-	0.06	0.20	0.15	0.74	0.42	0.31	-	0.06	0.25	0.23	0.26	0.10	0.07
	Sept.	0.30	-	0.07	0.08	0.2	0.15	-	-	0.30	0.51	0.11	0.49	0.04	0.02
5	Jul.		0.07		0.04		0.15		-		0.26		-		0.01
	Aug.	-	0.13	0.07	0.06	0.06	0.08	0.2	-	0.01	0.55	-	0.50	0.02	0.02
	Sept.	0.53	-	0.08	0.06	0.28	0.06	-	-	0.31	0.51	0.11	-	0.05	0.01
6	Jul.		-		0.04		0.11		-		0.30		-		-
	Aug.	-	0.09	0.06	0.04	0.01	0.04	-	-	0.01	0.64	-	0.64	0.01	0.01
	Sept.	0.26	-	0.06	0.04	0.14	0.01	-	-	0.30	0.48	0.10	-	0.03	-
7	Jul.		-		0.04		0.11		-		0.26		-		0.01
	Aug.	-	0.08	0.06	0.05	0.01	0.08	-	-	0.01	0.52	-	0.35	0.01	0.01
	Sept.	0.51	-	0.06	0.04	0.15	0.02	-	-	0.32	0.50	0.05	-	0.03	-
8	Jul.		-		0.04		0.13		-		0.29		-		0.01
	Aug.	-	-	0.06	0.04	0.02	0.04	-	-	0.01	0.62	0.30	0.62	0.01	0.01
	Sept.	0.45	-	0.06	0.04	0.19	0.02	-	-	0.31	0.54	0.08	-	0.04	-
9	Jul.		-		0.04		0.11		-		0.25		-		-
	Aug.	-	-	0.13	0.06	0.32	0.09	-	-	0.04	0.46	0.18	-	0.06	-
	Sept.	0.33	-	0.05	0.04	0.14	0.01	-	-	0.31	0.50	0.01	-	0.03	-
10	Jul.		-		0.03		0.13		-		0.22		-		-
	Aug.	-	-	0.06	0.04	0.01	0.02	0.2	-	0.01	0.46	-	-	-	-
	Sept.	0.35	-	0.03	0.04	0.01	0.01	-	-	0.39	0.50	0.04	-	-	-

<sup>a</sup> White and Liber (2018): Very high >1, High (0.7 – 0.99), Moderate (0.40 – 0.69), Low (0.10 – 0.39) and Very low (<0.1).



**Figure 2.4.** The cumulative risk for dissolved metals exposure to aquatic macroinvertebrates in both (A) 2018 and (B) 2019 monitoring years. HQs greater than 1.0 (horizontal line) indicate a potential risk.

For major ions, the HQs based on osmolarity showed that the risk in Vulture Lake resulted in values  $<1$  in both monitoring years (highest values for 2018 and 2019 were 0.26 and 0.25, respectively). Consequently, the HQs for the remaining monitoring locations downstream

registered even lower values than Sites 1 and 2, meaning that major ion concentrations represent only a minor risk of adverse effects to aquatic invertebrates.

#### **2.3.5. *Sediment chemistry***

Concerning total metal concentrations in sediment samples, only Co, Ni, As, Se and Mo displayed values that followed the general effluent pattern with higher values in Vulture Lake that decreased moving downstream along McClean Lake to Collins Creek. The remaining metals (Be, Al, Ti, V, Cr, Fe, Mn, Cu, Zn, Sr, Mo, Ag, Cd, Sn, Sb, Ba, Hg, Tl, Pb and U) did not show any pattern related to effluent distribution, suggesting that these elements more likely represented background concentrations for this system. Total organic carbon content in 2018 tended to be slightly higher than in 2019 at McClean Lake while remaining relatively similar in Vulture Lake. The percentages of TOC followed the general effluent pattern, except for Site 8. On the other hand, particle size composition showed that in both years, the content of sand was similar through the lake system, registering values > 80% at all monitoring locations. Regarding silt content, values were higher in 2018 than in 2019 and followed the effluent pattern. Details of sediment chemistry and metal concentrations are presented in appendices J to M.

#### **2.3.6. *Ecotoxicological risk in sediments***

For both years (2018 and 2019), the individual element risk calculations showed that V and Cd had very high or moderate HQs (Table 2.2). Neither V nor Cd followed the effluent distribution pattern of having higher concentrations in the higher exposure areas (Vulture Lake and McClean Lake Site 4), suggesting that the mill effluent had little influence on their presence in sediment. In 2018, except for Site 7, all monitoring locations registered HQs >0.4 for V. Likewise, in 2019, all sites displayed HQs between very high and moderate except for Sites 3 and 10. Regarding Cd, HQs >0.7 (high) were recorded in Vulture Lake in both years and (HQ>0.4) in Collins Creek (2018).

**Table 2.2.** Hazard quotients (HQs)<sup>a</sup> for aquatic ecotoxicological risk of select individual metals in sediment for 2018 and 2019.

Site	V		Cd	
	2018	2019	2018	2019
1	0.62	0.55	0.8	0.82
2	0.51	0.42	0.7	0.62
3	0.65	0.32	0.3	0.11
4	0.81	0.55	0.37	0.26
5	0.78	0.7	0.27	0.23
6	0.44	0.47	0.13	0.18
7	0.39	0.45	0.18	0.15
8	1.04	0.47	0.45	0.32
9	1.29	0.87	0.22	0.13
10	1.68	0.32	0.2	0.22

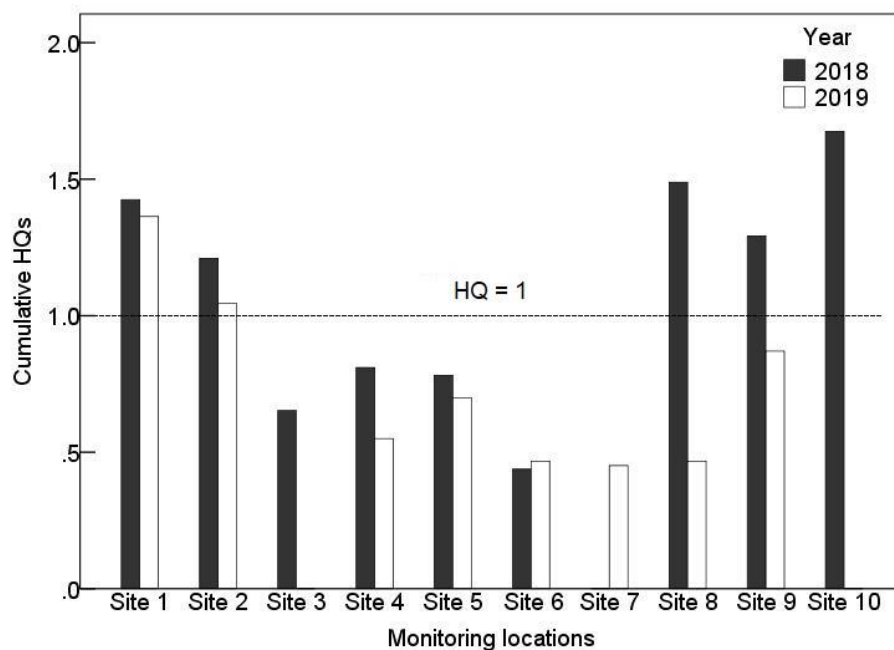
<sup>a</sup> White and Liber (2018): Very high >1, High (0.7 – 0.99), Moderate (0.40 – 0.69), Low (0.10 – 0.39) and Very low (<0.1).

Consequently, in 2018, the cumulative HQs for total metals in sediment resulted in values > 1 in Vulture Lake (Site 1 and Site 2), McClean Lake east basin (Site 9), Collins Creek (Site 8), and McClean Lake west basin (Site 10). For 2019, the cumulative HQs were > 1 in Vulture Lake only (Figure 2.5). The remaining eight monitoring locations registered cumulative HQs lower than 1.

### 2.3.7. *Prediction of ecotoxicological risk using sensor data*

Given that the risk of aqueous toxicity of individual elements was considered 'moderate' to 'very high' for Se, As, Ag, Cd, Tl, Fe and Hg, these elements were chosen for broader assessment using correlations with hand-held meter EC readings. Results revealed good correlation coefficients only for Se and EC ( $r = 0.94$ ) and As and EC ( $r = 0.93$ ). For the Se risk assessment, regression analysis with 80% of the dataset ( $\text{LnSe} = 1.296\text{LnEC} - 8.1962$ ) indicated that higher Se concentrations were associated with higher EC values ( $F_{1,30} = 254.41$ ,  $P < 0.001$ ,  $r^2 = 0.89$ , Figure 2.6A). Additionally, the model validation resulted in an RMSE of 11%, which was considered acceptable. For the As risk assessment, regression analysis with 80% of the dataset ( $\text{LnAs} =$

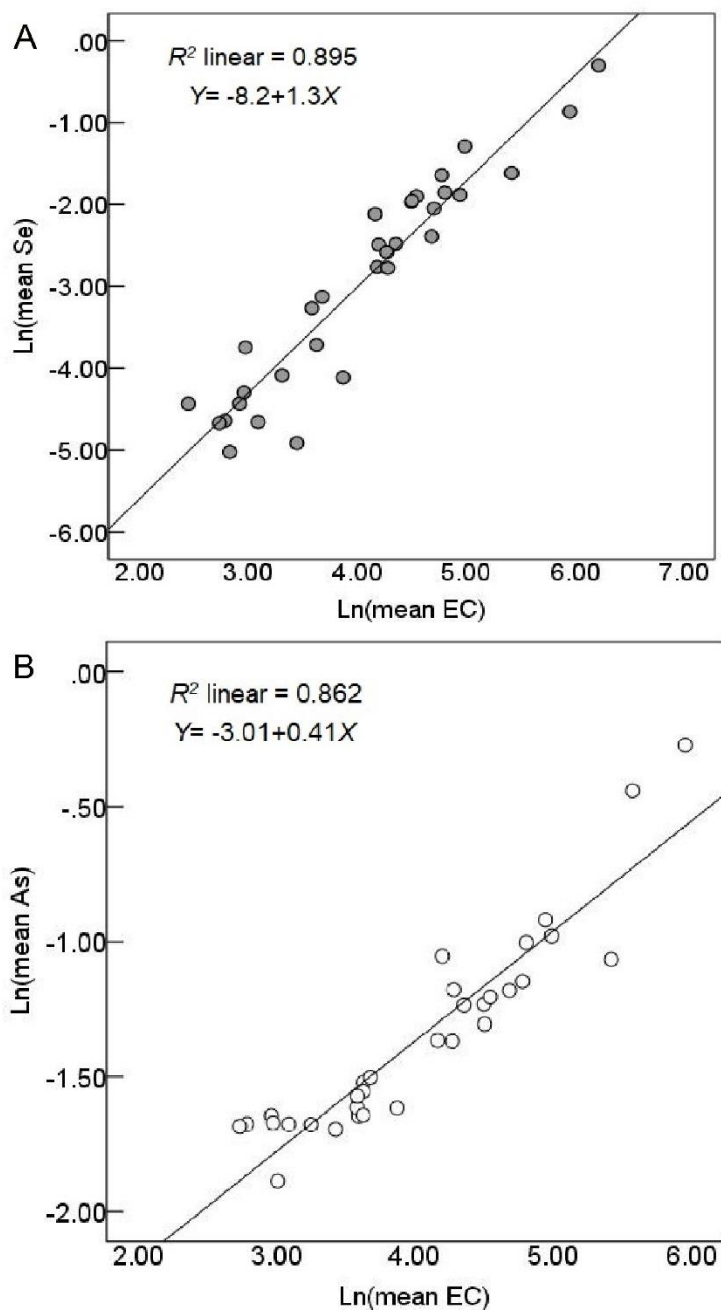
$0.409\ln EC - 3.0057$ ) showed higher As concentrations related to higher EC measurements ( $F_{1,30}=187.07$ ,  $P<0.001$ ,  $r^2 = 0.86$ , Figure 2.6B). Similarly, the validation of this model registered an RMSE of 16%, which was considered acceptable.



**Figure 2.5.** Cumulative risk for total metals exposure to aquatic macroinvertebrates during September 2018 and September 2019. HQs greater than 1.0 (horizontal line) indicate potential ecotoxicological risk.

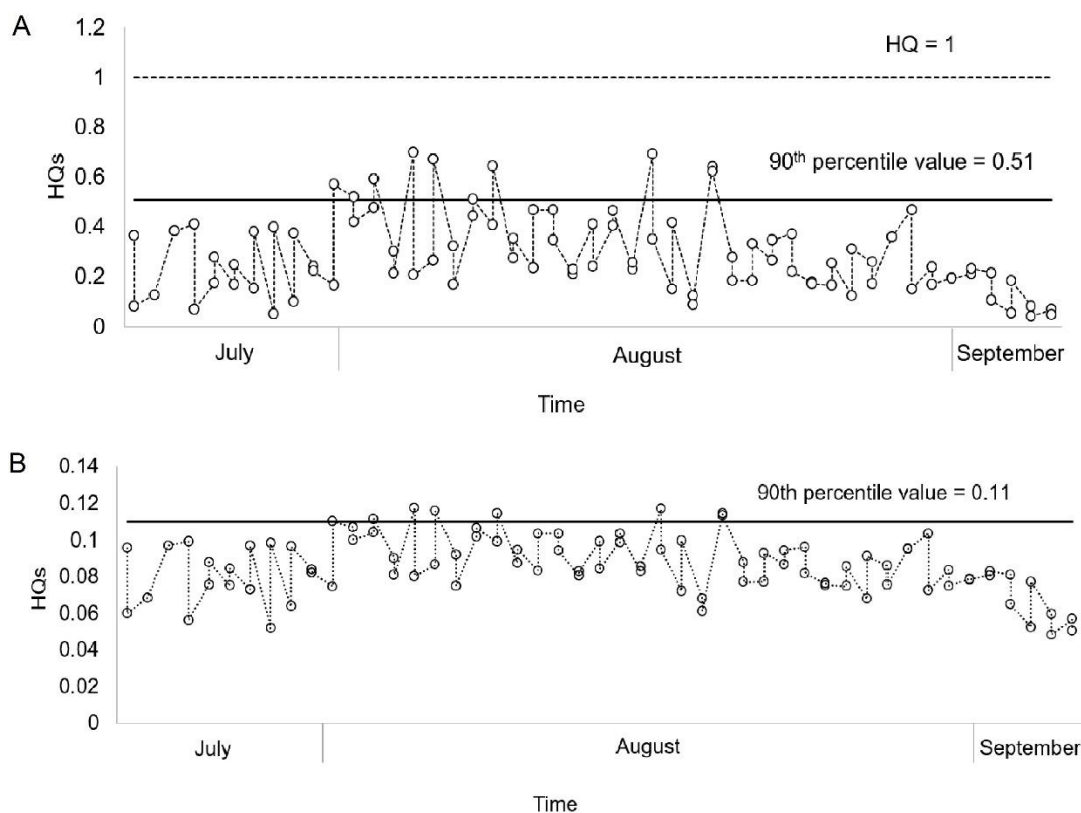
Therefore, it was possible to estimate variation in aqueous Se and As concentrations using sensor EC data from July to September 2019. The mean Se concentration estimated from sensor EC data in the effluent inflow from Vulture Lake to McClean Lake east basin (Site 4) was  $0.29 \mu\text{g Se/L}$ , while the 90<sup>th</sup> percentile value was  $0.51 \mu\text{g Se/L}$ . Since the water quality benchmark for Se is  $1 \mu\text{g Se/L}$ , the estimated risk quotients had the same values (0.29 and 0.51) (Figure 2.7A). For Site 8, the average Se concentration was estimated at  $0.09 \mu\text{g Se/L}$  and the 90<sup>th</sup> percentile value at  $0.16 \mu\text{g Se/L}$ . Similarly, the estimated HQs were 0.09 and 0.16, meaning that the Se concentrations at the inflow and outflow of McClean Lake do not represent a likely risk of adverse effects to aquatic invertebrates since all the estimated HQs were  $<1$ . Sensor EC data for site 4 (McClean Lake inflow) was also used to predict aqueous As concentrations. The mean As value was estimated at  $0.43 \mu\text{g As/L}$  and the 90<sup>th</sup> percentile at  $0.53 \mu\text{g As/L}$ . The risk associated with both

of these concentrations was  $HQ=0.09$  and  $HQ=0.11$ , respectively, when using  $5\text{ }\mu\text{g As/L}$  as the water quality benchmark (Figure 2.7B); consequently, all other sites in McClean Lake had lower HQs values. These results indicated that As concentrations in McClean Lake do not represent a likely risk of adverse effects to aquatic invertebrates.



**Figure 2.6.** Correlation between mean electrical conductivity (EC) values and mean aqueous selenium (A) and arsenic (B) concentrations (2018+2019 data) in McClean Lake east basin.





**Figure 2.7.** Estimated (A) selenium and (B) arsenic ecotoxicological risk (HQ) using sensor EC values for Site 4 in McClean Lake east basin. HQs greater than 1.0 (dashed line) indicate potential risk.

## 2.4. Discussion

Water quality monitoring sensors helped delineate the diluted effluent distribution in McClean Lake east basin using EC as a surrogate for effluent exposure. Data suggested that effluent distribution was highly variable and followed no easily predictable pattern across the monitoring locations (using the information available), except for the higher EC in Vulture Lake and the decreasing EC moving away from the McClean Lake inflow (site 4) and towards the outflow at Collins Creek. Vulture Lake (Site 1) is influenced mainly by inflow from the Sink Reservoir and showed a temporal decline in the EC values, which may be related to the shutdown of the McClean Lake mill from August 21<sup>st</sup> to September 8<sup>th</sup> (2019). Consequently, downstream in McClean Lake east basin, there was also a temporal decrease in the EC measurements despite the high variability in EC values. These variations were likely influenced by variable and

incomplete mixing of the inflow water from Vulture Lake, by the inflow of clean water from McClean Lake west basin, and by changing wind speed and direction. A similar pattern was observed at the outflow near Collins Creek, with EC values decreasing by the beginning of September 2019. Changes in effluent inflow volume can influence streamflow, lake level conditions and the chemical content of downstream surface water bodies (Plagnes *et al.*, 2016).

An essential aspect of using sensor units was that EC probes could detect variation in EC peaks during the monitoring period, confirming that the effluent distribution was uneven along McClean Lake east basin with periods with higher peaks of exposure to aquatic organisms. Similarly, Saab *et al.* (2019) suggested that sensors can better record spatial and temporal variations in contaminant concentrations or physicochemical parameters related to water quality and are therefore needed for better risk assessment.

With respect to routine physicochemical water quality, values obtained were mostly reasonably similar among the two monitoring years (2018, 2019), with higher concentrations in July and August and lower concentrations in September, which is consistent with the pattern of the EC sensor data. McClean Lake sites showed wider variations than Vulture Lake sites, especially for alkalinity, hardness and DOC. These results were generally consistent with conclusions drawn from previous monitoring studies (2011 to 2015) made at the McClean Lake operation by AREVA (2016a).

The individual risk outcomes for metals and trace elements in surface water indicated that most dissolved metals evaluated in the present study should be of little concern to aquatic organisms ('low' risk). Only seven metals were identified as being of 'moderate' to 'very high' risk (As, Se, Hg, Tl, Ag, Cd and Fe). The reason for the high As risk rating was because the CWQG is based on multiplying the 14-d EC50 (growth) value of 50 µg/L (Vocke et al., 1980) for the most sensitive organism, the algae *Scenedesmus obliquus*, by a safety factor of 0.1 (CCME, 1991), resulting in a guideline of 5 µg As/L (CCME, 2021). With regard to Se, currently, the CWQG is 1 µg/L (CCME, 2021) and was originally established by the Canadian Council of Resource and Environment Ministers (CCREM) based on field studies in the Great Lakes, which indicated waterborne Se concentrations from 5 to 10 µg/L caused acute lethality to predatory fish (CCREM, 1987). The CWQG for Se highlighted endpoints such as malformation of fish larvae and embryos

and the increased toxicity of biologically generated organic forms of Se. However, guidelines for the latter could not be accurately incorporated at the time and simply applied a safety factor of 0.1. Details for the SWQO and CWQG values used for the remaining metals (Hg, Tl, Ag, Cd and Fe) HQ calculations in this assessment are presented in appendix N.

Even though the risk outcomes of all metals were assessed individually, it is important to note that cumulative toxicity may be an issue and thus should be evaluated. Overall, cumulative HQs (using a concentration addition approach) for the different sites showed that Vulture Lake had HQs >1 in both monitoring years, and McClean Lake and Collins Creek had toxicological risks >1 once in August 2019. These results suggested a possible risk of adverse effects to aquatic invertebrates from cumulative metal exposure in Vulture Lake and possibly at some locations in McClean Lake and Collins Creek. Nevertheless, except for Ag, Cd and Fe, the CWQGs used in the present study to assess potential risk considered the most sensitive species to be organisms other than invertebrates. Therefore, the toxicity benchmarks used for this risk assessment are likely overly conservative for the evaluation performed here. Besides, the Ag, Cd and Fe benchmarks in the SWQOs were not updated or revised since they were adopted from the old CCREM (1987) guidelines. The cumulative HQs were >1 only once in 2019 for Sites 3, 5 and 6 (McClean Lake east basin) and Site 8 (Collins Creek) with values close to 1 (1.05 to 1.24) coming mostly from Cd and Fe, metals that did not follow the effluent distribution pattern. The latter suggests that the HQs for these sites likely reflected natural background concentrations. Finally, Muscatello & Janz (2008) concluded that concentrations of metals in Diptera (Ba, Cu, Mn, Ni and Zn), Trichoptera (Ba and V), Gastropoda (Cr, Fe and V) and Odonata (Cd, Cu and Sr) collected from Vulture Lake (the highest exposure area in the present study) were significantly less than concentrations in comparable organisms from their reference site (Indigo Lake).

The toxicological risk of major ions (anions and cations) concentrations was difficult to assess for individual ions, given that a CWQG is available only for two anions, Cl<sup>-</sup> (120 mg/L) and F<sup>-</sup> (0.12 mg/L) (CCME, 2021). Calculations based on the Mount et al., (2019) equation showed that even though Vulture Lake had higher HQs relative to McClean Lake and Collins Creek, major ions HQs (based on total osmolarity) were <1 at all monitoring sites, suggesting that the risk for aquatic invertebrates is low despite the HQ being calculated using an EC50 for total osmolarity.

The sediment risk assessment generated individual HQs for V and Cd, suggesting that these metals could have 'moderate' or 'very high' risk to aquatic invertebrates. Vanadium concentrations were similar across all sites yielding similar HQs, suggesting that these values represent natural background concentrations. Hazard Quotients for V were evaluated using Canadian Nuclear Safety Commission Working Benchmarks (Thompson et al., 2005), given that this metal does not have a CCME guideline. These authors reported that V and Cr are found in very low concentrations in uranium ore, and their releases with treated effluent are low. Additionally, Burnett-Seidel & Liber (2013) derived sediment quality values (SQVs) and confirmed that Cr, Cu, Pb, and V SQVs were similar to reference values indicating that these metals were not significantly influenced by the uranium operations in northern Saskatchewan. Conversely, Cd concentrations in Vulture Lake and at the McClean Lake outflow (Collins Creek) resulted in HQs of 'moderate' and 'high' risk. The benchmark used to evaluate the ecotoxicological risk of Cd was obtained from CCME (2021), which lists Canadian interim sediment quality guidelines (ISQGs) and probable effect levels (PELs) based on different field-collected sediment from multiple studies. In general, Cd concentrations in the present study were lower than the ISQG (0.6 mg/Kg), meaning that these values would rarely be associated with adverse biological effects (Environment Canada, 1997).

Regarding the cumulative HQs for metals in sediment, the risk was  $>1$  in Vulture Lake, at some sites in McClean Lake east basin, and at the reference site (McClean Lake west basin), which in theory suggests a possible risk of adverse effects (FCSAP, 2012). However, cumulative HQs were influenced mostly by V concentrations since they were usually higher than the toxicological benchmark concentration (TBC) at all study sites, especially in 2018. The TBC for V is clearly not appropriately derived and should be used with caution. On the other hand, Cd concentrations contributed to the HQs only at three monitoring sites, given that the remaining HQs were considered of low risk. Additionally, neither Thompson et al., (2005) nor Burnett-Seidel & Liber (2013) included Cd in their analysis to establish sediment quality benchmarks related to uranium mining activities in northern Saskatchewan, confirming the general lack of influence of uranium operations on the concentrations of this metal in downstream sediment. Laird et al., (2014) reported that lakes in the vicinity of the McClean Lake operation contain naturally metal-enriched sediments, particularly for As, Hg and V. Finally, sediment metal concentrations at reference sites in northern Saskatchewan, which represent natural background conditions, are known to

occasionally exceed existing sediment quality guidelines used in Canada (Thompson et al., 2005; CCME, 2021).

In the estimation of water HQs for selected elements, the Smart Water® monitoring system was combined with the risk assessment approach to help better estimate the concentrations and potential risk of Se and As in the aquatic environment, given that these elements are continuously discharged during the uranium milling process and can be heterogeneously distributed across McClean Lake. Even though temporal and spatial fluctuations in effluent distribution were detected with the sensors in McClean Lake, the estimated 90<sup>th</sup> percentile of the concentrations for Se (0.51 µg/L) and As (0.53 µg/L) at site 4 generated HQs <1 ( $HQ_{Se}=0.51$  and  $HQ_{As}=0.11$ ). HQs for the outflow at Collins Creek (Site 8) registered even lower values, suggesting that Se and As concentrations do not represent a likely risk of adverse effects to aquatic invertebrate communities in McClean Lake. These results are supported by Muscatello & Janz (2008), who concluded that it was not possible to identify adverse effects of Se in Vulture Lake at water and sediment concentrations of 0.43 µg/L and 0.54 µg/g dry weight, respectively, even though they found Se content in aquatic biota (e.g., forage fish) were elevated. Similar studies also have reported Se accumulation in prey organisms inhabiting aquatic systems with waterborne Se concentrations below 1 µg/L (Lemly, 1993; Muscatello et al., 2008). Additionally, some authors have concluded that sediment plays an important role in the accumulation of Se in aquatic systems (Saiki et al., 1993; Hamilton & Lemly 1999; Orr et al., 2006). For As, an assessment by Sprague and Vermaire (2018) in boreal lakes close to a mining operation in Ontario determined that reference lakes registered mean arsenic concentrations of 2.2 and 1.7 µg/L, values below the limit for the protection of aquatic life (5 µg/L) and above the concentration of 0.53 µg/L determined in the present study.

## **2.5. Conclusions**

Overall, autonomous sensors allowed for easy collection of very detailed exposure data (based on EC) allowing for a risk assessment with a much better understanding of the spatial and temporal variability of that risk in the McClean Lake east basin than which could be obtained with traditional sampling. This risk of metal toxicity was generally considered low and aqueous Se and As should not adversely affect aquatic invertebrates in McClean Lake. The cumulative risk of

aqueous metal toxicity for Vulture Lake and once for McClean Lake and Collins Creek was  $>1$ , suggesting that there may be a possible ecotoxicological risk to sensitive aquatic invertebrates. Similarly, the cumulative risk for metal-contaminated sediments was  $>1$  in Vulture Lake and at some McClean Lake east basin sites. However, some published toxicity benchmarks and the summed cumulative risk assessment approach used here appear to have been overly conservative. Additionally, both water and sediment quality benchmarks do not always include toxicity modifying factors and other confounding factors. For instance, physical stressors (e.g., habitat, scour), chemical stressors (e.g., ammonia, TOC, hardness), and biological stressors (e.g., competition, predation) (Chapman, 2018). However, Se is a persistent pollutant that can accumulate through the food chain to potentially impact the sustainability of fish populations (Lemly, 1997). The continuous effluent discharges to McClean Lake may increase Se concentrations in the system. Therefore, even though no apparent adverse effect on the aquatic invertebrate community was observed, this scenario could change with time. Continued environmental monitoring of the aquatic environment at McClean Lake is recommended to ensure that adverse ecological effects on aquatic biota are not observed in the future.

**CHAPTER 3: EFFECTS OF DILUTED EFFLUENT ON AQUATIC  
MACROINVERTEBRATE COMMUNITIES AT THE MCCLEAN LAKE URANIUM  
MILL IN NORTHERN SASKATCHEWAN**

## PREFACE

The main objective of Chapter 3 was to identify whether there is a potential effluent effect on macroinvertebrate communities in different parts of McClean Lake and at different times (2018, 2019). With the results from Chapter 2, it was possible to identify key elements of concern in water and sediment that may affect the macroinvertebrate communities in McClean Lake.

Chapter 3 was prepared in a manuscript style and will be submitted to the journal *Ecotoxicology and Environmental Safety* for publication.

Cupe-Flores, B., Mendes, M., Phillips, Iain., & Liber, K. (2022). Effects of Diluted Effluent on Aquatic Macroinvertebrate Communities at The McClean Lake Uranium Mill in Northern Saskatchewan. *Ecotoxicology and Environmental Safety*.

### Author Contributions:

Cupe-Flores, B (University of Saskatchewan): Performed and helped design the field and laboratory work (water, sediment and macroinvertebrate collection) and conducted the chironomid bioassay. Conducted the data analysis, generated tables and figures, and drafted the manuscript.

Mendes, M (University of Saskatchewan): Helped conduct and design the field and laboratory work (water, sediment and macroinvertebrate collection). Provided feedback and edits on the manuscript.

Phillips, I (Water Security Agency): Provided guidance and helped with the macroinvertebrate taxonomy and data analysis for macroinvertebrate samples (diversity indices, CCA and GAM). Gave feedback and edits on the manuscript.

Panigrahi, B (University of Saskatchewan): Helped perform the statistical analysis and figures (GAM models CCA plots). Provided feedback and edits on the manuscript.

Liber, Karsten (University of Saskatchewan): Helped design and implement the laboratory and fieldwork. Helped perform the fieldwork and provided feedback and edits on the manuscript.



## Abstract

Diluted treated effluent from the McClean Lake uranium milling operation in northern Saskatchewan is released into the east basin of McClean Lake, which could potentially cause a variety of both chemical and physical disturbances to the aquatic ecosystem. This study aimed to determine the potential effects of diluted effluent exposure (metals and major ions) to benthic macroinvertebrate communities in different parts of two connected lakes, McClean Lake and Vulture Lake. Ten monitoring locations were established in and upstream of McClean Lake to collect water, sediment and benthic macroinvertebrates in late summer-early fall 2018 and summer-early fall 2019. Complementary surface water bioassays were performed with larvae of the midge *Chironomus dilutus* using lake water from selected sites. Results indicated that metrics of macroinvertebrate communities (total abundance and Margalef index (MI)) did not follow the diluted effluent pattern. In general, higher total abundances were recorded at sites 4 and 9 in McClean Lake and site 8 at Collins Creek. In addition, Vulture Lake registered higher MI values compared to McClean Lake. The final model from the Generalized Additive Modelling (GAM) approach confirmed that electrical conductivity (EC), selenium (Se), and chloride (Cl) in water, and total organic carbon (TOC) and cadmium (Cd) in sediment are key elements that collectively may have influenced macroinvertebrate community composition at the study sites. Finally, across all test endpoints in the bioassays, exposure to lake water from Vulture Lake and McClean Lake had no statistically significant effects on *C. dilutus*.

### 3.1. Introduction

All currently active Canadian uranium mines and mills are located in the Athabasca Basin ecoregion in northern Saskatchewan. The geology of the Athabasca Basin results in natural concentrations of some metals and radionuclides in surface water and sediments that are higher than in other regions (Kilgour et al., 2018). The aquatic environments downstream of uranium mining and milling operations are exposed to a variety of both chemical and physical disturbances generating elevated concentrations of many different trace elements (e.g., arsenic [As], molybdenum [Mo], nickel [Ni], selenium [Se], and uranium [U]), as well as major ions (e.g., sulfate and ammonium) (deRosemond et al., 2005; Klaverkamp et al., 2002; Muscatello et al., 2006; Muscatello & Janz 2008; Pyle et al., 2001; Wiramanaden et al., 2009).

Treated effluent from the McClean Lake uranium milling operation is eventually released into the east basin of McClean Lake in northern Saskatchewan. The common repository for all wastewaters generated at the McClean Lake operation is the Sink/Vulture Treated Effluent Management System, which consists of Sink Reservoir, Vulture Lake and all structures and connecting pipelines from Sink Reservoir to the east basin of McClean Lake. The complete list of constituents of potential concern (COPC) at the McClean Lake operation includes the following water quality ions: ammonium ( $\text{NH}_4^+$ ), chloride ( $\text{Cl}^-$ ), fluoride ( $\text{F}^-$ ), potassium ( $\text{K}^+$ ), sulfate ( $\text{SO}_4^-$ ), and total dissolved solids (TDS), the following metals/metalloids: arsenic (As), cobalt (Co), copper (Cu), lead (Pb), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn); and the following radionuclides: U, thorium-230, radium-226, lead-210, and polonium-210 (AREVA, 2016b).

In the case of benthic communities in the McClean Lake east basin, a previous assessment done by AREVA indicated that they were numerically dominated by the taxa Chironomidae (~50-70%) and Pisidiidae clams ( $\geq 20\%$ ). These taxa are usually monitored since they possess many of the characteristics of 'ideal' sentinel organisms. These include being largely sedentary and sufficiently long-lived to allow simultaneous sampling of multiple age classes and developmental stages, thereby reflecting site-specific exposure to water-borne and sediment-accumulated contaminants (Mousavi et al., 2003; Bonada et al., 2006). Because most fish eat benthic organisms during at least part of their life cycle, benthic production influences fish production through

bottom-up effects (Boisclair & Leggett 1989). Additionally, benthic macroinvertebrate communities respond to multiple environmental factors, and there are many interactions among these factors, including water quality (Clews et al., 2014; Miserendino & Masi, 2010), substrate composition (Schröder et al., 2013), and the distribution and abundance of macrophytes (Declerck et al., 2005). Water quality, hydrodynamic conditions, and substrate conditions are the main abiotic factors influencing benthic macroinvertebrate communities (Yi et al., 2018). Therefore, assessing the potential effects of physical and chemical disturbances from the effluent releases at the McClean Lake site is relevant.

Given that mining effluents generally contain elevated levels of several metals and metalloids leading to localized increases in their concentrations, it is appropriate to examine the potential long-term effects of effluent discharges on lower trophic level organisms such as the midge, *Chironomus dilutus*, which plays a vital role in colonization and ecological succession of aquatic ecosystems (White, 2017). Chironomids are an ecologically diverse family of Dipterans and one of the most ubiquitous of all aquatic insects due to their physiological tolerance to environmental stress, such as modifications of salinity or temperature and reduced dissolved oxygen levels. Their increasing use in bioassays and toxicity testing is mainly due to this wide distribution and ecological importance, their short life-cycles, their ability to be reared in the laboratory, and the ease of identifying different stages (Anderson, 1977). In addition, they can be used for the evaluation of both water and sediment toxicity (Ibrahim et al., 1998). Furthermore, the Chironomidae is an invertebrate group that plays a vital role in colonization and ecological succession in the McClean Lake east basin (AREVA, 2016a).

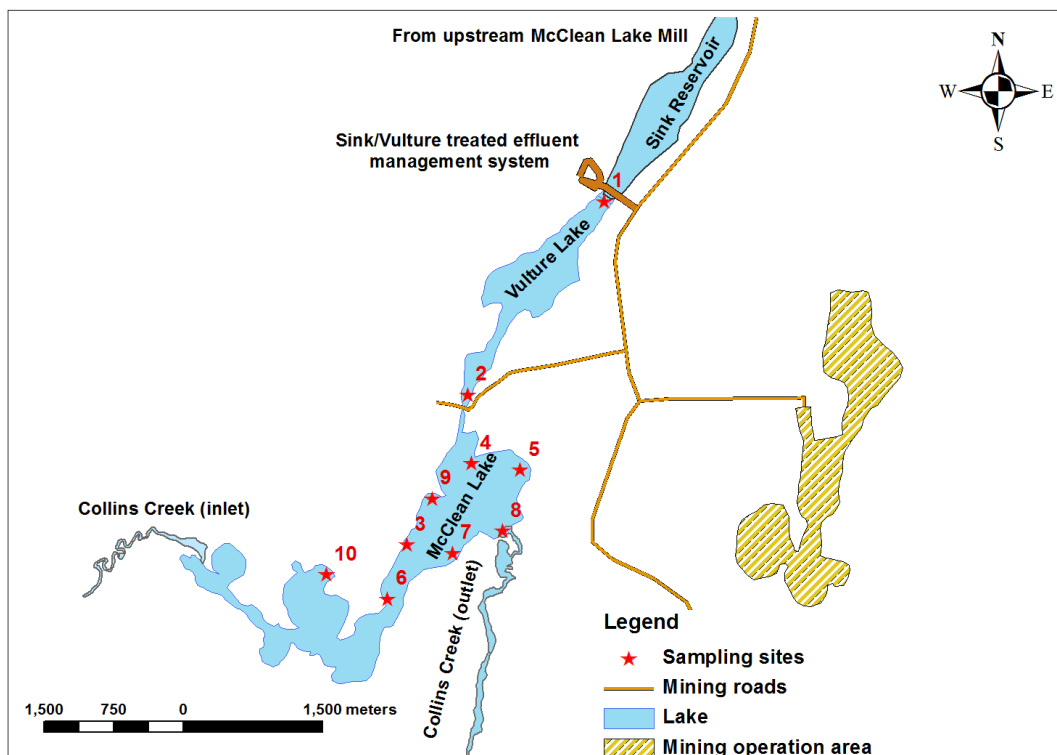
The objectives of this research project were to (1) identify whether there is a potential effluent effect on macroinvertebrate communities in different parts of McClean Lake and at different times (2018, 2019), and (2) to perform a bioassay to characterize the toxicity of surface water from diluted effluent-exposure sites to a representative freshwater midge species, *Chironomus dilutus*.

## 3.2. Materials and methods

### 3.2.1. Study sites

The study sites for this project were located downstream of the McClean Lake uranium milling operation in northern Saskatchewan, Canada. The monitoring locations were chosen to cover different areas within the downstream lakes, especially in the McClean Lake east basin. An effluent diffuser is located at the inflow to the McClean Lake east basin from Vulture Lake to enhance mixing of the diluted effluent. However, the diluted effluent mixing in McClean Lake is variable depending on season, meaning that contaminant exposure to macroinvertebrates in different parts of the lake may be highly variable. For this reason, the focus of this project was on McClean Lake east basin while also including data from Vulture Lake and Collins Creek (other primary exposure areas) as complementary information. Thus, 10 monitoring locations (Figure 3.1) were selected along Vulture Lake, McClean Lake east basin (exposure sites), Collins Creek (downstream site), and McClean Lake west basin (reference location) and sampled in late summer-early fall 2018 and summer-early fall 2019. In 2019, the reference site was moved a few hundred meters to the north in McClean Lake west basin. At the same time, site 8 was moved slightly from just inside Collins Creek to just outside the creek near McClean Lake's outflow to have more consistent benthic habitats.

All sites were located at a water depth between 0.6 and 1 m and always in locations with at least some detritus/organic material covering the otherwise mostly sandy sediment surface in McClean Lake. Vulture Lake contained a softer more organic-rich sediment. Both McClean Lake east and west basins are shallow with similar surface areas of 117.2 ha and 107.2 ha, respectively, and similar volumes, with  $1.62 \times 10^6 \text{ m}^3$  for the east basin and  $1.59 \times 10^6 \text{ m}^3$  for the west basin. Additionally, approx. 50% of both basins have water depths  $\leq 1 \text{ m}$ . Vulture Lake is also relatively shallow with mean and maximum water depths of 2.3 m and 3.4 m, respectively, a surface area of 73.5 ha and a volume of  $1.65 \times 10^6 \text{ m}^3$ . Over 40% of the total water volume ranges from 0 to 1 m. (AREVA 2016a).



**Figure 3.1.** Map of the study site showing the ten 2019 monitoring locations (red stars) along Vulture Lake, McClean Lake and Collins Creek in northern Saskatchewan, Canada.

### 3.2.2. Sample Collection

Water samples ( $n=3$  per site) were collected twice (August and September) in 2018, and three times (July, August and September) in 2019 at a water depth of ~50 cm at all monitoring locations, using a Wildco® 2.2-L acrylic Van Dorn horizontal beta water sampler (Wildlife Supply Company, FL USA). Water from the sampler was poured into a 1-L acid-washed container, and subsamples transferred and filtered through 0.45- $\mu\text{m}$  polyethersulfone membranes to two sets of 30-mL HDPE Nalgene™ bottles using 5-mL syringes. One set of samples was for major ions and physicochemical analysis. The other set was for subsequent metal and trace element analysis (acidified to a pH of  $\leq 2$  with 192  $\mu\text{L}$  of 69% ultra-pure nitric acid).

Sediment samples were collected in September 2018 and September 2019 at the same monitoring locations as water ( $n = 3$  per site) using a standard (15- x 15- x 15-cm) Ekman grab sampler. Processing included removing the top ~2 cm layer of the sediment grabs with a stainless-steel spoon and directly transferring the sediment into plastic vials. Both sediment and water

samples were refrigerated shortly after sampling and transported to the University of Saskatchewan Toxicology Centre (Saskatoon, SK) in ice-packed coolers.

Macroinvertebrate samples and periphyton were collected at the same monitoring locations as water and sediment using Hester-Dendy (H-D) multiplate artificial substrate samplers, which are selective mainly for mobile drift-prone species that colonize hard substrata (Hester and Dendy, 1962; EEM, 2012). The H-D samplers were built at the Toxicology Centre (University of Saskatchewan) and consisted of ten 10 x 10 cm Masonite plates. They were built with two different distances between plates (12 and 4 mm) to create more habitat diversity for macrofauna colonization. In the field, two sets of H-D samplers were tied with ropes to two steel frames anchored in the sediment and left in place for four weeks between August and September 2018 and seven weeks between July and September 2019. One set of H-D samplers ( $n = 4$  per site) was deployed to determine macroinvertebrate community composition. The other set ( $n = 4$  per site) was used to determine tissue metal concentrations in macroinvertebrates and periphyton. In 2019, glass plate samplers (five 20 x 20-cm plates) were deployed at the same locations for additional collection of natural periphyton communities. A standard Ekman grab sampler was used to collect surface sediment ( $n=3$  per site) as part of a complementary sampling protocol to better characterize macroinvertebrate community structure at the different locations.

The H-D and grab samples for macroinvertebrate community analysis, once collected, were preserved in ethanol (96%) and stored in double Ziploc bags. The H-D and grab samples for tissue analysis were retrieved and stored in double Ziploc bags containing site water and maintained cool until being processed immediately after return to the Toxicology Centre. Periphyton samples were obtained by scraping each artificial substrate/glass plate and storing the scraped material in plastic tubes (50 ml). All samples were transported to the University of Saskatchewan Toxicology Centre (Saskatoon, SK) in ice-packed coolers to be processed for macroinvertebrate community analysis in-house following the Canadian Environmental Effects Monitoring guidance document for benthic macroinvertebrate samples (EEM, 2012).

### **3.2.3. Characterization of water chemistry**

*In situ* water quality parameters (pH, EC, and DO) were measured at a water depth of ~50 cm in 2018 using a Sevensgo duo pH/conductivity meter (Mettler Toledo, NY, USA) and a S4 dissolved oxygen meter (Mettler Toledo, NY, USA), and in 2019 using a Thermo Scientific Orion Star™ A329 pH/ISE/conductivity/dissolved oxygen portable multiparameter meter (Thermo Scientific, KS, USA). Dissolved metals and trace elements analyses (B, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Ag, Cd, Sn, Sb, Ba, Hg, Tl, Pb and U) were performed in-house via inductively coupled plasma mass spectrometry (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, CA, USA). Analysis for specific major ions ( $\text{Na}^+$ ,  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{+2}$ ,  $\text{Ca}^{+2}$ ,  $\text{F}^-$ ,  $\text{Cl}^-$  and  $\text{SO}_4^{-2}$ ) was performed via ion chromatography with a Dionex ICS 2000, using an IonPac AS18 column (Thermo Fischer Scientific, CA, USA) in the Department of Soil Science at the University of Saskatchewan. Conventional water quality analyses (hardness, alkalinity and turbidity) were performed in-house at the Toxicology Centre. Dissolved organic carbon (DOC) content was analyzed using a Total Organic Carbon Analyzer TOC-VCPN (Shimadzu Corporation, Tokyo, Japan) in the Department of Soil Science.

### **3.2.4. Physicochemical characterization of sediment**

Sediment samples were dried at 60° C for 72 h, subsequently ground, hydrofluoric acid added to a subsample (47% - 51%), and the sample digested in a MARS-5 Microwave Accelerated Reaction System with closed Teflon vessels (CEM Corporation, NC, USA) for four hours. The reference material used for quality assurance analysis was marine sediment PACS-3 (NRC-CNRC, Ottawa, Canada). All elements of interest (same as for water) were analyzed using an Agilent 8800 ICP-MS QQQ Triple Quadrupole spectrometer (see details above). Total organic carbon (TOC) analysis and particle size (% sand, % silt and % clay) determination were performed in the Department of Soil Science. Sediment subsamples for TOC determination were pre-treated with approximately 1 mL of sulphurous acid (6%) to prevent analysis of inorganic carbon. Subsequently, samples were analyzed for TOC in a LECO C632 TOC analyzer (LECO Corporation, MI, USA).

### **3.2.5. *Characterization of aquatic macroinvertebrates***

In the Toxicology Centre, H-D samplers were disassembled, and each plate was gently scrubbed with a soft brush and rinsed with water to dislodge all periphyton and invertebrates. The slurry containing water, periphyton and macroinvertebrates was poured into a sorting tray, where all invertebrates were picked out by eye, identified to family, and counted following the EEM guidance document for benthic macroinvertebrate samples (EEM, 2012). Additionally, some macroinvertebrates were sorted for identification to the lowest practical level. Macroinvertebrate community data were used to calculate total abundance and the Margalef Index (MI) (Margalef 1958). The formula for MI is as follows:

$$MI = (S - 1)/\ln N \quad (1)$$

Where S is taxa richness, that is the number of taxa within a sample and N is the total number of individuals.

### **3.2.6. *Macroinvertebrates and periphyton tissue analysis***

Macroinvertebrate and periphyton samples were freeze-dried and then cold digested using nitric acid (1 mL Omnitrace Ultra, EMD Chemicals, WA, USA) and hydrogen peroxide (0.66 mL Super Pure; EMD Chemicals). The reference material used for digestions was Tort-3 (Lobster hepatopancreas, National Research Council, Canada). Elements of interest (same as for water and sediment) were analyzed using an Agilent 8800 ICP-MS QQQ Triple Quadrupole spectrometer at the Toxicology Centre. Quality assurance/quality control measures included an instrumental standard, a certified reference material and instrumental and method blanks. The instrumental standard reference material, natural water 1640a (National Institute of Standards and Technology, MA, USA) and the certified reference material (Tort-3), were run with all samples with an analytical accuracy always within  $\pm 20\%$  of certified values.

### **3.2.7. *Determination of Bioaccumulation Factors***

A Bioaccumulation Factor (BAF) is defined as the quotient obtained by dividing the concentration of a substance in an organism (or specified tissue) by its concentration in a specified exposure medium (e.g., food, sediment, and water) (ASTM, 2011). For this project, BAFs were



calculated using metal concentrations in whole macroinvertebrates as the numerator, and total metal concentrations in sediment, surface water or food (periphyton) as the denominator:

$$BAF = \frac{\text{Organism tissue concentration}}{\text{Water/sediment/food concentration}} \quad (2)$$

Contaminant concentrations in water were converted to mg/L, and concentrations in sediment and food were included in the calculations as mg/kg. All units cancelled out and BAFs could be compared among the various compartments. BAFs were determined only for metals that resulted in higher hazard quotients in a previous ecotoxicological assessment (Cupe-Flores et al., 2022). Average sediment and water metal concentrations were used for calculations assuming that macroinvertebrates had lived in the lake in a pseudo-steady state for most of the monitoring period.

### 3.2.8. *Electrical conductivity adjustment*

Electrical conductivity measurements were corrected to have meaningful data interpretation given that besides the amount and composition of ionic species, EC is strongly dependent on temperature. Thus, EC hand-held meter data were adjusted to a water temperature of 13°C, which was the median lake water temperature during both study periods. Adjustments of EC values were made using equations from Hayashi (2004).

$$EC_{t0} = EC_t / [1 - c(t - t_0)] \quad (3)$$

Where  $EC_{t0}$  is electrical conductivity at a standard temperature  $t_0$  and  $c$  is a constant given by:

$$c = a/[1 + a(t_0 - 25)] \quad (4)$$

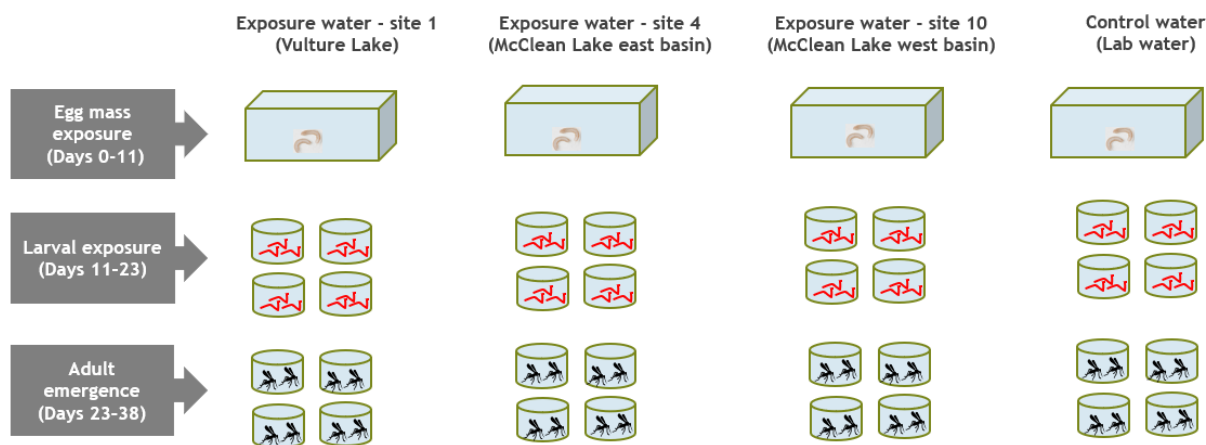
For equation (4),  $a$  (temperature compensation factor) was considered to be 0.0191 as recommended by Clesceri et al. (1998).

### 3.2.9. *Bioassay with Chironomus dilutus*

The bioassay using *C. dilutus* was conducted following Environment Canada Biological Test Method EPS 1/RM/32 guidelines (1997), with some modifications for the increased exposure duration and endpoints, according to White (2017). The *C. dilutus* used in this bioassay were

obtained from an in-house culture at the University of Saskatchewan Toxicology Centre raised in 15-L aquaria containing a thin layer of silica sand (particle size 200-400  $\mu\text{m}$ ) and aerated, carbon-filtered, bio-filtered municipal water from the City of Saskatoon. Aquaria were kept in an environmental chamber maintained at  $23 \pm 1^\circ\text{C}$  with a 16:8 h light:dark photoperiod and were fed 15 mL of a slurry of Nutrafin fish flakes (Rolf C. Hagen Inc., Montreal, QC, Canada) three times a week with weekly water changes. Lake water was collected in July and September 2019 using 4-L containers. Enough water was collected for both culturing and for water renewals during two complementary, long-term experiments. Exposure waters came from Vulture Lake (site 1), McClean Lake east basin (site 4), and McClean Lake west basin (site 10).

The experimental design consisted of a partial and a full lifecycle test of 27 and 38 days of exposure, respectively, to examine potential effluent effects on more sensitive sub-lethal endpoints such as larval development and adult emergence (Figure 3.2). The difference in the exposure time for the first bioassay (27 days) was because not enough lake water was sampled in July, and the test had to be initiated with second instar larvae. Egg masses had to be previously adapted in laboratory water adjusted to the same hardness as exposure waters (Site 1: 500 mg  $\text{CaCO}_3/\text{L}$ , Site 4: 67 mg  $\text{CaCO}_3/\text{L}$ , Site 10: 6 mg  $\text{CaCO}_3/\text{L}$ ). For the second bioassay, exposures started with egg masses because there was enough lake water sampled in September, and no adaptation steps were needed.



**Figure 3.2.** Experimental design of the bioassay with *Chironomus dilutus*.

To start the second bioassay, egg masses were isolated from adults from the in-house culture and placed into three 15-L aquaria (2-3 egg masses per tank) containing silica sand and

exposure waters (from sites 1, 4, and 10), as well as one tank with laboratory control water. Test organisms were therefore exposed from the egg mass stage until adult emergence to better mimic the environmental exposure scenario and account for possible effects on the earliest life stage. As soon as they were visible, second instar larvae were collected (11 days after addition of egg masses) to start the second phase of the bioassay.

The second phase (12 days of exposure), for both bioassays, was initiated with 80 larvae per treatment randomly isolated from the aquaria and transferred in groups of 10 into 300-mL lidded tall form glass beakers containing silica sand and the respective exposure or control water (250 ml). In order to investigate possible effects on both larval and adult endpoints, the eight replicates per treatment were divided so that half of the test vessels were taken down before pupation, while the rest continued the exposure through to adult emergence. Observations of survival and larval activity were recorded daily. Water was partially renewed every 2 days during the experiments by removing and replacing 150 ml of exposure water in each beaker. Exposure water was fully changed, and larvae were removed to new beakers every 8 days to avoid biofilm growth and ammonia buildup. Once pupation was observed (after 12 d of exposure), four test vessels for each treatment were terminated and survival recorded. Finally, all organisms were removed from their sediment cases and oven-dried before weighing.

To finalize the bioassays (15 days of exposure), the remaining four test vessels in each treatment continued with the exposure unchanged; however, daily observations were also expanded to include notes of the state of larval pupation and adult emergence. All successfully emerged adults were collected and removed from test vessels to determine sex and then individually oven-dried to calculate adult dry weights.

### **3.2.10. Data and statistical analysis**

Total abundances and the Margalef diversity index (MI) were used to describe the benthic macroinvertebrate community structure in the study area. The MI was calculated at the lowest practical taxonomic level using PRIMER-E Version 6.1.13 (Clarke and Warwick 2001). Canonical Correspondence Analysis (CCA) was applied using the "Vegan" package from the R Project for Statistical Computing Version 3.6.2 program (R Development Core Team 2013). This analysis

was carried out to describe relationships between benthic macroinvertebrates and physicochemical parameters and identify factors that may significantly influence the benthic community. A Generalized Additive Modeling (GAM) approach was followed to quantify relationships between the MI and physicochemical factors as presented by Yi et al. (2018). The general form for the GAM is shown in Equation. (5).

$$\alpha(E(Y)) = \beta_0 + f_1(x_1) + f_2(x_2) + \dots + f_n(x_n) \quad (5)$$

Where  $E(Y)$  is the expected value of the response variable  $Y$ ;  $\alpha$  is the link function;  $\beta_0$  is the intercept; and  $f_n$  is the smoothing function of the explanatory variable  $x_n$ .

A total of 10 physicochemical factors were chosen as possible explanatory variables, including the water quality variables, electrical conductivity (EC), selenium (Se), sulfate ( $\text{SO}_4^{+2}$ ), chloride ( $\text{Cl}^-$ ), and pH, as well as the sediment variables total organic carbon (TOC), silt, cadmium (Cd), and vanadium (V). In this study, GAMs were developed using the software package R (version 3.6.2), including the "mgcv" library (Wood, 2006). The Shapiro-Wilk method was used to check for normality. The test result had a w value of 0.95 and a p-value of 0.195 ( $>0.05$ ), implying that the data follow a normal distribution. Similarly, the calculated MI for all study sites also met the assumption of normal distribution. Hence, the Gaussian distribution was adopted in the GAM model. The GAM uses a link function to establish the relations between the mean of the response variable and a smoothed function of the explanatory variables. The relationship between the environmental factors and MI was examined first to check whether the effects of the single parameters on the index were significant ( $\alpha = 0.1$ ). Factors that had a significant effect on MI were retained in the model.

In order to determine whether the exposure water caused significant effects on the survival, growth and emergence of *C. dilutus* larvae and adults, the average percentage of survival and dry weight from each treatment were analyzed and compared using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. If data did not pass the Kolmogorov-Smirnov test or the assumption of homoscedasticity, significant differences among treatments were determined using Kruskal-Wallis one-way ANOVA on ranks followed by Dunnett's post-hoc test. All the

statistical analyses were run using SPSS 20 (SPSS Inc., IBM) with a 95% ( $\alpha=0.05$ ) confidence level.

### **3.3. Results**

#### **3.3.1. *Surface water chemistry***

Water chemistry parameters (mean  $\pm$  SD) for the monitoring locations during the two years (2018 and 2019) are reported in the appendices A to I. Briefly, the data revealed a decrease in total hardness and EC moving with distance from the effluent diffuser (site 4) in McClean Lake. Measurements for pH and DO were similar among sites, while alkalinity and turbidity varied among sites. Not surprisingly, major ions displayed a similar pattern to the EC measurements, with higher values in Vulture Lake, dropping at the inflow to McClean Lake and dropping to even lower values near the outlet at Collins Creek. Decreasing concentrations of major ions, especially calcium and sulfate, were largely responsible for the decrease in conductivity. The change in EC reflected a decreasing effluent influence on surface water quality with distance from the effluent diffuser.

Dissolved B, Ni, As, Se, Sr, Mo, Ba and U concentrations in the lake system correlated well with the effluent pattern ( $r>0.5$ ). Higher concentrations were present in Vulture Lake and decreased along McClean Lake east basin moving away from the effluent diffuser towards the outflow at Collins Creek. However, other metals such as Al, V, Cr, Mn, Co, Zn, Sr, Mo, Ag, Cd, Sn, Sb, Hg, Tl and Pb did not correlate with effluent concentration ( $r<0.5$ ) and did not display any pattern, suggesting that values at all sites represented normal background concentrations with negligible effluent impact.

#### **3.3.2. *Sediment chemistry***

Sediment chemistry results (mean  $\pm$  SD) for all monitoring locations are presented in appendices J to M. Total Co, Ni, As, Se and Mo concentrations in sediment displayed values that followed the general diluted effluent distribution pattern with higher values in Vulture Lake that decreased moving downstream along McClean Lake to Collins Creek. The remaining metals (Be, Al, Ti, V, Cr, Fe, Mn, Cu, Zn, Sr, Mo, Ag, Cd, Sn, Sb, Ba, Hg, Tl, Pb and U) did not show any

pattern related to effluent distribution, suggesting that these elements more likely represented background concentrations for this system.

Total organic carbon content in 2018 tended to be slightly higher than in 2019 at McClean Lake while remaining relatively similar in Vulture Lake. The percentages of TOC followed the general effluent pattern, except for site 8. On the other hand, particle size composition showed that the content of sand was similar through the lake system in both years, registering values higher than 80% at all monitoring locations. Silt content at all sites was higher in 2018 than in 2019 and largely followed the effluent pattern.

### **3.3.3. *Benthic community structure***

A total of 22 families belonging to 10 orders and six classes were identified in samples collected with both Hester-Dendy and grab samplers from the study sites. Overall, there were 16 arthropod families, two families of annelids, and four families of Mollusks (Table 3.1). Details of total macroinvertebrate community abundances are presented in appendices P to R. The most abundant taxa included *Polycentropus*, *Leptophlebia*, Chironomidae, *Hyalella*, *Sphaerium* and *Fossaria* (Table 3.2). Among these, the most consistently abundant taxon was Chironomidae. The six taxa summarized here accounted for 75.2 to 96.9 % of total abundances in 2018 and 44.5 to 99.7% in 2019. The low percentage for site 1 in 2019 was due to high abundances in a few taxa (e.g., *Promenetus* and *Centroptilum*) which were somewhat unique to that site. There were only minor differences in total abundances among sites in 2018. However, higher abundances were recorded in 2019, possibly linked to the longer colonization time (7 vs. 4 weeks) with colonization starting earlier in the season. Moving sites 8 and 10 to more representative habitats may also have contributed to higher abundances for those sites.

In addition, in 2019, a few taxa contributed to higher-than-average abundances at sites 4, 8 and 9. These differences were largely attributed to very high abundances of the taxa Chironomidae, *Polycentropus* and *Leptophlebia*. Consequently, the higher abundance in 2019, related to high numbers of a few taxa, resulted in lower diversity indices (Margalef index) in 2019 compared to 2018. Finally, diversity was highest for site 1 in both years; nothing else was particularly unique for the diversity values for the other six sites.

**Table 3.1.** Benthic macroinvertebrates taxa in Vulture Lake, McClean Lake and Collins Creek (2018-2019) collected with Hester Dendy and Ekman grab samplers

Phylum	Class	Order	Family	Genre	Code <sup>a</sup>
Arthropoda	Insecta	Odonata	Aeshnidae	<i>Aeshna</i>	AESH
			Coenagrionidae	<i>Coenagrion</i>	COEN
		Trichoptera	Phryganeidae	<i>Agrypnia</i>	AGRY
			Brachycentridae	<i>Brachycentrus</i>	BRACHY
			Polycentropodidae	<i>Polycentropus</i>	POLY
		Ephemeroptera	Leptoceridae	<i>Oecetis</i>	OEC
			Molannidae	<i>Molanna</i>	MOLA
			Leptophlebiidae	<i>Leptophlebia</i>	LEPHTO
			Baetidae	<i>Centroptilum</i>	CENTROP
			Heptageniidae	<i>Stenonema</i>	STENO
			Caenidae	<i>Caenis</i>	CAEN
			Ephemeridae	<i>Hexagenia</i>	HEXA
			Metretopodidae	<i>Siphloplecton</i>	SIPH
		Diptera	Chironomidae	-	CHIRO
		Coleoptera	Dytiscidae	<i>Ilybius</i>	ILY
				<i>Nebrioporus</i>	NEBRIO
				<i>Potamonectes</i>	POTA
	Crustacea	Amphipoda	Hyalellidae	<i>Hyalella</i>	HYALL
	Arachnida	Acari	-	-	ACARI
Annelida	Hirudinea	Rhynchobdellida	Glossiphoniidae	<i>Batracobdella</i>	BATRA
		Pharyngobdellida	Erpobdellidae	<i>Erpobdella</i>	ERPOB
	Oligochaeta	-	-	<i>Nephelopsis</i>	NEPHE
				-	OLIGO
Mollusca	Pelecypoda	Veneroida	Pisidiidae	<i>Sphaerium</i>	SPHA
	Gastropoda	Basommatophora	Lymnaeidae	<i>Fossaria</i>	FOSS
			Planorbidae	<i>Promenetus</i>	PROME
			Physidae	<i>Physa</i>	PHYSA

<sup>a</sup> Codes used in CCA plots.

**Table 3.2.** Total abundances and diversity results for macroinvertebrates collected with Hester Dendy samplers during the two monitoring years

Most abundant taxa	Year	Monitoring locations									
		Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
<i>Polycentropus</i>	2018	10	0	6	20	0	4	14	26	6	15
	2019	13	0	19	78	0	1	14	1770	38	4
<i>Leptophlebia</i>	2018	18	40	19	36	68	103	92	6	4	16
	2019	105	199	22	5	117	27	60	1334	91	7
Chironomidae	2018	56	71	70	121	84	60	71	10	66	7
	2019	142	186	129	1633	442	376	139	109	770	60
<i>Hyalella</i>	2018	0	0	11	7	2	9	4	7	7	0
	2019	0	1	66	21	1	22	22	23	51	32
<i>Sphaerium</i>	2018	22	35	0	3	7	0	9	8	0	0
	2019	71	0	0	0	5	3	6	45	0	0
<i>Fossaria</i>	2018	79	39	0	0	0	0	0	0	0	0
	2019	58	4	0	0	0	0	0	0	0	0
Total abundance of select taxa	2018	185	185	106	187	161	176	190	57	83	38
	2019	389	390	236	1737	565	429	241	3281	950	103
Combined percentage total abundance (%)	2018	75.2	87.3	80.3	81.0	94.7	91.2	96.9	81.4	84.7	86.4
	2019	44.5	91.8	96.7	99.5	97.8	92.7	96.0	99.7	98.3	91.2
Margalef Index <sup>a</sup>	2018	2.83	1.64	1.72	1.71	1.17	1.36	1.35	2.15	1.68	1.36
	2019	2.27	1.30	0.90	0.77	0.67	1.12	0.97	0.90	1.02	1.08

<sup>a</sup> Based on all taxa

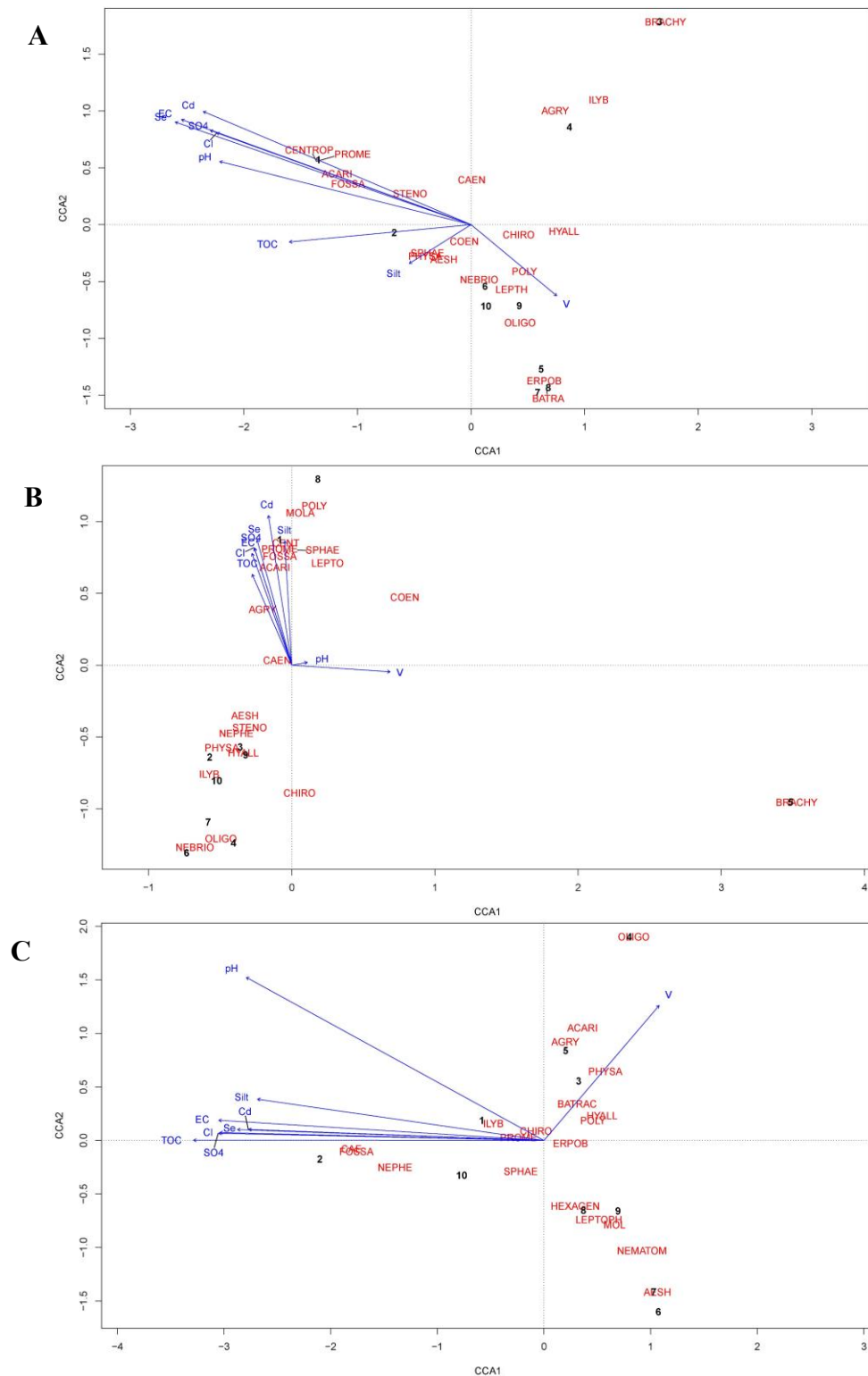
A CCA was conducted to investigate relationships between benthic macroinvertebrate taxa and water and sediment quality variables (Fig. 3.3A, 3.3B and 3.3C) for 2018 and 2019 data. The physicochemical parameters EC, Se, SO<sub>4</sub>, Cl, and pH in the water column and TOC, silt, Cd, and V in sediments were chosen for the analysis. Most of these parameters were associated with the treated diluted effluent, and Se, Cd and V were determined to present moderate to high theoretical ecotoxicological risk in a prior assessment (Cupe-Flores et al. 2022).

For H-D data from 2018 (Figure 3.3A), the CCA1 and CCA2 axes explained 22.2% and 20.1% of the variation, respectively. All the physicochemical parameters assessed had a negative correlation with CCA1 except V; so did TOC, V and silt with CCA2. Selenium was the water



quality parameter with the highest correlation coefficient ( $r=0.89$ ) on CCA1. The amount of variation in the macroinvertebrate community explained by CCA2 was low, with both EC and Se having a correlation coefficient of 0.31, the highest correlation on CCA2 (positive). The main water quality parameters appearing to drive the distribution of macroinvertebrates were EC, Se, Cl, SO<sub>4</sub> and Cd, given that these variables had a large correlation coefficient with axis CCA1. The taxa *Aeshna*, *Coenagrion*, *Centroptilum*, *Stenonema*, *Caenis*, *Nebrioporus*, *Sphaerium*, *Fossaria*, *Promenetus*, *Physa*, and Acari displayed a negative correlation with CCA 1, indicating that these taxa were positively correlated with EC, Se, Cl, SO<sub>4</sub> and Cd.

The H-D results from 2019 (Figure 3.3B) show macroinvertebrate data where CCA1 and CCA2 explained 22.9% and 19.9% of the variation, respectively. Cadmium had the highest correlation coefficient with axis CCA2 ( $r=0.67$ ). Similarly, the parameters EC, Se, SO<sub>4</sub>, Cl, and silt all had positive correlations of approximately  $r=0.5$  with CCA2, suggesting that those are the main factors driving the distribution of the taxa. The amount of variation in the macroinvertebrate community explained by axis CCA1 was low compared to CCA2. The taxa *Aeshna*, *Agrypnia*, *Molana*, *Caenis*, *Centroptilum*, *Coenagrion*, *Fossaria*, *Leptophlebia*, *Polycentropus*, *Promenetus*, *Sphaerium*, *Stenonema*, and Acari were positively correlated with EC, Se, SO<sub>4</sub>, Cl, silt and Cd. The other taxa were negatively correlated with these parameters. Differences in the two CCA plots (2018 vs. 2019) could possibly be explained by the slightly different deployment periods, longer colonization time, annual variation, and the slight change in the monitoring locations for sites 8 and 10.



**Figure 3.3.** CCA biplot of physicochemical parameters and macroinvertebrate taxa collected with Hester-Dendy samplers for 2018 (A) and 2019 (B), as well as with an Ekman grab sampler for

2019 (C). Monitoring sites numbers are shown in black, taxa in red, and physicochemical parameters in blue.

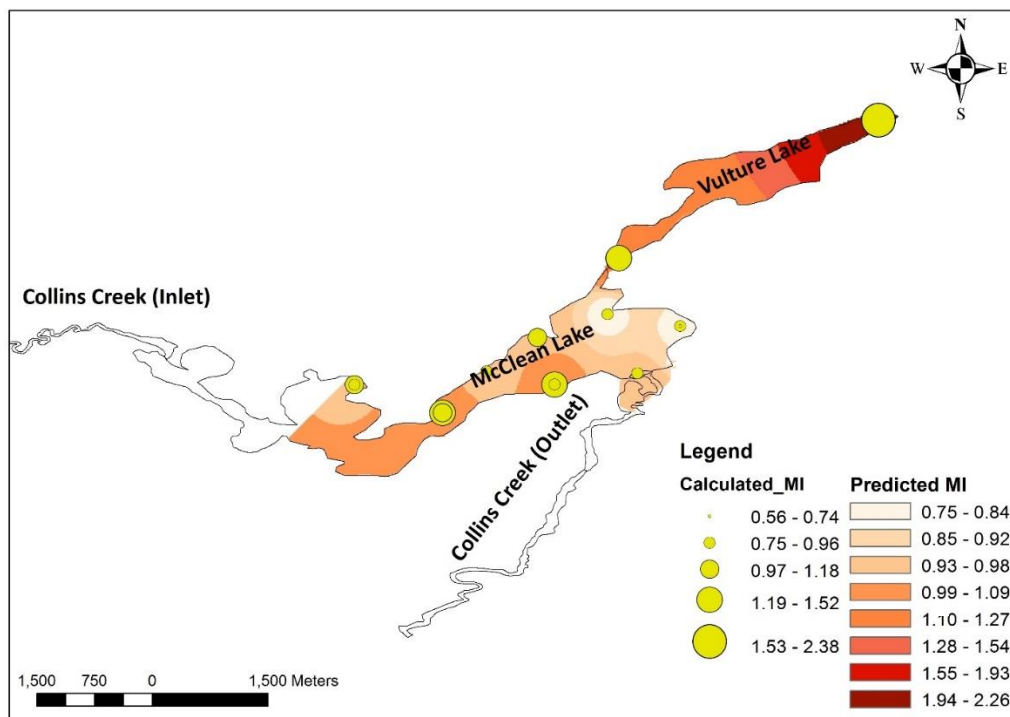
A CCA analysis was also performed using macroinvertebrate data collected in 2019 using Ekman grab samples (Figure 3.3C) to complement the community assessment. The axis CCA1 and CCA2 explained the 20.1% and 16.8% of the variation, respectively, and all parameters (EC, Se, SO<sub>4</sub>, Cl, pH, TOC, silt, and Cd), with the exception of V, were negatively correlated with CCA1. Overall, there was a good correlation between these variables and CCA1 ranged between -0.72 for silt and -0.88 for TOC. Only V had a low correlation ( $r=0.29$ ) with CCA2. Thus, the amount of variation in the macroinvertebrate community explained by axis CCA2 was low. The taxa *Nephelopsis*, *Caenis*, *Fossaria*, *Ilybus*, *Promenetus*, *Sphaerium*, and Chironomidae, showed a negative correlation with CCA1, meaning that these taxa were positively correlated with EC, Se, SO<sub>4</sub>, Cl, pH, TOC, silt, and Cd. Finally, higher abundances were generally observed for the H-D samples than the grab samples, especially for sites 4 and 8.

### **3.3.4. Effects of physicochemical factors on the macroinvertebrate community**

The relationship between each physicochemical variable and the Margalef diversity index (MI) was investigated using data from 2018. The variables EC, Se, pH, and Cd had significant correlations with MI ( $r>0.5$ ,  $p<0.05$ ), while SO<sub>4</sub>, Cl, TOC, V, and silt resulted in lower correlations ( $r<0.5$ ,  $p>0.05$ ). Seven different models were developed to determine which variables have effects on the macroinvertebrate community. Initially, model-1 included only Cd, then physicochemical variables such as Cl and EC were added to models 2 and 3, respectively, not showing much improvement. Afterwards, Se, SO<sub>4</sub> and TOC were added to model 4. However, SO<sub>4</sub> was removed to get model 5 since there was an improvement in the model (higher deviance explained). Lastly, SO<sub>4</sub>, pH and silt, as well as V, were added to create model 6 and model 7, respectively. Nevertheless, after adding these variables to the models, the model performance was not significant ( $p>0.05$ ). The final form of the model was model 5:  $MI \sim 1.69 + s(Cd) + s(Se) + s(EC) + s(Cl) + s(TOC)$ , where 's' represents the smoothing spline fit in the GAM function. The deviance explained by the model was 77.9% (Pearson,  $r^2=0.72$ ).

### 3.3.5. Validation of the model

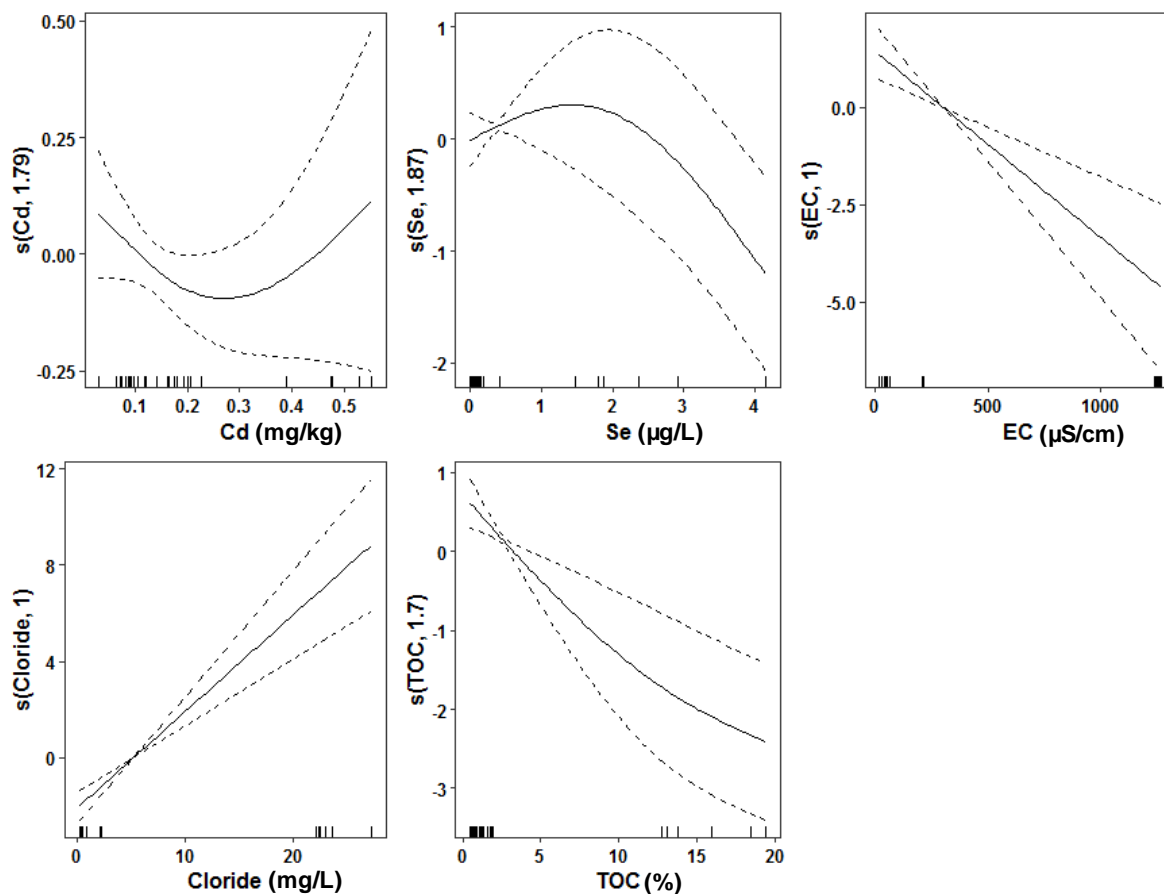
Macroinvertebrate data collected in 2019 were used to validate model 5 (developed using 2018 data) so that calculated MI values and the predicted MI values produced by this model were consistent. Monitoring sites with low calculated MI values corresponded with low predicted MI values. Similarly, sites with high calculated MI values corresponded with high-predicted MI values. Calculated MI and predicted MI reached a significantly high correlation (Pearson,  $r^2=0.90$ ;  $p < 0.001$ ) and showed a small mean squared error (MSE) of 0.015. Therefore, these data indicate that the model developed in this study can effectively simulate and estimate the actual distribution of the diversity index (MI) at McClean Lake. Additionally, the distribution of the diversity index was mapped using a GIS platform, thus depicting the diversity of macroinvertebrate fauna in McClean Lake (Figure 3.4).



**Figure 3.4.** Distribution of calculated and predicted macroinvertebrate taxa diversity indices (MI) in McClean Lake.

According to model 5, the response curves (Figure 3.5) showing the relationships between calculated MI and EC (edf = 1) and between MI and Cl (edf = 1) were linear, while calculated MI relative to TOC (edf = 1.70), Se (edf = 1.87), and Cd (edf = 1.78) had different response curves

showing a weakly non-linear relationship. The effective degree of freedom (edf) is a summary statistic of GAM that reflects the degree of non-linearity of a curve (Wood, 2006). An  $\text{edf} = 1$  is equivalent to a linear relationship,  $1 < \text{edf} \leq 2$  is considered a weakly non-linear relationship, and  $\text{edf} > 2$  implies a highly non-linear relationship (Stenseth et al., 2006; Litzow & Ciannelli 2007; Zuur et al., 2009). The vertical axis (effect) in Figure 3.5 shows a response curve indicating that the MI was negatively correlated with EC and positively correlated with Cl. The response curves showing the relationship between MI and Cd, TOC, and Se were somewhat unimodal. Because the scale of the study area was small, the range of values for each parameter was narrow. Therefore, the model can only describe changes in the MI within the ranges of these parameters.



**Figure 3.5.** Response curves for the Margalef diversity index (MI) to select physicochemical variables in the generalized additive model (GAM) analysis. The vertical axes indicate the relative influence of each explanatory variable on the prediction. Dotted lines indicate 95% confidence limits.

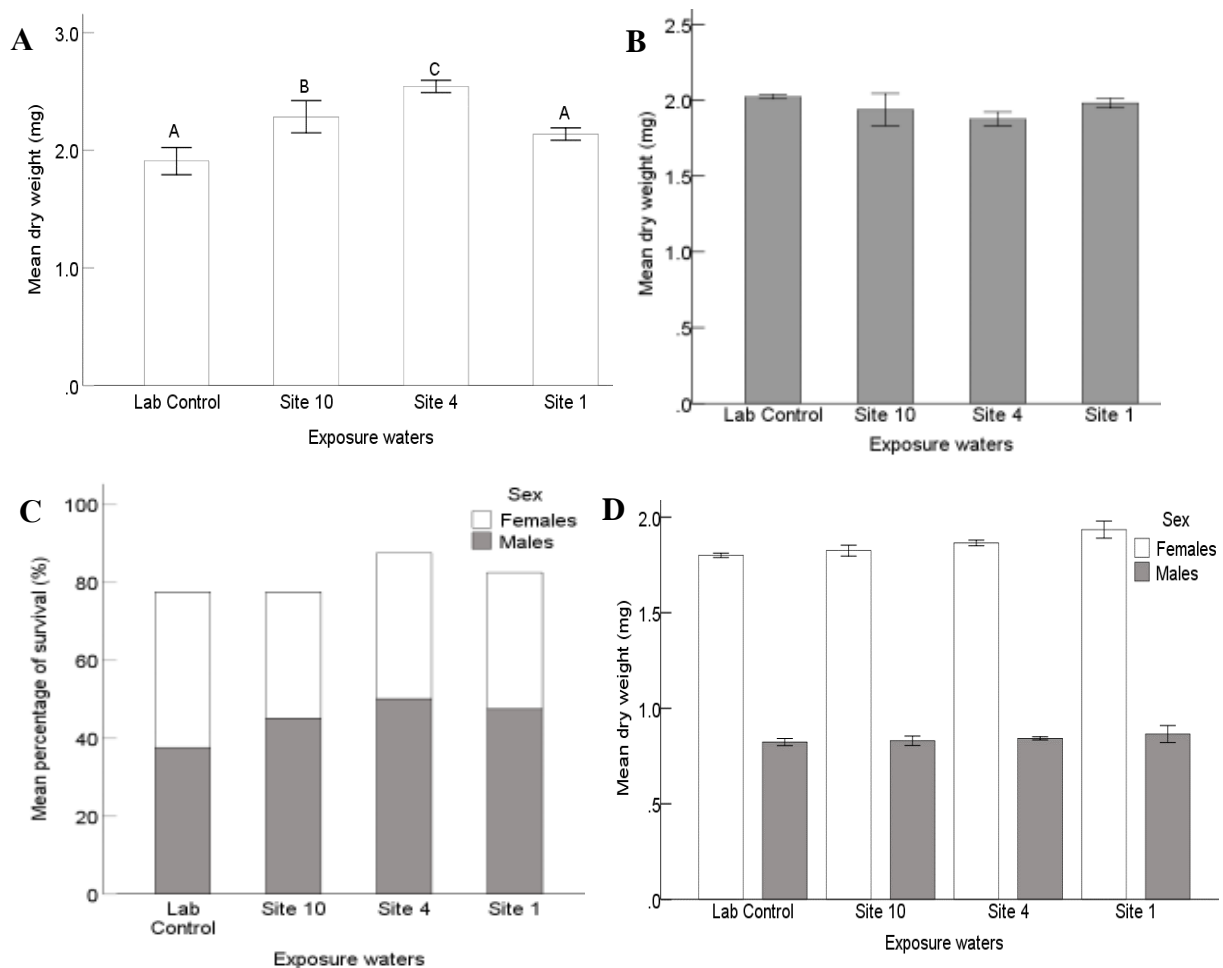
### 3.3.6. Bioaccumulation Factors

Bioaccumulation factors (BAFs) for the most abundant and representative macroinvertebrate family (Chironomidae) collected from Vulture Lake site 1 (high exposure site), McClean Lake east basin site 4 (close to the effluent diffuser), Collins Creek site 8 (outlet), and McClean Lake west basin site 10 (reference site) were calculated based on water and food exposure and focusing on As, Se and Hg concentrations in water and periphyton. Similarly, BAFs based on sediment and food for V and Cd in sediment and periphyton were calculated for *Sphaerium* and Chironomidae since there were not enough tissue masses for chironomids from Vulture Lake and Collins Creek. The elements As, Se, Hg, V, and Cd were included in this analysis because they resulted in moderate to high risk in an earlier ecotoxicological risk assessment (Cupe-Flores et al. 2022). Details of BAFs for other macroinvertebrate taxa are presented in appendices S to X.

Bioaccumulation factors for metals in macroinvertebrates for 2018 and 2019 based on water and food (periphyton from H-D samplers) showed that macroinvertebrate BAFs based on aqueous exposure concentrations were higher than BAFs based on periphyton (food) concentrations. Bioaccumulation factors were highest for Se (1,000 – 451,000), followed by As (65 – 34,000) and Hg (6 – 2,000), while BAFs based on food registered lower values (from 0.01 to 9.36). Furthermore, BAFs based on whole sediment and food (periphyton) were calculated using total concentrations of Cd and V analyzed in sediment and macroinvertebrates, both collected with an Ekman grab sampler in 2019. Bioaccumulation factors based on whole sediment metal concentrations were generally lower than BAFs based on water. BAFs were lower because exposure included non-bioavailable metals in the sediment. BAFs for V based on either sediment and food (ranged from 0.01 to 0.40) and were lower than Cd BAFs (ranged from 17 to 3,000), given that V concentrations were higher than the Cd concentration in sediments. Details of BAFs for Chironomidae and *Sphaerium* are presented in appendices Y and Z.

### 3.3.7. Chironomid bioassays

The survival of pre-adapted second instar chironomid larvae after 12 d of exposure to surface water from four sites ranged from 77.5% (site 4) to 100% (lab control) in the first bioassay. Mean larval dry weight showed significant differences between the four test waters (Figure 3.6A). Sites 4 and 10 registered higher dry weights than site 1 and the lab control.



**Figure 3.6.** (A) Mean ( $\pm$ SE) dry weight of chironomid larvae in the first bioassay after 12 days of exposure to lab control water and three site waters. Significant differences between site waters and the control are shown in different letters. (B) Mean ( $\pm$ SE) larval dry weight in the second bioassay after 12 days of exposure to lab control and site waters. (C) Mean ( $\pm$ SE) percent adult (male and female) emergence in the first bioassay. (D) Mean ( $\pm$ SE) dry weight comparison between female and male adult chironomids in the second bioassay.

In the second bioassay, where exposure started with egg masses without adaptation to exposure water conditions, differences in the time for egg masses to hatch/dissolve were observed among the control and the exposure waters after 3-4 days of starting the test. Larvae appeared to begin hatching within the first 48 h in the lake waters, while the lab control larvae hatched after 72 h. Hatching of the control water larvae appeared to be further delayed as the egg masses were

still visible and well-defined after 72 h, while the egg masses in the lake waters had almost completely disappeared in the following order site 2 > site 4 > site 10 within 72 h. Direct egg mass exposure to the lake water did not have any visible effects on hatching success or larval survival, as >200 larvae were counted in each of the aquaria. After 12 days of exposure, no statistically significant differences were observed in mean dry weight of larvae among the four treatments (Figure 3.6B).

Adult emergence success for the first bioassay (after 27 days of exposure) ranged from 78% to 89% between control water and lake water treatments. There were no significant effects on the survival of chironomids during the exposure time (Figure 3.6C). Furthermore, the ratio between female and male chironomids was relatively similar among treatments with values of 1.0 for the lab control and 0.7 for sites 10, 4, and 1. Of the organisms that died in the second bioassay, many started doing so at the pupation stage. This was observed not only in the exposure waters but also in the control water. Overall, survival was low, ranging from 30% to 70%. The reason for this is unknown. The female to male emergence ratios were similar in the lab control (1.25) and site 10 (1.23), but low in sites 4 and 1 water (0.12 and 0.28, respectively). By test termination (after 38 days of exposure), there were no statistically significant differences in dry weights among the lake waters and the control once the sex of emerged adults was considered (Figure 3.6D). Adult emergence in lake waters, especially sites 4 and 1 was faster than in the lab control water by 2-3 days. Due to the small number of replicates and surviving individuals, the test's statistical power was poor. Across all test endpoints, exposure to lake water from Vulture Lake and McClean Lake had no statistically significant effect on the survival and emergence of *C. dilutus*.

### **3.4. Discussion**

Overall, macroinvertebrate abundances as measured by Hester Dendy samplers were higher in 2019 than in 2018 at all monitoring locations likely due to the difference in the deployment period and duration. However, the total abundances did not correspond to the diluted effluent pattern, especially in 2019, since higher numbers were recorded in sites 4, 8 and 9, mostly coming from the taxa Chironomidae, *Leptophlebia*, and *Polycentropus*. One of the most sensitive community metrics for observing detrimental effects on aquatic macroinvertebrates is diversity. If diversity is affected, the most tolerant organisms will often remain and increase in abundance,



while the most sensitive organisms will disappear, thus affecting the community composition (Slooff, 1983). The lower Margalef indices (MI) for 2019 compared to 2018 were possibly influenced by the higher total abundances of a few taxa, especially for sites 4 and 5 that recorded the lowest MI. Interestingly, site 1 (the highest exposure site) in Vulture Lake registered the highest MI among the two monitoring years, suggesting that the MI was also not adversely affected by the diluted effluent. Additionally, two statistical methods, CCA and GAM, were used to identify the key physicochemical parameters that appear most likely to influence the benthic macroinvertebrate communities in McClean Lake. The CCA analysis of invertebrate abundances, physicochemical parameters (EC, SO<sub>4</sub>, Cl, and pH in the water column and TOC and silt in sediments) and metals (Se in water, and Cd and V in sediment) separated the different taxa of the Vulture Lake communities from the Collins Creek and the McClean Lake communities. The differences in the CCA plots were likely explained by the different deployment periods in 2018 and 2019.

According to model-5 of the GAM analysis, EC, Cl, and Se in water, and TOC and Cd in sediment were selected as key variables that in combination may have influenced the macroinvertebrate communities as measured by the Margalef diversity index (MI). Electrical conductivity and Cl are considered tracers for effluent dilution/mixing within the McClean Lake east basin, as displayed by the gradient in concentrations of these parameters among the study sites, with higher values in Vulture Lake (upstream) and lower values in Collins Creek (downstream) and McClean Lake west basin (reference site). The observation of a negative relationship between the MI and EC was similar to findings by Sowa et al. (2020), who found that the diversity of the macroinvertebrate taxa had a significant negative correlation with the electrical conductivity assessed in ponds. Conversely, the relationship between MI and Cl was positive in the present research. Similarly, a study evaluating acute toxicity of Cl to freshwater species found that the survival of *H. azteca* was positively correlated with chloride concentrations (Soucek & Kennedy, 2009). Total organic carbon content in sediment showed a negative, weakly non-linear relationship with MI. While organic matter in sediment is an important source of food for benthic macroinvertebrates, an overabundance may lead to reductions in species richness, abundance, and biomass due to oxygen depletion and build-up of toxic by-products (e.g., ammonia and sulfide) associated with the breakdown of these materials (Diaz & Rosenberg 1995; Gray et al., 2002).

Species richness typically shows a gradual decline over intermediate TOC ranges as a sensitive species start to disappear (Hyland et al., 2005).

Both Se in water and Cd in sediment resulted in a weakly non-linear relationship with MI, that were somewhat unimodal. The relationship between aqueous Se and MI showed a slight increase and then a dramatic decrease, a pattern that is similar to that seen in another study wherein the diversity index was significantly greater in the 0.12 µg/L Se treatment than in the 1.0 µg/L and the 8.9 µg/L treatments. That study concluded that high Se exposure (8.9 µg Se/L) significantly reduced macroinvertebrate diversity (Graves et al., 2019). On the other hand, Cd in sediment displayed a wider confidence interval than other variables, meaning that there was greater uncertainty regarding a possible influence of Cd on MI at higher Cd concentrations. A different study showed that despite high Cd levels in spiked sediment (up to 843.1 mg Cd/kg), the population densities of most taxa appeared to have been unaffected by the presence of Cd in sediment compared to Cd in overlying water (Warren et al., 1988).

The GAM analysis effectively simulated and estimated the macroinvertebrate diversity (MI) at the different study sites, suggesting that the combination of the five variables in model-5 could somehow be collectively influencing macroinvertebrate diversity. This was confirmed by the model validation, where sites with high calculated MI values corresponded with high model predicted MI values and sites with low calculated MI values corresponded with low model predicted MI values. Interestingly, sites 4 and 5 (close to the effluent diffuser) registered lower calculated and estimated MIs for 2019, which may be due to a combination of stressors as suggested by model-5, or due to minor habitat differences in this very sandy lake. In contrast, sites 1 and 2 (higher exposure areas) showed higher calculated and predicted MIs (Figure 3), most likely a result of the softer and more organic sediment in this lake, which should have provided a better and more diverse macroinvertebrate habitat. Finally, an important point regarding the GAM (model-5) is that it generally shows uncertainty from the use of the diversity index, the environmental variables selected in the model, and the structure of the GAM equation (Guisan & Thuiller, 2005; Wilhere, 2012). The calculations performed with the GAM are limited by the assumption that a unique subset of optimal variables exists. Sometimes there is no single best subset, meaning that some important factors may be ignored in the model (Neter et al., 1989). For instance, water hardness levels that were high (~500 mg CaCO<sub>3</sub>/L) in Vulture Lake could modify

the bioavailability and toxicity of some metals in aquatic systems through processes such as competition (e.g.,  $\text{Ca}^{+2}$ ) or complexation (e.g., carbonates) (Pascoe et al., 1986; Winner & Gauss, 1986; Spry & Wiener, 1991).

The BAFs for chironomids based on water exposure varied according to the monitoring site; BAFs were higher for Se, followed by As and Hg. BAFs for chironomids based on their food (periphyton) registered lower values. In macroinvertebrates, differences in metal bioavailability or metal species dominance in water can also influence the BAFs (Goulet & Thompson 2018). Even though Se BAFs for macroinvertebrates were usually higher than As and Hg BAFs at the sites monitored here, neither of these metals are known to have a detrimental effect on aquatic invertebrates at the concentrations present here, but Se can bioaccumulate to significant levels in higher order organisms. For instance, in a study conducted in northern Saskatchewan, 19 trace metals were analyzed, but only Se was present at a significantly higher concentration in pike eggs originating from an exposure site than those from the reference site (Muscatello et al., 2006). Another study by Muscatello and Janz (2008) conducted at the McClean Lake operation did not find differences in Se concentrations among the invertebrate groups and between plankton or periphyton from each site. However, Se concentrations in burbot (*Lota lota*) were significantly greater than in periphyton, Gastropoda and Hirudinea within the exposure sites. Metal BAFs for macroinvertebrates calculated from sediment and food/periphyton exposure concentrations (from sediment grab samples) resulted in V BAFs that were lower than Cd BAFs, probably because V concentrations were generally higher than Cd concentrations in sediments, even at the reference site. Thompson et al. (2005) reported that V concentrations in northern Saskatchewan are more likely to result from natural conditions than from uranium mining influence. Furthermore, Muscatello and Janz (2008) concluded that concentrations of some metals in Diptera (Ba, Cu, Mn, Ni, Zn), Trichoptera (Ba, V), Gastropoda (Cr, Fe, V) and Odonata (Cd, Cu, Sr) collected from Vulture Lake were significantly less than those from the reference site.

Finally, the surface water bioassays conducted here with Vulture Lake and McClean Lake water used a common test species, *C. dilutus*, that is reasonably representative of chironomid taxa found in McClean Lake. The differences observed in the mean larval dry weight for the first bioassay (Figure 3.6A) could possibly be explained by the comparable difference in survival of

test organisms since sites 10 and 4 registered lower larvae survival than site 1 and the control. Theoretically, lower survival translated into slightly more food and space availability and, consequently, greater dry weight for the remaining living larvae in sites 4 and 10 water. Besides that, results confirmed that McClean Lake water did not adversely affect the hatching, development, or adult emergence of *C. dilutus*. Developmental stages are generally more sensitive to contaminants than adult organisms (UNSCEAR, 1996; Adam-Guillermin et al., 2018). Also, organisms sufficiently stressed by contaminants for extended periods will likely have less energy available for both growth and reproduction than unstressed organisms (Liber et al., 1996). Exposure to environmental stressors often leads to a greater proportion of males in Chironomidae populations (Liber et al., 1996). However, no effects on sex ratio were observed in these bioassays.

### **3.5. Conclusion**

Neither the total abundances nor the diversity index (MI) of benthic macroinvertebrates corresponded to the diluted effluent pattern, meaning that there were possibly other factors influencing macroinvertebrate community composition. In this regard, the GAM model helped identify which variables could potentially influence macroinvertebrate community diversity measured with the MI at the study sites. As a predictive tool, the GAM model suggested that EC, Se, and Cl in water and TOC and Cd in sediment are key variables (stressors) that combined may have affected the macroinvertebrate community composition. In addition, the unique habitat characteristics of McClean Lake (very sandy) compared to Vulture Lake (soft organic sediment) likely resulted in different communities in these lakes. The BAFs for chironomids based on aqueous contaminant exposure varied across monitoring sites with higher values for Se than for As and Hg, and with sites with higher effluent exposure registering lower BAFs, and sites with lower effluent exposure registering higher BAFs. However, all BAFs were possibly influenced by exposure concentrations of metals in both water and sediment. Finally, the bioassay results did not show detrimental effects of surface water on chironomids. Overall, environmental monitoring of the aquatic environment in McClean Lake should continue to ensure that macroinvertebrate communities remain healthy, especially when considering aqueous Se, which could continue to bioaccumulate in the aquatic biota over time.

## **CHAPTER 4: GENERAL DISCUSSION**

#### **4.1. Project focus and objectives**

Treated effluent from the McClean Lake uranium milling operation is released into Sink Reservoir and subsequently flows through Vulture Lake and into the east basin of McClean Lake in Northern Saskatchewan, Canada. Although the concentrations of individual metals in the diluted effluent may not be toxicologically significant, the cumulative effect of these metals could potentially represent a toxicological risk to aquatic organisms, including benthic macroinvertebrates, a key component of the local aquatic food web. Because effluent is typically a complex mixture of constituents that are constantly changing in constituent concentrations, fluctuations and sporadic contaminant concentration peaks are likely to only be detected through relatively high-frequency or continuous measurements. In this context, applying autonomous sensor technology to monitor water quality in real-time is an extraordinary tool to better describe temporal changes in aqueous contaminant exposure to aquatic organisms.

This study aimed to delineate effluent exposure with a focus on combined metals and major ions to identify the potential cumulative risk to aquatic invertebrates and determine the variability of that risk over time using autonomous sensor technology, as well as to measure the impacts on the macroinvertebrate communities within McClean Lake east basin. The main objectives of this study were to (1) better understand if there were temporal and spatial variations in effluent distribution in the McClean Lake east basin using remote sensor technology, (2) estimate the associated risk from cumulative contaminant exposure (metals and major ions) to aquatic invertebrates in McClean Lake east basin, (3) predict the pattern of the toxicological risk of metals to aquatic invertebrates in McClean Lake east basin using remote sensor data, (4) determine the actual diluted effluent effect on macroinvertebrate communities in McClean Lake east basin, and (5) perform a bioassay to characterize the toxicity of surface water from effluent-exposure sites in McClean Lake to a representative freshwater species (*Chironomus dilutus*).

#### **4.2. Summary of findings**

##### **4.2.1. Effluent distribution**

The diluted effluent mixing in McClean Lake east basin was previously observed to be uneven and variable depending on season, based on EC as an effluent tracer. To understand better the effluent mixing pattern, six autonomous sensor units were deployed at all McClean Lake study

sites during the primary monitoring period (July, August and September 2019) to measure EC in real-time. Two sensor units were also deployed in Vulture Lake, one sensor unit in McClean Lake west basin and one in Collins Creek. Data suggested that effluent distribution was variable and followed no easily predictable pattern across the monitoring locations, except for the higher EC in Vulture Lake and decreasing EC moving away from the McClean Lake inflow (site 4) and towards the outflow at Collins Creek. Vulture Lake EC values ranged from 1,700 to 870  $\mu\text{S}/\text{cm}$ ; McClean Lake east basin site 4 (approx. 50 m from the diffuser inflow), from 420 to 48  $\mu\text{S}/\text{cm}$ ; and Collins Creek (site 8) from 225 to 22  $\mu\text{S}/\text{cm}$ . In general, EC measurements showed a temporal decline at all study sites from July to September 2019, which may be related to the shutdown of the McClean Lake mill from August 21<sup>st</sup> to September 8<sup>th</sup> (2019). Changes in the volume of effluent release from the mill could have influenced streamflow, lake levels and the concentration of constituents in downstream surface water bodies. In conclusion, it was possible to detect daily variations in EC peaks during the monitoring period using sensor technology, confirming that the effluent distribution was uneven and variable along the McClean Lake east basin with periods of higher peaks of exposure to aquatic organisms.

#### ***4.2.2. Ecotoxicological risk in surface water***

The toxicological assessment, based on HQs, was performed for 18 metals with available benchmarks. These benchmarks were mainly obtained from the Saskatchewan Surface Water Quality Objectives (SWQO). Results confirmed that only seven metals (As, Se, Hg, Tl, Ag, Cd and Fe) were identified as potentially having 'moderate' ( $\text{HQ} > 0.4$ ) to 'very high' ( $\text{HQ} > 1$ ) risk within the two monitoring years. Even though the risk outcomes of all metals were assessed individually, it is important to note that cumulative toxicity may be an issue and thus should be evaluated. Therefore, a concentration addition approach was used, summing the toxicity of each compound in a conservative, hypothetical scenario assuming that the risk from individual metals is additive. Results showed that Vulture Lake presented cumulative  $\text{HQs} > 1$  in both monitoring years, while McClean Lake and Collins Creek had cumulative  $\text{HQs} > 1$  only once in August 2019, suggesting a possible risk of adverse effects to aquatic macroinvertebrates in Vulture Lake and possibly at some locations in McClean Lake and Collins Creek.

The toxicological risk for major ions (anions and cations) was difficult to assess individually, given that a CWQG was available only for two anions (Cl and F). Therefore, benchmarks were calculated using a Mount et al. (2019) equation to estimate the chronic effects of major ions solutions (Na, Na/Ca, Mg/Na, Ca and their mixtures) on *C. dubia*. Results suggested that even though Vulture Lake had higher HQs relative to McClean Lake and Collins Creek, the HQs were <1. Thus, major ions at all the monitoring sites did not appear to represent a likely risk of adverse effects to aquatic invertebrates.

#### **4.2.3. *Ecotoxicological risk in sediment***

The risk assessment for sediment contaminants followed a similar approach as for water using both individual and cumulative HQs. For both years (2018 and 2019), the individual element risk calculations showed that only V and Cd had ‘very high’ (HQ>1) or ‘moderate’ (HQ>0.4) HQs. However, neither V nor Cd concentrations followed the diluted effluent distribution pattern of having higher concentrations in the higher exposure areas (Vulture Lake and McClean Lake site 4), suggesting that the mill effluent had little influence on their presence in sediment. Consequently, the existing toxicity benchmarks for V and Cd appear to be overconservative and more represent background concentrations in this region than actual sediment toxicity thresholds.

The cumulative HQs for metals in sediment were >1 in Vulture Lake, at some sites in McClean Lake east basin, and at the reference site (McClean Lake west basin), which in theory suggests a possible risk of adverse effects. However, cumulative HQs were influenced mostly by V concentrations since they were generally higher than the toxicological benchmark concentration at all study sites. Cadmium concentrations only contributed to the cumulative HQs at three monitoring sites, two in Vulture Lake in 2018 and 2019 and one in Collins Creek in 2019. The cumulative HQs at the remaining sites were considered of ‘low risk’.

#### **4.2.4. *Prediction of ecotoxicological risk using sensor data***

After better understanding the diluted effluent distribution based on sensor EC data and identifying metals associated with moderate to high risk, the Smart Water® monitoring system was combined with the risk assessment approach to help better estimate the concentrations and potential risk of metals in the aquatic environment. Correlation analysis was employed to describe



the relationship between hand-held meter EC measurements and ICP-MS metal concentrations with  $HQ > 0.4$ . Where significant relationships were established, linear regression equations were calculated to estimate concentrations of aqueous metals not measured directly from the water sampling but derived from EC sensor data. Results suggested that there was a significant relationship only for aqueous Se and EC ( $r = 0.94$ ) and As and EC ( $r = 0.93$ ). After applying regression equations, the estimated 90<sup>th</sup> percentile of the concentrations for Se ( $0.51 \mu\text{g/L}$ ) and As ( $0.53 \mu\text{g/L}$ ) generated  $HQs < 1$  ( $HQ_{Se} = 0.51$  and  $HQ_{As} = 0.11$ ) for the highest exposure area in McClean Lake east basin (site 4). The  $HQs$  for the outflow at Collins Creek (Site 8) registered even lower values, suggesting that Se and As concentrations do not represent a likely risk of adverse effects to aquatic invertebrate communities in McClean Lake or in Collins Creek. Therefore, it was possible to use EC sensor data to estimate concentrations of some metals and to calculate estimated  $HQs$  to determine the toxicological risk of aqueous contaminants like Se and As in the downstream aquatic environment.

#### **4.2.5. Effects on the macroinvertebrate community**

Results from the 2018 and 2019 Hester-Dendy samplers identified sixteen arthropod families, two annelid families, and four mollusc families at the study sites. The most abundant taxa included *Polycentropus*, *Leptophlebia*, Chironomidae, *Hyalella*, *Sphaerium*, and *Fossaria*. There were only minor differences in total abundances among the monitoring sites in 2018. However, higher abundances were recorded in 2019, possibly linked to the longer colonization time (7 vs. 4 weeks) and the slight relocation of sites 8 and 10 to more representative habitats. The higher abundances in 2019, related to high abundances of a few taxa, resulted in lower diversity indices (Margalef index) in 2019 compared to 2018. Neither the total abundances nor the diversity index for benthic macroinvertebrates corresponded to the diluted effluent pattern.

The CCA analysis of invertebrate abundances, physicochemical parameters (EC,  $\text{SO}_4$ , Cl, and pH in the water column, and TOC and silt in sediments) and metals (Se in water, and Cd and V in sediment) separated the different taxa in the Vulture Lake communities from the Collins Creek and the McClean Lake communities. However, one of the most sensitive community metrics for observing detrimental effects on macroinvertebrates is diversity. If diversity is affected, the most tolerant organisms will often remain and increase in abundance while the most sensitive

organisms will disappear thus affecting the community composition (Slooff, 1983). The relationship between the Margalef diversity index (MI) and potential stressors (physicochemical variables/metals) was investigated using data from 2018. Seven different Generalized Additive Model (GAM) were developed to determine which variables may affect the macroinvertebrate communities. The best model from the GAM analysis included EC, Se, Cl, TOC, and Cd, and showed that these are key variables that combined may have affected the macroinvertebrate community composition at the different study sites. According to the GAM model, MI was negatively correlated with EC and positively correlated with Cl. The response curve showing the relationship between MI and Cd, TOC, and Se was somewhat unimodal. After validating the model with data from 2019, it was observed that the calculated and predicted MI reached a significantly high correlation and displayed a small mean squared error. These data indicated that the model developed in this study could effectively simulate and estimate the actual range of the diversity indices (MI) for McClean Lake.

#### **4.2.6. Bioassay**

It was appropriate to examine the potential long-term effects of Vulture Lake and McClean Lake water on lower trophic level organisms, such as the midge *Chironomus dilutus*. This invertebrate belongs to the ‘Chironomidae’ family, which plays a key role in the food web in McClean Lake. The bioassay results confirmed that McClean Lake water (site 4) did not adversely affect the hatching, larval development, pupation, and adult emergence of *C. dilutus*. Across all test endpoints, lake water from high exposure sites in Vulture Lake and McClean Lake had no statistically significant effect on the survival and emergence of *C. dilutus*.

#### **4.3. Integration of the risk assessment to the aqueous environment of McClean Lake**

The ecotoxicological risk assessment of metals in water showed that the study sites registered HQs with ‘moderate’ to ‘high’ risk for As, Se, Fe, Tl, Hg, Ag and Cd. Concentrations of all of these metals were generally higher in Vulture Lake. However, only As and Se followed the observed diluted effluent pattern of having higher values in Vulture Lake and McClean Lake east basin Site 4. When considering the combined risk from individual metals to be additive, the cumulative HQs had a similar pattern, registering higher values in Vulture Lake in both monitoring years (maximum HQ=9). However, some McClean Lake and Collins Creek locations had

cumulative HQs slightly  $>1$ , but only once (September 2019). In theory, there was a possible risk of adverse effects to benthic macroinvertebrates in Vulture Lake and possibly at some locations in McClean Lake and Collins Creek. However, this outcome resulted when using the SCWQG benchmarks, which directly adopt the CWQGs. In these guidelines, except for Ag, Cd and Fe, the CWQGs are driven by organisms other than invertebrates as the most sensitive species. For instance, fish are the most sensitive taxon for Hg and Se, algae for As, and an aquatic plant for Tl. The SWQO for Ag and Cd have not been updated and are still based on the old CCREM (1987) benchmarks, while for Fe, its higher concentrations at the reference site suggested that this metal was likely reflecting natural background conditions. For major ions, the assessment showed that they do not represent a likely risk of adverse effects to aquatic macroinvertebrates. These results were also supported by the water bioassay, which confirmed that McClean Lake water from high exposure sites did not adversely affect the hatching, larval development, pupation, or adult emergence of *C. dilutus* (Chironomidae play a key role in the food web in McClean Lake). Some factors like water hardness and the concentrations of other trace elements in Vulture Lake could have also have modified the bioavailability and decreased toxicity of some metals.

As for aqueous As and Se, their concentrations followed the diluted effluent pattern. Thus, these metals had a good correlation with EC. This outcome allowed using EC sensor data to estimate HQs and thus determine the predicted ecotoxicological risk of these metals. The estimated 90<sup>th</sup> percentile of the lake water concentrations for Se and As generated HQs  $<1$ . The HQs for the outflow at Collins Creek (Site 8) registered even lower values, suggesting that aqueous Se and As concentrations do not represent a likely risk of adverse effects to aquatic macroinvertebrate communities in McClean Lake or Collins Creek. However, Se is persistent and can bioaccumulate through the food chain to potentially impact the reproductive success of fish and the sustainability of fish populations (Lemly, 1997). The continuous diluted effluent release to McClean Lake may continue to increase Se load to McClean Lake and thus possibly lead to increased Se concentrations at some sites. Therefore, even though no apparent adverse effect on the macroinvertebrate community was observed in this study, this scenario could change with time. Consequently, it is important to continue to monitor the aquatic environment at McClean Lake to ensure that adverse ecological effects on aquatic biota are not observed in the future.

Results of the risk assessment for metals in sediments showed that V and Cd had HQs >1 in Vulture Lake, McClean Lake, Collins Creek and the reference site (McClean Lake west basin). Vanadium concentrations were similar across all sites, yielding similar HQs, suggesting that these values represented natural background concentrations. Burnett-Seidel and Liber (2013) and Thompson et al. (2005) reported that the presence of V in Northern Saskatchewan was not significantly influenced by the uranium operations and that this metal is found in very low concentrations in uranium ore; therefore, its release with treated effluent is low. Cadmium registered HQs >1 only in Vulture Lake, with no pattern in McClean Lake east basin, suggesting a lack of influence of diluted effluent on Cd in the downstream sediment. Cadmium concentrations, in general, were lower than the benchmark (the Canadian interim sediment quality guideline of 0.6 mg/kg), suggesting that the concentrations of Cd at the study sites should not be associated with adverse biological effects (Environment Canada, 1997). Overall, cumulative HQs for metals in sediment were >1 in Vulture Lake, at some sites in McClean Lake east basin, and at the reference site, which in theory suggests a possible risk of adverse effects at some sites. However, the cumulative HQs were influenced mostly by V concentrations since they were usually higher than the benchmark concentration at all study sites, given that lakes near the McClean Lake operation contain naturally metal-enriched sediments, particularly for As, Hg and V (Laird et al., 2014).

After performing the macroinvertebrate community assessment, it was observed that both the total abundances and the Margalef index (MI) did not correspond to the diluted effluent pattern. The (MI), a species diversity index and an indicator of the sensitivity of macroinvertebrates to different contaminants (Slooff, 1983), was likely influenced by EC, Se, and Cl in water and by TOC and Cd in sediment, as predicted by the best GAM model developed in this study. This model could effectively simulate and estimate the macroinvertebrate diversity at different McClean Lake sites.

Overall, the legitimate toxicity benchmarks used in this water and sediment risk assessment were probably overly protective and maybe somewhat inappropriate for this study location (McClean Lake). Despite that, the diluted effluent exposure, depending on the season and the maintenance and care period at the McClean Lake operation, could affect the contaminant inflow to McClean Lake and downstream sites. Therefore, contaminant exposure to aquatic organisms could increase during some periods as detected by the autonomous sensors. In addition, the fact

that neither the total abundances nor the diversity index (MI) for benthic macroinvertebrates corresponded to the diluted effluent pattern indicated that there were possibly other factors influencing macroinvertebrate community composition. In this regard, the GAM model helped identify which variables could potentially affect macroinvertebrate community diversity at these study sites. In addition, the unique habitat characteristics of McClean Lake (very sandy) compared to Vulture Lake (soft organic sediment) likely resulted in different communities in these lakes. Finally, the data generated from this research are useful to complement the regular monitoring programs for both the effluent release and the chemistry and biology of the receiving environment at McClean Lake. The ecotoxicological assessment approach, if effectively utilized to integrate information and identify possible cause-effect relationships, could be used to identify emerging toxicological issues before they become an environmental and/or regulatory concern.

#### **4.4. Limitations of the research and recommendations for future work**

##### **4.4.1. *Challenges with the use of sensor technology***

Not all water quality parameters can be monitored online in real-time. Lee et al. (2012) prepared a list of only 34 parameters that can be monitored online using commercially available wireless sensors (Banna et al., 2013). However, with time and the advancement of technology, more sophisticated and automated sensors can now measure more parameters. For instance, the latest Smart Water® sensors from Libelium (2021) (Smart Water Xtreme sensors, Smart Water PRO, and Smart Water Ions) can now measure at least 20 variables, including suspended solids, total dissolved solids, many different ions, chlorophyll, and dissolved organic matter, among others. One of the problems with these devices is that many sensors and systems can be expensive, especially when they are first released to the market. Dong et al. (2015) suggests that for the development of multi-parameter, high-performance sensors, further research on biosensors, micro-sensors, nanotechnology sensors, and smart sensors incorporated into a programmable microprocessor should be considered.

Additionally, most sensors/probes placed in surface water (e.g., lake, river and sea) will eventually become covered with biofilm and other organisms and coatings after some time (site and limnology specific). Such biofouling can be directly related to the bioproductivity, water chemistry and other environmental conditions at the site of study. Unfortunately, no unique

solution currently exists to control biofouling, and the choice of the method to maintain clean the sensor probes will have to take into account characteristics of each specific monitoring location. According to O'Flynn (2010), there are different ways to prevent biofouling, such as manual cleaning, choosing certain construction materials, painting probes with antifouling coatings, and using electric fields. In addition, there are some more recent anti-fouling methods available, including optical technology, inductive technology, automated brushes, copper coverage, and LED UV systems (Libelium, 2021), which are part of more recent sensor technologies.

Finally, the weather conditions could also affect sensor readings and the sensor devices themselves. The monitoring data could be compromised with bad weather conditions like heavy rain, high wind speed, heavy fog, etc. For instance, heavy rain might cause sediment particles and microalgae resuspension, which could promote biofouling or interfere with readings. Strong wind could move (depending on the anchoring method) the sensors from their original and desired place of deployment. This movement might also cause damage to the sensor probes or the unit itself. Moreover, foggy conditions, sometimes combined with rain, may cause an interference between the wireless sensor communication and the data delivery to other wireless devices. Finally, the desired location of the monitoring station (sensor unit) could be challenging to choose if the cellular or WIFI signal is not strong or good enough in the area to provide dependable wireless communication to and from the sensors.

#### **4.4.2. *Challenges with the use of a Generalized Additive Model (GAM)***

There is uncertainty when using a GAM prediction, and it comes from both the input data and the model output (Cayuela et al., 2011; Lin et al., 2015). In this study, the uncertainty in the ecological input data mainly results from measurement inaccuracies for collected water, sediment and macroinvertebrates, taxonomic uncertainty in macroinvertebrate identification, and sampling efforts that is related to the methodology chosen (e.g. Hester Dendy or Ekman grab samplers).

The GAM uncertainty usually derives from the input data, in this case the Margalef diversity index (MI), the environmental variables selected or available for use in the model (e.g., EC, Se, Cl, Cd, and TOC), and the structure of the GAM equation (Guisan & Thuiller, 2005; Wilhere, 2012), the latter of which was a result of choosing one out of seven possible models. The selected model (GAM model-5) itself could also be a source of uncertainty because the steps to

calculate the selected GAM model were limited by assuming that a unique subset of optimal variables exists (Neter et al., 1989). In this sense, seven different combinations of physicochemical variables were found to affect the MI. However, the decision to choose the best model was based on, besides the statistical significance, getting the lowest MRSE (root mean squared error).

In many cases, there is no single best subset of variables or factors that influence the MI, which means that some important factors may be ignored in the model (Neter et al., 1989). For example, other metals in the assessment resulted in  $HQ > 1$  like As, Hg, Fe in water or V in sediments. Consequently, it was necessary to compare all the possible models with similar variables to select the most suitable combination, as was done in this study for model-5. Thus, uncertainty was minimized, especially uncertainty caused by model parameters and different distribution prediction approaches (Austin, 2007; Guisan & Thuiller, 2005; Lin et al., 2015; Guisan & Zimmermann, 2000). Finally, when the scale of the study area is relatively small, like in this study at McClean Lake, the range of values for each parameter tend to be narrow. Therefore, the selected model could only describe changes in the MI within the ranges of these parameters.

#### **4.4.3. Future research**

The mostly continuous, diluted effluent discharge to McClean Lake may further increase metal and major ion concentrations in the lake system. Thus, even though no apparent adverse effect on the macroinvertebrate community was observed in this study, this scenario could change with time. For that reason, continued environmental monitoring of the aquatic environment (water and sediment) and biota (periphyton, aquatic invertebrates and fish) at McClean Lake are recommended to ensure that adverse ecological effects are not observed in the future. Additionally, since surface water quality in McClean Lake was not detrimental to the development and emergence of *Chironomus dilutus*, this could be further investigated by more extensive field surveys and additional bioassays with other, possibly more sensitive species, including native chironomids and planktonic species such as water fleas (daphnids) that are already present within the lake. Finally, it is important to consider pore-water metal concentrations in future assessments since it could be useful for predicting the toxicity of metal-contaminated sediment to benthic organisms. Whole-sediment toxicity tests could also be utilized.

One way to possibly improve the water quality monitoring in future work might consist of collecting more sensor EC data to more completely estimate HQs for As and Se. In this research, the sensor deployment was done in 2018 and 2019. However, data for 2018 was considered only preliminary work due to a few technical issues that were then corrected in 2019. Sensor EC data for the toxicological assessment was recorded for seven weeks only. In this sense, this EC data could be collected every year, including having longer monitoring periods ranging from late spring to early fall (the open water season). With more EC data from sensors, it would be possible to see the broader seasonal variability in effluent distribution and associated HQs. Moreover, having a bigger dataset is always more useful since it usually decreases the uncertainty associated with the measurements. In addition, sensor developers are creating new and more sophisticated units and probes all the time that can be taken into consideration for future deployments. For instance, Smart Water Xtreme® sensors, among others, from Libelium incorporate an integrate temperature sensor to compensate measurements, have an inside calibration, very low power consumption, better sleep mode, and have an optical and anti-fouling system (Libelium, 2021). Consequently, calibrations, deployment, data collection and cleaning of sensor probes would theoretically take less time and resources.

#### **4.5. Concluding statements**

This research was designed to conduct an ecotoxicological risk assessment on McClean Lake east basin while having complementary information from Vulture Lake (upstream site) and Collins Creek (downstream site). Results clearly demonstrate that the cumulative risk assessment approach utilized overly conservative water and sediment quality benchmarks obtained from published Canadian guidelines and Saskatchewan objectives. Effluent exposure fluctuations were identified with the autonomous sensors and sensor results allowed for a more detailed estimation of Se and As risk to aquatic macroinvertebrates; that risk was generally considered low. Moreover, there was no evidence of an adverse biological effect of surface water from high exposure sites in both Vulture and McClean Lakes on *C. dilutus*. Additionally, the GAM analysis, as a predictive tool, suggested that EC, Se, and Cl in water and TOC and Cd in sediment are key variables that combined could influence macroinvertebrate community diversity as measured by the Margalef diversity index (MI).



Overall, environmental monitoring of the aquatic environment in McClean Lake should continue to ensure that aquatic communities remain healthy, especially when considering aqueous Se which could continue to bioaccumulate in the aquatic biota over time. Therefore, the results of this thesis will hopefully assist Orano Canada Ltd. by providing new data, some of it complementary information to their regular environmental monitoring, making suggestions for key monitoring variables for use in their risk assessments, and/or for providing an idea for predictive models that may help prevent contaminant concentrations in the aquatic environment of McClean Lake operation from reaching toxic levels.

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## **APPENDICES**



### Appendix A. Hand-held meter measurements in surface water 2018-2019

2018	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10	
August	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
pH	7.71	0.01	7.63	0.00	7.09	0.03	7.31	0.03	7.18	0.02	7.05	0.03	7.17	0.12	7.33	0.01	7.06	0.02	7.05	0.01
DO	10.56	0.11	9.99	0.50	9.61	0.39	10.64	0.07	10.80	0.28	9.80	0.20	9.71	0.47	10.16	0.45	9.01	0.24	9.12	0.06
EC	1216.40	1.73	1235.71	4.36	66.61	0.25	500.27	1.53	65.64	0.85	18.62	0.07	31.49	0.15	27.46	0.53	262.96	1.53	17.02	0.14
T	15.83	0.21	17.37	1.33	16.60	0.89	15.63	0.21	15.63	0.12	15.47	0.55	16.40	0.10	17.50	0.26	14.17	0.35	15.20	0.36
September	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
pH	7.02	0.10	7.06	0.05	7.23	0.03	7.12	0.07	7.24	0.01	7.10	0.06	7.19	0.02	7.21	0.01	7.23	0.02	7.26	0.04
DO	12.35	0.43	12.25	0.35	12.01	0.42	12.89	0.45	12.45	0.29	12.36	0.64	12.66	0.15	11.92	0.36	12.25	0.35	12.60	0.54
EC	904.62	5.13	900.00	4.16	64.43	0.56	225.44	4.53	146.65	1.54	89.82	0.17	94.16	0.21	118.81	0.06	90.30	1.25	11.63	0.02
T	4.57	0.15	5.03	0.59	5.13	0.68	3.43	0.06	3.30	0.35	4.43	0.49	4.17	0.12	3.14	0.23	3.93	0.15	4.57	0.60
2019	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10	
July	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
pH	7.41	0.02	7.98	0.01	7.22	0.03	7.36	0.03	7.25	0.05	7.11	0.05	7.18	0.02	7.28	0.03	7.29	0.02	7.14	0.03
DO	8.56	0.18	8.33	0.16	8.69	0.02	8.94	0.01	8.09	1.20	8.65	0.18	8.85	0.08	8.93	0.06	8.97	0.07	8.92	0.09
EC	1496.47	7.23	1550.36	3.06	36.42	0.11	122.13	2.51	37.64	0.31	25.80	0.07	36.03	0.18	30.86	0.12	37.64	0.06	20.33	0.05
T	21.63	0.12	23.30	0.17	21.60	0.44	20.27	0.06	19.40	0.00	19.60	0.17	20.20	0.26	20.93	0.06	20.93	0.15	21.17	0.15
August	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
pH	7.18	0.03	7.16	0.01	7.19	0.02	7.51	0.02	7.92	0.02	6.95	0.02	7.04	0.01	7.36	0.03	7.21	0.02	7.04	0.02
DO	9.33	0.01	9.24	0.03	9.63	0.02	9.93	0.04	9.95	0.04	9.20	0.05	9.22	0.02	9.81	0.02	9.71	0.05	9.35	0.01
EC	1289.81	1.15	1255.60	1.73	110.9	0.63	384.18	2.05	77.92	0.06	39.66	1.30	71.62	0.09	36.11	0.31	108.15	1.05	19.65	0.11
T	16.13	0.06	16.23	0.06	16.94	0.06	17.83	0.06	18.07	0.06	16.33	0.06	16.27	0.06	18.07	0.06	16.83	0.06	16.77	0.06
September	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
pH	7.35	0.01	7.25	0.03	6.84	0.02	7.05	0.03	6.88	0.01	6.79	0.06	6.86	0.03	6.95	0.01	6.82	0.02	6.84	0.03
DO	10.54	0.07	10.50	0.06	10.23	0.04	10.16	0.21	9.91	0.03	10.10	0.03	10.04	0.02	10.27	0.02	10.21	0.34	10.01	0.03
EC	1064.89	5.86	1031.01	3.06	19.39	0.21	140.24	0.51	72.36	0.35	16.28	0.03	37.70	0.35	48.05	0.23	22.04	0.07	15.46	0.11
T	11.97	0.12	12.13	0.06	11.37	0.06	12.10	0.26	11.13	0.25	11.14	0.06	11.9	0.00	11.67	0.15	11.77	0.06	11.34	0.06

Units: pH (pH units), DO (mg/L), EC ( $\mu\text{S/cm}$ ), and T ( $^{\circ}\text{C}$ ).

### Appendix B. Hardness, Alkalinity and Turbidity values in surface water for 2018 and 2019

Locations	Period	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10	
	2018	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Hardness (mg CaCO <sub>3</sub> /L)	Aug.	511.33	2.31	498.67	6.11	14.67	1.15	172.67	1.15	14.67	1.15	10.67	1.15	10.67	3.06	12.67	3.06	91.33	2.31	9.33	1.15
	Sept.	502.67	4.62	494.67	19.43	25.33	1.15	52.00	8.00	63.33	1.15	37.33	1.15	36.00	2.00	53.33	4.62	31.33	1.15	5.33	1.15
Alkalinity (mg CaCO <sub>3</sub> /L)	Aug.	18.00	0.00	22.00	7.21	13.33	1.15	14.00	2.00	14.00	0.00	13.33	1.15	10.67	3.06	13.33	1.15	14.67	1.15	13.33	1.15
	Sept.	15.33	4.16	14.67	1.15	13.33	1.15	12.67	1.15	11.33	1.15	11.33	2.31	12.67	1.15	14.00	3.46	14.00	1.00	12.33	0.58
Turbidity (NTU)	Aug.	0.78	0.16	0.68	0.10	1.07	0.32	0.42	0.28	1.37	0.21	1.03	0.15	1.27	0.21	1.50	0.50	0.92	0.59	1.33	0.12
	Sept.	0.43	0.08	0.78	0.19	0.60	0.10	0.25	0.09	0.52	0.10	0.32	0.10	0.47	0.25	0.53	0.24	0.37	0.28	0.47	0.08
Dissolved Organic Carbon (mg/L)	Aug.	2.86	0.14	3.91	1.77	5.77	0.50	5.67	1.15	6.86	0.07	5.95	0.11	5.93	0.08	5.79	0.06	5.77	0.06	3.89	3.08
	Sept.	2.70	0.07	2.87	0.10	4.68	0.06	4.66	0.07	4.78	0.02	5.08	0.24	4.71	0.04	4.73	0.09	5.33	0.08	4.61	0.02
	2019	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Hardness (mg CaCO <sub>3</sub> /L)	Jul.	554.67	5.03	517.33	25.01	16.67	1.15	41.33	3.06	13.67	1.53	19.33	4.16	10.33	0.58	16.00	2.00	15.00	1.00	6.33	1.53
	Aug.	511.33	2.31	498.67	6.11	14.67	1.15	172.67	1.15	14.67	1.15	10.67	1.15	10.67	3.06	12.67	3.06	91.33	2.31	9.33	1.15
	Sept.	432.00	10.00	428.67	25.32	10.67	1.15	47.33	6.11	47.33	6.11	6.00	0.00	12.67	2.31	14.00	2.00	15.33	1.15	6.33	0.58
Alkalinity (mg CaCO <sub>3</sub> /L)	Jul.	6.67	0.58	7.67	2.89	13.33	2.31	6.00	1.00	9.67	2.89	10.00	1.00	6.00	2.00	8.00	1.73	7.00	2.65	4.00	2.00
	Aug.	18.00	0.00	22.00	7.21	13.33	1.15	14.00	2.00	14.00	0.00	13.33	1.15	10.67	3.06	13.33	1.15	14.67	1.15	13.33	1.15
	Sept.	7.67	0.58	10.67	0.58	11.33	1.15	14.67	2.31	12.67	1.15	12.00	3.46	14.00	4.00	8.67	1.15	8.67	1.15	7.00	0.00
Turbidity (NTU)	Jul.	1.90	0.05	2.45	0.37	0.71	0.04	1.14	0.14	0.81	0.15	0.88	0.03	0.64	0.11	0.68	0.08	0.60	0.08	0.67	0.06
	Aug.	0.78	0.16	0.68	0.10	1.07	0.32	0.42	0.28	1.37	0.21	1.03	0.15	1.27	0.21	1.50	0.50	0.92	0.59	1.33	0.12

### Appendix C. Anion concentrations in surface water for 2018 and 2019

Fluoride (F <sup>-</sup> )					Chloride (Cl <sup>-</sup> )				Sulfate (SO <sub>4</sub> <sup>2-</sup> )			
2018												
mg/L	August		September		August		September		August		September	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Site 1	0.19	0.03	0.11	0.11	33.64	0.08	6.85	1.16	518.38	40.23	147.75	18.97
Site 2	0.20	0.01	0.18	0.004	32.77	0.15	25.83	2.68	542.67	11.13	480.28	33.94
Site 3	<LOQ	-	<LOQ	-	1.47	0.09	1.81	0.04	21.57	1.58	29.32	0.68
Site 4	0.016	0.016	<LOQ	-	12.69	0.24	3.39	0.39	224.78	4.38	58.99	7.16
Site 5	<LOQ	-	<LOQ	-	1.30	0.13	0.55	0.07	20.80	2.25	9.03	1.28
Site 6	<LOQ	-	<LOQ	-	0.38	0.01	1.62	0.53	0.89	0.04	23.87	16.04
Site 7	<LOQ	-	<LOQ	-	0.63	0.03	2.79	0.09	5.31	0.10	47.54	1.42
Site 8	<LOQ	-	<LOQ	-	0.52	0.01	0.79	0.19	3.49	0.07	14.39	4.80
Site 9	<LOQ	-	<LOQ	-	5.93	0.12	0.10	0.02	105.42	1.76	0.84	0.41
Site 10	<LOQ	-	<LOQ	-	0.06	0.05	0.06	0.01	<LOQ	-	<LOQ	-
2019												
mg/L	July		September		July		September		July		September	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Site 1	0.32	0.312	0.13	0.054	26.619	4.961	22.51	0.467	555.37	92.66	652.46	15.12
Site 2	0.77	0.001	0.08	0.015	23.596	0.094	21.04	0.360	497.21	2.17	613.27	7.77
Site 3	0.24	0.002	0.21	0.011	0.366	0.003	0.32	0.002	3.52	0.03	2.03	0.15
Site 4	0.24	0.003	0.22	0.002	2.032	0.020	2.35	0.028	36.12	0.41	57.95	0.32
Site 5	0.24	0.004	0.22	0.012	0.420	0.005	1.22	0.041	4.48	0.19	26.82	0.79
Site 6	0.19	0.085	0.20	0.002	0.256	0.001	0.33	0.068	1.34	0.01	0.65	0.03
Site 7	0.23	0.001	0.22	0.009	0.380	0.002	0.46	0.048	3.84	0.04	5.41	0.19
Site 8	0.23	0.001	0.23	0.003	0.328	0.009	0.32	0.018	2.68	0.04	2.00	0.34
Site 9	0.24	0.010	0.22	0.015	0.395	0.014	0.35	0.022	4.03	0.04	2.75	0.15
Site 10	0.10	0.120	0.23	0.002	0.209	0.013	0.25	0.020	0.19	0.00	0.32	0.03

<LOQ: Concentrations lower limit of quantification.

### Appendix D. Cation concentrations in surface water for 2018 and 2019

Sodium (Na <sup>+</sup> )				Potassium (K <sup>+</sup> )				Magnesium (Mg <sup>2+</sup> )				Calcium (Ca <sup>2+</sup> )				
2018																
mg/L	August		September		August		September		August		September		August		September	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Site 1	64.17	0.28	64.09	0.18	9.50	0.05	9.47	0.02	3.90	0.02	3.89	0.01	199.85	1.23	200.37	0.55
Site 2	61.97	0.12	62.19	0.200	9.24	0.00	9.28	0.04	3.91	0.01	3.94	0.02	196.40	0.25	197.53	0.68
Site 3	3.19	0.14	3.20	0.13	0.65	0.02	0.65	0.01	0.41	0.01	0.41	0.01	8.52	0.48	8.58	0.45
Site 4	23.134	0.429	23.20	0.39	3.52	0.03	3.54	0.04	1.65	0.01	1.67	0.03	74.32	0.91	74.61	0.69
Site 5	3.14	0.22	3.13	0.22	0.63	0.01	0.60	0.03	0.36	0.02	0.36	0.02	8.21	0.74	8.23	0.70
Site 6	1.09	0.00	1.08	0.00	0.31	0.02	0.29	0.00	0.20	0.00	0.21	0.00	1.28	0.03	1.30	0.04
Site 7	1.56	0.03	1.53	0.02	0.37	0.01	0.36	0.00	0.25	0.00	0.25	0.00	2.79	0.08	2.81	0.06
Site 8	1.36	0.01	1.37	0.02	0.33	0.00	0.33	0.01	0.19	0.00	0.19	0.01	2.18	0.06	2.19	0.04
Site 9	11.85	0.05	11.88	0.09	1.89	0.02	1.88	0.01	0.96	0.01	0.97	0.01	37.80	0.22	37.94	0.27
Site 10	1.02	0.01	1.02	0.01	0.29	0.01	0.29	0.01	0.21	0.00	0.21	0.00	1.11	0.04	1.12	0.04
2019																
mg/L	July		September		July		September		July		September		July		September	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Site 1	61.47	27.61	51.00	0.20	6.50	3.02	5.34	0.05	0.08	0.09	<LOQ	-	173.82	77.76	161.50	1.88
Site 2	53.25	18.48	48.65	0.163	5.64	1.97	5.06	0.075	0.05	0.04	<LOQ	-	151.83	52.33	154.71	1.023
Site 3	1.52	0.01	1.06	0.05	0.24	0.00	0.20	0.01	0.22	0.00	0.15	0.01	3.18	0.02	2.03	0.03
Site 4	5.704	0.128	5.69	0.08	0.757	0.027	0.84	0.01	0.571	0.016	<LOQ	-	14.675	0.397	17.04	0.26
Site 5	1.67	0.02	3.22	0.11	0.26	0.01	0.50	0.01	0.20	0.00	<LOQ	-	3.63	0.07	9.08	0.32
Site 6	1.23	0.01	0.96	0.02	0.20	0.01	0.22	0.03	0.46	0.01	0.39	0.00	1.96	0.03	1.35	0.01
Site 7	1.57	0.02	1.41	0.07	0.25	0.00	0.26	0.01	0.22	0.00	0.14	0.00	3.29	0.05	3.06	0.05
Site 8	1.41	0.03	1.09	0.05	0.22	0.01	0.21	0.01	0.22	0.00	0.24	0.12	2.78	0.01	1.94	0.24
Site 9	1.58	0.00	1.16	0.02	0.25	0.00	0.23	0.00	0.21	0.00	0.17	0.00	3.32	0.02	2.32	0.02
Site 10	1.03	0.01	0.94	0.04	0.21	0.01	0.21	0.00	0.45	0.00	0.41	0.00	1.36	0.00	1.28	0.01

<LOQ: Concentrations lower than limit of quantification.

### Appendix E. Mean metal(oids) concentrations in surface water for August 2018

µg/L	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>B</b>	350.73	4.39	346.35	5.76	16.41	2.46	130.07	2.23	14.29	1.80	1.94	0.11	4.64	0.20	3.53	0.36	65.59	2.40	1.20	0.35
<b>Al</b>	4.95	0.24	3.59	0.37	10.97	1.29	4.34	0.32	16.14	1.17	12.44	0.16	13.76	1.95	12.33	0.35	5.96	0.50	12.83	0.31
<b>Ti</b>	0.001	0.01	0.001	0.001	0.16	0.03	0.03	0.01	0.24	0.03	0.23	0.02	0.19	0.06	0.22	0.05	0.08	0.05	0.26	0.05
<b>V</b>	0.03	0.001	0.04	0.001	0.09	0.01	0.03	0.001	0.11	0.01	0.10	0.01	0.10	0.001	0.12	0.01	0.05	0.001	0.11	0.01
<b>Cr</b>	0.12	0.03	0.15	0.05	0.24	0.05	0.15	0.05	0.20	0.08	0.16	0.14	0.33	0.39	0.20	0.02	0.16	0.07	0.16	0.01
<b>Mn</b>	0.20	0.01	2.07	0.33	143.72	11.53	19.45	3.97	181.47	9.88	193.19	2.41	175.08	5.88	196.40	7.15	55.29	3.86	202.80	5.24
<b>Fe</b>	5.62	0.66	29.19	0.55	2.16	0.13	21.89	1.81	3.29	0.11	2.29	0.05	2.06	0.16	2.34	0.06	11.74	2.03	2.29	0.20
<b>Co</b>	0.08	0.01	0.24	0.02	0.01	0.001	0.07	0.01	0.02	0.001	0.01	0.001	0.01	0.001	0.01	0.001	0.03	0.004	0.01	0.001
<b>Ni</b>	9.57	0.21	8.92	0.11	0.59	0.07	3.13	0.02	0.51	0.03	0.25	0.20	0.29	0.09	0.18	0.01	1.62	0.02	0.12	0.03
<b>Cu</b>	0.15	0.05	0.14	0.04	0.18	0.01	0.11	0.02	0.16	0.06	0.22	0.16	0.36	0.19	0.14	0.04	0.11	0.04	0.14	0.01
<b>Zn</b>	3.07	0.82	2.70	0.36	3.19	0.72	2.98	0.68	3.05	1.01	1.99	0.46	2.53	2.53	2.10	0.34	3.08	1.21	2.07	0.76
<b>As</b>	2.45	0.05	2.44	0.07	0.35	0.03	1.01	0.04	0.37	0.03	0.29	0.02	0.29	0.03	0.30	0.01	0.64	0.03	0.29	0.03
<b>Se</b>	2.61	0.12	2.40	0.02	0.08	0.04	0.74	0.03	0.06	0.02	0.01	0.01	0.01	0.001	0.02	0.01	0.32	0.02	0.01	0.01
<b>Sr</b>	148.96	1.86	146.43	0.89	17.45	1.01	61.12	0.54	15.60	0.76	11.72	0.30	12.69	0.29	11.40	0.22	37.35	0.66	11.86	0.27
<b>Mo</b>	15.92	0.14	18.18	0.29	1.30	0.07	6.91	0.12	1.13	0.09	0.04	0.00	0.34	0.02	0.34	0.03	3.60	0.08	<LOQ	-
<b>Ag</b>	0.001	0.001	0.001	0.001	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-
<b>Cd</b>	0.03	0.003	0.03	0.001	0.01	-	0.01	0.001	<LOQ	-	<LOQ	-	<LOQ	-	0.01	-	0.01	0.001	<LOQ	-
<b>Sn</b>	0.01	0.01	0.003	0.003	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-
<b>Sb</b>	0.06	0.001	0.06	0.001	0.01	0.0004	0.03	0.001	0.01	0.001	0.01	0.0001	0.01	0.001	0.01	0.001	0.01	0.002	0.001	0.001
<b>Ba</b>	31.71	0.21	31.28	0.28	5.31	0.81	14.08	0.21	4.12	0.14	3.10	0.01	3.58	0.11	3.15	0.58	9.70	0.12	3.21	0.13
<b>Hg</b>	0.03	0.01	0.02	0.001	0.01	0.001	0.01	0.001	0.01	0.00	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	0.01	0.01
<b>Tl</b>	0.29	0.01	0.22	0.01	0.02	0.001	0.08	0.001	0.01	0.001	0.01	0.01	0.01	0.01	0.01	0.01	0.05	0.002	<LOQ	-
<b>Pb</b>	0.03	0.01	0.02	0.001	0.03	0.001	0.03	0.01	0.02	0.01	0.02	0.01	0.04	0.02	0.02	0.001	0.04	0.005	0.01	0.001
<b>U</b>	0.12	0.01	0.15	0.01	0.03	0.001	0.06	0.00	0.03	0.00	0.02	0.00	0.02	0.01	0.03	0.001	0.04	0.001	0.02	0.001

<LOQ: Concentrations lower than limit of quantification.

### Appendix F. Mean metal(oids) concentrations in surface water for September 2018

µg/L	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>B</b>	340.32	7.04	315.71	9.99	17.04	1.84	33.50	4.43	45.60	1.31	24.23	1.22	25.77	0.34	35.83	1.34	23.55	1.72	1.12	0.04
<b>Al</b>	7.02	0.38	7.26	2.22	9.57	2.72	8.94	0.29	10.21	0.74	9.29	1.07	11.38	0.69	10.79	1.82	10.82	0.54	11.96	0.89
<b>Ti</b>	0.09	0.05	0.28	0.31	0.27	0.09	0.15	0.05	0.23	0.05	0.24	0.04	0.25	0.10	0.24	0.08	0.26	0.05	0.21	0.06
<b>V</b>	0.05	0.03	0.05	0.01	0.13	0.11	0.08	0.02	0.09	0.02	0.09	0.01	0.09	0.00	0.10	0.03	0.09	0.04	0.10	0.01
<b>Cr</b>	0.29	0.11	0.43	0.12	0.57	0.00	0.74	0.17	2.23	0.61	0.57	0.03	1.39	0.33	1.38	0.23	0.51	0.09	0.97	0.90
<b>Mn</b>	32.78	0.84	36.96	0.93	6.75	1.52	7.88	0.48	9.53	0.44	7.18	0.07	7.29	0.01	8.13	0.18	6.54	0.20	5.85	0.04
<b>Fe</b>	17.52	0.55	18.34	2.95	98.97	2.67	90.26	4.41	91.68	0.32	89.62	0.36	96.96	2.76	91.80	1.49	94.44	3.07	118.76	4.39
<b>Co</b>	0.28	0.04	0.29	0.00	0.08	0.10	0.03	0.01	0.04	0.003	0.03	0.01	0.03	0.002	0.03	0.01	0.03	0.002	0.02	0.001
<b>Ni</b>	10.26	2.22	7.94	0.24	1.03	0.03	1.29	0.08	1.62	0.08	1.08	0.01	1.13	0.03	1.27	0.02	1.14	0.08	0.79	0.09
<b>Cu</b>	0.20	0.27	0.09	0.02	0.37	0.47	0.15	0.09	0.41	0.15	0.11	0.03	0.46	0.03	0.36	0.16	0.08	0.02	0.20	0.16
<b>Zn</b>	5.18	3.39	3.16	0.43	4.37	2.07	2.57	0.50	3.36	1.64	2.80	0.82	3.18	0.79	2.47	1.43	3.12	0.52	2.50	0.37
<b>As</b>	2.28	0.02	2.08	0.13	0.25	0.05	0.34	0.02	0.38	0.02	0.29	0.01	0.30	0.02	0.32	0.01	0.27	0.02	0.17	0.02
<b>Se</b>	2.18	0.02	2.08	0.10	0.12	0.02	0.20	0.02	0.28	0.04	0.14	0.03	0.15	0.02	0.19	0.01	0.14	0.04	0.01	0.01
<b>Sr</b>	152.57	0.36	144.75	2.15	15.96	0.40	22.09	1.60	26.54	1.01	18.79	0.18	19.75	0.07	23.43	0.28	18.18	0.50	8.82	0.08
<b>Mo</b>	17.48	0.13	18.28	0.30	1.25	0.52	1.90	0.23	2.57	0.13	1.35	0.04	1.51	0.005	2.02	0.10	1.35	0.08	0.11	0.06
<b>Ag</b>	0.06	0.01	0.03	0.03	0.06	0.02	0.03	0.01	0.05	0.02	0.03	0.02	0.05	0.02	0.04	0.01	0.03	0.01	0.04	0.02
<b>Cd</b>	0.03	0.004	0.03	0.002	0.00	0.00	0.00	0.001	0.00	0.003	0.00	0.001	0.00	0.001	0.00	0.002	0.00	0.002	0.00	0.001
<b>Sn</b>	0.08	0.05	0.06	0.05	0.12	0.09	0.08	0.01	0.08	0.02	0.08	0.01	0.09	0.004	0.09	0.01	0.08	0.01	0.07	0.01
<b>Sb</b>	0.66	1.03	0.06	0.00	0.01	0.00	0.02	0.02	0.02	0.005	0.01	0.002	0.01	0.004	0.03	0.003	0.02	0.02	0.02	0.02
<b>Ba</b>	31.32	0.23	30.59	0.47	4.03	0.36	5.43	0.44	6.56	0.01	4.86	0.41	5.07	0.05	6.35	0.73	4.51	0.23	2.26	0.03
<b>Hg</b>	0.00	0.004	<LOQ	<LOQ	0.01	0.01	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
<b>Tl</b>	0.31	0.005	0.26	0.01	0.02	0.004	0.03	0.003	0.04	0.001	0.02	0.001	0.03	0.002	0.03	0.0001	0.02	0.002	0.00002	0.00002
<b>Pb</b>	0.07	0.06	0.05	0.01	0.07	0.07	0.09	0.10	0.03	0.003	0.03	0.01	0.03	0.01	0.02	0.01	0.02	0.01	0.01	0.004
<b>U</b>	0.17	0.01	0.17	0.01	0.04	0.01	0.03	0.002	0.04	0.01	0.02	0.0003	0.04	0.002	0.04	0.01	0.06	0.06	0.02	0.002

<LOQ: Concentrations lower than limit of quantification.

### Appendix G. Mean metal(oids) concentrations in surface water for July 2019

µg/L	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>B</b>	314.77	11.44	306.58	5.03	4.34	0.01	23.78	0.91	5.78	0.04	2.53	0.04	4.68	0.42	3.68	0.22	4.53	0.14	1.42	0.14
<b>Al</b>	11.36	1.47	14.21	0.60	6.03	0.24	7.14	0.63	7.14	1.18	6.73	0.59	7.39	1.01	6.95	0.19	7.17	0.87	6.64	0.35
<b>Ti</b>	1.19	0.17	0.72	0.27	0.12	0.03	0.10	0.02	0.16	0.03	0.12	0.02	0.13	0.05	0.13	0.01	0.12	0.02	0.13	0.01
<b>V</b>	1.32	0.37	0.58	0.12	0.05	0.01	0.06	0.01	0.04	0.01	0.03	0.01	0.03	0.00	0.09	0.09	0.05	0.05	0.01	0.01
<b>Cr</b>	0.08	0.08	0.16	0.07	0.36	0.12	0.44	0.11	0.32	0.03	0.46	0.06	0.56	0.21	0.41	0.22	0.25	0.11	0.23	0.01
<b>Mn</b>	42.17	2.36	69.85	2.68	1.60	0.08	5.69	0.15	1.79	0.29	2.24	0.16	2.30	1.06	2.74	0.25	2.21	0.16	1.91	0.05
<b>Fe</b>	20.75	9.14	21.12	2.73	78.67	0.26	68.26	2.46	78.81	5.37	90.57	4.10	79.21	4.11	87.30	5.58	75.83	3.05	65.14	1.79
<b>Co</b>	0.40	0.04	0.67	0.06	0.01	0.004	0.04	0.001	0.02	0.01	0.02	0.00	0.02	0.01	0.02	0.001	0.01	0.001	0.01	0.001
<b>Ni</b>	10.84	0.43	9.72	0.36	0.23	0.05	0.86	0.03	0.24	12.69	0.31	0.28	0.53	0.46	0.24	0.05	0.24	0.03	0.09	0.04
<b>Cu</b>	1.29	1.25	0.38	0.27	0.38	0.27	0.29	0.13	0.12	20.70	0.34	0.25	1.18	1.79	0.25	0.18	0.12	0.04	0.12	0.06
<b>Zn</b>	8.11	1.81	7.80	2.02	3.85	0.57	5.02	0.36	5.45	2.04	4.08	0.92	5.53	6.62	4.73	3.00	4.29	1.09	5.46	1.49
<b>As</b>	2.81	0.13	2.66	0.12	0.19	0.01	0.37	0.01	0.21	0.01	0.19	0.01	0.20	0.01	0.18	0.03	0.19	0.02	0.15	0.01
<b>Se</b>	4.14	1.56	2.92	1.57	0.19	0.19	0.16	0.07	0.15	0.01	0.11	0.11	0.11	0.11	0.13	0.13	0.11	0.11	0.13	0.13
<b>Sr</b>	166.67	0.25	169.66	0.43	11.33	0.26	20.58	0.64	10.35	0.07	10.19	0.10	10.56	0.14	9.96	0.14	10.49	0.07	9.46	0.10
<b>Mo</b>	20.64	0.25	19.12	0.22	0.93	0.03	3.39	0.03	1.16	0.03	0.44	0.02	0.92	0.04	0.73	0.02	0.97	0.05	0.05	0.01
<b>Ag</b>	0.01	0.00	0.01	0.01	<LOQ	-	<LOQ	-	0.01	0.01	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-
<b>Cd</b>	0.06	0.06	0.07	0.05	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-
<b>Sn</b>	<LOQ	-	<LOQ	-	0.07	0.05	0.02	0.02	0.59	0.95	0.03	0.02	0.01	0.01	0.03	0.004	0.04	0.02	0.07	0.02
<b>Sb</b>	0.11	0.01	0.10	0.03	0.005	0.005	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.00	0.01	0.001	0.01	0.01	<LOQ	-
<b>Ba</b>	33.08	0.45	32.94	0.70	2.30	0.03	4.20	0.18	2.09	0.06	2.17	0.08	2.35	0.04	2.24	0.10	2.14	0.06	2.09	0.02
<b>Hg</b>	0.09	0.03	0.07	0.03	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-
<b>TI</b>	0.43	0.04	0.34	0.04	0.01	0.001	0.02	0.003	0.01	0.001	<LOQ	-	0.01	0.01	<LOQ	-	0.01	0.01	<LOQ	-
<b>Pb</b>	0.10	0.05	0.07	0.03	0.03	0.03	0.03	0.02	0.01	2.22	0.02	0.02	0.06	0.07	0.02	0.01	0.02	0.02	0.02	0.02
<b>U</b>	0.17	0.02	0.18	0.01	0.02	0.005	0.05	0.003	0.03	0.01	0.02	0.001	0.02	0.01	0.02	0.01	0.02	0.001	0.01	0.01

<LOQ: Concentrations lower than limit of quantification.

### Appendix H. Mean metal(oids) concentrations in surface water for August 2019

µg/L	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>B</b>	286.32	10.59	276.62	10.30	16.97	0.66	68.07	2.69	9.94	0.86	4.14	0.33	9.32	0.49	3.42	0.23	14.60	0.79	0.69	0.07
<b>Al</b>	6.61	0.82	7.21	0.27	6.27	6.08	5.03	0.39	7.08	2.34	7.88	0.57	5.50	0.66	6.84	0.82	6.96	1.65	5.61	0.45
<b>Ti</b>	0.07	0.02	0.10	0.03	0.24	0.05	0.12	0.07	0.24	0.13	0.22	0.08	0.15	0.01	0.16	0.05	0.15	0.05	0.15	0.003
<b>V</b>	0.06	0.00	0.07	0.00	0.09	0.01	0.07	0.002	0.08	0.01	0.08	0.001	0.07	0.02	0.07	0.003	0.06	0.003	0.04	0.01
<b>Cr</b>	0.33	0.22	0.26	0.06	0.27	0.06	0.23	0.04	0.13	0.06	0.24	0.19	0.14	0.02	0.09	0.03	0.16	0.08	0.07	0.03
<b>Mn</b>	74.98	1.88	86.07	1.23	5.59	1.02	22.57	0.22	3.66	2.45	2.76	0.13	2.50	0.12	2.06	0.07	4.18	0.31	1.24	0.08
<b>Fe</b>	17.00	1.32	26.97	3.60	142.31	10.27	73.55	5.31	164.67	38.99	191.31	1.82	154.81	1.58	185.21	1.89	137.81	3.10	138.22	0.42
<b>Co</b>	0.54	0.02	0.58	0.03	0.03	0.003	0.09	0.004	0.02	0.01	0.02	0.002	0.02	0.002	0.01	0.002	0.02	0.002	0.01	0.001
<b>Ni</b>	8.21	0.09	7.84	0.13	0.50	0.05	1.69	0.05	0.35	0.04	0.19	0.03	0.31	0.01	0.15	0.01	0.43	0.04	0.07	0.03
<b>Cu</b>	0.13	0.02	0.20	0.13	0.20	0.06	0.12	0.03	0.20	0.16	0.15	0.04	0.13	0.03	0.11	0.01	0.14	0.04	0.12	0.03
<b>Zn</b>	5.13	2.31	5.10	1.72	3.26	0.97	3.67	1.01	2.15	0.07	2.14	1.40	3.31	0.52	2.86	0.13	2.34	1.46	1.19	0.54
<b>As</b>	2.80	0.01	2.60	0.06	0.33	0.01	0.76	0.02	0.29	0.01	0.22	0.01	0.25	0.01	0.21	0.01	0.31	0.02	0.19	0.01
<b>Se</b>	2.37	0.01	1.87	0.04	0.13	0.02	0.42	0.01	0.08	0.03	0.04	0.01	0.08	0.02	0.04	0.01	0.09	0.03	0.02	0.01
<b>Sr</b>	161.40	2.34	153.92	0.79	19.30	0.65	45.09	0.32	15.28	0.03	12.94	0.33	15.82	0.82	11.87	0.18	19.18	0.55	11.00	0.59
<b>Mo</b>	21.41	0.30	20.16	0.53	2.18	0.10	6.15	0.02	2.00	0.06	0.74	0.03	1.42	0.10	0.67	0.06	2.04	0.13	0.06	0.01
<b>Ag</b>	0.02	0.005	0.01	0.001	0.01	0.003	0.01	0.01	0.01	0.01	0.01	0.001	0.01	0.01	<LOQ	-	<LOQ	-	<LOQ	-
<b>Cd</b>	0.05	0.01	0.05	0.002	0.01	0.003	0.02	0.004	0.01	0.002	0.01	0.004	0.01	0.000	0.01	0.01	<LOQ	-	<LOQ	-
<b>Sn</b>	0.03	0.01	0.07	0.07	0.03	0.01	0.03	0.004	0.32	0.52	0.02	0.004	0.02	0.002	0.01	0.01	0.01	0.01	0.01	0.005
<b>Sb</b>	0.10	0.01	0.10	0.01	0.03	0.00	0.04	0.003	0.03	0.01	0.03	0.004	0.03	0.002	0.02	0.01	0.02	0.01	0.02	0.002
<b>Ba</b>	33.39	0.42	33.81	0.67	4.51	0.36	10.29	0.62	2.95	0.06	2.86	0.03	3.58	0.22	2.38	0.03	4.40	0.48	2.42	0.12
<b>Hg</b>	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-
<b>Tl</b>	0.27	0.01	0.23	0.01	0.02	0.003	0.06	0.003	0.01	0.003	0.01	0.01	0.01	0.003	<LOQ	-	0.01	0.001	<LOQ	-
<b>Pb</b>	0.06	0.04	0.03	0.02	0.02	0.004	0.01	0.01	0.02	0.03	0.02	0.01	0.01	0.001	0.001	0.001	<LOQ	-	0.01	0.001
<b>U</b>	0.19	0.02	0.18	0.01	0.04	0.002	0.06	0.003	0.04	0.01	0.02	0.004	0.03	0.01	0.01	0.001	0.02	0.002	0.01	0.001

<LOQ: Concentrations lower than limit of quantification.



### Appendix I. Mean metal(oids) concentrations in surface water for September 2019

µg/L	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>Al</b>	7.30	1.36	9.85	1.18	11.29	0.65	9.48	27.30	13.12	1.92	13.96	3.57	17.10	7.48	21.47	15.53	12.75	1.32	14.08	1.19
<b>Ti</b>	0.05	0.02	0.04	0.04	0.11	0.02	0.28	0.36	0.15	0.02	0.16	0.03	0.48	0.45	0.54	0.60	0.18	0.06	0.23	0.08
<b>V</b>	0.12	0.01	0.13	0.01	0.11	0.02	0.14	0.05	0.11	0.01	0.10	0.01	0.16	0.07	0.20	0.14	0.11	0.00	0.10	0.01
<b>Cr</b>	0.76	0.30	0.63	0.22	0.30	0.04	0.29	5.13	0.80	0.21	1.03	0.79	2.33	0.48	4.18	2.31	1.89	0.50	1.27	0.42
<b>Mn</b>	68.92	0.70	63.71	0.45	3.19	0.07	10.82	0.67	9.42	0.73	3.42	0.18	8.23	6.14	4.49	11.23	4.27	0.21	2.32	0.04
<b>Fe</b>	27.74	1.55	42.37	4.18	139.70	1.92	152.40	88.51	152.23	9.07	143.32	1.23	148.96	146.68	161.11	429.78	150.89	5.80	148.58	3.12
<b>Co</b>	0.37	0.01	0.35	0.004	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-
<b>Ni</b>	7.05	0.16	6.49	0.11	0.49	0.12	1.12	0.20	0.83	0.18	0.33	0.07	0.41	0.05	0.48	0.09	0.35	0.07	0.31	0.14
<b>Cu</b>	0.32	0.17	0.14	0.01	0.13	0.01	0.45	0.46	0.26	0.09	0.26	0.20	0.57	0.29	0.44	1.09	0.43	0.08	0.46	0.09
<b>Zn</b>	2.95	1.78	3.03	1.70	1.57	1.41	1.55	1.17	2.15	1.00	2.63	1.69	1.57	0.24	2.05	0.58	1.33	0.29	2.38	1.16
<b>As</b>	2.40	0.05	2.30	0.06	0.19	0.01	0.40	0.03	0.31	0.04	0.19	0.01	0.22	0.02	0.20	0.02	0.19	0.01	0.19	0.01
<b>Se</b>	1.80	0.03	1.48	0.001	0.01	0.01	0.15	0.02	0.06	0.004	0.01	0.004	0.02	0.01	0.02	0.01	0.01	0.001	0.01	0.01
<b>Sr</b>	135.37	2.97	129.23	3.22	8.36	0.08	19.46	0.05	13.23	0.70	8.12	0.25	9.15	0.14	8.08	0.07	8.68	0.11	8.38	0.15
<b>Mo</b>	22.31	0.73	20.75	0.16	0.30	0.02	2.98	0.56	1.56	0.05	0.26	0.14	0.87	0.21	0.93	0.16	0.64	0.04	0.30	0.16
<b>Ag</b>	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-
<b>Cd</b>	0.04	0.004	0.04	0.01	<LOQ	-	0.01	0.01	0.01	0.01	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	0.01	0.01
<b>Sn</b>	0.04	0.01	0.03	0.01	0.02	0.002	0.04	0.06	0.01	0.002	0.05	0.08	0.12	0.08	0.19	0.11	0.12	0.07	0.12	0.03
<b>Sb</b>	0.09	0.01	0.08	0.002	0.01	0.001	0.02	0.004	0.05	0.06	0.02	0.00	0.02	0.01	0.02	0.01	0.01	0.001	0.02	0.00
<b>Ba</b>	29.97	0.92	28.89	0.78	2.53	0.08	5.52	0.30	3.89	0.18	2.49	0.11	2.89	0.11	2.61	0.17	2.66	0.05	2.61	0.08
<b>Hg</b>	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-
<b>Tl</b>	0.19	0.01	0.17	0.01	<LOQ	-	0.02	0.001	0.01	0.003	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-	<LOQ	-
<b>Pb</b>	0.03	0.004	0.02	0.01	0.02	0.01	0.07	0.10	0.03	0.02	0.03	0.04	0.09	0.03	0.15	0.10	0.08	0.02	0.09	0.06
<b>U</b>	0.25	0.02	0.21	0.003	<LOQ	-	0.03	0.02	0.03	0.02	0.01	-	0.02	0.02	0.01	0.003	0.02	0.02	0.02	0.02

<LOQ: Concentrations lower than limit of quantification.

### Appendix J. Mean metal(oids) concentrations for sediment 2018

	mg/kg	Al	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Sr	Mo	Ag	Cd	Sn	Sb	Ba	Hg	Tl	Pb	U
Site 1	Mean	17249.79	925.66	21.88	9.14	113.80	12449.53	10.07	53.03	4.26	28.44	19.54	7.12	69.59	56.16	<LOQ	0.48	0.47	0.21	482.40	0.03	1.61	7.15	5.49
	SD	2251.72	199.16	2.14	0.72	9.68	1557.78	1.75	10.44	0.55	5.11	0.29	0.45	10.86	14.68	<LOQ	0.02	0.15	0.01	27.89	0.01	0.26	0.58	1.17
Site 2	Mean	17768.42	816.68	17.82	7.64	89.03	11289.04	7.32	54.35	4.18	28.45	12.00	7.79	91.18	47.86	<LOQ	0.42	0.40	0.25	296.80	0.02	1.40	7.22	5.24
	SD	1841.65	138.19	2.45	0.95	13.22	1672.67	3.16	18.37	1.77	1.88	1.36	3.99	18.35	0.00	<LOQ	0.02	0.06	0.10	62.62	0.01	0.60	1.89	1.03
Site 3	Mean	21407.35	960.66	23.00	13.33	274.49	18867.04	2.06	4.75	0.60	9.69	2.19	1.28	139.09	12.05	<LOQ	0.18	0.64	0.05	388.46	0.01	0.39	10.55	0.99
	SD	3045.66	84.65	2.86	3.35	99.76	4780.98	0.37	0.77	0.24	1.73	0.85	0.57	1.74	5.34	<LOQ	0.03	0.10	0.01	25.23	0.00	0.02	0.38	0.30
Site 4	Mean	14695.19	620.44	28.50	11.26	345.82	36993.92	3.87	17.05	1.57	22.19	9.91	4.06	89.09	52.68	<LOQ	0.22	0.64	0.21	262.99	0.02	0.43	8.98	3.00
	SD	4169.28	103.74	10.59	1.82	275.62	24339.53	2.21	9.33	1.13	11.69	6.51	2.04	11.97	31.87	<LOQ	0.07	0.19	0.10	39.81	0.01	0.06	0.90	1.68
Site 5	Mean	17754.54	653.40	27.54	8.83	378.84	25035.50	2.15	6.88	0.86	16.14	5.79	1.82	101.82	21.01	<LOQ	0.16	0.50	0.07	345.95	0.01	0.40	10.06	1.37
	SD	4645.19	98.85	5.49	1.13	238.91	13214.07	0.81	2.74	0.36	4.86	2.99	1.29	13.95	10.14	<LOQ	0.02	0.17	0.01	46.01	0.00	0.02	0.99	0.41
Site 6	Mean	15905.56	419.48	15.41	5.14	259.44	15030.33	1.40	2.48	0.18	13.76	2.07	1.97	124.23	8.04	<LOQ	0.08	0.47	0.02	346.73	0.00	0.32	8.27	0.66
	SD	3531.93	110.70	3.77	3.13	65.10	805.72	0.26	1.08	0.21	12.47	0.12	0.57	22.44	0.90	<LOQ	0.01	0.12	0.01	19.71	0.00	0.01	0.19	0.05
Site 7	Mean	16256.70	435.81	13.90	2.58	553.68	9317.06	1.50	3.02	0.28	6.67	2.23	2.72	141.14	13.05	<LOQ	0.11	0.52	0.01	416.55	0.00	0.43	10.55	0.52
	SD	2824.42	95.71	2.73	1.27	84.01	1486.77	0.26	0.66	0.00	3.02	0.29	0.32	26.13	1.75	<LOQ	0.02	0.09	0.00	80.78	0.00	0.02	0.14	0.07
Site 8	Mean	13183.92	655.82	45.48	13.33	241.62	46911.49	4.51	18.57	3.75	34.71	10.51	4.05	73.16	68.74	<LOQ	0.27	0.80	0.22	218.52	0.04	0.32	6.90	3.53
	SD	1340.40	67.71	9.50	2.54	39.42	18220.43	0.89	1.77	0.10	8.20	3.75	0.35	10.64	34.87	<LOQ	0.04	0.11	0.09	21.77	0.01	0.02	1.13	0.67
Site 9	Mean	14570.90	605.58	36.59	10.90	848.43	31418.65	2.91	9.80	0.40	28.43	7.35	2.23	109.42	43.32	<LOQ	0.13	0.66	0.06	387.28	0.00	0.42	9.68	1.08
	SD	2415.96	14.12	2.26	2.27	80.36	897.08	0.09	3.72	0.14	17.09	0.32	0.38	8.91	2.17	-	0.01	0.10	0.01	57.31	0.00	0.01	0.34	0.03
Site 10	Mean	14342.52	383.49	58.97	11.73	1191.68	92151.05	3.83	2.63	0.74	46.90	15.54	2.36	102.24	6.54	<LOQ	0.12	0.59	0.03	316.96	0.00	0.28	7.65	0.93
	SD	2687.97	69.96	9.39	3.19	440.50	15031.07	0.87	0.87	0.62	8.71	2.38	0.36	13.03	0.87	<LOQ	0.02	0.12	0.02	12.44	0.00	0.03	0.12	0.25

<LOQ: Concentrations lower than limit of quantification.

### Appendix K. Mean metal(oids) concentrations for sediment 2019

	mg/kg	Be	Al	Ti	V	Cr	Fe	Mn	Co	Ni	Cu	Zn	As	Se	Sr	Mo	Ag	Cd	Sn	Sb	Ba	Hg	Tl	Pb	U
Site 1	Mean	0.81	21519.86	918.94	19.25	9.65	147.03	14939.84	15.39	91.56	6.06	39.47	32.52	11.77	115.61	97.72	0.05	0.49	0.29	0.30	694.46	0.01	1.93	9.12	6.91
	SD	0.10	2844.35	122.54	2.66	1.34	3.94	2143.33	0.99	1.36	0.12	4.56	5.86	0.36	17.29	19.43	0.01	0.09	0.11	0.10	210.07	0.01	0.12	1.39	0.38
Site 2	Mean	0.69	19636.00	780.00	14.80	7.00	184.32	9646.89	8.74	63.60	5.11	32.83	18.19	9.74	108.43	87.64	0.07	0.37	0.32	0.25	237.21	0.01	1.10	7.32	5.76
	SD	0.04	551.07	34.30	0.40	2.06	35.83	3497.79	1.53	12.47	0.88	5.02	1.93	4.08	4.58	15.83	0.01	0.17	0.03	0.04	6.49	0.01	0.37	0.35	0.38
Site 3	Mean	0.81	24114.13	371.15	11.26	8.08	541.35	11391.39	1.95	4.37	1.61	11.82	3.36	0.06	132.83	9.08	0.03	0.06	0.12	0.02	341.67	0.01	0.38	9.86	0.59
	SD	0.06	1253.97	141.85	3.05	1.83	31.95	1446.51	0.21	0.75	0.64	3.62	0.31	0.08	1.48	1.11	0.03	0.03	0.10	0.01	14.81	0.00	0.02	0.44	0.20
Site 4	Mean	0.83	35421.85	433.40	19.36	3.70	164.42	21307.13	1.94	7.31	1.71	20.18	6.87	0.37	216.36	33.40	0.03	0.15	0.17	0.09	484.13	0.01	0.44	12.43	1.22
	SD	0.30	10070.42	34.17	2.98	1.76	78.79	5855.63	0.51	1.36	0.20	3.32	2.66	0.02	68.57	14.33	0.02	0.07	0.00	0.04	231.56	0.00	0.17	4.10	0.25
Site 5	Mean	1.08	25215.11	818.41	24.64	11.46	192.87	17120.22	2.31	6.46	1.83	21.84	4.43	0.21	159.40	18.89	0.05	0.14	0.36	0.07	400.27	0.01	0.45	13.71	0.88
	SD	0.31	1732.14	171.95	3.44	1.89	23.63	1791.30	0.15	0.90	0.20	2.72	0.50	0.10	22.43	0.85	0.01	0.04	0.07	0.01	75.25	0.01	0.08	2.49	0.13
Site 6	Mean	0.82	29460.60	490.67	16.45	6.63	614.20	24847.68	2.44	3.17	2.42	22.84	6.42	0.10	145.38	7.32	0.03	0.11	0.16	0.06	314.44	0.01	0.36	9.42	0.55
	SD	0.19	3021.77	299.27	4.90	1.71	119.90	13559.58	0.73	0.88	1.44	8.60	5.71	0.05	10.42	2.32	0.01	0.02	0.02	0.01	16.87	0.01	0.06	0.98	0.10
Site 7	Mean	0.89	31729.71	805.22	15.87	7.68	670.42	11819.88	2.67	5.95	1.19	13.31	2.13	0.07	166.01	8.90	0.05	0.09	0.22	0.05	359.30	0.01	0.40	11.32	0.65
	SD	0.12	7989.10	69.81	1.76	1.72	52.90	461.37	0.15	0.46	0.32	0.91	0.07	0.01	40.24	0.49	0.01	0.02	0.10	0.03	41.30	0.00	0.04	0.41	0.02
Site 8	Mean	0.68	26814.25	970.93	16.45	9.47	533.66	13228.20	3.08	6.00	1.07	13.55	2.72	0.11	146.44	15.69	0.08	0.19	0.42	0.04	325.31	0.01	0.37	10.15	0.93
	SD	0.03	4414.33	290.46	2.17	2.46	114.98	1243.88	0.32	0.51	0.15	3.65	0.11	0.06	29.88	0.57	0.04	0.04	0.23	0.03	21.45	0.00	0.04	0.41	0.16
Site 9	Mean	0.60	24646.46	388.50	30.63	6.45	102.72	23590.52	1.45	5.76	1.07	19.02	4.41	0.21	136.75	19.23	0.12	0.08	0.09	0.06	262.83	0.01	0.30	8.44	0.76
	SD	0.05	1313.20	62.42	5.73	2.39	2.62	5032.63	0.25	1.89	0.14	6.57	1.23	0.21	4.87	4.14	0.15	0.01	0.04	0.02	19.75	0.00	0.08	0.72	0.07
Site 10	Mean	0.56	21921.87	326.69	11.41	1.25	363.77	13619.10	1.35	1.66	1.28	14.73	2.68	0.05	134.29	0.48	0.05	0.13	0.07	0.07	312.89	0.01	0.34	9.87	0.59
	SD	0.11	867.05	10.29	1.44	0.76	81.45	714.89	0.14	0.26	0.92	7.19	0.21	0.03	2.40	0.04	0.02	0.05	0.03	0.04	24.50	0.01	0.03	0.97	0.19

### Appendix L. Mean particle size in sediments 2018-2019

Site	Gravelly sand		Sand				Silt				Clay			
	2018		2019		2018		2019		2018		2019		2018-2019	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	0.00	0.00	0.00	0.00	80.33	0.58	83.81	0.72	23.75	2.09	16.20	0.72	0.00	0.00
2	0.00	0.00	0.00	0.00	82.56	3.72	89.53	0.61	17.44	3.72	10.47	0.61	0.00	0.00
3	0.71	1.23	0.00	0.00	95.61	1.31	97.95	1.26	3.68	1.22	2.05	1.26	0.00	0.00
4	0.00	0.00	1.99	1.35	89.60	3.43	95.74	1.33	10.40	3.43	2.28	0.43	0.00	0.00
5	0.00	0.00	0.00	0.00	92.85	0.92	94.62	2.46	7.16	0.91	5.38	2.46	0.00	0.00
6	0.61	0.73	0.14	0.25	98.37	0.72	97.28	0.42	1.03	0.25	2.57	0.63	0.00	0.00
7	0.00	0.00	0.00	0.00	98.38	0.14	97.90	0.42	1.62	0.14	2.10	0.42	0.00	0.00
8	1.97	0.84	1.45	1.72	88.87	0.38	96.29	1.08	9.17	0.72	2.26	0.91	0.00	0.00
9	0.00	0.00	2.51	2.96	97.66	0.30	95.76	2.63	2.35	0.30	1.73	0.37	0.00	0.00
10	1.00	1.07	0.00	0.00	94.59	1.22	96.84	0.92	4.41	2.24	3.16	0.92	0.00	0.00

### Appendix M. Mean total organic carbon (TOC) in sediments 2018-2019

TOC	Percentage (%)	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
2018	Mean-Samples (%TOC)	13.17	15.34	1.11	6.57	5.78	1.24	1.03	21.07	1.73	1.43
	SD	0.35	5.65	0.31	4.96	3.14	0.46	0.15	1.87	0.21	0.58
2019	Mean-Samples (%TOC)	13.23	17.92	0.60	1.53	1.64	0.98	0.68	0.93	0.63	0.64
	SD	0.53	1.80	0.07	0.46	0.28	0.22	0.08	0.28	0.02	0.12

## Appendix N. Values used in toxicological risk assessment calculations

Canadian Water Quality Guideline values used in toxicological risk assessment					
Dissolved metal		Value (µg/L)	Reference	Type	Details
Mercury	Hg*	0.026	CCME 1991	Safety factor (0.1x)	60-d LOEL <i>Pimephales promelas</i> = 0.26 µg Hg/L
Thallium	Tl	0.8	CCME 1991	Safety factor (0.1x)	14-d EC50 <i>Lemna minor</i> = 8 µg/L
Silver	Ag*	0.1	CCREM 1987	Equation (Hardness)	Based on 1980k US EPA Guideline
Cadmium	Cd*	0.017	CCME 1996	Safety factor (0.1x)	21-day EC16 <i>Daphnia magna</i> =0.17 µg/L
Iron	Fe*	300	CCREM 1987	Unknown	Based on 1978 ON IJC Guideline

\*Guidelines adopted for the Saskatchewan Water Quality Objectives (2015).

CCME: Canadian Council of Ministers of the Environment Guideline.

CCRM: Canadian Council of Resource and Environment Ministers Guideline.

ON IJC: Ontario Great Lakes Water Quality International Joint Commission Guideline.

US EPA: United States Environmental Protection Agency Guideline.

**Appendix O. Instrumental quality assurance/quality control (QA/QC) information for aqueous  
Se and As in 2019**

Concentrations (ug/L)	September 2019		August 2019		July 2019	
	75 -> 75 As [ He ]	78 -> 78 Se [ H2 ]	75 -> 75 As [ He ]	78 -> 78 Se [ H2 ]	75 -> 75 As [ He ]	78 -> 78 Se [ He ]
1640a-1	7.93	20.24	7.83	19.88	8.61	22.66
1640a-2	7.75	19.94	8.05	20.17	8.46	21.19
1640a-3	7.80	20.17	7.60	19.74	8.33	20.78
1640a-4	7.53	20.03	7.47	19.66	8.06	19.60
1640a certified value	8.08	20.13	8.08	20.13	8.08	20.13
	As	Se	As	Se	As	Se
Recovery percentage %	98.26	100.57	97.02	98.74	106.68	112.59
Recovery percentage %	96.02	99.04	99.71	100.20	104.77	105.26
Recovery percentage %	96.64	100.22	94.14	98.04	103.10	103.21
Recovery percentage %	93.23	99.52	92.46	97.65	99.82	97.36
Average of recovery percentage	95.77	99.90	95.83	98.66	103.59	104.61
SD	1.71	0.55	3.20	1.12	2.91	6.29

1640a = Certified reference material, trace elements in natural water, National Institute of Standards and Technology.

**Appendix P. Total abundance of benthic macroinvertebrates collected by Hester Dendy  
samplers in 2018**

Taxa	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Total
<i>Aeshna</i>	3	4	0	0	0	3	0	0	3	4	17
<i>Coenagrion</i>	10	5	1	5	4	3	0	6	1	1	36
<i>Agrypnia</i>	5	0	7	23	0	0	0	0	0	0	35
<i>Brachycentrus</i>	0	0	4	0	0	0	0	0	0	0	4
<i>Polycentropus</i>	10	0	6	20	0	4	14	26	6	15	101
<i>Leptophlebia</i>	18	40	19	36	68	103	92	6	4	16	402
<i>Centropilum</i>	5	0	0	0	0	0	0	0	0	0	5
<i>Stenonema</i>	3	6	0	2	0	0	0	0	0	0	11
<i>Caenis</i>	6	1	4	0	0	3	0	0	3	0	17
Chironomidae	56	71	70	121	84	60	71	10	66	7	616
<i>Ilybius</i>	0	1	10	14	1	0	0	0	0	0	26
<i>Nebrioporus</i>	0	1	0	0	0	7	0	0	0	0	8
<i>Hyaella</i>	0	0	11	7	2	9	4	7	7	0	47
<i>Batracobdella</i>	0	0	0	0	0	0	2	4	0	0	6
<i>Erpobdella</i>	0	0	0	0	3	0	3	0	0	0	6
<i>Sphaerium</i>	22	35	0	3	7	0	9	8	0	0	84
<i>Fossaria</i>	79	39	0	0	0	0	0	0	0	0	118
<i>Promenetus</i>	7	0	0	0	0	0	0	0	0	0	7
<i>Physa</i>	1	3	0	0	0	1	1	0	0	0	6
Oligochaeta	0	1	0	0	1	0	0	3	8	1	14
Acari	21	5	0	0	0	0	0	0	0	0	26
<b>Total</b>	<b>246</b>	<b>212</b>	<b>132</b>	<b>231</b>	<b>170</b>	<b>193</b>	<b>196</b>	<b>70</b>	<b>98</b>	<b>44</b>	<b>1592</b>

**Appendix Q. Total abundance of benthic macroinvertebrates collected by Hester Dendy  
samplers in 2019**

Taxa	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Total
<i>Aeshna</i>	3	3	0	0	0	0	0	1	8	5	20
<i>Agrypnia</i>	7	0	0	0	0	2	0	0	0	0	9
<i>Molanna</i>	5	0	0	0	0	0	0	4	0	0	9
<i>Nephelopsis</i>	3	5	1	0	0	1	4	1	4	3	22
<i>Brachycentrus</i>	0	0	0	0	2	0	0	0	0	0	2
<i>Caenis</i>	13	1	4	0	1	3	0	0	3	1	26
<i>Centroptilum</i>	319	1	3	0	2	0	0	0	0	0	325
Chironomidae	142	186	129	1633	442	376	139	109	770	60	3986
<i>Coenagrion</i>	21	0	0	0	8	0	0	5	1	0	35
<i>Fossaria</i>	58	4	0	0	0	0	0	0	0	0	62
<i>Hyalella</i>	0	1	66	21	1	22	22	23	51	32	239
<i>Ilybius</i>	2	14	0	0	0	9	0	0	0	0	25
<i>Leptophlebia</i>	105	199	22	5	117	27	60	1334	91	7	1967
Oligochaeta	0	0	0	5	0	2	3	0	0	0	10
<i>Physa</i>	1	3	0	0	0	1	1	0	0	0	6
<i>Polycentropus</i>	13	0	19	78	0	1	14	1770	38	4	1937
<i>Nebrioporus</i>	0	0	0	0	0	16	1	0	0	1	18
<i>Promenetus</i>	61	0	0	1	0	0	1	0	0	0	63
<i>Sphaerium</i>	71	0	0	0	5	3	6	45	0	0	130
<i>Stenonema</i>	2	1	0	3	0	0	0	0	0	0	6
Acari	48	7	0	0	0	0	0	0	0	0	55
<b>Total</b>	<b>874</b>	<b>425</b>	<b>244</b>	<b>1746</b>	<b>578</b>	<b>463</b>	<b>251</b>	<b>3292</b>	<b>966</b>	<b>113</b>	<b>8897</b>



**Appendix R. Total abundance of benthic macroinvertebrates collected by Ekman grab  
samplers in 2019**

Taxa	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10	Total
<i>Aeshna</i>	0	0	0	0	0	0	1	0	0	0	1
<i>Agrypnia</i>	1	0	0	1	3	0	0	0	0	0	5
<i>Molanna</i>	0	0	1	0	1	0	6	6	0	0	14
<i>Nephelopsis</i>	0	1	0	0	0	0	0	0	0	1	2
<i>Batracobdella</i>	0	0	0	0	2	0	0	1	0	0	3
<i>Erpobdella</i>	0	0	0	3	1	0	1	2	4	5	16
<i>Caenis</i>	0	26	0	0	3	0	0	0	0	1	30
Chironomidae	210	140	5	90	251	58	85	124	80	45	1036
<i>Fossaria</i>	14	57	0	0	0	0	0	0	0	0	71
<i>Hyalella</i>	0	0	80	195	68	4	149	20	81	90	687
<i>Ilybius</i>	0	2	2	0	1	0	0	1	0	0	6
<i>Leptophlebia</i>	0	0	1	0	0	0	5	19	0	1	26
Oligochaeta	0	0	0	8	0	0	0	0	0	0	8
<i>Physa</i>	0	0	5	2	0	0	1	0	0	0	8
<i>Polycentropus</i>	2	0	0	7	7	0	3	14	0	0	33
<i>Promenetus</i>	126	20	6	1	42	0	25	26	0	4	250
<i>Sphaerium</i>	231	218	44	16	64	19	87	414	16	24	1133
Acari	0	0	0	1	4	0	0	0	0	0	5
<i>Hexagenia</i>	0	0	0	0	0	0	0	7	0	1	8
Total	584	464	144	324	447	81	363	634	181	172	<b>3394</b>

## Appendix S. Water BAFs for macroinvertebrates collected with H-D samplers in 2018

### A. Vulture Lake (Site 1)

BAF	<i>Hydroaracnida</i>	<i>Agripnia</i>	<i>Leptophlebia</i>	<i>Helobdella</i>	<i>Promenetus</i>	<i>Fossaria</i>	Ephemeroptera	<i>Polycentropus</i>	<i>Corixidae</i>	<i>Sphaerium</i>	<i>Sphaeridae</i>	<i>Coenagrionidae</i>
As	1426	745	1155	1291	290	723	1004	1041	1820	1045	1066	2090
Se	1917	6254	4408	6158	576	3390	2079	7514	3340	423	759	2337
Hg	1938	1118	768	811	94	1605	420	193	594	1009	241	448

### B. McClean Lake east basin (Site 4)

BAF	<i>Agripnia</i>	<i>Helobdella</i>	<i>Zygoptera</i>	<i>Ilybius</i>	Ephemeroptera	<i>Physa</i>	<i>Hyalrella azteca</i>
As	1650	2161	7238	1594	4508	3723	3862
Se	13523	10893	3850	8772	6377	9929	2911
Hg	3077	91	-	2354	431	491	-

### C. Collins Creek (Site 8)

BAF	<i>Helobdella</i>	<i>Ilybius</i>	<i>Hyalrella azteca</i>
As	2959	17696	2714
Se	15464	6869	3255
Hg	-	-	-

### D. McClean Lake west basin (Site 10)

BAF	<i>Coenagrionidae</i>	<i>Agripnia</i>	Ephemeroptera	<i>Leptophlebia</i>	<i>Polycentropus</i>	<i>Tricoptera</i>	<i>Physa</i>	Chironomidae
As	3004	3651	10556	2648	4154	737	11345	27288
Se	64512	94583	72679	15394	31258	41557	112480	130150
Hg	983	18076	1188	-	2343	31873	1555	2197

## Appendix T. Water BAFs for macroinvertebrates collected with H-D samplers in 2019

### A. Vulture Lake (Site 1)

BAF	<i>Leptophlebia</i>	<i>Centropilum</i>	<i>Caenis</i>	<i>Stenonema</i>	<i>Coenagrionidae</i>	<i>Ilybius</i>	<i>Sphaerium</i>	<i>Physa</i>	<i>Fossaria</i>	Mites	<i>Ashena</i>	<i>Coenagrionidae</i>	<i>Ashena</i>	<i>Agrypnia</i>
As	1788	678	1506	103	3.8	10	150	3512	1247	9414	561	430	629	606
Se	3721	3577	8164	704	102	120	213	5011	5064	149803	5127	5906	4941	17764
Hg	265916	80263	44751	-	11240	-	-	1063154	52877	-	63456	-	228747	-

### B. McClean Lake east basin (Site 4)

BAF	<i>Leptophlebia</i>	<i>Sphaerium</i>	<i>Aeshna</i>
As	256	1772	2090
Se	3896	8816	15767
Hg	-	-	-

### C. Collins Creek (Site 8)

BAF	<i>Polycentropus</i>	<i>Leptophlebia</i>	<i>Sphaerium</i>	<i>Ashena</i>	<i>Coenagrionidae</i>	<i>Aeshna</i>	<i>Hyalella azteca</i>
As	3218	519	3356	2456	1999	1416	180
Se	40216	6919	16863	17941	39432	15658	3278
Hg	-	-	-	-	-	-	-

### D. McClean Lake west basin (Site 10)

BAF	<i>Caenis</i>	<i>Aeshna</i>	<i>Hyalella azteca</i>	<i>Agrypnia</i>
As	62	3661	347	1549
Se	-	14256	5731	6381
Hg	-	-	-	-

## Appendix U. Food BAFs for macroinvertebrates collected with H-D samplers in 2018

### A. Vulture Lake (Site 1)

BAF	<i>Hydroaracnida</i>	<i>Agripnia</i>	<i>Leptophlebia</i>	<i>Helobdella</i>	<i>Promenetus</i>	<i>Fossaria</i>	Ephemeroptera	<i>Polycentropus</i>	<i>Corixidae</i>	<i>Sphaerium</i>	<i>Sphaeridae</i>	<i>Coenagrionidae</i>
As	0.18	0.09	0.14	0.16	0.04	0.09	0.13	0.13	0.23	0.13	0.13	0.26
Se	0.75	2.43	1.71	2.39	0.22	1.32	0.81	2.92	1.30	0.16	0.29	0.91
Hg	0.64	0.37	0.25	0.27	0.03	0.53	0.14	0.06	0.20	0.33	0.08	0.15

### B. McClean Lake east basin (Site 4)

BAF	<i>Agripnia</i>	<i>Helobdella</i>	<i>Zygoptera</i>	<i>Ilybius</i>	Ephemeroptera	<i>Physa</i>	<i>Hyalella azteca</i>
As	0.08	0.10	0.34	0.07	0.21	0.17	0.18
Se	1.18	0.95	0.34	0.76	0.56	0.86	0.25
Hg	0.07	0.00	-	0.05	0.01	0.01	-

### C. Collins Creek (Site 8)

BAF	<i>Helobdella</i>	<i>Ilibius</i>	<i>Hyalella azteca</i>
As	0.11	0.68	0.11
Se	1.22	0.54	0.26
Hg	-	-	-

### D. McClean Lake west basin (Site 10)

BAF	<i>Coenagrionidae</i>	<i>Agripnia</i>	Ephemeroptera	<i>Leptophlebia</i>	<i>Polycentropus</i>	<i>Tricoptera</i>	<i>Physa</i>
As	0.08	0.10	0.29	0.07	0.11	0.02	0.31
Se	0.76	1.12	0.86	0.18	0.37	0.49	1.33
Hg	-	-	-	-	-	-	-

## Appendix V. Food BAFs for macroinvertebrates collected with H-D samplers in 2019

### A. Vulture Lake (Site 1)

BAF	<i>Leptophlebia</i>	<i>Centroptilum</i>	<i>Caenis</i>	<i>Stenonema</i>	<i>Coenagrionidae</i>	<i>Ilybius</i>	<i>Sphaerium</i>	<i>Physa</i>	<i>Fossaria</i>	Mites	<i>Ashena</i>	<i>Coenagrionidae</i>	<i>Ashena</i>	<i>Agrypnia</i>
As	0.22	0.08	0.19	0.01	0.00	0.00	0.02	0.44	0.16	1.17	0.07	0.05	0.08	0.08
Se	0.97	0.93	2.13	0.18	0.03	0.03	0.06	1.31	1.32	39.11	1.34	1.54	1.29	4.64
Hg	626.53	189.11	105.44	-	26.48	-	-	2504.92	124.59	-	149.51	-	538.96	-

### B. McClean Lake east basin (Site 4)

BAF	<i>Leptophlebia</i>	<i>Bivalve</i>	<i>Aeshna</i>
As	0.01	0.06	0.07
Se	0.17	0.39	0.70
Hg	-	-	1371.26

### C. Collins Creek (Site 8)

BAF	<i>Polycentropus</i>	<i>Leptophlebia</i>	<i>Sphaerium</i>	<i>Ashena</i>	<i>Coenagrionidae</i>	<i>Aeshna</i>	<i>Hyaella azteca</i>
As	0.04	0.01	0.05	0.03	0.03	0.02	0.00
Se	0.83	0.14	0.35	0.37	0.82	0.32	0.07
Hg	-	-	-	1044.87	-	-	-

### D. McClean Lake west basin (Site 10)

BAF	<i>Caenis</i>	<i>Aeshna</i>	<i>Hyaella azteca</i>	<i>Agrypnia</i>
As	0.00	0.04	0.00	0.02
Se	-	0.78	0.32	0.35
Hg	-	-	-	-

## Appendix W. Sediment BAFs for macroinvertebrates collected with grab samplers in 2019

### A. Vulture Lake (Site 1)

BAF	<i>Sphaerium</i>	<i>Agrypnia</i>
V	0.02	0.25
Cd	170	689

### B. McClean Lake east basin (Site 4)

BAF	<i>Hyalella azteca</i>	<i>Hyalella azteca</i>	<i>Chironomidae</i>
V	0.30	0.16	0.03
Cd	3017	2020	797

### C. Collins Creek (Site 8)

BAF	<i>Sphaerium</i>	<i>Sphaerium</i>
V	0.06	0.03
Cd	245	255

### D. McClean Lake west basin (Site 10)

BAF	<i>Sphaerium</i>	Chironomid	<i>Promenetes umbilicatellus</i>
V	0.34	0.41	0.41
Cd	85	211	911

## Appendix X. Food BAFs for macroinvertebrates collected with grab samplers in 2019

### A. Vulture Lake (Site 1)

BAF	<i>Sphaerium</i>	<i>Agrypnia</i>	<i>Sphaerium</i>
V	0.03	0.36	0.06
Cd	213	863	242

### B. McClean Lake east basin (Site 4)

BAF	<i>Hyalella azteca</i>	<i>Hyalella azteca</i>
V	0.22	0.12
Cd	1006	674

### C. Collins Creek (Site 8)

BAF	<i>Sphaerium</i>	<i>Sphaerium</i>
V	0.03	0.01
Cd	170	177

### D. McClean Lake west basin (Site 10)

BAF	<i>Sphaerium</i>	Chironomidae	<i>Promenetus umbilicatellus</i>
V	0.10	0.12	0.12
Cd	17	42	183

**Appendix Y. Comparison of bioaccumulation factors (BAFs) for Chironomidae in 2018 and 2019 using both water and periphyton (food) as the denominator in the calculation.**

Monitoring period			2018			2019		
Location		BAF	Se	As	Hg	Se	As	Hg
Vulture Lake	Site 1	water	1,133	1,796	254	4,324	1,079	2,837
		food	0.44	0.22	0.08	1.13	0.13	6.68
McClellan Lake east basin	Site 4	water	18,607	182	279	4,933	65	-
		food	1.62	0.01	0.01	0.22	0.002	-
Collins Creek	Site 8	water	10,072	18,348	-	451,664	34,436	-
		food	0.80	0.71	-	9.36	0.46	-
McClellan Lake west basin	Site 10	water	130,150	27,288	2,198	20,635	2,707	-
		food	1.54	0.75	-	1.13	0.03	-

**Appendix Z. Comparison of bioaccumulation factors (BAFs) for Chironomidae and *Sphaerium* in 2018 and 2019 using both sediment and periphyton (food) as the denominator in the calculation.**

Monitoring locations		Vulture Lake	McClellan Lake East basin	Collins Creek	McClellan Lake West basin
		Site 1	Site 4	Site 8	Site 10
Species		<i>Sphaerium</i>	Chironomidae	<i>Sphaerium</i>	Chironomidae
V	sediment	0.022	0.026	0.056	0.407
	food	0.032	0.019	0.028	0.123
Cd	sediment	170	798	245	211
	food	213	266	170	43



## **Appendix AA. Autonomous Sensors Programming and Calibration**

The model for surface water quality monitoring (Smart Water units) works through the use of Wasmote Plug & Sense® sensor devices. These devices have been developed to facilitate remote environmental monitoring, allowing solar-powered units to be deployed in the field with attached probes (up to 6) that can measure pH, dissolved oxygen (DO), electrical conductivity (EC), oxidation reduction potential (ORP), turbidity and temperature (T) (Libelium Technical guide, 2018). This technology is used here to evaluate contaminant risk downstream of a uranium mill.

Programming was performed within the Wasmote Pro IDE (Integrated Development Environment) using the Wasmote Pro API (Application Programming Interface) from Libelium®. The Wasmote IDE is the software development kit used to write and upload code (firmware) to the sensor units and monitor serial output (real-time measurements) and debugging. The Wasmote API contains libraries necessary for compiling programs and is constantly updated whenever improvements are made. The software Wasmote IDE v.38, provided by Libelium®, was used as the template code for programming to measure EC, pH, DO, turbidity and temperature.

Probes were calibrated against standard solutions to ensure accuracy and liability of results. The Smart Water units were connected to a computer via a USB cable, and the template code from the Wasmote v.38 software was uploaded to each unit. The probes were individually connected to each unit and calibrated according to the Smart Water technical manual (Libelium 2018). Values were measured in millivolts (mV) and then converted into a respective probe unit (i.e., C°, NTU, pH log scale, percent of dissolved oxygen, and  $\mu\text{S}/\text{cm}$ ) by the instrument. After calibration, the values obtained were inserted into each node's programming code. Then, EC sensor probe measurements were compared with parallel measurements taken with calibrated hand-held meters to known EC standard solutions (9.72, 84, 100, 1000 and 1413  $\mu\text{S}/\text{cm}$ ), allowing the validation of the sensor EC probes. The final code was based on the original template (Wasmote v.38) with modifications to accommodate the specific needs of this project.