THE INFLUENCE OF WATER QUALITY CHARACTERISTICS ON VANADIUM TOXICITY TO MODEL AQUATIC ORGANISMS

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ABSTRACT

Vanadium (V) is a contaminant of emerging concern for the Alberta oil sands region that could present a risk for aquatic organisms. Petroleum coke (PC) has been experimentally used to treat oil sands process-affected water (OSPW) to reduce organic toxicants. However, PC contains up to 1,000 mg of V per kg of PC, and during OSPW treatment V leaches from coke reaching levels of up to 7 mg/L in "treated" OSPW, a concentration that is toxic to aquatic organisms. Little information is available on how common water quality variables affect the toxicity of V to aquatic organisms. Furthermore, there is no clear understanding of the mechanism(s) of toxicity of V in aquatic organisms. Vanadium is a transition metal with several oxidation states, and could potentially elicit its toxicity through either ion imbalance or oxidative stress. Therefore, the objectives of this research were to (i) investigate the influence of key water chemistry variables representative of the Alberta oil sands region on V toxicity to freshwater organisms, and (ii) determine if ion imbalance and oxidative stress are part of its mechanism of toxicity. To describe how water chemistry influences V toxicity to two representative freshwater organisms, *Daphnia pulex* and *Oncorhynchus mykiss*, descriptive relationships were developed between those parameters that differ the most between OSPW and the Athabasca River. Results indicate that an increase in pH increases V acute toxicity to both species, whereas increasing alkalinity and sulphate ameliorate V toxicity to both species. Sodium only causes amelioration of V toxicity to daphnids above 325 mg/L. The mechanistic studies with *Daphnia magna* and *O. mykiss* suggest that concentrations of V close to their respective median lethal concentration (LC_{50}) cause sodium imbalance in both species, as well as calcium imbalance in rainbow trout, and oxidative stress in *O. mykiss*. In conclusion, the influence of pH, alkalinity and sulphate on V toxicity should be considered when creating new acute water quality guidelines or local

benchmarks for V. The mechanism of V toxicity to aquatic organisms includes ion imbalance and oxidative stress, but further mechanistic research will be needed to increase knowledge on the ecological risks of V contamination, which will enable the formulation of possible mitigation strategies.

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LIST OF ABBREVIATIONS

A	Sodium or calcium concentration in tissue digest (µg/mL based on an equation presented by Roy (2009), Chapter 4)
AER	Alberta Energy Regulator
AIC	Akaike information criterion
Al	Aluminium
ANOVA	Analysis of variance
AOSR	Alberta Oil Sands Region
AR	Athabasca River
ARW	Athabasca river water
ATP	Adenosine triphosphate
ATRF	Aquatic toxicology research facility
As	Arsenic
ASTM	American society for testing and materials
B	Boron
Ba	Barium
BIC	Bayesian information criterion
BLM	Biotic ligand model
C	Carbon
Ca	Calcium ion
Ca-ATPase	Calcium ATPase
CaCl	Calcium chloride
$CaCO_3$	Calcium carbonate

$CaSO_4$	Calcium sulphate
CAPP	Canada's Oil and Natural Gas Producers
$CaSO_4 \cdot 2H_2O$	Gypsum/calcium sulphate
CAT	Catalase
CHO_2Na	Sodium formate
Cr	Chromium
CCME	Canadian Council of Ministers of the Environment
Cd	Cadmium
CEPA	Canadian Environmental Protection Act
$C_2H_4O_2$	Glacial acetic acid
CI	Confidence interval
Cl	Chloride ion
cm	Centimetre
Co	Cobalt ion
CO_2	Carbon dioxide
cpm	Counts per minute
Cr	Chromium
CT	Composite tailings
Cu	Copper
CuOH	Hydrated copper ion
CWQG	Canadian Water Quality Guideline
d	Day
DeCl	Carbon-filtered, bio-filtered municipal Saskatoon City water

DIDS	4, 40-diisothiocyanatostilbene-2, 20-disulfonic acid
DNA	Deoxyribonucleic acid
d.w.	Dry weight
DO	Dissolved oxygen
DOC	Dissolved organic carbon
EC_x	Effective concentration estimated to cause an effect on x% of exposed organisms
ECCC	Environment and Climate Change Canada
ETMFs	Exposure and toxicity modifying factors
FA	Fulvic Acid
Fe	Iron
g	Grams
x g	Times gravity
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione S-transferase
h	Hour
H	Proton
H-ATPase	Proton ATPase
H_2O	Molecular Water
H_2O_2	Hydrogen peroxide

HC	Health Canada
HC_5	Hazardous concentration to 5% of species tested
HCO_3/ CO_3	Bicarbonate ion
HNO_3	Nitric acid
HPO_4	Phosphate ion
HSO_4	Hydrogen sulphate ion
$H_2VO_4^-$ /HVO_4^{2-}	Vanadium oxyanions
IC	Inhibition concentration
IC	Ion chromatography
ICP-MS	Inductively coupled plasma-mass spectrometry
ICP-OES	Inductively coupled plasma-optical emission spectrometry
KCl	Potassium chloride
kg	Kilogram
$KHCO_3$	Potassium bicarbonate
L	Litre
La	lanthanum
LC_{50}	Median lethal concentration
m	Wet mass of the animal(s)
MDA	Malondialdehyde
MFT	Mature fine talings
mg	Milligram
mg/kg	Milligram per kilogram
mg/L	Milligrams per liter

Mg-ATPase	Magnesium ATPase
$MgSO_4$	Magnesium sulphate
min	Minute(s)
ml	Milliliter
MLR	Multiple linear regression
MLSB	Mildred Lake Settling Basin
mM	Millimolar (mmol/L)
Mn	Manganese
Mo	Molybdenum
mol	Mole
Mo	Molybdenum
MS-222	Tricaine methanesulfonate
n	Number of replicate
N	Normal concentration
N_2	Nitrogen
NA	Naphthenic acid
N/A	Not available
Na	Sodium
^{22}Na	Radiolabelled sodium
NAD(P)H	Reduced form of Nicotinamide adenine dinucleotide phosphate
$NaHCO_3$	Sodium bicarbonate
Nb	Niobium
NaOH	Sodium hydroxide/caustic soda

NH_3	Ammonia
NH_4	Ammonium
NKA	Sodium/Potassium ATPase
$NaVO_3$	Sodium metavanadate
ng	Nanogram
Ni	Nickel
NiOH	Nickel hydroxide
NMCP	North Mine Centre Pond
O_2	Molecular oxygen
$O_2^{\bullet-}$	Superoxide anion
OECD	Organisation for Economic Cooperation and Development
OSPW	Oil sands process-affected water
p	Probability
PAH	Polycyclic aromatic hydrocarbons
Pb	Lead
PC	Petroleum coke
pH	Potential of hydrogen
ppt	Parts per trillion
QA/QC	Quality assurance/quality control
RAMP	Regional aquatics monitoring program
RO	Reverse osmosis
ROS	Reactive oxygen species
SA	Specific activity of the exposure water (cpm of ^{22}Na/µM of total sodium)

Sb	Antimony
SD	Standard deviation
Se	Selenium
SE	Standard error
SLC26	Sulphate antiporter
SLRS	Standard reference material
SSD	Species Sensitivity Distributions
SO_4	Sulphate ion
$^{35}SO_4$	Radiolabelled sulphate
SOD	Superoxide dismutase
Sr	Strontium
t	Time
Ta	Tantalum
TBARS	Thiobarbituric acid reactive substances
TDS	Total dissolved solids
V	Vanadium / (Note: V was also used in Chapter 4 as volume in mL of tissue digest based on an equation presented by Roy (2009))
V(V)	Vanadate
V(IV)	Vanadyl
VIF	Variance inflation factors
$VO^{2+}/VO(OH)^{+}$	Vanadyl ions
W	Tungsten
WHO	World Health Organization

WQC	Water quality criteria
WQG	Water quality guideline
wt.	Weight
YCT	Yeast, cerophyll and trout-chow
Zn	Zinc
°C	Degrees Celsius
µCi	Microcurie
µg	Microgram
µL	Microliter
µm	Micrometre
µS/cm	Micro Sieverts per centimetre
1640a	Standard reference material

NOTE TO READERS

This thesis is organized and formatted to follow the University of Saskatchewan College of Graduate and Postdoctoral Studies guidelines for a manuscript-style thesis. Chapter 1 is a general introduction and literature review, including the project goal and objectives, and Chapter 5 contains a general discussion and conclusions that integrates all the remaining. Chapters 2, 3, and 4 are organized as manuscripts for publication in peer-reviewed scientific journals. Chapter 2 was published in Ecotoxicology and Environmental Safety. Chapter 3 was submitted to Environmental Toxicology and Chemistry, and Chapter 4 will be submitted to Aquatic Toxicology. As a result of the manuscript-style format, there is some repetition of information in the introduction and materials and methods sections of the thesis. The tables, figures, supporting information and references cited in these research chapters have been reformatted here to follow a thesis style. References cited in each chapter are combined and listed in the References section of the thesis. Supporting information associated with research chapters are presented in the Appendix section after the reference section.

The author contributions included:

Mr. Esteban Gillio Meina (University of Saskatchewan) managed and conducted all experiments, collected and analyzed lab data, performed all chemical, statistical, modelling and speciation analysis, and drafted each of the manuscripts and the thesis.

Ms. Katherine Raes (University of Saskatchewan) assisted with some of the exposure studies, lab data collection, and some water chemistry analyses (only Chapter 2).

Dr. Karsten Liber (University of Saskatchewan) developed the project, provided scientific guidance and input, supervised the research, reviewed the manuscripts, provided comments and editorial corrections of this thesis, and secured the funding for this research project.

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CHAPTER 1: GENERAL INTRODUCTION

1. General introduction

Industrial activities in the Alberta oil sands region have increased dramatically in the last two decades. The higher demand for bitumen and its refinement products has led to an increase in the production of two main by-products, known as petroleum coke (PC, a solid by-product) and oil sands process water (OSPW, a liquid by-product) (Allen, 2008a). Both products pose environmental risks since OSPW contains high concentrations of naphthenic acids (NA), and coke presents elevated concentrations of leachable trace elements (Puttaswamy et al., 2010). As a result of this elevated concentration of toxicants in PC, the provincial government approved regulations demanding the permanent storage of PC on site. To reduce the stockpiling of PC and potential environmental contamination, Syncrude Ltd. proposed the use of PC on-site to treat OSPW toxicity as a wet-landscaping technique. When in contact with OSPW, PC significantly reduces the concentration of NAs, making PC a potential OSPW remediation tool (Zubot et al., 2012). However, once PC is in contact with aqueous media, such as OSPW, trace elements, particularly vanadium (V), are released. If sufficiently elevated, V is toxic to various aquatic organisms (Schiffer and Liber, 2017a). Previous research has rarely assessed how exposure and toxicity modifying factors (ETMFs) like pH, alkalinity, or major ions (sodium (Na), chloride (Cl), sulphate (SO_4) or bicarbonate (HCO_3)) alter V toxicity to aquatic organisms. Therefore, using a combination of toxicity testing using regionally representative invertebrate and fish species and geochemical speciation modelling, the goal of this research is to better understand how ETMFs

alter V toxicity under both short- and long-term exposure conditions and to elucidate the mechanism(s) of V toxicity to aquatic species.

1.1. Oil sands location

Oil sands are sandy deposits that contain a thick form of petroleum called bitumen. Globally, these deposits represent one of the world's major petroleum reservoirs (CAPP, 2017, 2012; Lim and Thomas, 1980). The most abundant oil sand deposits are located in the provinces of Alberta and Saskatchewan, Canada, and in the Orinoco Region, Venezuela. Smaller deposits occur in China, the United States, Peru, Trinidad, Madagascar, the Balkan States, Russia, Romania, and the Philippines (Chalaturnyk et al., 2002). Formed in the Lower Cretaceous age (Deroo and Powell, 1978; Hein et al., 2001; Wightman et al., 1989) the Albertan Oil Sands are located in the northeast part of the province of Alberta (Hein et al., 2001, see Figure 1.1), with a small intrusion occurring in the northwest part of Saskatchewan (Prebble et al., 2009; Schramm et al. 2010). Together, these deposits cover approximately 142,000 km^2 (Riva et al., 2018; Yeh et al., 2015). The oil sands reservoir in the Athabasca area is the largest and, to date, the most exploited Canadian deposit (Figure 1.1). There are two main towns located in the area; Fort MacKay and Fort McMurray, with a total population close to 80,000, more than 50% of whom are directly employed by the oil sands projects (Regional Municipality of Wood Buffalo, 2012).

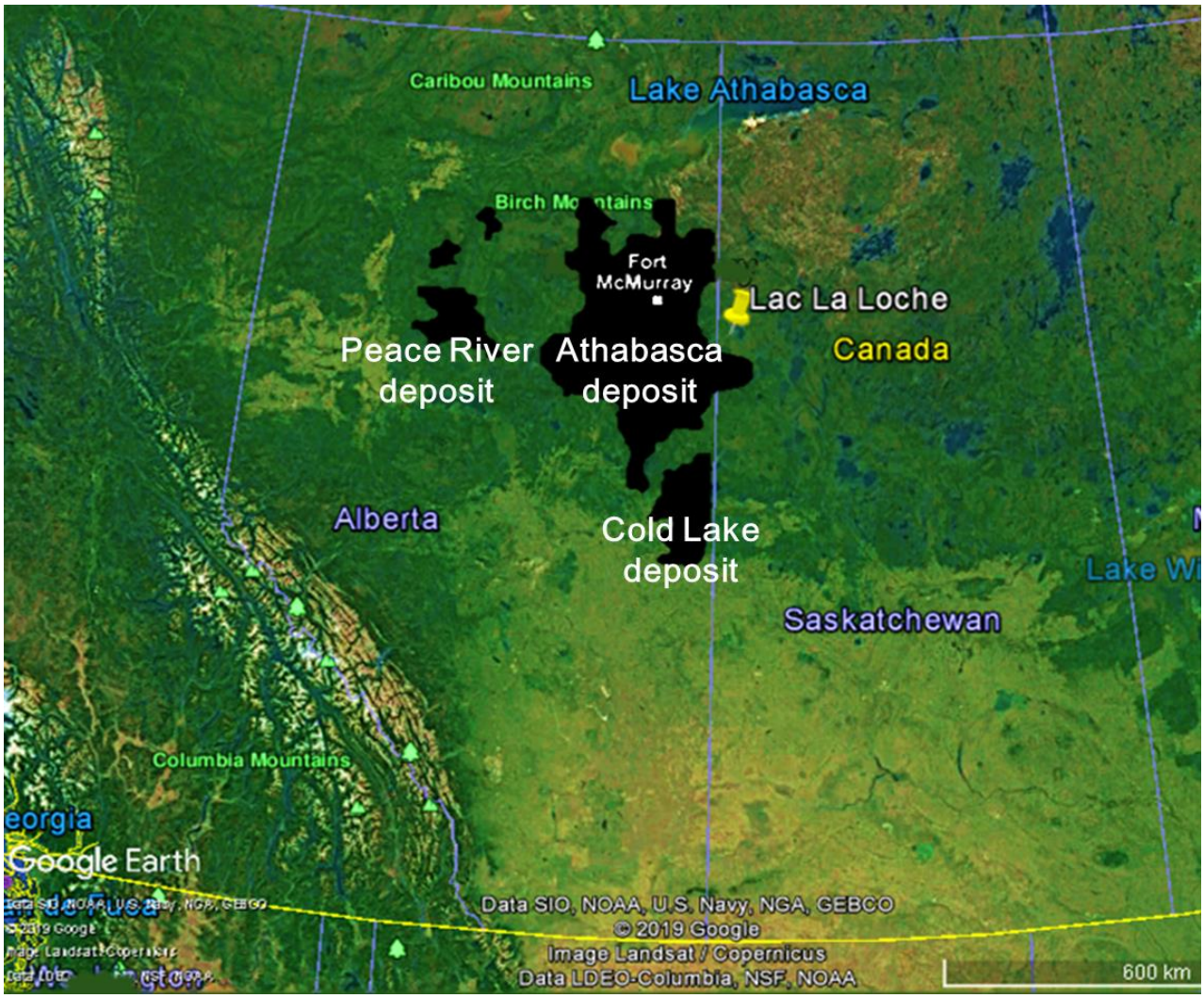

Figure 1.1: Oil sands exploitation and exploration sites in Alberta and Saskatchewan. Map obtained from Google Earth on January 24, 2020. Oil sands deposit (sizes are approximated) were obtained from CAPP (2013).

1.2. Oil sands mining process

Oil sands comprise a natural mixture of clay, sand, water, bitumen and mineral solids. In Canada, oil sands occur beneath boreal forest and muskeg (Allen, 2008a; Chalaturnyk et al., 2002; Deroo and Powell, 1978; Hein et al., 2001; Lim and Thomas, 1980; Rogner, 1997; Zubot et al., 2012). Annual production of crude oil from the Alberta oil sands in 2016 was 3.9 million barrels per day (mbpd) and is estimated to grow to 5.4 mbpd by 2030 (CAPP, 2017). Two primary processes are used to exploit the Alberta Oil Sands bitumen, open pit mining for 20% of the bitumen reserves and *in situ* extraction for 80% of bitumen reserves, Chen et al., 1999; Mahmoudkhani et al., 2012). Open pit mining is used to access deposits up to a depth of 75 m, whereas *in situ* extraction is used to mine deeper deposits (Lim and Thomas, 1980; Masliyah et al., 2004). Bitumen exploitation is economically feasible in Canada because naturally-occurring bitumen contained in sand grains are surrounded by a thin layer of water, leaving the bitumen in the centre of the pore and facilitating its extraction (Masliyah et al., 2004). Once extracted, the bitumen from the open-pit mining is broken into smaller pieces and mixed with hot water to form a slurry (Albion et al., 2009; Sanders et al., 2004). The slurry is then mixed with caustic soda (NaOH) to separate the bitumen from the associated water and sand. At this point, the slurry is also aerated to promote the attachment of bitumen to air bubbles (Romanova et al., 2006). The addition of NaOH also causes the liberation of natural surfactants, like carboxylates, sulphates, or sulphonates, from the bitumen, which reduces the interfacial tension between the bitumen and the air bubbles enhancing the bitumen attachment to the air (Babadagli et al., 2008; Schramm and Smith, 1985). This mix is then sent to a primary separation vessel where unwanted materials settle out of the slurry and are pumped to settling basins known as tailings ponds (Lim and Thomas, 1980).

The froth in the separation vessel contains bitumen, air, solids (clay, silt and sand) and remnant water. This mixture is sent to a defrother vessel where it is heated and deaerated for further separation (Humphreys R. D., 1999). Naphtha is then added to reduce the density of the bitumen, thereby separating the bitumen from the water via centrifugation (Schramm and Kwak, 1988). The liquid residues from the separation vessel and the centrifugation are sent to tailings ponds. The liquid tailings in the settling ponds are referred to as oil sands process or process-affected waters (OSPW).

The collected bitumen is refined through a primary and a secondary process to derive synthetic and enriched products such as gasoline, diesel and kerosene, and to remove undesirable compounds such as sulphur, nitrogen and trace metals (Gosselin et al., 2010). One of these steps, called coking, heats the bitumen to break the carbon (C)-C bonds of heavy C chains. Coking also concentrates the remaining carbon, trace metals and other impurities in a solid, carbonaceous by-product known as petroleum coke (PC; Bryers, 1995). A major portion of the C content in the PC originates from asphaltenes (Scott and Fedorak, 2004). The asphaltenes are the highest molecular weight fraction of the crude oil and possess a high content of ash (3.25%), which contains vanadyl porphyrins, nickel (Ni) and iron (Fe, Wightman et al., 1989).

1.2.1 Petroleum coke (PC)

The coking process generates two types of PC; fluid PC (produced by Syncrude Ltd.) and delayed PC (produced by Suncor Energy Inc. and the other Oil Sands companies) (Bryers, 1995; Furimsky, 1998). Fluid PC consists of mainly fine particles, whereas delayed PC is coarse-grained (Kessler and Hendry, 2006). Nevertheless, both types have concentrations of sulphur that range from 4 to 7% dry weight (d.w.) , Ni concentration of 1.5% d.w. and a concentration of V of about

5% d.w. (Bejarano et al., 2001; Di Panfilo and Egiebor, 1995) Furimsky, 1998; Gosselin et al., 2010; Scott and Fedorak, 2004). In particular, Syncrude's PC presents a concentration of V of 1,200 mg/kg, mainly as organo-metallic complexes at a concentration of 220 mg/kg of bitumen (Zubot et al., 2012).

1.3. Environmental concerns

In 2017, the annual production of PC averaged slightly more than 10 million tonnes (Alberta Energy Regulator (AER), 2017). Due to the high concentrations of sulphur in PC (6-9% d.w.), the Energy Resources Conservation Board of Alberta requires oil sands companies to store all PC on-site (ERCB, 2012; Puttaswamy and Liber, 2012, 2011). It is expected that 1 billion m^3 of PC will be generated over the lifetime of the Alberta Oil Sands (Fedorak and Coy, 2006). The public, regulators, and the industry have concerns about the potential environmental impact that could arise from the leaching of contaminants from these PC stockpiles as the result of rain or snow melts, and from the high concetration of organic toxicants and salts present in OSPW (Puttaswamy and Liber, 2011; Squires, 2005). To reduce the aforementioned environmental risks, it has been suggested that PC be used in the treatment of OSPW to reduce the concentration of organic toxicants, which are known to be the primary source of toxicity of the liquid by-product (Baker et al., 2012; Zubot et al., 2012). However, several studies have shown that the use of PC for these purposes could pose environmental risks. For example, under aerobic conditions PC stimulated heterotrophic bacteria to convert sulphur from coke into sulphate, which was then released to the aqueous phase (Fedorak and Coy, 2006). Sulphate concentrations (477 and 597 mg SO_4^{-2}/L) within the range found in OSPW caused chronic lethality to 20% of exposed *Pimephales*

promelas (fathead minnow) and *Oncorhynchus mykiss* (rainbow trout; Government of British Columbia, 2013; Wang et al., 2016).

In addition, the use of PC as a surface layer in constructed wetlands promoted the liberation of Ni and other trace metals into sediment pore water (Baker et al., 2012). In the same study, V and Ni concentrations in the green algae *Chara* sp. were three- and one- fold higher, respectively, in specimens collected from constructed wetlands, compared to *Chara* sp. collected from reference wetlands. Also, adult dragonflies from wetlands containing PC had elevated whole-body V; and dragonfly larvae in PC-treated wetlands accumulated more lanthanum (La) and yttrium (Y) than in the reference wetlands (Baker et al., 2012). In a comprehensive study, Puttaswamy and Liber (2012, 2011) and Puttaswamy et al. (2010) found that undiluted PC leachates were highly toxic to the freshwater crustacean *Ceriodaphnia dubia*. The authors found that V and Ni concentrations (the lowest concentration of V found in a shallow lysimeter's leachate was 1.7 mg/L and the lowest concentration of Ni in the leachate of a deeper lysimeter was 14 µg/L) were at least three and four times higher, respectively, than their associated median lethal concentration (LC_{50}) for *C. dubia* (the LC_{50} for V was 0.55 mg/L and for Ni 3.8 µg/L). These low LC_{50}s presented by V and Ni were higher than the other metals measured in the leachates (aluminum (Al), boron (B), barium (Ba), Fe, manganese (Mn), molybdenum (Mo), selenium (Se), strontium (Sr), and zinc (Zn)), and thus V and Ni were deemed responsible for the toxicity.

From these studies, it becomes clear that coke is enriched with contaminants that, once in contact with aqueous media, can leach and become bioavailable and potentially toxic to aquatic organisms. Contaminants like V seem to have a high chance of getting released from PC into the aqueous media, increasing its chances to reach freshwater.

1.4. Oil sands process-affected water (OSPW)

Bitumen extraction and upgrading processes generate OSPW that is delivered into tailings ponds and retained on site. Additionally, site precipitation, runoff and aquifer-depressurization water are kept and mixed with the OSPW (Allen, 2008a, 2008b; Bailey et al., 1995). The only site water returned to the Athabasca River is treated sanitary wastewater effluent (Zubot et al., 2012).

1.4.1 Physical and chemical characteristics of OSPW

OSPW in tailings ponds consists of a mixture of residual water, clay, silt, sand, unrecoverable bitumen, and water-soluble organic and inorganic compounds (He et al., 2010). Based on settling times and physical characteristics, OSPW in tailings ponds can be divided into three fractions: rapid-settling sand particles, an aqueous suspension of fine particles, and a clarified water superficial layer. The superficial layer contains residual bitumen and is recovered for reuse in the oil sands process (Allen, 2008a, 2008b). The fine particles that do not settle quickly form a stable slurry-structure known as mature fine tailings (MFT), which can remain stable for several decades (Chalaturnyk et al., 2002).

It is a common practice within the oil sands companies to add gypsum ($CaSO_4 \cdot 2H_2O$) as a coagulant to the tailings to enhance the settling of fine tailings, forming composite tailings (CT), thus maximizing the solid tailings waste (Renault et al., 2001). The CT and the coarse sediments are deposited into mine pits for later use in land reclamation (Banijamali and Meadows, 2011). Unfortunately, the consolidation process does not target the ions present in the OSPW. Therefore, there is marked retention of Na, SO_4, Cl, trace elements, and organic compounds in the aqueous phase (Renault et al., 2001). Allen (2008a) reviewed and summarized the water chemistry of OSPW (Table 1.1). The OSPW's high salinity (e.g. Na = 820 mg/L, SO_4 = 416 mg/L, Cl = 540

mg/L and conductivity as high as 2400 µS/cm) and the high content of organic compounds commonly known as NAs, which concentrations can reach as high as 130 mg/L) present an environmental concern among the industry and regulators.

Table 1.1: Chemical profile of oil sands process water from recent and long-term monitoring (Zubot *et al*., 2012; Allen, 2008a; MacKinnon *et al*., 2005). N/A means data were not available. Pooled data from different tailings ponds.

Water chemistry	Data from 1986-2004 range/mean	Data from 2012-2013 range/mean
pH	7.7 - 8.8	8.0
Alkalinity[a]	635 - 779	679
Water hardness[a]	91 - 112	75 - 90
Conductivity[b]	1,506 – 2,400	3,514
TDS[c]	1,900 – 2,221	1,900 – 2,221
DO[d]	N/A[g]	7.6 - 7.4
Organics (mg/L)		
PAHs[e]	0.01	0.01
NA[f]	40 - 130	68 - 82
Major ions (mg/L)		
Sodium	250 - 820	840
Magnesium	10	10.6
Calcium	14	15.8
Chloride	80 - 540	527
Bicarbonate	650 - 950	821
Sulphate	218 - 416	384
Trace metals (mg/L)		
Aluminium	0.1 - 0.5	0.6
Arsenic	0.01 - 0.02	N/A[g]
Chromium	0.01 - 2.00	N/A[g]
Copper	0.01 - 0.90	N/A[g]
Iron	0.8 – 3.0	0.1
Lead	0.04 - 0.19	N/A[g]
Nickel	0.01 - 2.80	N/A[g]
Molybdenum	N/A[g]	0.1
Vanadium	N/A[g]	1.0E-3
Zinc	0.01 - 3.20	N/A[g]

[a] Units are in mg/L as $CaCO_3$
[b] Units are in µS/cm
[c]TDS = Total dissolved solids; units are in mg/L
[d]DO = Dissolved oxygen; units are in mg/L
[e]PAHs = Polycyclic aromatic hydrocarbons
[f]NA = Naphthenic acids
[g]N/A = Not available

1.4.1.1. Naphthenic acids

During the bitumen extraction process, alkyl-substituted cyclic and aliphatic carboxylic acids known as NAs are released into the OSPW (Allen, 2008a; Clemente and Fedorak, 2005). Naphthenic acids can form bonds with clays in OSPW through hydrogen bonds between the carboxylic acid groups and water molecules on the clay surface, van der Waal's interactions and electrostatic-dipole (Zou et al., 1997). NAs are soluble in neutral or slightly alkaline conditions, such as in oil sands tailings ponds (Table 1.1), where they exist in the ionized forms of carboxylate sodium salts (Clemente and Fedorak, 2005; Zubot et al., 2012). Additionally, some NAs are resistant to photodegradation and biodegradation due to their high molecular mass and complex structure (McMartin, 2003; Scott et al., 2005). Bitumen from the Alberta Oil Sands region contains between 2-4% NAs d.w., depending on the reservoir (Clemente et al., 2003; Headley et al., 2007). Naphthenic acids are undesirable in the refining process due to their corrosive capacity (Fan, 1991; Slavcheva et al., 1999) and because they increase the acidity of the petroleum products, thereby lowering their commercial value (Quagraine et al., 2005).

1.4.2 Oil sands process-affected water toxicity

Various studies have demonstrated that NAs are toxic to aquatic organisms. For example, when the midge *C. dilutus* was chronically exposed for 10 d to 70 mg of NA/L, the wet body mass was reduced and growth, pupation, and larval emergence were delayed (Anderson et al., 2012, 2011). Melvin et al. (2013) showed that when northern leopard frog tadpoles, *Lithobates pipiens*, were exposed to 1 and 2 mg/L of NAs for 75 d, growth and development decreased, and there was a significant reduction in stored glycogen and an increase in triglycerides levels. More recently,

through fractionation Morandi et al. (2015) found that NAs with 15 to 20 C chain presented the highest toxicity to fathead minnow embryos.

Although NA toxicity has received considerable attention in recent years, high salinity typically co-occurs with NAs in OSPW. To date, bioassays have been conducted to assess the toxicity of NAs in combination with elevated salt content. Still, little information exists regarding the influence of salt (composition or content) on NA toxicity. For example, fathead minnows exposed to 25 mg/L of NAs and elevated conductivity levels showed significant inhibition of spawning and a reduction of male secondary characteristics, and testosterone and 11-ketotestosterone levels. Additionally, females showed a significant decrease in plasma 17β-estradiol (Kavanagh et al., 2011). However, when fathead minnows were first pre-acclimated to salinity levels similar to that of OSPW and later exposed to 40 mg/L of NAs, then fish showed a significant reduction in spawning. These results suggested that reproduction impairment was caused by NAs and not salinity (Kavanagh et al., 2011).

Salinity can become toxic to many freshwater aquatic organisms. Recently, White (2017) suggested that salinity, especially Na, Cl, and HCO_3, are the main factors impeding the potential of aquatic organisms, especially *C. dubia*, to establish in Base Mine Lake. Whereas, metals in OSPW were below Canadian water quality guidelines except for boron (B) and Ni (White, 2017). Similarly, freshwater and salt-water acclimated *Daphnia magna*, *Hyalella azteca*, freshwater cultured fathead minnows, and saltwater-held *Morone saxatilis* (striped bass) were exposed to effluent collected from irrigation drain waters. The effluent contained high chloride and sulphate concentrations with a salinity of 22 g/L. An effluent dilution to 26% killed half of the freshwater adapted fathead minnows, whereas the LC_{50} for *Daphnia pulex* and the freshwater- and saltwater-cultured *H. azteca* was between the 25% and 50% of whole effluent. Besides, the range of salinity

tested failed to stress *H. azteca*, but 8 to 10 g/L of Instant Ocean salt were acutely toxic to *D. magna* and fathead minnow (Ingersoll *et al*., 1992). *Perca flavescens* (yellow perch) were exposed to OSPW amended with sodium sulphate ($NaSO_4$) to evaluate the effects of NAs on gill morphology as a function of salinity. Even though high salinity alone can cause mortality to freshwater organisms, when it was combined with increasing NA concentrations from 0.9 to 6.8 mg/L there was a significantly greater structural and inflammatory alteration in the gill compared to NA-only exposed fish (Nero et al., 2006).

In summary, previous studies have shown that salinity alone, or in combination with contaminants such as NAs, can cause significant toxicity to many freshwater organisms. Salinity causes significant physiological stress to unacclimated aquatic organisms that could potentiate toxicant actions. Alternatively, it is possible that the ions that comprise salinity can compete with a given toxicant for the site of action, thereby protecting aquatic organisms against uptake and toxicity.

1.4.3 The use of PC to reduce OSPW toxicity

It is the responsibility of the companies exploiting the oil sands that they ensure that at the end of the life of a project, the area operated is restored to an ecological status close to its former conditions. This phase is known as the reclamation phase of the project, and it is a mandatory requirement imposed by the Canadian federal and provincial governments (Gosselin et al., 2010). The proposed plan for the AOSR area is to incorporate the reclaimed end pit lakes to the closure landscape to restore it to the best attainable environmental conditions (CEMA 2014; Government of Alberta, 2015). There are presently two main reclamation techniques applied to tailings ponds: dry and wet landscapes. Dry landscape is constructed with dewatered fine tailings, sand and a

cover layer of soil, whereas wet landscape features end-pit lakes that contain fluid fine tails capped with process water and river water (Allen, 2008a). These reclamation techniques are described by others (Harris, 2007; Mikula et al., 1996; van den Heuvel et al., 1999; World Wide Fund (WWF), 2010) and will not be reviewed in detail in this work.

In looking to improve reclamation techniques, oil and gas companies, such as Syncrude, promote research to determine the environmental risks related to bitumen exploitation. Due to the increase in production and stockpile of PC (116,000,000 tonnes of stored PC as of 2017; AER, 2017), Syncrude implemented a multi-year pilot project in which PC is being used to treat OSPW. This project is based on the idea of using PC as charcoal to reduce the concentration of NAs (Small et al., 2012), and consequently, reduce the toxicity of OSPW. The reclamation technique using PC starts when the excess PC generated in the upgrading process is mixed with the process water and hydro-transported to the PC storage areas (Allen, 2008a; Puttaswamy and Liber, 2011; Squires, 2005; Syncrude Canada Ltd., 2012). Syncrude's proposed reclaimed landscape will have three PC storage areas designated Coke Cell 5, Mildred Lake Settling Basin (MLSB), and North Mine Centre Pond (NMCP). The MLSB is currently receiving PC and the NMCP will continue to receive PC after the Mildred Lake facility is closed (Puttaswamy and Liber, 2011). Although PC can absorb NAs from OSPW, it has been demonstrated that once PC is in contact with OPSW, it releases the trace metals stored within its structure (Puttaswamy et al., 2010).

Zubot et al. (2012) investigated the use of PC to reduce NAs from OSPW. They found that using PC significantly decreased the concentration of NAs in treated OSPW, thereby reducing its acute toxicity to *Vibrio fischeri* (Microtox®), *C. dubia* and rainbow trout. Nevertheless, although it is feasible to use PC to reduce the concentration of NAs in OSPW, Zubot et al. (2012) also showed that Mo and V are released from PC to the aqueous phase. Mo concentration in OSPW

increased from <0.1 to 0.8 mg/L and V increased from <0.05 to >7 mg/L within the first 100 d. These results are consistent with Puttaswamy et al. (2010), where they ascertained that weathering favoured the release of V from PC stock-piled in lysimeters located onsite. Therefore, if Syncrude intends to implement the treatment of OSPW with PC as a reclamation tool to reduce the organic toxicants present in OSPW before releasing it to the Athabasca River, the risks posed by the unexpected release of trace metals from PC into OSPW needs to be assessed. This scenario is particularly true for V since little is known about its effect on model and regional aquatic species, or how environmental variables modify its bioavailability and toxicity. Therefore, it is important to focus future research on how OSPW and Athabasca River water quality characteristics could influence V toxicity, and which V species are relevant in aquatic ecosystems that presents these ranges of water quality characteristics (Table 1.1 and 1.2).

1.5. Athabasca River watershed

Starting at the Columbia Ice Fields (Jasper National Park) and ending to the northeast in Athabasca Lake, the Athabasca River (AR) is a 6^{th} order stream that runs through almost the entire Province of Alberta (Culp et al., 2005; Squires et al., 2009). Its basin covers a surface of 157,000 km^2 flowing through different physiographic ecoprovinces such as the Rocky Mountains, Great Plains, Athabasca plains and Bear-Slave-Churchill Upland (Culp et al., 2005) and several towns including Hinton, Whitecourt, Athabasca and Fort McMurray (Mackenzie River Basin Board, 2003a). The AR, together with the Peace and the Slave Rivers form the Peace-Athabasca-Slave River system, which is part of the Mackenzie River Basin, covering approximately 20 % of Canada's total land mass. The Mackenzie River Basin is of worldwide importance since it accounts for 60 % of the freshwater that flows into the Arctic Ocean, significantly regulating the circulation

of the world's oceans currents and climate systems (Dubé et al., 2013; Mackenzie River Basin Board, 2003a, 2003b). The AR and its tributaries mainly discharge their waters to the Athabasca Delta, then Athabasca Lake and the Peace Athabasca Delta. Through this delta, water from the Athabasca River reaches the Slave River indirectly. Therefore, any adverse effects on the AR have the potential to reach the Slave River and downstream areas. Between 1976 and 2006, there has been marked industrialization of the areas drained by the AR. During this period, agriculture, forestry, pulp and paper industries and the Alberta Oil sands were conceived and/or expanded (Squires et al., 2009). In particular, in the lower Athabasca basin, there has been a significant increase in forest harvesting, agricultural intensity and oil sands development. The oil sands operations located north of Fort McMurray is the primary industry of the area, with Suncor and Syncrude representing the two largest surface mining developers (Dubé and Wilson, 2013; Squires et al., 2009).

1.5.1 Water chemistry

Alberta Environment (2009) has comprehensively summarized the water chemistry of the AR from 1960 to 2007 at the town of Athabasca and for six years (2002-2008) at the city of Fort McMurray (Table 1.2). In addition, (Regional Aquatic Monitoring Program (RAMP), 2017, 2013) has summarized the surface water chemistry of the oil sands area from 2012 (Table 1.2).

Table 1.2: Water chemistry profile of the Athabasca River at Athabasca (1960 to 2007, 2012) and Fort McMurray (2002 to 2008), Alberta, Canada.

Water chemistry	ABE[a]. 1960-2007 Athabasca Site range/mean	ABE. 2002-2008 Fort McMurray range/mean	RAMP[b] 2012 Athabasca range/mean
pH	7.9 - 7.9	8.1	8.0 - 8.5
Alkalinity[c]	123 - 127	123.5	85 - 110
Hardness[c]	139 - 140	130	90 - 120
Conductivity[d]	290 - 294	291	218 - 291
TDS[e]	148 - 171	170	156 - 187
DO[f]	9 - 10	11.6	8.1 - 8.7
Organics (mg/L)			
PAHs[g]	N/A[i]	N/A[g]	3.0E-4 - 3.0E-3
NA[h]	N/A[i]	N/A[g]	0.02 - 0.1
Major ions (mg/L)			
Sodium	7 - 10	14.3	8 - 14
Magnesium	10	36.1	6 - 9
Calcium	38 - 39	36.1	18 - 33
Chloride	2.4 - 2.5	3.6	2.3 - 15.4
Bicarbonate	141 - 153	151	N/A[i]
Sulphate	26 - 29	33	6 - 27
Trace metals (mg/L)			
Aluminium	7.4E-3	9.8E-3	1 - 4.8
Arsenic	4.0E-4	4.8E-4	9.0E-4 - 1.6E-3
Chromium	2.4E-4	3.2E-4	1.4E-3 - 5.3E-3
Copper	9.0E-4	9.1E-4	3.0E-3 - 4.0E-3
Iron	0.1	0.1	1.2 - 4.3
Lead	5.4E-5	9.0E-05	<4.0E-3
Nickel	1.0E-3	1.1E-3	N/A[i]
Vanadium	2.2E-4	2.9E-4	N/A[i]
Zinc	3.3E-3	2.6E-3	N/A[i]

[a] Alberta Environment
[b] Regional Aquatics Monitoring Program
[c] Units are in mg/L as $CaCO_3$
[d] Units are in µS/cm
[e] TDS = Total dissolved solids; units are in mg/L
[f] DO = Dissolved oxygen; units are in mg/L
[g] PAHs = Polycyclic aromatic hydrocarbons
[h] NA = Naphthenic acids
[i] N/A = Not available

1.5.2 How toxicants in OSPW could behave in the Athabasca River

Oil sands companies ultimately need to release the treated OSPW back to the AR or incorporate the end-pit lakes to the reclaimed landscape. However, there are marked differences between the water chemistry of OSPW and the AR (Tables 1.1 and 1.2) and these differences need to be considered regarding how they could alter the bioavailability and toxicity of contaminants in treated OSPW. For instance, in a set of standardized toxicity tests with *D. pulex*, Ni toxicity decreased when calcium (Ca) concentrations increased due to cation competition at the biotic ligand (Kozlova et al., 2009). The AR has 3-fold more Ca than OSPW (Table 1.1 and 1.2), and this could serve to protect aquatic organisms from Ni potentially released from coke-amended OSPW. An increase of chloride (Cl) levels in AR water due to OSPW release could increase metals toxicity, as was shown by Erickson et al. (1998) where the 96-h LC_{50} for fathead minnows exposed to Ag decreased from 7.8 to 5.0 when NaCl was increased to 11.7 mg/L. OSPW has higher concentrations of HCO_3 than AR water. Higher HCO_3 levels favour the formation of nickel hydroxide (NiOH) over Ni, increasing its bioavailability and toxicity to aquatic invertebrates (Puttaswamy and Liber, 2012). Thus, these examples show how a release of OSPW could potentially cause a modification of water chemistry and major ions in the AR, which could eventually modify metal toxicity in the aquatic environment.

1.6. Vanadium

Vanadium is a transition metal and the 22nd most abundant element in the Earth's crust (Etcheverry et al., 2009; García, 2006), and it is a trace element in the petroleum and mining industries. In bitumen, V concentrations can range from 19 to 240 mg/L (Ernst and Garside, 1987; Gosselin et al., 2010; International Atomic Energy Agency, 1993). Vanadium is present as a trace

element in minerals such as vanadinite, chileite, patronite and carnotite, and in titaniferous magnetites (WHO, 2001). This trace element is commercially exploited in Chile, Finland, Namibia, Norway, South Africa, the United States, Russia, and China; and it is in this last country where the biggest reserves are present. It is mainly used as an additive in the fabrication of steel, plastics, ceramics, rubber, as a catalyst in the sulphuric acid fabrication, in pigments and inks, as a colouring agent, to harden steel tools, and even as ultraviolet filtering protection in glasses (García, 2006; WHO, 2001). In 2018, Shell Co. launched a pilot project where they intend to use V in batteries to power green energy resources (CBC News, 2018). Approximately 65,000 tonnes of V annually enters the environment from natural sources (volcanic eruptions, wild forest fires, or wind-blow continental dust) and 200,000 tonnes from anthropogenic sources, with the metallurgical and oil industries being the major emission sources (ECCC & HC, 2010; WHO, 2001).

The role of V in animal physiology is still under investigation because V may be an essential element for some bacteria, algae, lichens, fungi, invertebrates and vertebrate species (Etcheverry and Cortizo, 1998; Rehder, 2015). It binds to the active sites of nitrogenases in the nitrogen-fixing bacterium *Azotobacter* to reduce N_2 to NH_4. Also, V is present in the active sites of enzymes in sea algae and lichens. Ascidians accumulate V in specialized cells called vanadocytes, located in the exoplasm, and V(IV) was found in *Amanita muscaria* (Rehder, 2003, 1991). Additionally, V plays an essential role in the physiology of some vertebrates. For instance, a deficiency of V in rats and chickens can retard growth, impair reproduction, alter lipid metabolism, and inhibit Na/K ATPase (NKA) activity in the kidneys, brain and heart (Etcheverry and Cortizo, 1998). Vanadium also plays a key role in thyroid function. Thyroid hormones are responsible for regulating bone and cartilage metabolism. In organisms with hypothyroidism, bone

formation and resorption can be reduced. Rats fed with 2 ng of V per gram of diet showed an increase in thyroid weight and thyroid to body weight, which affected their body growth compared with the control group (Badmaev et al., 1999; French and Jones, 1992).

1.6.1. Vanadium chemistry in aquatic environments

Vanadium has several oxidation states, and in the aquatic environment, it commonly occurs as V(III), V(IV) and V(V), with V(V) typically being the most abundant. The oxidative state of V is strongly related to the pH of the surrounding media (García, 2006; Rehder, 1991). The oxidation state and bioavailability of V are influenced by pH, dissolved oxygen (DO), organic matter and the concentrations of other trace elements, but pH significantly determines which V oxidation state will be the most abundant (Figure 1.2). Under strong acidic conditions (pH< 3), the free cation V(III) predominates and forms a soluble precipitate of vanadium oxide. At higher pHs (5-10), vanadate (V(V)) and vanadyl (V(IV)) are the dominant oxidation states (Rehder, 1991). The concentration of dissolved oxygen also contributes to the speciation of V. Under slightly reducing conditions V(IV) is prevalent as VO^{2+}, and if reducing conditions increase with increasing acidity, V(IV) is reduced to V(III) (Szalay and Szilágyi, 1967). Under aerobic conditions, V(V) is the most abundant species occurring primarily as VO_4^-, since V(IV) is rapidly oxidized to V(V) (Rehder, 1991; Zhao et al., 2006).

Similar to arsenic (As), Se, chromium (Cr), Mo, and antimony (Sb), oxygen bonds to vanadate through covalent bonds, forming the $H_2VO_4^-$ and HVO_4^{2-} oxyanions; giving high stability and solubility to the molecule (Crans et al., 1998; Fairbrother et al., 2007; Wehrli and Stumm, 1989). Oxyanions are dominant in oxygenated waters with a pH that varies between 6 and 9 (Figure

1.2, Rehder, 1991; Wehrli and Stumm, 1989). Therefore, in OSPW or AR, where the pH is around 7.4-8.4 and the DO is high, the vanadate oxyanions will be the dominant and most stable V species.

Vanadyl ions (VO^{2+} and $VO(OH)^{+}$) are more thermodynamically stable at pH conditions lower than 6. In this conditions, the presence of oxalates forms complexes with vanadyl that significantly increase its solubility (Brumsack and Gieskes, 1983; Szalay and Szilágyi, 1967; Wanty and Goldhaber, 1992). However, in situations where DO concentrations are low and there is an abundance of organic matter or inorganic compounds, vanadyl will absorb to particulates phase and sediments (Wehrli and Stumm, 1989). Therefore, understanding the fate of V in water chemistry representative of OSPW and the AR will help to inform decisions related to the environmental management and site reclamation of oil sands and discharge of PC-treated OSPW.

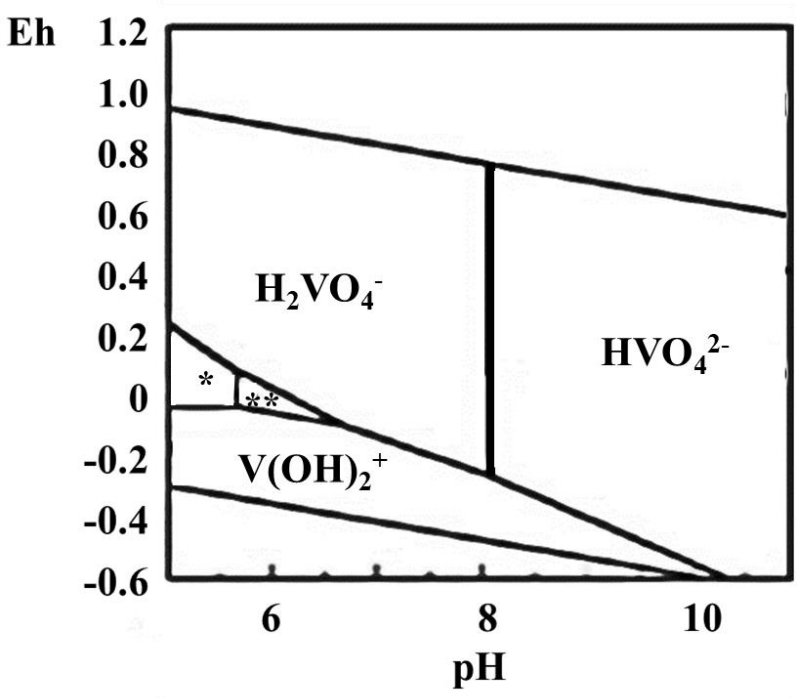

Figure 1.2: Speciation scheme of vanadium with variation of pH and reduction potential (Eh). Modified from Wehrli and Stumm, 1989. * represents VO^{2+} and ** represents HVO_2^+.

1.6.2. Vanadium toxicity

As a result of the substantial amount of PC stockpiled in the Alberta oil sands area and the intent to use this by-product in reclamation, increasing loads of anthropogenic V are likely to be introduced into regional aquatic systems. The release of V could cause direct or indirect effects on aquatic organisms (lethality and impact at a population level, respectively). Therefore, the following sections focus on understanding the direct and indirect toxic effects of V to aquatic organisms.

1.6.2.1. Direct toxic effects to aquatic organisms

Vanadium has been shown to have direct effects on different aquatic species, and some examples are presented here. When V concentrations ranged from 0.04 to 3 mg/L in a whole-lake system, there was a significant reduction of the overall photosynthesis in those lakes where cyanobacteria were the most abundant species and Bacillariophyta and Chrysophyta were the least abundant (Nalewajko et al., 1995). *Scenedesmus quadricauda*, a species of green algae, showed significant growth inhibition, and reductions in total chlorophyll, chlorophyll a and chlorophyll b content when exposed to 2.24 mg V/L. Lee et al. (1979) showed that when *Anabaena flos-aquae*, a filamentous cyanobacteria, was exposed to 0.1 mg/L of V, its growth was completely suppressed. However, 1 mg V/L only slightly retarded the growth of the green algae *Chlorella pyrenoidosa* and *Scenedesmus obliquus*.

Vanadium has been demonstrated to be toxic to various invertebrate species. Vanadium was acutely toxic to *D. magna* exposed to 3.4-4.8 mg V/L (LC_{50}); however V did not impair reproduction but instead caused mortality (Beusen and Neven, 1987). In another study, V concentrations close to 3.1 mg/L (LC_{50}) caused a marked depression in the feeding rate of *D.*

magna . Puttaswamy and Liber (2011) found significant toxicity of V when *C. dubia* was exposed for 7 d to 0.55 mg/L of V (LC_{50}). It is evident that V is toxic to both freshwater and saltwater fish species. For instance, V concentrations ranging between 2.9-5.3 and 6.1-10.2 mg/L were acutely toxic to *Danio rerio* (zebrafish) and *Poecilia reticulata* (guppy), respectively, over a 96-h period. Also, zebrafish were more susceptible than guppies to V within the above-tested concentrations, showing a higher mortality (Beusen and Neven, 1987). In another study, adult *Nuria danricus* (flying barb) exposed to V concentrations above 0.5 mg/L downgraded the swimming ability and balancing patterns compared to the control group, showing a higher rate of erratic movements, increased frequency of surface breathing and vertical migration rates. When concentrations of V were above 1 mg/L, feeding impairment was observed, and this V concentration led to lethargy and eventually death (Abbasi, 1988). Ernst and Garside (1987) described the effect of V on alevins (newly hatched) and yearling *Salvelinus fontinalis* (brook trout). They found that after 96 h of exposure, alevins were 3 times less sensitive than yearlings to V toxicity. Alevins were acutely affected when exposed to 24 mg of V/ L and the yearlings to 7 mg of V/ L. A potential explanation of the difference in sensitivity between alevins and yearlings could be that V like many other metals produce toxicity at the gill level; and at the time of exposure alevins did not have fully developed gills (Evans, 1987; González et al., 1996). Also, when *Clarias batrachus* (catfish) were exposed to 5, 10 and 15 mg/L of V for 4 and 30 days, significant accumulation of V in kidney (the principal target), liver, gill, and intestine occurred, showing an increasing trend with increased V concentration (Ray et al., 1990). *Jordanella floridae* (American flagfish) larvae exposed for 96 h to 0.17 mg/L of V showed a significant reduction in growth and survival rate (Holdway and Sprague, 1979). Additionally, Taylor et al. (1985) found that the seawater species *Limanda limanda* (dab) exposed to 27.8 mg/L ammonium metavanadate for 96 h, experienced acute

toxicity. Recently, Schiffer and Liber (2017a, 2017b) found that V is acutely and chronically toxic to several aquatic organisms that are key elements in a healthy trophic network. These authors suggested that concentrations of V between 1 and 7 mg/L were acutely toxic to aquatic organisms, especially those that belong to the lower trophic levels, such as daphnids. For instance, the 48-h median lethal concentrations (LC_{50}s) for different daphnids ranged from 0.60 to 2.17 mg/L (Schiffer and Liber, 2017a, 2017b). For rainbow trout and fathead minnow, the 48-h LC_{50}s were 14.8 and 4 mg V/ L, respectively. The chronic V LC_{50}s ranged between 0.08 and 0.44 mg V / L for daphnids (21-d), and for rainbow trout and fathead minnow were 4.3 and 0.5 mg V/ L, respectively (28-d).

1.6.2.2. Indirect toxic effects to aquatic organisms

Vanadium has been shown to exhibit indirect but large-scale effects to aquatic organisms, and some examples are presented here. An in-situ study in Darby Creek, Pennsylvania, USA, showed that a concentration of 4 mg/L of V caused a significant shift of the algal community, reducing diatoms and increasing the blue-green algae *Schizothrix calcicola* and *Microcoleus vaginatus* (Patrick, 1978). An increase in abundance of some cyanobacteria can cause the release of phycotoxins that could cause severe toxicity to invertebrates, fish, and livestock, and potentially severe health consequences to human populations if contaminated food or affected water supplies are ingested (Gorham and Carmichael, 1979; Pérez-Linares et al., 2003). Thus, an increase in V concentration in aquatic environments could promote undesirable algal species and have severe indirect effects on the ecosystem and human health.

In addition, concentrations of V ranging from 21 to 70 mg/L severely affected populations of the oligochaete *Pristina leidyi* (Smith et al., 1991). *Pristina leidyi* feed principally on green

algae, diatoms, bacteria and detritus; *P. leidyi* is, in turn, an important source of food for caddisflies, dragonflies, beetle larvae and fish (Streit, 1978). Therefore, once V is released to the receiving environment, it has the potential to impact different aquatic species from the bottom and/ or the top of the food web if elevated environmental concentrations are reached. However, the aquatic toxicity of V remains poorly understood because relatively few aquatic species have been tested to date. Furthermore, there has been limited investigation on the effects of toxicity modifying factors on V toxicity to aquatic organisms, and how V elicits that toxicity.

1.6.3. Vanadium and exposure and toxicity modifying factors (ETMFs)

The ionic contents of OSPW and AR water are very different from each other (Table 1.1 and 1.2). Little is known about the effect of the variation in ETMFs on V availability in aquatic systems. For example, Puttaswamy and Liber (2011) showed that at pH 9.5 there was an abundance of V oxyanions, which appeared to be the cause of the toxic effects. Finally, Puttaswamy and Liber (2012) demonstrated that inorganic anions had a significant influence on the amount of V released from coke, but differed on how they influenced V toxicity to *C. dubia*. For instance, when the concentration of HCO_3 increased from 50 to 250 mg/L the release of V increased, but when the concentration of SO_4 increased from 96 to 300 mg/L, the release of V did not change. However, only an increase in sulphate from 96 to 300 mg/L caused a non significant but marked amelioration of V toxicity (LC_{50}s increased from 879 to 1155 µg V/L), potentially indicating that V and SO_4 could be competing for the same uptake route in *C. dubia*. In another study, Ernst and Garside (1987) found that when diluted sulphuric acid was added to the stock solution V toxicity to *S. fontinalis* was decreased, and when instead of sulphuric acid sodium hydroxide was added, V toxicity to *S. fontinalis* increased. These results suggests that pH could be one of the main drivers

of V toxicity to aquatic organisms. Therefore, the effects of trace metals like V on aquatic organisms should be studied.

Similarly, it is not known how differences in salinity between OSPW and AR water may affect V bioavailability. For instance, a comparative study showed that there is an increase in dissolved V (as vanadate) with an increase in salinity under oxic conditions and under neutral to slightly basic conditions (pH 7-9). These mixing conditions are commonly found in estuaries but can occur when salty OSPW is diluted by the AR (Shiller and Boyle, 1987). It is interesting and important to understand how a variation in salinity can affect V availability. For example, when the effect of V and salinity to the brackish water hydroid *Cordylophora caspia* was studied, there was an increase in V toxicity with decreasing salinity, affecting reproduction and consequently causing an impact on the population (Ringelband, 2001). At low salinities, V ions exist mainly as mono or divanadates (Pettersson, 1994), which are known to be potent phosphatase inhibitors. As salinity increases, V forms tetra- or pentavanadates. These species are less effective inhibitors of phosphatases because their structure does not mimic the phosphate ion (Ringelband, 2001). Therefore, if a scenario is proposed where OSPW with high salinity is introduced into a water body, such as the AR, with low salinity, through runoff, seepage or an accidental spill, the decrease in salinity of the OSPW that will happen when OPSW is diluted in the ARW should cause a change in V speciation, potentially increasing V toxicity.

There is little data on V speciation with varying water chemistry as described above. The presence of HCO_3 in solution favoured V solubility and when the concentration of HCO_3 was increased, HVO_4^{2-} species were predominant over $H_2VO_4^-$. Also, when chloride or sulphate was present in solution, $H_2VO_4^-$ dominated V speciation (Puttaswamy and Liber, 2012).

It has been demonstrated that metals with similar electronic configurations in the outer orbital, and similar crystalline structure, will behave similarly among these varying conditions (Kaiser, 1979). Vanadium shares a similar crystalline structure with tantalum (Ta), tungsten (W), niobium (Nb), Mo and Cr. All of these elements are members of groups 5 and 6 from the periodic table, and they can cause deleterious effects on gill, kidney and intestine by inhibiting ionic balance and causing a consequential alteration of osmoregulation, and oxidative stress (Atli and Canli, 2011; Valko et al., 2005). Therefore, we can infer the behaviour of V in varying aquatic environments by observing the behaviour of structurally similar chemicals. For example, the acute toxicity of Cr (VI) to rainbow trout was significantly increased by a 10-fold change when pH was reduced from 7.8 to 6.5 (van der Putte et al., 1981). This increase in toxicity was strictly related to Cr speciation. It was demonstrated that at pH 6.5 $HCrO_4$ predominates over CrO_4, whereas at pH 7-7.8 the reverse occurs. Therefore, it seems that $HCrO_4$ is mainly responsible for Cr toxicity in aquatic environments. Variations in salinity also influence Cr toxicity due to a reduction in metal bioavailability. For instance, when *Oreochromis niloticus* (Nile tilapia) were exposed to 2 and 8 g/L salinity alone, the activity of the NKA in their gills increased. However, when the exposure was combined with 1 mg Cr/L, the increase in NKA activity was significantly counteracted at 2 g/L, but not at 8 g/L suggesting that the increase in salinity somehow compensated Cr toxicity to Nile tilapia (Baysoy et al., 2013). It is also interesting that a combination of salinity and temperature can alter oxyanion toxicities. For example, when *Praunus flexuosus* were exposed to Cr in combination with increasing salinity ranging from 4 to 27 ppt, the LT_{50} increased with increasing salinity (i.e., Cr toxicity decreased). However, when this phenomenon was combined with temperature, *P. flexuosus* survival decreased as temperature increased for all the salinities tested (McLusky and Hagerman, 1987).

Variation in ETMFs had affected other metals, as well. For instance, an increase in water hardness reduced the toxicity of copper (Cu) to both *D. magna* and fathead minnows because the metal competed with Ca (one of the main drivers of water harness) for the binding site at the gill level (Santore et al., 2001). Another major modifier of metal toxicity to aquatic organisms is water pH. For example, exposure of brook trout and rainbow trout to a reduction of pH from 6.5 to 4.8 caused an increase in Na and Cl losses. However, when fish were exposed to 333 µg Al/L, the effect was exacerbated, resulting in many cases in increase mortality (Wood and McDonald, 1987). These authors and others suggested that speciation was the main driver of Al toxicity to aquatic organism (Helliweli et al., 1983; Leivestad et al., 1987; Poléo, 1995; Wood and McDonald, 1987). Also, an increase in pH increased the acute toxicity of Zn, cadmium (Cd) and Cu to rainbow trout. When pH increased from 4.7 to 7.0, the 96-h LC_{50}s of Zn, Cd and Cu decreased from 671 to 66, 28 to <0.5, and 66 to 2.8 µg/L, respectively (Cusimano et al., 1986).

By comparing V to similar transition metals, it is possible to predict that a variation in water chemistry between conditions representative of the AR water and OSPW will modify V toxicity. Thus, it is necessary to study the behaviour of V within a gradient of key water chemistry variables, each of which has the potential to modify V bioavailability and toxicity.

1.6.3.1 Vanadium and a mixture of ETMFs

It has been shown that the presence of an isolated ETMFs in the test media affects metal toxicity and thus could modify V toxicity (Brix et al., 2017; Meyer et al., 1999; van der Putte et al., 1981). However, the environment presents a combination of ETMFs that together could elicit a different effect on V toxicity. So far, there has been little discussion about how a combination of ETMFs affects metal toxicity. For instance, when *D. magna* was chronically exposed to zinc

together with a mixture of varying pH, hardness and dissolved organic carbon (DOC), the toxicity of Zn was significantly modified (Heijerick et al., 2003). An increase in DOC alone decreased Zn toxicity, whereas when DOC was combined with increasing pH, the decrease became more pronounced at greater pHs. This alteration of Zn toxicity could be explained by an enhancement of the interactive negative forces of DOC as pH increases, which favours the attraction of Zn ions to DOC, reducing Zn bioavailability (Heijerick et al., 2003). Similarly, Waiwood and Beamish (1978) indicated that water hardness and pH modified Cu lethality to rainbow trout. The authors found that an increase in water hardness from 60 to 360 mg/L as $CaCO_3$ at pH 6 reduced Cu toxicity to rainbow trout by almost five times (LC_{20} increased from 22 to 100 µg Cu/ L). When the effect caused by the increase of water hardness was combined with a change in pH from 6 to 8, the LC_{20} increased from 100 to almost 300 µg Cu/ L, reducing Cu toxicity to rainbow trout even more. These results can be explained by the effect of pH on Cu speciation where at high pH there is a reduction in the proportion of hydrated copper ion (CuOH) and other Cu species, and an increase in water hardness will cause an increase in competition between CuOH and Cu and Ca and magnesium (Mg) for the biotic ligand. These results were confirmed when the pH of the test water was held at 6, but water hardness increased from 60 to 360 mg/L as $CaCO_3$, and the relative concentrations of CuOH and Cu remained constant. These species were the most predominant, confirming that CuOH and Cu were mainly responsible for Cu toxicity to aquatic organisms. In another relevant study, Stendahl and Sprague (1982) found that only pH had a significant altering effect on vanadium toxicity to rainbow trout when fish were kept in a combination of varying water hardness and pH.

Overall, these studies highlight the importance of understanding how a mixture of toxicity modifying factors affects V toxicity to be able to attain conclusions that better represents what is happening in the environment.

1.6.4 Modelling tools for the evaluation of the influence of multiple ETMFs on V toxicity to freshwater organisms

In the environment, multiple ETMFs may influence metal toxicity differently than when an individual water quality variable is tested (Di Toro et al., 2001; Santore et al., 2001). During the past decades, research advanced on understanding how ETMFs influenced the toxicity of cationic metal to aquatic organisms. This collection of research allowed the creation of modelling tools that enable to predict how the influence of combinations of ETMFs could affect cationic metal toxic to aquatic organisms. The BLM is an empirical modelling tool that models the toxicity of metals to aquatic organisms, taking into account the biological availability of metal to aquatic organisms and the effects of water chemistry on metal speciation. The theory that supports BLM modelling is based on that metals compete with naturally occurring ions (Ca, sodium (Na), Mg, proton (H), Cl, SO_4, HCO_3) and can form complexes with other ligands like organic matter, which will influence the interaction at the site of action of toxicity (the ligand) (Di Toro et al., 2001). Additionally, BLM theory indicates that metal toxicity occurs when the concentration of a metal at the biotic ligand (e.g., gills in fish) exceeds a threshold concentration (Di Toro et al., 2001, 2000; Niyogi and Wood, 2004). During the past 30 years, different BLM models have been created, ranging from mechanistic based BLM to empirical models (Brix et al., 2020). The mechanistic models are based on measuring the concentration of metals accumulated in the gill for each scenario of a modified water chemistry condition and the empirical BLM models are a

mathematical derivation of the stability constants from known toxicity data and a broad range of water chemistry measurements (de Schamphelaere et al., 2005; de Schamphelaere and Janssen, 2002; Niyogi and Wood, 2004). These two approaches present difficulties for regulators because the first requires a high number of animals and human resources, and the second requires complicated calculations and a big volume of data that sometimes make the model unsuitable for the scenario being investigated. Consequently, an alternative empirical and user-friendly tool known as Multiple Linear Regression (MLR) is becoming a valuable resource for the development of CWQGs for V (Brix et al., 2020, 2017; DeForest et al., 2018; Esbaugh et al., 2011). Based on the hardness-based water quality criteria (WQC), MLR incorporates both a high number of ETMFs and toxicity data for a pool of aquatic organisms (DeForest et al., 2018). The MLR has presented equal accuracy to BLM in developing a WQC for Cu toxicity to four daphnid species and the fathead minnows. The predicted MLR WQC was within a factor of two from the BLM criteria, confirming that MLR could be a valuable and user-friendly tool for regulators (Brix et al., 2017). Because no current model can predict how ETMFs influence V toxicity to aquatic organisms, it is imperative to develop a model for V that can facilitate the future creation of a site-specific CWQG-PAL.

1.6.5 Canadian water quality guidelines for the protection of aquatic life from metals

Canadian Water Quality Guidelines for the Protection of the Aquatic Life (CWQG-PAL) were proposed by the Canadian Council of Ministers of the Environment (CCME) to protect freshwater and marine life from anthropogenic stressors including chemical inputs or changes to physical properties of the environment (CCME, 2007). The CWQG-PALs are a numerical limit or a narrative statement that works as generic national recommendations (CCME, 2007, 2003). They

are created with the most current scientific information available for a particular stressor (e.g. Ni, Cu, V) at that time (CCME, 2007). The process of deriving CCME guidelines evolved from the extrapolation from the lowest available and acceptable toxicity endpoint (type B guidelines) to a more sound statistical distribution based approach, where all the available and acceptable data is used (type A guidelines; CCME, 2007; Schiffer and Liber, 2017b). Once a guideline is derived, it is important to identify and quantify the influence of potential modifying factors like pH, alkalinity, and water hardness among others, to determine how or if any of the factors affect the stressor's toxicity to aquatic organisms. These modifying factors are generally known as ETMFs. The incorporation of ETMFs to CWQG-PAL derivations will allow the user to apply guidelines with more certainty according to the site-specific characteristics. Some examples of CWQGs-PAL for metals that were modified by ETMFs include Ni, Cu, and lead (Pb) (CCME, 2019). Their long-term exposure CCME CWQGs-PAL were modified due to the effect of water hardness within a range between 60 and 180 mg/L as $CaCO_3$ because Mg and Ca compete with these metals for the active si at the aquatic organisms (Niyogi and Wood, 2004; Santore et al., 2001).

In 2016, Environment and Climate Change Canada (ECCC) released a federal environmental type A quality guideline for V using only eight toxicity values available until 2010 with a recommended V chronic concentration of 0.12 mg/L for freshwater organisms (ECCC, 2016). Recently, Schiffer and Liber (2017b) expanded EC work by developing more completed acute and chronic species sensitivity distribution (SSD) models. Their findings indicated that the calculated acute hazardous concentration endangering 5% of the species (HC_5) was 0.64, and the chronic was 0.05 mg V/L. This new chronic HC_5 is lower than the water quality guideline of 0.12 mg V/L developed by ECCC for V, indicating that the federal guideline may need to be revised in the future. This work advanced this knowledge by investigating how ETMFs representative of the

Alberta oil sands region influenced the acute and chronic toxicity of vanadium to model aquatic organisms.

1.7. Potential mechanism of action of V

To date, the mechanism of uptake of V into aquatic organisms and the main target(s) of toxic action have received limited attention. One study demonstrated that when adult rainbow trout were exposed for 96 h to V (3 to 22.5 mg V/L), gill hyperplasia (abnormal cell growth) occurred in all exposed fish, with 98.5 % of the trout showing thickening of the secondary lamellae. The liver was not affected, suggesting that the liver was not a target organ (Giles et al., 1979). However, juvenile rainbow trout exposed to V for 96 h (5.3 to 16.8 mg V/L, pH ranging from 6 to 9) showed histopathological effects in the kidney, such as cloudy swelling, hydropic degeneration and hyaline degeneration. When V concentrations were reduced to 3 and 4 mg/L, there was a reduction in the histopathological effects, with gills showing only hyaline and hydropic degeneration (Giles et al., 1979). Adult catfish exposed to 5, 10 and 15 mg/L of V for 4 and 30 d accumulated V in the kidney, liver, gills and intestine, with tissue concentrations increasing with exposure concentration (Ray et al., 1990). These authors indicated that the main target of V was the kidney, followed by liver, gill and intestine in both acute and chronic exposures. The findings of Ray et al. (1990) contradict the conclusion of Giles et al. (1979) that the liver is not a target for V. This discrepancy could be related to interspecific variation, differences in feeding behaviour, or duration of exposure. Bishayee and Chatterjee (1994) indicated that V also caused a significant increase in lipid peroxidation in the liver, kidney and brain, probably through the NADPH-dependant system.

The mechanism of action of V inside the gills is postulated to be mainly through inhibition of NKA. For instance, microsomal preparations of *Anguilla anguilla* (eel) gill tissue were exposed

to orthovanadate concentrations ranging from 0.184 µg/L to 183.91 mg /L (Bell and Sargent, 1979), and it was found that V acted as a potent inhibitor of NKA and as a result it could be inferred that V interferes with the normal phosphorylation of NKA by ATP (Bell and Sargent, 1979). Orthovanadate has a similar structure to phosphate, which plays a key role in ATP synthesis. Phosphate and sulphate cross animal membranes through anionic protein carriers and it has been hypothesized that V, when present as an oxyanion, use these proteins as transporters (Avila et al., 2000; Brix et al., 2001; Codd et al., 2001; Ogle and Knight, 1996; Pomeroy and Haskin, 1954; Rainbow, 1997). For example, vanadate, which resembles phosphate inhibited phosphate uptake, whereas chemicals such as molybdate or chromate that have a similar chemical structure as sulphate, inhibited more pronouncedly the uptake of sulphate than phosphate (Davidson et al., 2007). Several studies have shown that Cr and V inhibit NKA, but there is still no clear consensus as to how this inhibition happens. For instance, phosphate might be replaced by orthovanadate. A replacement of phosphate could potentially stop ATP synthesis, and therefore alter NKA activity, decreasing the intracellular Na concentration affecting the internal ion balance in gill cells. NKA could also be a major target of V in invertebrates. Microsomal preparation of the freshwater water hydroid (*Cordylophora caspia*) exposed to metavanadate concentrations ranging from 1.36E-03 to 136 mg of V/ L showed a significant reduction of NKA activity (Ringelband and Karbe, 1996). However, it still unclear if V toxicity mechanism is through a direct action (inhibiting the NKA by blocking a specific site on the enzyme), or an indirect effect causing an internal ion imbalance due to the blockage of another enzyme that ultimately affects the NKA. For instance, Lin and Randall (1993) exposed rainbow trout gills to 18.39 mg/L of V, which caused a reduction of the activity of H-ATPase by a 60%. Lew and Simon (1991) found that vanadate added to the cultivation media, inhibits exocytosis in *Saccharomyces cervisiae*, a species of yeast, by inhibiting the H-ATPase on

the plasma membrane. These last studies confirmed the inhibition of NKA or another related enzyme, but they do not elucidate the mechanism of action of V. Additionally, orthovanadate could potentially interfere with other mechanisms where phosphate is indispensable, causing for instance inhibition of the ATP phosphohydrolases, ribonucleases, adenylate kinase, phosphofructokinase, squalene synthetase, glyceraldehydes-3-phosphate dehydrogenases, glucose-6-phosphatase and phosphotyrosyl-protein phosphatases (Nechay, 1984). Other works suggest that V toxicity could also be due to the ability of vanadyl to generate reactive oxygen species, which can cause cell death by DNA damage or lipid peroxidation (Sharma and Talukder, 1987; Soares et al., 2008; Valko et al., 2005).

1.8. Test organisms

1.8.1. *Daphnia pulex* and *Daphnia magna*

Daphnia pulex is a commonly used animal model in toxicity testing and is an ideal species to study the toxicity of V in aquatic environments varying in water quality characteristics. *Daphnia pulex* has a ubiquitous distribution, being widely distributed in lakes, ponds, and slow-moving water sections of rivers and streams (Smith and Work, 2001). Daphnids have a high sensitivity to pollution caused by industrial chemicals and play an important role in the aquatic food networks, feeding on algae and bacteria and being the prey of several fish species (Smith and Work, 2001). Their use in toxicity testing is therefore ecologically relevant. As laboratory organisms, they are easy to maintain, highly adaptable to laboratory water, and they provide quick acute (48-h) and chronic tests (21-d) results. Also, there are well-established protocols for the use of *D. pulex* in toxicity testing. More specific to investigating OSPW, *D. pulex* is also resilient to a wide range of pH (6.0 – 8.5), with an optimal pH for reproduction between 7 and 8.7. Besides, they tolerate levels

of water hardness between 10 and 250 mg/L. Finally, Moore (1952) indicated that *D. pulex* presents a broad tolerance of salinity, being present within 0.5 to 13.14 ppt. This resilience to wide variations in pH, salinity and water hardness makes them ideal candidates to study how alterations in the water quality characteristics representative of OSPW could affect the toxicity of V to aquatic organisms (Davis and Ozburn, 1969; ECCC, 1990a; Smith and Work, 2001; Weber and Pirow, 2009).

Daphnia magna tolerance to the variation of water chemistry is more restricted than *D. pulex*. For instance, *D. magna* can resist water hardness as low as 80 mg/L as $CaCO_3$, whereas the *D. pulex* water hardness lowest limit is 10 mg/L as $CaCO_3$ (ECCC, 1990a). *Daphnia magna* can inhabit water bodies with lower dissolved oxygen (DO) as compared to *D. pulex*. The former can support water bodies with 3 mg/L of DO and the latter 5 mg/L (ECCC, 1990a). Although *D. magna* is less adaptable than *D. pulex* to wide variations in water chemistry conditions, they have been widely used for mechanistic toxicology, especially the effects of metals on ion imbalance and oxidative stress (Barata et al., 2005b; Clifford and McGeer, 2009; Glover and Wood, 2005). Also, has been *D. magna* extensively used to create models to explain how water chemistry variables like water hardness, or pH affects metal toxicity (de Schamphelaere et al., 2005; de Schamphelaere and Janssen, 2002; Pane et al., 2003; Tan and Wang, 2011). Thus, *D. magna* was a suitable invertebrate model to study the mechanisms of toxicity of vanadium.

1.8.2. *Oncorhynchus mykiss* (rainbow trout)

Rainbow trout is one of the most commonly used fish species in toxicity testing for regulatory and research purposes. This species is originally from western North America, but it has been introduced in several cool, freshwater bodies among all Canadian provinces and even in

Latin America (ECCC, 1990b). Rainbow trout play an important role in many Canadian aquatic food webs (COSEWIC, 2014), and they can tolerate water temperatures from 0 to 25 °C, with an optimum range between 10 and 15 °C (Wolf and Rumsey, 1985). Similar to *D. pulex*, rainbow trout can tolerate a range of water quality characteristics, making them a suitable species to study the toxicity of V under different environmental conditions. For instance, rainbow trout can survive in waters of pH ranging from 4.0 to 9.5, although its optimal pH ranges from 6.0 to 8.5 (ECCC, 1990b; Molony, 2001; Wilkie and Wood, 1991), and alkalinity levels from 10 to 400 as $CaCO_3$ (FAO 2018). Salinity tolerance of this species has been recorded between 0 and 30 ppt when fish were gradually adapted (Molony, 2001). For all the above reasons, rainbow trout is, therefore, appropriate to assess how water quality characteristics and major ion concentrations representative of OSPW could affect V toxicity to fish.

1.9. Project summary and rationale

Recently, a pilot project has been carried out to evaluate the use of PC to reduce organic toxicants from OSPW. Although this was proven to be successful, during the treatment, V is released to the treated OPSW reaching concentrations up to 7 mg V/L within the first 100 days, which showed to be toxic to aquatic organisms (Schiffer and Liber, 2017a, 2017b; Zubot et al., 2012). At this time, little information is available on how ETMFs influence V toxicity to aquatic organisms, and what the associated mechanism of V toxicity is. Lack of such a knowledge hinders the development of a site-specific CWQG-PAL for V and a better understanding of the ecological risks of V contamination, delaying the formulation of sound mitigation strategies.

1.9.1. Project objectives and null hypotheses

The overall goal of this thesis was to quantify the influence of ETMFs representative of the Alberta oil sands region on V toxicity to model aquatic organisms, and develop predictive relationships between these variables and V toxicity (Chapter 2 and 3). Additionally, this thesis proposed to investigate the mechanism of toxicity of V to *D. magna* and *O. mykiss* (Chapter 4). The objectives of this thesis by chapter were to:

Chapter 2

i) Assess how water quality characteristics representative of OSPW and Athabasca River Water (ARW) influence the acute and chronic toxicity of V to *D. pulex*; ii) develop predictive relationships between V and the most influential water quality variables; iii) verify if V cause reproduction impairment in long-term (21-d) exposure; and iv) determine which species of V are principally responsible for V toxicity under the different conditions tested.

H_o: Vanadium toxicity to D. pulex is not influenced by surface water quality characteristics representative of the Alberta oil sands region.

Chapter 3

i) Assess how water quality characteristics representative of OSPW and ARW influence the acute toxicity of V to *O. mykiss*; ii) develop predictive relationships between V and the most influential water quality variables; and iii) compare and contrast the models obtained for *O. mykiss* and *D. pulex*.

H_o: Vanadium toxicity to O.mykiss is not influenced by surface water quality characteristics representative of the Alberta oil sands region.

Chapter 4

i) Investigate the mechanism(s) of toxicity of V to *D. magna* and *O. mykiss*. In particular, investigate if V causes either ion imbalance or oxidative stress in *D. magna* and *O. mykiss*.

H_o: The mechanism of toxicity of V is neither disruption of ion homeostasis nor oxidative stress in D. magna and O. mykiss.

CHAPTER 2: MODELS FOR THE ACUTE AND CHRONIC AQUEOUS TOXICITY OF VANADIUM TO DAPHNIA PULEX UNDER A RANGE OF WATER CHEMISTRY CONDITIONS

Preface

This chapter describes the influence of water quality characteristics, or exposure and toxicity modifying factors (ETMFs), representative of the Alberta oil sands region on the toxicity of vanadium (V) to the model organism *Daphnia pulex*. Vanadium gets enriched in petroleum coke (PC), which is a solid carbonaceous by-product of the bitumen mining process. Past studies have shown that V leaches from PC once in contact with water, including oil sands process-affected water (OSPW), reaching concentrations that are toxic to several aquatic organisms.

Therefore, a set of acute and chronic toxicity tests were conducted where individual representative ETMFs were modified to evaluate how these variables influenced V toxicity to *D. pulex*. The variables that differed the most between the Athabasca River (AR) and OSPW were chosen. Combinations of these variables were also tested to determine if, when combined, these variables caused a different effect on V toxicity to *D. pulex* than when they were tested alone. Vanadium species were modelled to determine which chemical species were the most relevant within the ranges of the ETMFs tested. Among the variables tested, only pH, alkalinity, sulphate and sodium (only when sodium was above 300 mg/L) significantly influenced V toxicity to *D. pulex*. Additionally, only the combination of sodium and sulphate showed a marked decrease in V toxicity when compared to the effects of each individual variable on V toxicity to *D. pulex*. In all

the tests conducted here, V oxyanions were the chemical species of V that were the most abundant and most probably responsible for V toxicity. Overall, new water quality guidelines for the protection of aquatic life for V should incorporate the influential effect of pH, alkalinity and sulphate.

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2.1. Abstract

Alberta's oil sands petroleum coke (PC) generation has in recent years surpassed 10 million tonnes. Petroleum coke has been proposed as an industrial-scale sorbent to reduce concentrations of organic chemicals in oil sands process-affected water (OSPW). However, PC contains up to 1,000 mg of vanadium (V) per kg of PC, and during the treatment, it leaches from coke reaching levels of up to 7 mg/L in "treated" OSPW. Little information is available on how common water quality variables affect the toxicity of V to aquatic organisms. Here descriptive relationships are presented to describe how site-specific surface water characteristics representative of the Alberta oil sands region influence the toxicity of V to *Daphnia pulex*. Results revealed that when *D. pulex* was exposed to an increase in pH, a threshold relationship was found where acute V toxicity increased from a lethal median concentration (LC_{50}) of 1.7 to 1.2 mg V/ L between pH 6 and 7 and

then levelled off at around 1 mg V/L. When alkalinity (from 75 to 541 mg/L as $CaCO_3$) and sulphate (from 54 to 394 mg/L) increased, the acute toxicity of V decreased slightly with LC_{50}s changing from 0.6 to 1.6, and 0.9 to 1.4, respectively. When the length of V exposure was extended (from 2 to 21 d), only an increase of sulphate from 135 to 480 mg/L caused a slight increase in V toxicity from a LC_{50} of 0.6 to 0.4 mg V/L, the opposite trend seen in the acute exposures. In addition, the influence of two OSPW representative mixtures of increasing sodium and sulphate, and increasing alkalinity and sulphate on V acute toxicity to *D. pulex* were evaluated; only the mixture of increasing sodium (from 18 to 536 mg/L) and sulphate (from 55 to 242 mg/L) caused a slight decrease in V acute toxicity (LC_{50} 1.0 to 2.1 mg V/L). Evidence is presented that variations in surface water chemistry can affect V toxicity to daphnids, although only to a small degree (i.e. within a maximum factor of 2 in all cases evaluated here). These relationships should be considered when creating new water quality guidelines or local benchmarks for V.

2.2. Introduction

Canada's oil sands, located principally in the province of Alberta, is the third largest global reservoir of crude oil (CAPP, 2017). Industrial activities in the Alberta Oil Sands region have been increasing over the last two decades and are forecasted to triple in the upcoming decade (AER, 2015; Millington, 2017). The increase in industrialization is promoting higher production of bitumen, which when refined leads to the production of liquid and solid by-products respectively known as oil sand process-affected water (OSPW) and petroleum coke (PC) (Allen, 2008a, 2008b; Bryers, 1995; Cantley et al., 1977; Chalaturnyk et al., 2002; Deroo and Powell, 1978; Hein et al., 2001; Rogner, 1997; Zubot et al., 2012). Until 2015, the oil sands industry had accumulated 1.18 trillion litres of tailings, including OSPW, which have largely been stored on-site (Mcneill and

Lothian, 2017; Scott et al., 2008). It has been previously demonstrated that the toxicity of OSPW is largely caused by naphthenic acids (NAs) and other acid-extractable organic compounds (Anderson et al., 2012 (a); Anderson et al., 2012 (b); Morandi et al., 2015; Toor et al., 2013). Anderson et al. (2012a) and He et al. (2012) found that activated carbon (C) can reduce the toxicity of OSPW, but price-wise it is an unfeasible option for the industry. However, it has been shown that PC produced using a fluidized coking process does contain adsorbent properties similar to that of commercially available activated C (Zubot et al., 2012). The industry is investigating the use of PC (80% of its weight is C) to treat OSPW (Small et al., 2012). In 2016, the total annual PC production of the region surpassed 10 million tonnes (AER, 2016). Because of its value as an energy resource, some operators are required to store their produced PC on-site as a potential future energy resource (AER, 2016; Puttaswamy and Liber, 2012, 2011). To further assess potential value added benefits of PC, Syncrude Canada Ltd. is assessing the use of PC to treat OSPW for safe return to the environment (i.e., toxicity removal). Although Zubot *et al.* (2012) showed that it is feasible to use PC to reduce the concentration of NAs in OSPW, the authors also indicated that molybdenum (Mo) and vanadium (V) are released from PC to the aqueous phase. Molybdenum concentration in OSPW increased from <0.1 to 0.8 mg/L and V increased from <0.05 to >7 mg/L, only a few days after experimental commencement. These results are consistent with the findings of Puttaswamy et al. (2010), who showed that weathering favoured the release of V from PC stock-piled in lysimeters located onsite. Interestingly, Zubot et al. (2012) found that V is a non-conservative ion and concentrations decreased with increased pore water residence time. After 300 days from test initiation, concentrations were less than 1 mg/L. The initial increase in V concentration in OSPW may represent a potential concern for oil sands companies due to an incomplete understanding of V toxicity to aquatic organisms. The final concentration of V in

treated and/or aged OSPW could be higher than the median background concentration of V (0.5 – 3 µg/L) found in most freshwater environments in Canada (ECCC and HC, 2010; Hirayama et al., 1992; Rehder, 2015).

There is limited research available on the effects of V on aquatic organisms compared to data for many other metals. Recently, it has been demonstrated that the 48-h median lethal concentrations (LC_{50}s) for *Cerodaphnia dubia* and *Daphnia pulex* range from 0.60 to 2.17 mg/L, respectively (Schiffer and Liber, 2017a). These findings are in agreement with earlier publications. For instance, concentrations between 3.4 to 4.8 mg of V/L caused lethality to *Daphnia magna*, but did not cause reproductive impairment when exposed for 23 d (Beusen and Neven, 1987). Concentrations of 0.55 mg/L of V for 7 d were lethal to *C. dubia* (Puttaswamy et al., 2010).

Historically, the limited availability of peer-reviewed publications on V toxicity to aquatic organism for several years has hindered the creation of a sound Canadian water quality guideline for V for the protection of the aquatic life (CWQGs-PAL). However, recently Schiffer and Liber (2017b) presented acute and chronic species sensitivity distribution (SSD) data for V, which is the preferred method for the development of CWQGs (CCME 2007). Despite these recent data, there is very limited information on how exposure and toxicity modifying factors (ETMFs) could influence the toxicity of V to aquatic organism. The incorporation of ETMFs into the CWQGs-PAL is recommended by CCME to allow the user to select the guidelines that best resemble the site characteristics (CCME, 2007).

Ernst and Garside (1987) found that when diluted sulphuric acid was added to the stock solution, V toxicity decreased, suggesting that a variation in pH severely affects V toxicity to *Salvelinus fontinalis*. Similarly, Puttaswamy and Liber (2011) showed that at pH 9.5 the toxicity of V in PC leachate to *C. dubia* was dominant than at 5.5; and at pH 9.5, there was a predominance

of V oxyanions ($H_2VO_4^-$ and HVO_4^{2-}) in the leachate. Puttaswamy and Liber (2012) also found that when the concentration of sulphate was high, V toxicity to *C. dubia* was ameliorated. Chemical speciation analysis has shown that V is present as several different chemical species over a range of pH that could explain the variation in V toxicity to aquatic organisms (Rehder, 1991; Wehrli and Stumm, 1989). However, little is known about how other water chemistry variables, or mixtures of these variables, may affect V toxicity. Water quality variables like alkalinity, bicarbonate (HCO_3), sodium (Na) and chloride (Cl) markedly differ between OSPW and the Athabasca River (AR) (AE, 2009; Allen, 2008a; ERCB, 2009; MacKinnon et al., 2005; RAMP, 2013; Scott and Fedorak, 2004; Zubot et al., 2012). Therefore, if the oil sands industry some day wants to discharge PC-treated OSPW into the surrounding environment, it is imperative to develop mathematical relationships that will aid in understanding how the variation in water quality between OSPW and AR could alter the toxicity of V to aquatic organisms.

The objectives of this study were to: i) assess how exposure and toxicity modifying factors representative of OSPW and AR, influence the acute and chronic toxicity of vanadium to *D. pulex*; ii) develop predictive relationships between vanadium toxicity and the most influential water quality variables; iii) determine which chemical species of V are principally responsible for V toxicity under the different conditions tested; and iv) assess how mixtures of key water quality variables influence V toxicity to *D. pulex.*

2.3. Materials and methods

2.3.1. Chemicals

The chemicals used for preparing the different concentrations of vanadium and water quality ions were of analytical grade. Sodium metavanadate ($NaVO_3$, anhydrous, min. purity 96%)

was purchased from Strem Chemicals Inc. (Newburyport, MA, USA). Magnesium sulphate was purchased from Sigma-Aldrich, (Oakville, ON, Canada) and the remaining chemicals were obtained from Fisher Scientific (Ottawa, ON, Canada).

2.3.2. Test organisms

Daphnia spp., including *D. pulex,* have a higher sensibility to V than several model and regional fish species (Schiffer and Liber, 2017b; Smith and Work, 2001), and they are widely distributed in lakes, ponds, and slow-moving water sections of rivers and streams. *Daphnia pulex* is also resistant to a wide range of pH (6.0 – 8.5) and can tolerate levels of water hardness between 10 and 250 mg/L as $CaCO_3$. These characteristics make *D. pulex* an ideal model species to study how alterations in the water quality characteristics representative of OSPW and AR could affect the toxicity of V to aquatic organisms (Davis and Ozburn, 1969; ECCC, 1990a; Smith and Work, 2001; Weber and Pirow, 2009).

2.3.3. Culturing

Daphnia pulex were obtained from an in-house culture maintained in an environmental chamber at the Toxicology Centre, University of Saskatchewan (Saskatoon, SK). Daphnids were cultured in 2-L glass jars in groups of 30-40 individuals following Environment Canada and Climate Change (ECCC) and American Society for Testing and Materials (ASTM) protocols (ASTM, 2004; ECCC, 1990). The temperature in the environmental chamber was constant at 24 $\pm$ 1 ºC, with a 16 h light: 8 h dark photoperiod (light intensity was above 450 lux). The culture medium was prepared based on Schiffer and Liber (2017a), but modified to reach the desired ion concentrations or pH. Basically, a ratio of 70% C-filtered, bio-filtered municipal Saskatoon City

water (DeCl) and 30% reverse osmosis (RO) water was used to simulate Athabasca River water (ARW) and was periodically adjusted slightly (DeCl:RO ratio modified) to cope with small seasonal changes in the source water. The simulated ARW was aerated for at least 24 h before its use in culturing or in toxicity tests. The pH in the culture jars was maintained at 7.5 – 8.5.

A literature review was performed to choose the water chemistry variables that differ the most between the ARW and the OSPW, and document their respective ranges (Table 2.1). These ranges were also used to acclimate the daphnids to the new water chemistry conditions prior to a V toxicity test and also used for the duration of a test. The ranges in water chemistry conditions were obtained by adding specific salts that contained the ions of interest to the prepared ARW (Table A1, A2). In the experiment where the effect of pH on V toxicity was tested, glacial-acetic acid or sodium hydroxide was used to reach the desired pH. Additionally, the concentrations of all variables of interest in the prepared ARW were measured throughout all tests (Table A3-A6).

Table 2.1: Ranges in water chemistry of Athabasca River water (ARW) and Oil Sands Process Water (OSPW) obtained from literature review (AE, 2009; Allen 2008a; MacKinnon et al., 2005; RAMP, 2013; Zubot et al., 2012)**.**

Source	pH	Alkalinity [a]	Hardness [a]	Sodium [b]	Chloride [b]	Bicarbonate [b]	Sulphate [b]
OSPW	7.7 - 8.8	635 - 779	75 - 112	250 - 840	80 - 540	650 - 950	218 - 416
ARW	6.9 - 8.5	71 - 157	75 - 176	7 - 53	2 - 49	140 - 192	6 - 56

[a] concentration as mg/L as $CaCO_3$
[b] concentration as mg/L

Neonates obtained from the stock culture were transferred to 2-L glass jars and acclimated for at least 10 d to the modified test water (with a new ion concentration or pH, and without V); the 3rd brood was collected and used in the toxicity tests (Table 2.2). Even though the range of pH chosen for the acute and chronic tests should have been appropriate for *D. pulex* (Davis and Ozburn, 1969), a minor modification was made for daphnids adjusted to pH levels lower than 8. At these pH levels, water renewals were performed daily to reduce the stress that could be caused

by decomposition by-products generated by the algae (food) or the daphnids. During culturing, daphnids were fed daily with a mixture of algae (70% *Raphidocelis subcapitata* and 30% *Chlamydomonas spp.*) and 4 mL of YCT (Clifford and McGeer, 2009). Selenium (2 µg/L) and vitamin B_{12} (2 µg/L) were added to the culture jars, as they are essential to the overall health of *D. pulex* (ECCC, 1990a).

Table 2.2: Nominal test levels for water chemistry variables and major ions (mg/L) representative of the Athabasca River and oil sands process water used in acute, chronic, and mixture studies. Numbers in bold indicate the water chemistry of the in-house prepared Athabasca River water that corresponds to approximate environmental concentrations.

Test /Variable	Salt used	Nominal levels
Acute (48-h)		
Na	CHO_2Na	**10**-35-120-220-320-420
Cl	KCl	**10**-35-120-420
SO_4	$MgSO_4$	**55**-100-300-340-380
HCO_3	$KHCO_3$	**70**-160-250-410
pH	-	6-6.5-7-**8**-9
Hardness	$CaSO_4 2H_2O/MgSO_4$	70-**105**-140
Alkalinity	$NaHCO_3$	70-**100**-150-200-300-600
Chronic (21-h)		
SO_4	$MgSO_4$	100-200-450
pH	-	7-**8**-9
Alkalinity	$NaHCO_3$	70-**100**-200
Mixture (48-h)		
SO_4/Na	$MgSO_4/CHO_2Na$	**55**-100-146-192-246/**18**-120-220-320-420-540
SO_4/Na	$MgSO_4/CHO_2Na$	146-192-247/120-120-120
Alkalinity/ SO_4	$NaHCO_3/MgSO_4$	70-150-300/100-200-300

2.3.4. Toxicity testing procedures

Neonates less than 24-h old collected from an in-house culture were used in all tests described below. Before starting the acute V toxicity tests, the tolerance of *D. pulex* to the water

chemistry conditions being evaluated was tested by exposing daphnids to low, medium and high concentrations of each variable for 48 h without V.

2.3.4.1. Acute toxicity testing procedures

Following ECCC (1990), *D. pulex* neonates were exposed for 48 h without the addition of food. Based on results from Schiffer and Liber (2017) who found an LC_{50} for *D. pulex* of 2.23 mg V/L, daphnids were exposed to an untreated control and 5 geometrically increasing concentration (0.25, 0.5, 1, 2 and 4 mg/L of V), with five replicates per concentration, and 10 neonates per experimental unit. Because it was expected that alkalinity could ameliorate V toxicity due to potential competition between HCO_3 and V, the range of V test concentrations were shifted upwards. For alkalinities of 300 mg/L as $CaCO_3$, the aforementioned V range was modified to 0.5 to 8 mg/L, and for 600 mg/L as $CaCO_3$ V range was changed to 1 to 16 mg/L. To minimize a direct pH effect, pH was adjusted before each test initiation with glacial-acetic acid ($C_2H_4O_2$) or sodium hydroxide (NaOH). Test vessels consisted of 125-mL acid-washed glass jars with polypropylene lids. Jars were filled to maximum capacity to reduce the presence of air bubbles, thereby minimizing pH drift. Mortality was defined as lack of movement following gentle prodding and was recorded at 48 h. A test was considered acceptable when mortality in the control group was $\leq$ 10%. All tests met the above mentioned requirements.

To avoid disturbing the closed test jars at the beginning of the test, chemistry replicates (additional test vessels) with ten neonates were prepared for each treatment and the control group. Water samples (20 mL) were randomly collected from chemistry replicates at the beginning and end of the test (48 h) in triplicates for analysis of temperature, dissolved oxygen (DO), pH, alkalinity and total hardness. Measurements of pH were taken from test solutions using an OPRIM

PerpHect Log R meter model 370. Temperature and DO concentrations were measured using an ORION DO meter model 835 (ORION Research, Beverly, MA, USA). Alkalinity and hardness were measured using a Hach digital titrator model 16,900 (Hach Company, Loveland, CO, USA).

2.3.4.2. Chronic toxicity testing procedures

The chronic exposures were performed using the ranges in water quality variables that significantly altered V acute toxicity to *D. pulex* (Table 2.2). Water chemistry measurements were performed as described under acute toxicity procedures. Survival and reproduction tests (21 d) for *D. pulex* were conducted as static-renewal tests, following standardized testing protocols (ASTM, 2004; OECD 2012). The test vessels used in these tests were the same as for the acute exposure tests. The ranges of V concentrations for these tests were based on previous work done by Schiffer and Liber (2017). Third-brood neonates of *D. pulex* (≤ 24 h) were placed individually into each test vessel, with ten replicates for each test concentration and a control group. Complete water renewal occurred every second day except for the test done at pH 7, where water was changed on a daily bases to maintain pH within appropriate limits. Fresh stock solutions were prepared the day before and a serial dilution of the stock solution was prepared the day of each renewal. Daphnids were transferred to new test solutions containing a mixture of *R. subcapita* and *Chlamydomonas spp.* at a 2:1 ratio as a food source, and B_{12} and selenium as micro-nutrients. Neonates obtained from test organisms were counted and discarded daily. Mortality was defined as lack of movement following gentle prodding and was recorded daily. A test was considered acceptable when mortality in the control group was $\underline{\leq}$ 20% and 60% of surviving adults produced at least three broods with a total of at least 15 neonates per adult (ASTM, 2004; OECD, 2012).

Temperature and DO were monitored before and after each water renewal in the control, low, medium and high V treatments. Additionally, water samples (20 mL) were randomly collected at the beginning, weekly, and the end of each test in triplicates from control, low, medium and high V treatments for analysis of pH, DO, conductivity, alkalinity and water hardness. Samples for V analysis (5 mL) were randomly collected from three replicates from the control and all V treatments after the samples for water chemistry were collected. Weekly samples were taken from both old and new test solutions. To avoid disturbing the closed test jars at the beginning of the test, chemical replicates (additional test vessels) were prepared for each treatment and the control group for the collection of samples for water quality analysis. One neonate was placed in each chemical replicate to reproduce the test jar conditions.

2.3.4.3. Mixture toxicity testing procedure

Mixture toxicity tests (48-h) were performed with a combination of two water quality variables and a range in V concentrations similar to those used in the acute toxicity test. These mixtures of variables were chosen by combining individual water quality variables that had a significant effect on V toxicity to *D. pulex* (Table 2.2). The exposures were performed and the water samples collected as described in the acute toxicity testing section above.

2.3.5. Chemical analysis

All water samples were filtered using syringe filters with a 0.45-µm polyesthersulfone membrane (VWR International, Mississauga, ON, Canada) before major ion and V analyses. Samples for V analysis were acidified to 2% acid with high purity nitric acid (HNO_3) (Fisher Scientific, Ottawa, ON, Canada). Dissolved concentration of inorganic cations (Na) and anions

(Cl, HCO_3, SO_4) were analyzed in-house using ion chromatography (Dionex ICS-3000, Sunnyvale, CA, USA). Samples collected for V analysis were measured in-house using an inductively coupled plasma mass spectrometer (ICP-MS) equipped with collision cell technology (X Series II, Thermo Electron, Mississauga, ON, Canada). The accuracy of the ICP-MS measurements was evaluated with a river-water standard reference material (SLRS-5) and natural water standard reference material (1640a), purchased from the National Research Council (Ottawa, ON, Canada), and National Institute of Standard and Technology (Gaithersburg, MD, USA), respectively. The accuracy obtained from both reference materials was always $\geq$ 85%. Reproducibility of reference materials (SLRS-5 and 1640a) and precision of duplicates exceeded 90% in all cases. The ICP-MS limit of detection for V was 0.05 µg/L.

2.3.6. Statistical analyses and vanadium speciation

The acute and chronic LC_{50} estimates were calculated with 95% confidence intervals using either the moving average TOXCALC 5.0 (Tidepool Scientific, CA, USA) or Spearman-Karber method, version 1.5 (Hamilton et al., 1977). Regression models between each water quality variable and acute or chronic V toxicity (as LC_{50}) were obtained using curve estimation in SPSS version 20.0 (IBM, CA, USA) and the model that best fitted the measured dataset was chosen. The regression models used in the current paper can be obtained from: https://www.ibm.com/support/knowledgecenter/en/SSLVMB_23.0.0/spss/base/curve_estimation_models.html

The inhibition concentration (IC) approach, version 2.0 (Norbert-King, 1993), was used to estimate the concentrations that caused 10%, 20% and 50% inhibition in reproduction of the daphnia population. Significant differences between *D. pulex* reproductive output of the control

and V treatments in the chronic tests were determined by two-way analysis of variance (ANOVA), followed by the post-hoc parametric Tukey's test for pairwise comparison. In all cases $\alpha = 0.05$.

The dissolved chemical speciation of V was modelled using Visual MINTEQ 3.1 (KTH, Department of Land and Water Resources, Stockholm, Sweden) as described in detail at (https://vminteq.lwr.kth.se/download/). To model V speciation, the mean measured concentrations of dissolved V and the ions evaluated in each acute exposure scenario were used.

2.4. Results

2.4.1. Exposure conditions and test acceptability

Daphnids were successfully adapted to the tested water quality conditions and none of those variables, when evaluated independently (without V), caused mortality to *D. pulex* after 48 h of exposure at any of the concentrations tested. Experimentally, the measured modified water quality conditions and the V concentrations utilized in the toxicity tests were reasonably similar to the nominal concentrations (Table A4-A6). In addition, there was no substantial loss in aqueous V concentration between the beginning and the end of any of the tests performed. However, to derive the descriptive models only measured data were used. The only parameter that showed a marked change was conductivity. The variation in this parameter was due to the use of sodium metavanadate ($NaVO_3$) as a source of V and the salts chosen as a source of sodium, alkalinity and SO_4. However, according to the overall experimental conditions recorded in each test and the speciation modelling performed (see section 2.4.5 below), the oxyanion species (HVO_4^{2-} and $H_2VO_4^-$) of the monomeric V(V) were dominant within the conductivity ranges measured in these tests. Mortality of the control daphnids was always below 10%.

2.4.2. Effects of water quality on acute and chronic V toxicity

Between the ranges tested, neither Cl, nor HCO_3, nor water hardness had a significant effect on acute V toxicity to *D. pulex* with mean LC_{50} values of 0.97, 0.99 and 0.95 mg V/L, respectively (Figure A1).

The effect of pH on acute V toxicity to *D. pulex* was substantial between pH 6.3 and 7.2, with the 48-h LC_{50} decreasing from 1.74 to 1.15 mg/L, as pH increased. However, between pH 7.2 and 8.8, the toxicity of V to *D. pulex* changed only slightly (Figure 2.1a). This influence of pH on V toxicity to *D. pulex* was best described as a S-curve (IBM-SPSS, 2018) with an inflection point at pH 7 (r^2 = 0.796; p = 0.042, Table 2.3). When the exposure duration was extended to 21 d, there was still no significant change in V toxicity between pH 7.2 and 9.1, displaying a mean LC_{50} of 0.34 mg V/L (r^2 = 0.218; p = 0.691, Figure 2.1a). Unfortunately, it was not possible to conduct a successful chronic toxicity test with *D. pulex* below pH 7.2 because mortality surpassed acceptability criteria after the fourth day.

The acute toxicity of V to *D. pulex* decreased from a 48-h LC_{50} of 0.64 to 1.59 mg V/L as the alkalinity increased from 75 to 541 mg/L as $CaCO_3$ (Figure 2.1b), described with a S-curve (IBM-SPSS, 2018; r^2 = 0.916; p = 0.003; Table 2.3). However, under chronic exposure, alkalinity did not have a significant effect (r^2 = 0.686; p = 0.378) on V toxicity within the range tested (74-216 mg/L as $CaCO_3$). Thus, the 21-d mean LC_{50} was calculated as 0.38 mg V/L (Figure 2.1b).

Between 54 (ARW concentration) and 99 mg/L of SO_4, the acute V toxicity showed no change with a mean 48-h LC_{50} of 0.90 mg V/L. However, when the concentration of SO_4 increased from 99 to 394 mg/L, the acute toxicity of V to *D. pulex* decreased, reaching to a maximum LC_{50} of 1.39 mg V/L (Figure 2.1c). The effect of SO_4 between 99 and 394 mg/L on acute V toxicity to *D. pulex* was best described with a power curve (IBM-SPSS, 2018; r^2 = 0.842; p = 0.028, Table

2.3). When the exposure period was extended to 21 d, the effect of SO_4 between 134 to 480 mg/L on chronic V toxicity followed an opposite trend with a slight inverse relationship (IBM-SPSS, 2018; $r^2 = 0.998$; $p = 0.031$, Figure 2.1c, Table 2.3).

An increase in Na between 18 (ARW concentration) and 325 mg/L in the test water displayed no discernible effect on acute V toxicity to *D. pulex*, with a mean 48-h LC_{50} of 0.98 mg V/L ($r^2 = 0.001$; $p = 0.982$, Figure 2.1d). However, when the concentration of sodium increased from 325 to 473 mg/L, the toxicity of V to *D. pulex* doubled, with the 48-h LC_{50} dropping to 0.43 mg V/L (Figure 2.1d). This increase in V toxicity was described by a linear curve (IBM-SPSS, 2018; Table 2.3).

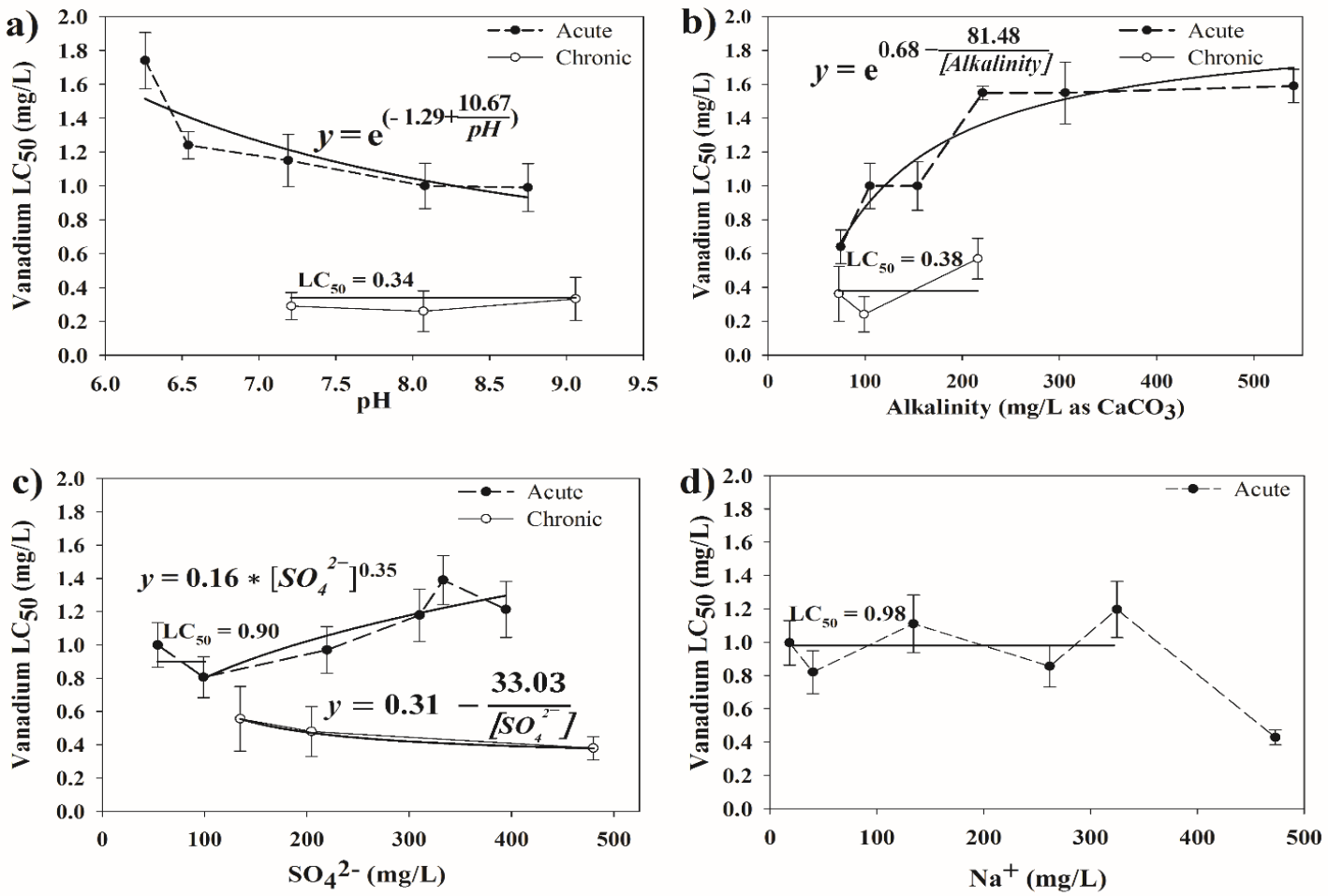

Figure 2.1: Descriptive models for the acute and chronic effects of increasing: a) pH; b) alkalinity; c) sulphate; and d) sodium, representative of Athabasca River water and OSPW on vanadium toxicity to *Daphnia pulex*. Error bars represent the 95% confidence intervals of the LC50 estimates. Thick solid line describes either the statistical model or the geometric mean obtained from the experimental LC50 values (mg/L).

Table 2.3: Summary table of acute, chronic and mixture toxicity models for vanadium to *Daphnia pulex* developed for a range of alkalinity, pH, sulphate and sodium under 48-h or 21-d tests.

Test /Variable	Equation or Geometric mean	r^2	p [a]
Acute (48-h)			
pH	**y = exp (-1.29 + (10.67/[pH])**	0.796	0.042
Alkalinity	**y = exp (0.68 – (81.48/[Alkalinity]))**	0.916	0.003
SO_4	**y = 0.16*[SO_4] ^ 0.35**	0.842	0.028
Na (18-325 mg/L)	**0.98**	0.001	0.982
Na (>325 mg/L)	**y = -0.01*[Na] + 3.65**	N/A	N/A
Chronic (21-d)			
pH	**0.34**	0.218	0.691
Alkalinity	**0.38**	0.686	0.378
SO_4	**y = 0.31 – (33.03/[SO_4])**	0.998	0.031
Mixture (48-h)			
↑ SO_4/ ↑ Na	y = exp (-0.12 + 0.13 [Na/ SO_4]) [b]	0.629	0.600
↑ SO_4/ Na	**0.81**	0.993	0.053
↑ SO_4/ ↑ Alkalinity	**1.91**	0.936	0.163

[a] p = p-value obtained for the mathematical model
N/A = not applicable
[b] Not bolded represents the model that best fit the data, but it was not statistically significant

2.4.3. Effects of V on Daphnia pulex reproduction

In all the chronic toxicity tests performed, the EC_{50} estimates for reproduction were higher than the corresponding 21-d LC50 estimates (Table A7). Also, in all the chronic toxicity tests performed neither V, nor the water quality variables tested, had any significant effect in the overall number of neonates per adult *D. pulex* (p>0.05). However, the adults held at 1mg V/L faced early

mortality, which resulted in a lower accumulated number of neonates produced in that treatment at the end of the 21 d of exposure.

2.4.4. Effects of ion mixtures on the acute toxicity of V

Mostly, the effects of the different combinations of ions tested did not show a significant trend in V acute toxicity to *D. pulex* (Figure 2.2). The exception was when increasing Na and increasing SO_4 were combined, there was a non-significant upward trend displaying an approximate doubling in LC_{50} from 18 mg/L Na + 55 mg/L SO_4 to 536 mg/L Na + 242 mg/L SO_4 (r^2 = 0.629, p = 0.600; Figure 2.2a, Table 2.3). This pattern most closely followed that seen for SO_4 alone, but with a slightly greater amelioration effect. At moderate levels (Figure 2.2b) the combination of Na and SO_4 caused no change in acute V toxicity (r^2 = 0.993, p = 0.053), reflecting the trends observed for the ions alone, at similar concentrations. At ≥ 199 mg/L of SO_4 (Figure 2.2a), there was an ameliorating effect on acute V toxicity due to SO_4, despite the fact that Na was above 440 mg/L which was predicted, when present alone, to double acute V toxicity to daphnids (LC_{50} dropped from 1 to 0.4 mg V/L when Na increased from 325 to 473 mg Na/L, Figure 2.1d). Additionally, in the three combinations of increasing alkalinity and increasing SO_4 tested here (alkalinity/ SO_4 80/87, 186/210, and 311/280 Figure 2.2c), the 48-h LC_{50}s for V approximately doubled (to a mean of 1.92 mg V/L) compared to the LC_{50}s of the individual parameters (Figure 2.1b and 2.1c). Also, at low alkalinity (80 mg/L as $CaCO_3$) and low SO_4 (87 mg/L) levels, SO_4 appeared to have ameliorated the acute V toxicity that was observed (i.e., at low concentrations of HCO_3 and CO_3, when alkalinity alone was below 100 mg/L as $CaCO_3$ (Figure 2.1b). Overall, there

was no upward- or downward-trend within the ranges of alkalinity plus SO_4 tested here (r^2 = 0.936; p = 0.163 Figure 2.2c).

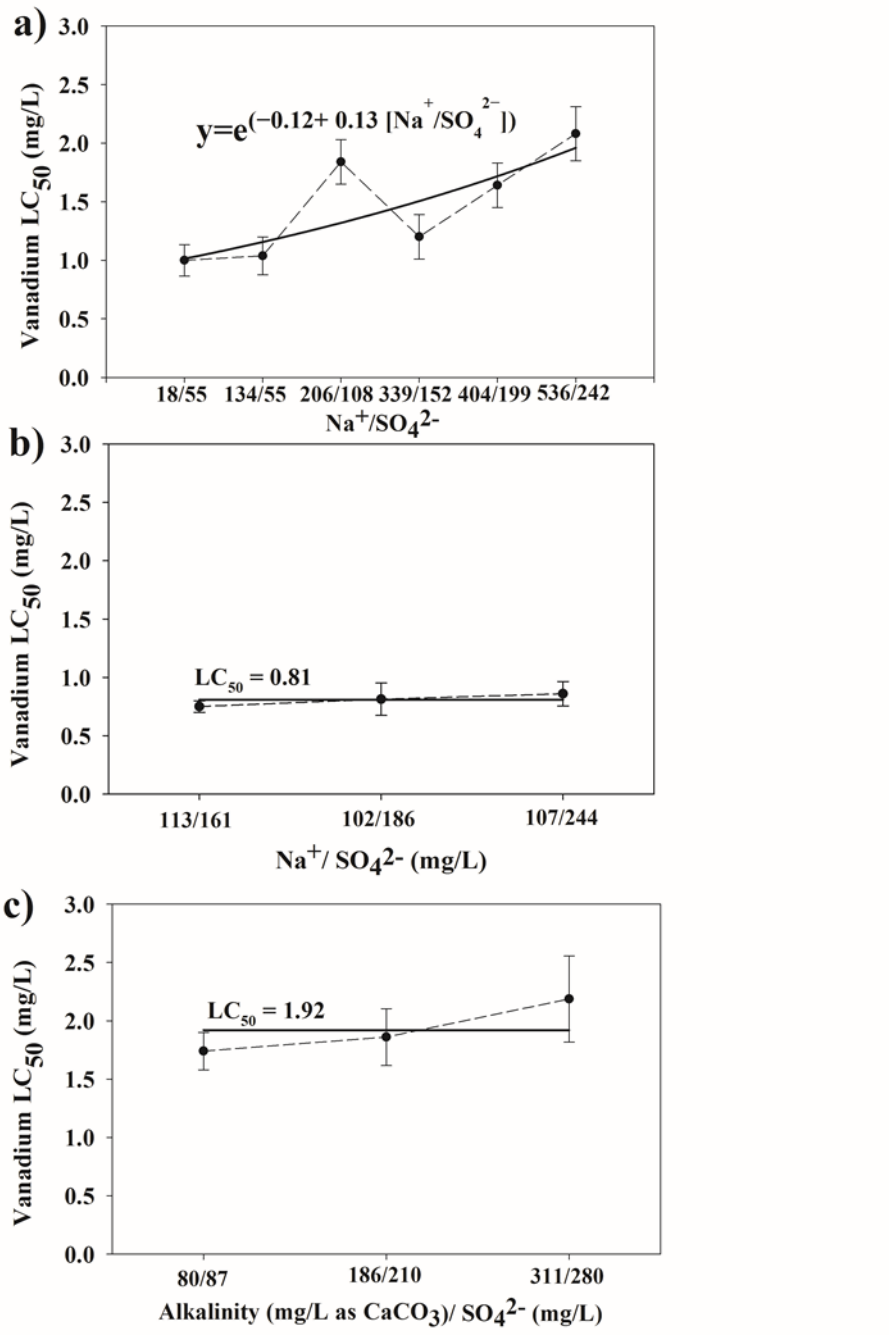

Figure 2.2: Descriptive models for the effect of mixtures of: a) increasing sodium and sulphate; b) fixed sodium and increasing sulphate; and c) increasing alkalinity and increasing sulphate representative of diluted OSPW on acute vanadium toxicity to *Daphnia pulex*. Error bars represent the 95% confidence intervals of the LC_{50} estimates. Thick solid line describes either the statistical model or the geometric mean obtained from the experimental LC_{50} values (mg/L).

2.4.5. Effects of water quality on V speciation

In all the toxicity tests conducted, HVO_4^{2-} and $H_2VO_4^-$ were the predominant V species. Also, pH was the only water quality characteristic that markedly influenced V speciation. At a pH of 6.23, $H_2VO_4^-$ represented 97.94% and HVO_4^{2-} 0.43% of total V (Figure 2.3). As pH increased to 8.80, the proportion of $H_2VO_4^-$ decreased to 42.10%, with a concomitant increase of HVO_4^{2-} to 57.68%, reaching approximately a 1:1 ratio at pH 8.80 (Figure 2.3). Within the concentration ranges of SO_4, alkalinity and Na tested here, the speciation modelling showed that there were only minor variations in the percentage of both V species (at a pH of approximately 8.2), with HVO_4^{2-} accounting for 14-30% and $H_2VO_4^-$ for 70-86 % of the total V present (data not shown).

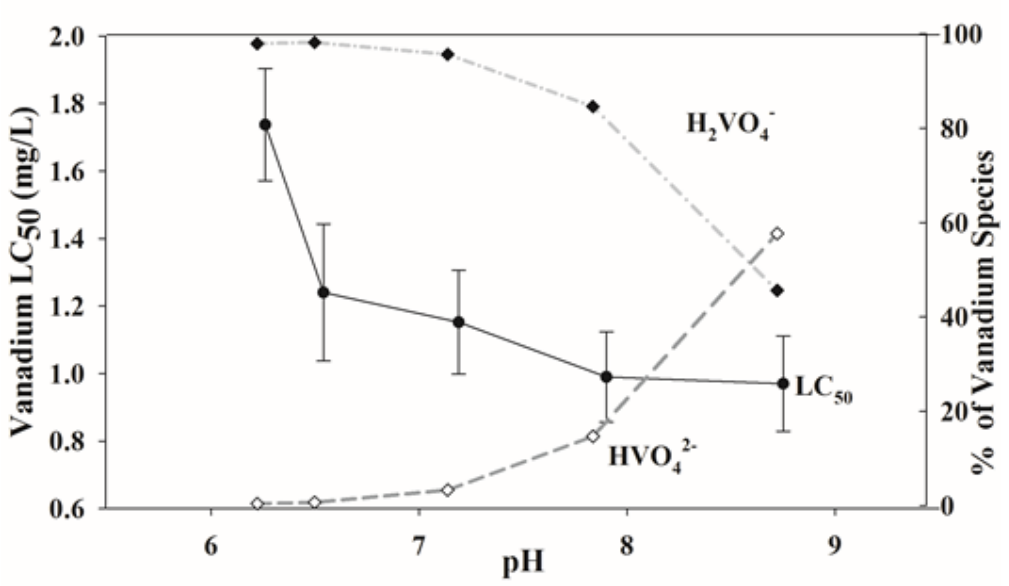

Figure 2.3: Change in dissolved vanadium speciation with pH and associated acute vanadium toxicity to *Daphnia pulex*. Dashed gray lines describes the speciation distributions obtained with Visual MINTEQ.

2.5. Discussion

Canadian water quality guidelines for the protection of aquatic life (CWQGs-PAL) are management tools used to help ensure that the introduction of substances into aquatic ecosystems do not degrade Canadian fresh and marine waters (CCME, 2007). An important step in the derivation of these guidelines is the identification and quantification of ETMFs to understand how water quality characteristics like pH, alkalinity and water hardness, among others, influence the substance's toxicity to aquatic organisms. The incorporation of ETMFs into CWQGs-PAL derivation allows the user to apply guidelines to a specific site with more certainty according to the site characteristics. In May 2016, ECCC released a federal environmental quality guideline for V by applying a species sensitivity distribution (SSD) to freshwater toxicity data and calculated a chronic value of 0.12 mg V/L for freshwater environments (ECCC, 2016). This guideline was created with only eight toxicity values published up until 2010 and did not consider ETMFs. Recently, Schiffer and Liber (2017b) expanded this V toxicity database and developed SSD models for both the acute and chronic toxicity of V to aquatic organisms. The calculated acute hazardous concentration endangering 5% of the species (HC_5) was 0.64 mg V/L, and the chronic HC_5 was 0.05 mg V/L (markedly lower than that of 0.12 mg V/L obtained by ECCC). The work presented here advanced this knowledge by investigating how water quality characteristics representative of the Alberta Oil Sands region influenced acute and chronic V toxicity to *D. pulex* (one of the most sensitive species to V). From the results, two main conclusions were drawn: First, some ETMFs do modify V toxicity, which was predominantly expressed as lethality, and second, pH was the most important ETMF that influenced acute V toxicity.

There are a few existing CCME CWQGs-PAL for metals that are corrected due to the effect of ETMFs in long-term exposures. Nickel (Ni), copper (Cu) and lead (Pb) long-term toxicity to

freshwater organisms are modified by water hardness within the range of 60 to 180 mg/L as $CaCO_3$ (CCME, 2018). For example, an increase in magnesium (Mg) will reduce Ni toxicity in daphnids due to competition in uptake (Niyogi and Wood, 2004). Similarly, an increase in calcium (Ca) due to an increase in water hardness will reduce Cu toxicity to both *Daphnia magna* and *Pimephales promelas* as a result of a competition between the metal and the ion for the binding site at the gill (Santore et al., 2001). Lead competes with both Ca and Mg for the binding site at the rainbow trout's gill, reducing its toxicity (Rogers et al., 2003; Rogers and Wood, 2004). The CCME CWQGs-PAL for aluminum (Al) is modified by pH. When pH is below 6.5, a guideline of 5 µg Al/L has been established, and when pH is above 6.5, the guideline for Al increases to 100 µg/L. Speciation was determined to be the main driver of Al toxicity to aquatic organism (Helliweli et al., 1983; Leivestad et al., 1987; Poléo, 1995).

Similar to Al, pH plays a significant role in V speciation and toxicity to aquatic organisms. Research done by Stendahl and Sprague (1982) found that the acute toxicity of V to *Oncorhynchus mykiss* (rainbow trout) decreased with a reduction in pH from 8.8 to 5.5. The authors suggested that this reduction was related to the change in V species. Within these pH values, HVO_4^{2-} and $H_2VO_4^-$ coexist and dominate the V species present. These species will also be the dominant and the most mobile species in well-aerated water bodies with a pH closer to 8 (Larsson et al., 2013; Rehder, 1991; Szalay and Szilágyi, 1967), like the Athabasca River, OPSW, or our test water. As described above, our findings suggest that there is an amelioration of V toxicity to *D. pulex* as pH decreased from 8.80 to 6.23. Speciation modelling for these tests indicated that the proportion of HVO_4^{2-} decreased from almost 57.68% at pH 8.8 to 0.4% at pH 6.23. Therefore, one plausible explanation for the decrease in V toxicity to daphnids as pH decreased could be that HVO_4^{2-} is the slightly more toxic V species to aquatic organism. However, since both HVO_4^{2-} and $H_2VO_4^-$ were

always present under the water quality conditions tested here, it is not possible to confirm whether one V species is more toxic to *D. pulex* than the other. Changes in pH also influence the acute toxicity of other metals like zinc (Zn), cadmium (Cd) and Cu to rainbow trout. When pH increased from 4.7 to 7.0, the 96-h LC_{50}s for Zn, Cd and Cu decreased from 671 to 66, 28 to <0.5, and 66 to 2.8 µg/L, respectively (Cusimano et al., 1986). In these cases, higher concentrations of metal were required to elicit toxicity at lower pH levels due to a competition between the cationic metal and H^+ for the ligand at the gill surface. This is not the case for V when pH is adjusted from 9 to 6 because the chemical structure of the V oxyanions hinders any possibility of uptake by H transporters and thus any competition with H (Rehder, 1991).

While pH had a clear influence on V toxicity to *D. pulex*, so did sulphate and alkalinity, and to a lesser extent sodium. For example, an increase in sulphate or alkalinity decreased acute V toxicity to *D. pulex*, whereas an increase in sodium above 300 mg/L increased acute V toxicity to *D. pulex*. Acclimation of daphnids to these increased ion concentrations may induce physiologic changes in the ionocytes to help them cope with the new environmental conditions (Baysoy et al., 2013; Soucek and Kennedy, 2005). These changes may involve an increase in ion transporters, down-regulation of enzymes like the sodium-potassium ATPase (NAK), or down-regulation of the H-VATPase (Piermarini and Evans, 2001, 2000). For instance, a 500-fold increase in water sodium concentration caused a 4-fold increase in sodium efflux in the fish *Platichthys flesus* (Motais et al., 1966). This higher salty water triggers regulatory mechanisms to restore salt balance (Maetz, 1971). Sulphate plays a key role in several cellular functions, like ion homeostasis in both mammalian and non-mammalian organisms, and bicarbonate is formed by carbon-anhydrase to maintain the acid-base balance in freshwater organism (Piłsyk and Paszewski, 2009; Schaechinger and Oliver, 2007). The amelioration of V toxicity to daphnids by these compounds could possibly

be explained by the orthovanadate species (HVO_4^{2-} and $H_2VO_4^-$) having a similar chemical structure to SO_4 and HCO_3 (Evans and Brusewitz, 1994; Rehder, 1991; Wehrli and Stumm, 1989). However, there is still debate as to whether SO_4 has a gill transporter that facilitates its uptake. Current research suggests that SO_4 is introduced to the animal through SO_4 permease and is removed by the antiporter SLC26 (Bayaa et al., 2009; Griffith, 2017). The antiporter SLC26 has been identified in fish gills, but there is no indication yet that SO_4 permeases are found in fish or invertebrates. Because of the similarity in structure between SO_4/HCO_3 and V, the latter could use the same carriers to get into and out of freshwater organisms (Avila et al., 2000; Brix et al., 2001; Codd et al., 2001; Ogle and Knight, 1996; Pomeroy and Haskin, 1954; Rainbow, 1997). An acclimation to higher SO_4 levels induced the up-regulation of SLC26 in freshwater elasmobranches (Piermarini et al., 2002), so that could possibly also occur in daphnia, and thus help the efflux of V, reducing V toxicity to *D. pulex*.

The mechanism(s) of toxicity of V to aquatic organisms are still unclear, but there are two main hypotheses: sodium imbalance due to inhibition of NAK, and oxidative stress. Studies done with brackish water hydroids and with vertebrates have found that V inhibits NAK (Bell and Sargent, 1979; Cantley et al., 1977; Ringelband, 2001; Valko et al., 2005). The increase in water sodium concentration above 300 mg/L in the present study, could have caused a decrease in NAK activity in daphnids as a coping mechanism to adapt to higher sodium concentrations. Conversely, the presence of V in the test water could have inhibited NAK in daphnids, thus impeded their physiological adaptation, causing an ion imbalance and thus lethality. However, the concentration of Na in the test water only reached 420 mg/L, which is almost nine times lower than the internal Na concentration. So, if V is not inhibiting the influx of Na, then V could be allowing Na to exit

the animal due to osmotic difference created by the interior and exterior Na concentrations. Further research is warranted to better understand the mechanisms of toxicity of V to aquatic organisms.

When *D. pulex* was exposed to increasing sulphate in the test water for 21 d, the chronic toxicity of V to *D. pulex* slightly increased. This increase in V toxicity could also be related to having more, or more active, permeases, resulting from chronic SO_4 exposure, allowing V to get into the daphnids, and thus increasing V toxicity. A parallelism could be drawn for alkalinity to explain the lack of ameliorating effect in the long-term alkalinity exposure, compared to the acute alkalinity exposure. However, it is possible that there could be other explanations for this phenomenon related to either the mechanism of uptake or toxicity of V.

To our knowledge, this is the first published research on the influence of water quality variables on V toxicity to aquatic organisms. Contrary to the influence on acute V toxicity to *D. pulex* seen for each water chemistry variable separately, some combination of these variables (sulphate and alkalinity, or sulphate and sodium) had only a minor effect on acute V toxicity and only when the concentrations of these variables combined were high. The mixture of high SO_4 and high Na ameliorated the acute V toxicity to daphnids, likely due to the presence of SO_4. Sulphate appears to have counteracted the increase in the acute V toxicity caused by high Na (325 mg/L), which could possibly be related to a combined effect of competition for the uptake carrier between V and SO_4, and a potential harmful role of V in sodium homeostasis. When both SO_4 and alkalinity were tested together, the combination of these ions had an ameliorating effect on acute V toxicity, with an LC_{50} that was almost double than that obtained when each ion was tested alone. This may be suggestive of an additive, ameliorating effect of these anions (hydrogen sulphate (HSO_4), SO_4, HCO_3, and carbonate (CO_3)) at low to intermediate concentrations, resulting from competition with the uptake of V. Further mechanistic studies are warranted to fully understand the mechanism

of toxicity of V. However, because sulphate modifies both acute V toxicity to *D. pulex* when alone and when in combination with another water quality characteristic, SO_4 could be considered as a second ETMF of importance in modifying acute V toxicity to freshwater Crustacea.

Counter-ions play an important role in the design and results interpretation of toxicity tests that evaluate the influence of water chemistry on metal toxicity. When salts are used, there is always a possibility that the presence of the counter-ion could influence or confound the toxicity caused by the metal ion of interest. For example, Gopalapillai et al. (2013) found that when SO_4 or Cl was the counter-ion present in calcium sulphate ($CaSO_4$) or calcium chloride (CaCl), they modified the influential effect that Ca had on the inhibition of root growth in *Lemna minor L.* caused by Ni. In our studies, Mg was always the counterion of SO_4, and it reached a maximum concentration of around 175 mg/L at the highest level of SO_4 tested (480 mg SO_4/L). Mount et al. (1997) exposed *D. magna* to magnesium sulphate ($MgSO_4$, same salt used here), and they reported a 48-h LC_{50} for $MgSO_4$ of 1,820 mg/L. In this concentration of $MgSO_4$, Mg is almost four times higher than the concentration of Mg used in the tests conducted here. Furthermore, the concentrations of Mg in both OSPW and AR are substantially lower than the concentrations present in the tests conducted here (Mg in OSPW averaged 12 mg/L and in AR 8.1 mg/L, Allen (2008a); White and Liber (2018)). Thus, Mg should not be significantly influencing the toxicity of V to *D. pulex* in the SO_4 tests. Another significant factor not studied here that can influence metal toxicity and is often considered for the correction of CWQGs-PAL is dissolved organic carbon (DOC). Cationic metals like Cu, Cd, or Pb, when present at the range of pH tested here, tend to complex with DOC, reducing their bioavailability (Di Toro et al., 2001). Within the ranges of pH representative of OSPW, AR and our test water, V is predominantly present as oxyanions, which hinders complexation with DOC. However, if pH of the water was below 6, the vanadyl ion

(protonated V) would start gaining predominance over the oxyanion species (Andersson et al., 2000; Rehder, 1991). Under such mildly acidic conditions, fulvic or humic acids could complex vanadyl, reducing the availability of dissolved V (Szalay and Szilágyi, 1967; Wang and Mulligan, 2006).

In conclusion, as indicated by Di Toro et al. (2001), "the use of total metal concentration to evaluate risk to aquatic organisms is well known to be inaccurate." Based on the work presented here, future development of a short-term CWQGs-PAL for V should consider the influence of pH and possibly SO_4 on the acute V toxicity to freshwater organisms. To better account for the influence of ETMFs on metal bioavailability, scientists have, over the past two decades, developed the Biotic Ligand Model (BLM) (Di Toro et al., 2000; Santore et al., 2001). The BLM is a modelling tool that is based on the theory that metal competition, complexation and interaction at the site of action of toxicity (the ligand) needs to be considered when evaluating metal toxicity to aquatic organisms (Di Toro et al., 2001). While a BLM could possibly be developed, such a model presently does not exist. It would, therefore, be wise in the future to investigate the applicability of the BML for calculation of CWQGs-PALs for V, or initially use the equations developed here as ETMFs of acute V toxicity in aquatic systems within the Alberta oil sands region.

CHAPTER 3: MULTIPLE LINEAR REGRESSION MODELLING PREDICT THE EFFECTS OF SURFACE WATER CHEMISTRY ON ACUTE VANADIUM TOXICITY TO MODEL FRESHWATER ORGANISMS.

Preface

To continue with the investigation done in Chapter 2, a set of acute toxicity tests where pH, alkalinity, sulphate (SO_4) and sodium were modelled to evaluate how these ETMFs influenced V toxicity to *O. mykiss*. The models created for *O. mykiss* were compared to those obtained in Chapter 2 for daphnids. To assess how combinations of the 96-h median lethal concentration (LC_{50}) and associated water chemistry from the above-mentioned acute toxicity tests, multiple linear regression (MLR) models for *O. mykiss* and *Daphnia pulex* were developed. The MLR models predicted that pH and alkalinity played a key modifying role over V toxicity to both species, whereas SO_4 only influenced V toxicity to *D. pulex*. The MLR predictions were highly accurate since they predicted that acute LC_{50}s for both species were within a factor of two from the observed LC_{50}s. Overall, MLR models for aquatic organisms should be considered for creating new local benchmarks or water quality guidelines for V for the protection of aquatic life.

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3.1. Abstract

Multiple Linear Regression (MLR) modelling has been successfully used to predict how water chemistry variables influence the toxicity of cationic metals to aquatic organisms. To date, no MLR model exists for vanadium (V). Recent research has indicated that an increase in pH (from 6 to 9), or high concentrations of sodium (>300 mg Na/L), increase V toxicity to *Daphnia pulex*, whereas increases in alkalinity (> 100 mg as $CaCO_3$) and sulphate (>100 mg SO_4/L) reduce V toxicity. How these variables influence V toxicity to *Oncorhynchus mykiss* was still unknown. Results presented here showed that increasing pH from 6.2 to 8.9, decreased the 96-h median lethal concentration (LC_{50}) for V toxicity to *O. mykiss* from 15.6 to 6.0 mg V/L. An alkalinity increase from 71 to 330 mg/L as $CaCO_3$, increased the 96-h LC_{50} from 5.7 to 9.0 mg V/L, whereas when sulphate rose from 150 to 250 mg/L, the LC_{50} increased from 7.1 to 7.4 mg V/L followed by a decrease to 6.4 mg V/L. Sodium (between 100 and 336 mg/L) showed no effect on V toxicity to *O. mykiss*. The toxicity patterns for *O. mykiss* were similar to those observed for *D. pulex*, except for that of sulphate, potentially indicating different mechanisms of V uptake or regulation in the two species. The individual LC_{50}s and associated water chemistry were combined to develop an MLR model for *O. mykiss* and *D. pulex*. Alkalinity and pH modified V toxicity to both species, whereas sulphate only influenced V toxicity to *D. pulex*. Overall, MLR models should be considered for creating new local benchmarks or water quality guidelines for V.

3.2. Introduction

Vanadium (V), a trace metal widely distributed in the Earth's crust, has become an element of concern for the Alberta Oil Sands region (CBC News, 2018). High concentrations of V can be introduced in reclaimed end-pit lakes if petroleum coke (PC) is used as a substrate (Gosselin et al.,

2010; Zubot et al., 2012). Petroleum coke is enriched with V during the bitumen coking process. When V-enriched PC is used to complex organic chemicals in oil sands process-affected water (OSPW) stored in tailings ponds, V is released to the OSPW, reaching concentrations up to 7 mg V/L within the first 100 days of treatment (Allen, 2008a; Chalaturnyk et al., 2002; Zubot et al., 2012). Previous publications have indicated that concentrations up to 7 mg V/L, V can cause acute and chronic toxicity to aquatic organisms from various trophic levels (Bell and Sargent, 1979; Beusen and Neven, 1987; Gillio Meina et al., 2019; Puttaswamy and Liber, 2011; Schiffer and Liber, 2017b, 2017a). For example, the 48-h median lethal concentrations (LC_{50}s) for *Ceriodaphnia dubia* and *Daphnia pulex* were found to be between 0.6 and 2.5 mg V/L, whereas 96-h LC_{50}s of *Pimephales promelas* (fathead minnow) and *Oncorhynchus mykiss* (rainbow trout) were found to be between 4 and 7 mg V/L (Puttaswamy et al., 2010; Schiffer and Liber, 2017b, 2017a; Stendahl and Sprague, 1982).

Only a small number of peer-reviewed publications have investigated V toxicity to aquatic organisms (CCME, 2019). As a result, the Canadian Council of Ministers of the Environment (CCME) has not yet been able to create Canadian water quality guidelines for V to protect aquatic life (CWQG-PAL). Although Schiffer and Liber (2017b) recently published an acute and chronic species sensitivity distributions (SSDs) useful for the development of CWQGs-PAL (CCME, 2007), this research did not consider the influence of exposure or toxicity modifying factors (ETMFs) on V toxicity to aquatic organisms. Incorporating ETMFs is critical in applying guidelines to a specific site because the particular influence of water chemistry on the toxicity of a chemical is accounted for (CCME, 2007, CCME, 2003).

Several publications have suggested that ETMFs, such as water hardness, alkalinity, pH, and dissolved organic carbon (DOC), modify metal toxicity to aquatic organisms by reducing or

enhancing their toxicity (de Schamphelaere et al., 2005; Heijerick et al., 2003; Meyer et al., 1999). For example, in a set of standardized toxicity tests with *D. pulex*, nickel (Ni) toxicity decreased by a factor of about 18 when calcium (Ca) concentrations increased by about 48.1 mg/L, due to increased competition between nickel (Ni) and Ca for binding to the biotic ligand (Kozlova et al., 2009). Cusimano et al. (1986) found that when pH increased from 4.7 to 7.0, the 96-h LC_{50}s for zinc (Zn), cadmium (Cd), and copper (Cu) for *O. mykiss* decreased from 671 to 66, 28 to <0.5, and 66 to 2.8 µg/L, respectively. Additionally, an increase in water hardness from 25 to 120 mg/L as $CaCO_3$, in DOC from 0 to 4 mg/L and in pH from 6 to 8 increased the 7-d EC_{10} for growth of *P. promelas* from 93 to 4,672 µg of total aluminum (Al)/L (Gensemer et al., 2018). The modifying effect of water hardness on Al is mostly due to the competition between the hardness cations (Ca and magnesium (Mg)) and cationic metals, whereas DOC forms complexes between fulvic or humic acids and cationic metals like Cu, Cd, or lead (Pb). Conversely, the variation in pH changes metal speciation, which, in turn, influences metal toxicity. For instance, Stendahl and Sprague (1982) showed that acute V toxicity in *O. mykiss* was ameliorated by a decrease in pH from 8.8 to 5.5, and the authors suggested that a change in speciation was the main factor. Gillio Meina et al. (2019) recently showed that modifications in pH, alkalinity, and sulphate (SO_4) influences V toxicity to *D. pulex*. Briefly, an increase in pH from 6 to 9 increased V toxicity to *D. pulex*, probably as a result of changes in V speciation. In contrast, an increase in alkalinity from 70 to 350 mg/L as $CaCO_3$ and in SO_4 from 55 to 540 mg/L reduced the toxicity of V to *D. pulex*, possibly due to competition between the V oxyanions (HVO_4^{2-} and $H_2VO_4^-$) and bicarbonate (HCO_3), or SO_4 for binding to the biotic ligand. When *D. pulex* were exposed to a combination of alkalinity between 80 and 311 mg/L as $CaCO_3$ and SO_4 between 87 and 280 mg/L, the mean 48-h LC_{50} doubled the 48-h LC_{50} obtained when one water quality variable (either alkalinity or SO_4) was

tested at a time (Gillio Meina et al., 2019). These results show that pH, alkalinity and SO_4 are the most influential ETMFs for V toxicity to *D. pulex*. However, there is no information on how these ETMs influence the toxicity of V to model fish species. Nor is it known if the influence of a combination of several ETMFs could differ from the effects of the individual ETMFs on V toxicity to both invertebrate and fish species.

In the environment, there are multiple ETMFs whose combinations may be different from the influence of the individual ETMFs on metal toxicity (Di Toro et al., 2001; Santore et al., 2001). Also, because the counter ions of the metal salts used in toxicity tests can create undesirable confounding effects, evaluating multiple ETMFs in a laboratory scenario is complicated and may misrepresent environmental conditions (Gopalapillai et al., 2013). Thus, to overcome this difficulty, scientists have developed a computational approach known as the Biotic Ligand Model (BLM) that models the toxicity of metals to aquatic organisms, accounting for the effects of water chemistry on metal speciation and the biological availability of metals to aquatic organisms. The BLM is based on the assumption that the metal concentration that reaches the biotic ligand is influenced by competition with naturally occurring ions (Ca, sodium (Na), Mg, proton (H), Cl, SO_4, HCO_3) and other ligands (e.g., DOC), and that toxicity occurs when the concentration of a metal at the biotic ligand (e.g., gills in fish) exceeds the threshold concentration (Di Toro et al., 2001, 2000; Niyogi and Wood, 2004). There are two approaches to developing a BLM model: one is based on measuring the concentration of metals accumulated in the gill for each scenario of a modified water chemistry condition; the second is a mathematical derivation of the stability constants from known toxicity data and a broad range of water chemistry measurements (de Schamphelaere et al., 2005; de Schamphelaere and Janssen, 2002; Niyogi and Wood, 2004). However, these two approaches often present difficulties for regulators: the first requires a

substantial number of animals and human labour, and the second requires complicated calculations that sometimes make the model unsuitable for the scenario being investigated. Consequently, a third tool (Multiple Linear Regression, MLR) is gaining popularity and could become a valuable resource for the development of CWQGs for V (Brix et al., 2017; DeForest et al., 2018; Esbaugh et al., 2011). The MLR model is created from the hardness-based water quality criteria (WQC) but incorporates both a higher number of ETMFs and information on the potential interaction among these key variables (DeForest et al., 2018). Compared to BLM, MLR has been equally accurate in developing a WQC for Cu toxicity to four daphnid species and the fathead minnows. The predicted MLR WQC was within a factor of two from the BLM criteria, confirming that MLR could be a valuable and user-friendly tool for regulators (DeForest et al., 2018). Because no current model can predict how ETMFs influence V toxicity to aquatic organisms, it is imperative to develop a model that can facilitate the future creation of a site-specific CWQG-PAL.

The objectives of the present study were to (i) investigate how individual ETMFs representative of the Alberta oil sands region modify the acute toxicity of V to *O. mykiss*; (ii) develop predictive relationships between V and the most influential water quality variables, and compare these relationships with those found for *D. pulex* by Gillio Meina et al. (2019; Chapter 2); (iii) develop an MLR model that describes the influence of ETMFs on V toxicity to aquatic organisms by combining the recent results presented by Gillio Meina et al. (2019; Chapter 2) and those obtained from objectives (i) and (ii) above.

3.3. Materials and methods

3.3.1. Chemicals

Sodium metavanadate ($NaVO_3$, anhydrous, min. purity 96%) and magnesium sulphate ($MgSO_4$) were purchased from Strem Chemicals Inc. (Newburyport, MA, USA) and Sigma-Aldrich, (Oakville, ON, Canada), respectively. The remaining chemicals were obtained from Fisher Scientific (Ottawa, ON, Canada). All the chemicals used were of analytical grade.

3.3.2. Animal culturing and holding

Oncorhynchus mykiss (rainbow trout) eyed-eggs were purchased from TroutLodge Ltd. (Bonney Lake, WA, USA) and reared at the Aquatic Toxicology Research Facility (ATRF), Toxicology Centre, University of Saskatchewan. Once the larvae hatched, they were transferred to 20-L tanks in an environmental chamber in the Toxicology Centre. The temperature in the environmental chamber was 15 $\pm$ 1 ºC, with a photoperiod of 16 h light: 8 h dark (light intensity was approximately 400 lux). A ratio of 70% bio-filtered, C-filtered municipal Saskatoon city water (DeCl), and 30% reverse osmosis (RO) water served as culture water. This ratio was slightly adjusted periodically (DeCl:RO ratio modified by less than 25%) to cope with small seasonal changes in the chemistry of the source water. This culture water was used to simulate Athabasca River water (ARW). The simulated ARW was prepared in 50-L plastic carboys and aerated for at least 24 h before being used in culturing or for toxicity tests to remove any chlorine from the water. The ranges in the water chemistry variables between the ARW and the OSPW (Table 3.1) chosen for investigation were based on previous research conducted on *D. pulex* by Gillio Meina et al. (2019; Chapter 2). Briefly, the ions of interest were added to the simulated ARW to obtain the ranges in water chemistry conditions for acclimation and toxicity tests using their specific salts

(Table A1-A2). When the effect of pH on V toxicity was tested, glacial-acetic acid ($C_2H_4O_2$) or sodium hydroxide (NaOH) was used to reach the desired pH. The pH in the fish aquaria was maintained at 7.5 – 8.5, except for two groups of larvae that were adjusted to pH 6 and pH 9. Fish larvae were acclimated (without V) until they reached the swim-up stage (approximately 12 d). During acclimation and through the toxicity test, larvae were not fed, following Environment and Climate Change (ECCC) protocols (ECCC, 1998). Water changes were periodically conducted to maintain low levels of ammonia (<0.02 mg/L) and high dissolved oxygen (≥ 8.0 mg/L). The V stock solutions were prepared the day before a toxicity test began in 50-L plastic carboys and then diluted with culture water to reach the desired V concentrations via serial dilution (a factor of two was used). Fish holding and testing was done in accordance to Animal Use Protocol #20140036 (University of Saskatchewan).

Table 3.1: Nominal test levels for water chemistry variables and major ions (mg/L) representative of the Athabasca River and oil sands process-affected water (OSPW) used in acute toxicity tests.

Test /Variable	Salt used	Nominal levels
pH	-	6-7-**8**-9
Alkalinity	$NaHCO_3$	70-**100**-200-300
Na	CHO_2Na	100-200-300-400
SO_4	$MgSO_4$	100-200-300-380

Numbers in bold indicate the water chemistry of the in-house prepared Athabasca River water that corresponds to approximate environmental concentrations.

3.3.3. Toxicity testing procedures

The *O. mykiss* toxicity tests were initiated when more than 50% of the larvae reached the swim-up stage. Following (ECCC, 1990b) protocols and the recent work of Schiffer and Liber (2017), *O. mykiss* larvae were exposed for 96 h to a control (untreated) and four geometrically increasing concentrations of V ranging from 2.18 to 35 mg V /L, with five replicates per level and 10 larvae per experimental unit. The pH of the test water was adjusted before each test initiation

with $C_2H_4O_2$ or NaOH to minimize unintended confounding effects of pH on the influence of an ETMF on V toxicity. Test vessels consisted of 4-L acid-washed glass jars with polypropylene lids. Except when the effect of pH on V toxicity was tested, a homemade biofilter containing cotton batten imbibed with Stability® water conditioner (Seachem Laboratories, Madison, GA, USA) and an internal air stone was installed inside the jars to help control ammonia levels (Figure B1). The jars were filled to maximum capacity and tightly sealed to reduce the presence of air bubbles and avoid pH drift. When the influence of pH was tested, the biofilter was installed without aeration, because bubbling was accidentally creating an overhead space between the lid and the test water. Mortality was defined as a lack of movement following gentle prodding and was recorded at 96 h. A test was considered acceptable when mortality in the control group was $\leq$ 10%. All tests met the requirements outlined earlier.

3.3.4. Chemical analysis

Water samples (20 mL) were randomly collected from each treatment at the beginning and end of the test (96 h) in triplicates for analysis of temperature, dissolved oxygen (DO), pH, alkalinity, water hardness, and ammonia (Table B1). Measurements of pH were recorded using an OPRIM PerpHect Log R meter model 370. Temperature and DO concentrations were measured using an ORION DO meter model 835 (ORION Research, Beverly, MA, USA). Alkalinity and hardness were measured using a Hach digital titrator model 16900 (Hach Company, Loveland, CO, USA). Ammonia was measured using an Orion Aquafast®II meter (Thermo Electron, Waltham, MA, USA).

Before any major ion and V analyses were performed, samples were filtered using a syringe filter with a 0.45-µm polyethersulfone membrane (VWR International, Mississauga, ON, Canada).

Additionally, samples for V analysis were acidified to 2% acid with high purity HNO_3 (Fisher Scientific, Ottawa, ON, Canada). The dissolved concentrations of Na and SO_4 were measured in-house using ion chromatography (Dionex ICS-3000, Sunnyvale, CA, USA). Samples collected for V analysis were measured in-house using an inductively coupled plasma mass spectrometer (ICP-MS) equipped with collision cell technology (Agilent Technologies 8800 ICP-MS Triple Quad, Mississauga, ON, Canada). The limit of detection for V was 0.05 µg V/L. River-water standard reference material (SLRS-5; National Research Council, Ottawa, ON, Canada) and natural water standard reference material (1640a, National Institute of Standard and Technology, Gaithersburg, MD, USA) were used in QA/QC for ICP-MS measurements. The accuracy of SLRS-5 and 1640a analyses were always $\geq$ 85%, and the precision of duplicates exceeded 90% in all cases.

3.3.5. Multiple linear regression (MLR) models

The Multiple Linear Regression (MLR) analyses were conducted as described by Brix et al. (2017). The ETMFs used to develop the V MLR were pH, alkalinity and SO_4 because these variables were found to have the most influence on V acute toxicity to daphnids within ranges of water chemistry representative of both the Athabasca River and OSPW (Gillio Meina et al., 2019; Chapter 2). Alkalinity, sulphate and V LC_{50}s were log-transformed (pH was not log-transformed because it is a log-scaled value) and then incorporated into a stepwise MLR analysis following the basic model (Eq. 3.1):

$$\ln(\text{toxicity}) = b_0 + b_1*\text{pH} + b_2*\ln(\text{alkalinity}) + b_3*\ln[SO_4] + \text{error} \quad \text{(Eq. 3.1)}$$

Additionally, any possible interactions between these water quality variables were tested following the basic model (Eq. 3.2):

$$\ln(\text{toxicity}) = b_0 + b_1*pH + b_2*\ln(\text{alkalinity}) + b_3*\ln[SO_4] + b_4*(pH*\ln(\text{alkalinity})) + b_5*(pH*\ln[SO_4]) + b_6*(\ln(\text{alkalinity})*\ln[SO_4]) + \text{error} \quad \text{(Eq. 3.2)}$$

where b_0 is the intercept and b_1-b_6 are the slopes of the mathematical model.

The models that best fitted the data were determined by comparing the adjusted r^2, and the Akaike (AIC) and Bayesian (BIC) information criteria. The model with the highest adjusted r^2 and the smallest AIC or BIC was considered the best-fitted model. Additionally, variance inflation factors (VIFs) were calculated for the independent variables to assess any potential collinearity between them (Brix et al., 2017; DeForest et al., 2018). In the case of collinearity between the independent variables, a mean centering will be conducted, and the model will be adjusted accordingly. The predicted values from the stepwise MLR models were plotted against the observed LC_{50}s along a one-to-one line to visually determine the goodness-of-fit of each final model.

The MLR models for *D. pulex* and *O. mykiss* were validated by using the water chemistry and LC_{50}s previously reported by Schiffer and Liber (2017b, 2017a) and Stendahl and Sprague (1982). Because *D. dentifera* presents a very similar physiology and size to *D. pulex*, the observed LC_{50} concentration and water chemistry presented by Schiffer and Liber (2017b) were also included.

3.3.6. Statistical analyses, inter-specific comparison and V speciation

The moving average TOXCALC 5.0 (Tidepool Scientific, CA, USA) or Spearman-Karber method version 1.5 (Hamilton et al., 1977) were used to calculate the acute LC_{50} estimates with 95% confidence intervals. The curve estimation of SPSS version 20.0 (IBM, CA, USA) was used to obtain the regression models between each water quality variable and acute V toxicity (as LC_{50})

to *O. mykiss*, and the model was chosen according to the best fitted dataset. The regression models used in the current paper can be obtained from the following web address: https://www.ibm.com/support/knowledgecenter/en/SSLVMB_23.0.0/spss/base/curve_estimation_models.html

The regression models obtained for each combination of a water chemistry variable and V for *O. mykiss* were then compared with those for *D. pulex* recently published by Gillio Meina et al. (2019; Chapter 2). Multiple linear regression models and all the statistic parameters described in section 2.5 were also obtained using SPSS. Assumptions of normality and homogeneity of variance were checked using Shapiro-Wilk's W test. Equality of variance was assumed when there was a random distribution of the residuals in standardized residuals versus standardized predicted values plots.

The speciation of V was modelled from the mean measured concentration of dissolved V and the ions evaluated in each acute exposure scenario using Visual MINTEQ (KTH, Department of Land and Water Resources, Stockholm, Sweden), as described in detail at (https://vminteq.lwr.kth.se/download/).

3.4. Results

3.4.1. Exposure conditions and test acceptability

Oncorhynchus mykiss swim-up larvae successfully adapted to the tested water quality conditions, and none of the water quality variables evaluated (without V) caused mortality to the fish during the acclimation period (12 d). In all the combinations of water chemistry variables and V concentrations tested, the nominal concentrations were reasonably similar to the measured concentrations, except for the final measured concentration of sodium, which was lower than the

nominal concentration. Thus, to derive the descriptive models, only the measured data were used. The only parameter that showed a marked change was conductivity, which was due both to the use of sodium metavanadate ($NaVO_3$) as a source of V and to the salts chosen as a source of sodium (sodium formate) and alkalinity (sodium bicarbonate). Despite the increase in conductivity, the speciation results obtained from Visual MINTEQ modelling indicated that the oxyanions (HVO_4^{2-} and $H_2VO_4^-$) of monomeric V(V) were the dominant species in all the studies conducted here. The mortality of the control fish was always below 10%.

3.4.2. Effects of ETMFs on acute V toxicity to Oncorhynchus mykiss

The models developed here for *O. mykiss* were compared with the models previously obtained for *D. pulex* by Gillio Meina et al. (2019, Figure 2.1). The relationships between water chemistry and the toxicity of V to *D. pulex* are reproduced here to facilitate the comparison between both species (details about the regressions for *D. pulex* can be found in Chapter 2). The effect of pH on acute V toxicity to rainbow trout followed an S-curve with a slightly non-significant (IBM-SPSS, 2018) inflection point at pH 7.1 (Figure 3.1a, $r^2 = 0.895$; $p = 0.054$, Table 3.2). Briefly, as pH increased from 6.2 to 8.99, the 96-h LC_{50} for V for rainbow trout showed a constant curvilinear decrease from 15.6 to 6.00 mg/L, increasing V toxicity to *O. mykiss*.

Alkalinity slightly ameliorated the acute toxicity of V to rainbow trout, following a non-significant linear regression (IBM-SPSS, 2018; Figure 3.1b; $r^2 = 0.854$; $p = 0.076$; Table 3.2). The effect of alkalinity on acute V toxicity to *O. mykiss* showed an increase of only 1.6-fold from an LC_{50} of 5.66 mg V/L at 71 mg/L as $CaCO_3$ to an LC_{50} of 9.02 at 330 mg/L as $CaCO_3$ (Figure 3.1b).

The influence of SO_4 on acute V toxicity to rainbow trout followed a significant quadratic relationship (IBM-SPSS, 2018; $r^2 = 0.998$; $p = 0.024$, Figure 3.1c; Table 3.2). An increase in SO_4

from 150 to 250 mg/L decreased acute V toxicity from an LC_{50} of 7.31 to LC_{50} of 7.39 mg V/L. However, when SO_4 was above 250 mg/L, the acute toxicity of V to rainbow trout slightly increased from an LC_{50} of 7.39 to an LC_{50} of 6.36 mg V/L.

The results also indicated that the increase in Na at levels below 336 mg Na/L for *O. mykiss* did not affect the acute V toxicity, displaying a 96-h LC_{50} mean of 9.25 mg V/L (Figure 3.1d, Table 3.2).

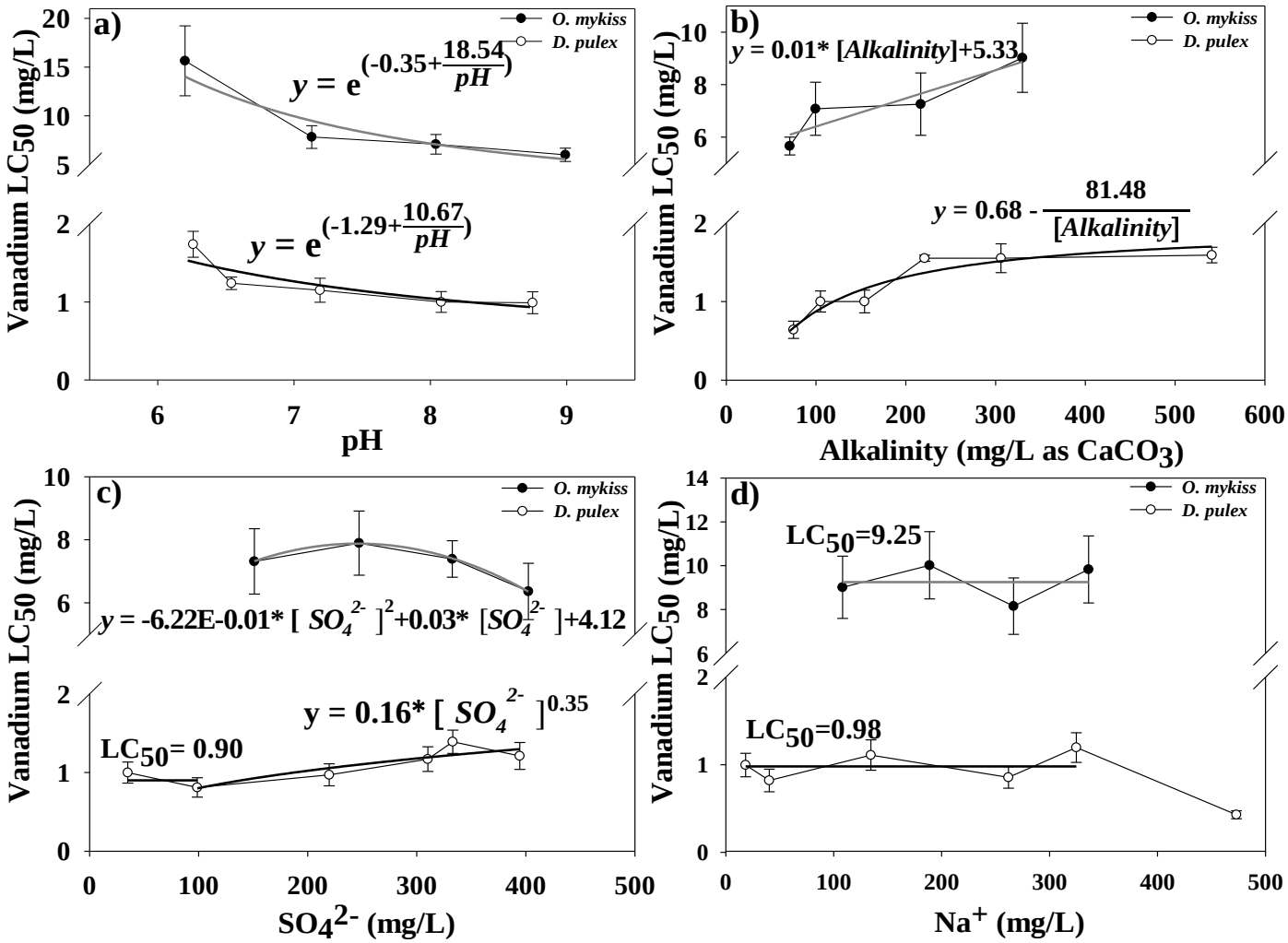

Figure 3.1: Descriptive models for the acute effects of increasing: a) pH; b) alkalinity; c) sulphate; and d) sodium, representative of Athabasca River water and OSPW on vanadium toxicity to *Oncorhynchus mykiss* and *Daphnia pulex*. Brackets represent the concentration of sulphate in mg/L or alkalinity in mg/L as $CaCO_3$. Error bars represent the 95% confidence intervals of the LC_{50} estimates. Thick solid lines describe either the statistical model or the geometric mean obtained from the experimental LC_{50} values (mg/L).

Table 3.2: Summary table of acute toxicity models for vanadium to *Oncorhynchus mykiss* developed for a range of alkalinity, pH, sulphate, and sodium concentrations under 96-h exposures.

Test/Variable	Equation or Geometric Mean	r^2	p[a]
pH	y = exp (-0.35 + (18.54/[pH]))[b]	0.895	0.054
Alkalinity	y= 0.01*[Alkalinity]+5.33[b]	0.854	0.076
Sulphate	**$y = -6.22E\text{-}0.01*[SO_4]^2+0.03*[SO_4]+4.12$**	0.998	0.024
Sodium	**9.25**	0.005	0.927

[a] p = p-value obtained for the mathematical model.
N/A = not applicable.
[b] Not bolded represents the model that best fit the data, but it was not statistically significant.

3.4.3. Multiple linear regression model (MLR) for V

The best-fitted MLR model obtained for *D. pulex* slightly differed from the one for *O. mykiss*: the daphnid model presented interactions between pH and alkalinity and between alkalinity and SO_4 with a slight mean centering correction to reduce collinearity between the individual variables, whereas the trout model included only the individual effects of pH and alkalinity (Table 3.3). These models predicted LC_{50} values for *D. pulex* and *O. mykiss* that were within a factor of two from the observed LC_{50}s (Figure 3.2a and 3.2b). The AIC and BIC for the models mentioned above for daphnids and trout were the lowest, confirming their best fit. The AIC for *D. pulex* was 54.5, whereas it was 48.1 for trout; On the other hand, the BIC was 56.1 for daphnids and 49.0 for trout (Table B2). Analysis of the adjusted r^2 between the models with and without the interaction of ETMFs indicated that the best-fitted daphnid model had interactions and presented an adjusted r^2 of 0.601, whereas the best-fitted trout model had no interaction and presented an adjusted r^2 of

0.711 (Table B2). The calculated VIFs for both models were below 2.0, indicating a low correlation among the independent variables. For the daphnid model, the VIFs for pH, alkalinity, and SO_4 were 1.37, 1.46, and 1.19, respectively, whereas the trout model's VIFs for pH and alkalinity equalled 1.27 (Table B3).

The calculated acute LC_{50}s for V with MLR for *D. pulex* and *O. mykiss* for a worst-case scenario (pH = 9, alkalinity = 70 mg/L as $CaCO_3$ and sulphate = 54 mg/L) were very similar to those observed acute LC_{50}s obtained for *D. pulex* when pH was 8.0, alkalinity was 75 mg/L as $CaCO_3$, and sulphate 54 mg/L (Chapter 2), and for *O. mykiss* when water pH was 7.8, and alkalinity was 71 mg/L as $CaCO_3$ (this Chapter). The MLR models for *D. pulex* and *O. mykiss* accurately predicted a similar acute LC_{50} for V for *D. dentifera* (2% difference, Schiffer and Liber (2017b)) and *O. mykiss* (0% difference, Stendahl and Sprague (1982)), but estimated two-fold lower acute LC_{50}s for V for *D. pulex* and *O. mykiss* than those calculated by Schiffer and Liber (2017b, 2017a) (Table 3.4).

Table 3.3: Stepwise multiple linear regressions models for *Daphnia pulex* and *Oncorhynchus mykiss*.

Species	Multiple Linear Regression model	*n*	r^2	p^a
D. pulex	ln(tox) = -0.01 + 0.65*[(pH-7.88)*(ln (alkalinity)-4.77)] - 0.40*[(ln (alkalinity)-4.77)*(ln [SO_4]-4.43)	17	0.651	0.001
O. mykiss	ln (tox) = 3.19 - 0.36*pH + 0.35*[ln (alkalinity)]	12	0.764	0.002

[a] *p* = p-value obtained for the mathematical model.

Table 3.4: Validation of the multiple linear regression models for *Daphnia pulex* and *Oncorhynchus mykiss* by comparing a worst-case scenario as proposed by the CCME (Canadian Council of Ministers of the Environment, 2007) to an observed toxicity median concentrations (LC_{50}) and tested on data from previously peer-reviewed publications.

Model tested	pH	Alkalinity	Sulphate	Modelled LC50 (mg/L)	Observed LC50 (mg/L)	Reference
D. pulex (WCS)	9.0	70	54	0.6	0.6[a]	Gillio Meina et al. (2019)
D. pulex	8.3	98	33	0.9	2.2	Schiffer and Liber (2017b)
D. dentifera	8.2	92	33	0.9	0.9	Schiffer and Liber (2017b)
O. mykiss (WCS)	9.0	70	-	4.4	5.7[b]	This study
O. mykiss	8.8	96	-	5.3	5.4	Stendahl and Sprague (1982)
O. mykiss	8.0	89	-	6.9	14.8	Schiffer and Liber (2017a)
WCS = Worst Case Scenario (CCME, 2007)	9	70	54			

[a] Observed LC_{50} was obtained with a water pH of 8.0, an alkalinity of 75 mg/L as $CaCO_3$, and a sulphate concentration of 54 mg/L.

[b] Observed LC_{50} was obtained with a water pH of 7.8 and an alkalinity of 71 mg/L as $CaCO_3$.

- Not applicable in the model.

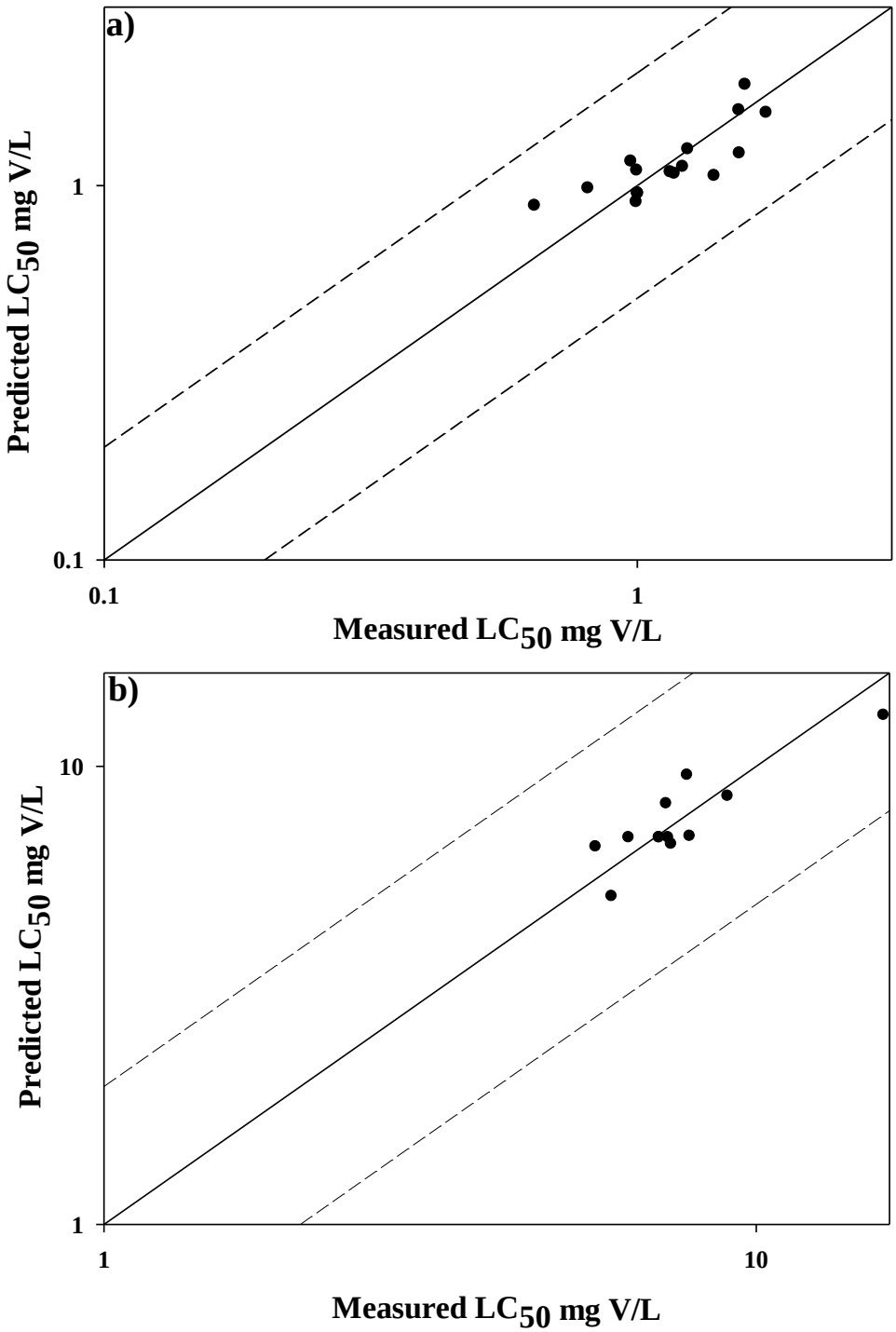

Figure 3.2: Predicted vs. measured acute LC_{50}s for a) *Daphnia pulex* and b) *Oncorhynchus mykiss* obtained by applying stepwise multiple linear regression modelling. The solid line represents a line of perfect agreement between measured and predicted LC_{50}s, and dashed lines represent ± a factor of two from the line of perfect agreement.

3.5. Discussion

The results presented here reinforce the findings of Gillio Meina et al. (2019), who reported that V toxicity to *D. pulex* was influenced by pH, alkalinity, SO_4, and, to a lesser extent, by Na. Although pH, alkalinity, and Na modified V toxicity to *O. mykiss* in a similar manner as for *D. pulex*, both *O. mykiss* and *D. pulex* presented slightly different models for SO_4. Because the relationships between these water variables and V toxicity to daphnids are thoroughly discussed by Gillio Meina et al. (2019) (Chapter 2), here we focus on the similarities and differences between the models obtained for *D. pulex* and *O. mykiss*.

Among the individual variables tested, pH was the most influential ETMF modifying V acute toxicity to *O. mykiss*. The models for the influences of pH on V toxicity to rainbow trout, and alkalinity on V toxicity to rainbow trout, were marginally non-significant ($p = 0.054$ and $p = 0.076$, respectively) at $\alpha = 0.05$. Had the α value been set at 0.1, that would have turned both individual relationships significant. However, it was decided in the present study to leave α at 0.05 to protect for statistical error I. The models presented for pH and alkalinity in Figure 3.1, even though statistically non-significant, show relationships that are likely biologically significant. Here, an increase in pH from 6 to 9 substantially increased V toxicity to *O. mykiss*. Water pH plays a major role in V speciation, modifying the chemical structure of V and the proportion of different V species (Rehder, 1991). Between pH 6 and 9 (the range most commonly found in the majority of aquatic environments), the oxyanions HVO_4^{2-} and $H_2VO_4^-$ are the most abundant species of V. The chemical structure of these oxyanions could facilitate the uptake of V through anionic carriers in the gills of these aquatic organisms, like the carriers used by SO_4, HCO_3, and/or phosphate HPO_4 (Avila et al., 2000; Brix et al., 2001; Codd et al., 2001; Ogle and Knight, 1996; Pomeroy and Haskin, 1954; Rainbow, 1997). This hypothesis is supported by the results found here for *O.*

mykiss and in Chapter 2 for *D. pulex*, since in both cases when pH remained constant at around 8 and V species remained approximately constant, V toxicity to *O. mykiss* or *D. pulex* decreased as alkalinity increased, and V toxicity to *D. pulex* decreased with an increase of SO_4 in the test water. The decrease in V toxicity to these organisms observed with increasing alkalinity is probably the result of an enhanced competition between the V oxyanions and HCO_3 and/or CO_3 for binding to the biotic ligand or the anion transporter used to get into the animal. Furthermore, HCO_3 and SO_4 present very similar chemical structures, and permeases have been suggested as the potential carriers for both HCO_3 and SO_4, and thus probably for V as well (Avila et al., 2000; Brix et al., 2001; Codd et al., 2001; Ogle and Knight, 1996; Pomeroy and Haskin, 1954; Rainbow, 1997). In Chapter 2, results indicated that in daphnids, SO_4 seems to compete with the V oxyanions for the same anionic carriers within the range of SO_4 concentrations tested (100 - 400 mg SO_4/L). However, in the results presented here for rainbow trout, the pattern was not clear because although SO_4 had an ameliorating effect on V toxicity between 150 and 250 mg/L, above 250 mg/L, SO_4 slightly enhanced V toxicity to fish. These findings could either imply that V is entering the fish through additional anion carriers once the SO_4 carriers reach maximum transport capacity, or that concentrations of SO_4 above 250 mg/L are stressful for *O. mykiss,* and the exposure to V causes additional physiological stress. Hobe (1987) found that high SO_4 concentrations in water jeopardized the ion homeostasis of fish, altering the rate, transfer, or electroneutrality of other ions, potentially explaining the enhanced V toxicity to *O. mykiss* seen above 250 mg SO_4/L.

Sodium did not affect V toxicity to *O. mykiss* when its concentration increased from 18 to 336 mg Na/L. This result may be indicating that either the Na concentrations inside the animal are not being altered by V, or the efflux of Na is being compensated by a higher Na influx facilitated by increasing Na concentration in the test water. Sodium imbalance has been proposed to be one

of the mechanisms of toxicity of V by the inhibition of the Na/K ATPase (NAK) in brackish water hydroids and fish (Cantley et al. 1977; Bell and Sargent 1979; Ringelband 2001; Valko et al. 2005). If V inhibits NAK, Na could use another transporter to get into the animal to maintain the ionic homeostasis. In addition to NAK, freshwater invertebrates and fish present Na cotransporters, which simultaneously introduce Na and the SO_4, HCO_3 or HPO_4 into the animal. Thus, V inhibition of Na influx could be compensated by this co-transporter, and thus not showing an influence of water Na on V toxicity. However, in *D. pulex,* when sodium concentration was above 325 mg/L, the toxicity of V almost doubled. A plausible explanation for this increase in toxicity could be that beyond a certain concentration of Na^+ in the exposure water, Na uptake increases may be facilitating the co-transport of the V oxyanions, due to similarities in structure with SO_4, HCO_3 or HPO_4 (Bianchini and Wood 2008; Griffith 2017). Lethality was not increased in rainbow trout at concentrations of Na above 300 mg/L, but an increase in lethality as Na increased was observed in daphnids. The difference between the regression models for Na for *O. mykiss* and *D. pulex* (Chapter 2) can be explained by differences in the uptake mechanisms for V between these organisms. These hypotheses should be further investigated using anion carriers and NAK inhibitors to better understand the mechanism of uptake and toxicity of V.

One limitation of the tests presented here is that they showed the influence of individual ETMFs on V toxicity under controlled laboratory conditions. In the natural environment, several ETMFs are present at the same time, possibly altering the individual influence of ETMFs on V toxicity to aquatic organisms. To address this issue, scientists have developed modelling tools like BLM and MLR to predict metal toxicity at sites with a specific water chemistry profile (Brix et al., 2017; Di Toro et al., 2001; Paquin et al., 2002). Although the BLM model has been successfully developed for cationic metals like Cu, silver (Ag), Zn, Ni, Cd, lead (Pb), and cobalt (Co), to our

knowledge, there is still no BLM model for anions like V oxyanions (Niyogi and Wood, 2004). Additionally, BLM applications for the development of CWQG-PAL are limited because there is still an overall perception among regulators that BLM is complicated to implement and requires measurements of several water quality parameters (Brix et al., 2017). The application of MLR has been successfully developed for Cu, Al, and Pb (Brix et al., 2017; DeForest et al., 2018; Esbaugh et al., 2011). When compared to the BLM approach, MLR has been able to predict Cu toxicity to several aquatic species under a wide range of water chemistries as accurately as BLM, allowing MLR to be applied in the development of water quality criteria (WQC) for Cu (Brix et al., 2017). With the toxicity and water chemistry data obtained here for *D. pulex* and *O. mykiss*, it was possible to develop an MLR model for each species for the first time (at least, to our knowledge). The resultant best-fitted MLR model for *D. pulex* differed from the one obtained for *O. mykiss*. For *D. pulex*, the best-fitted MLR model was based on the interaction between pH and alkalinity, and that of alkalinity and SO_4, whereas the best-fitted model for *O. mykiss* incorporated only the individual effects of pH and alkalinity. Thus, pH and alkalinity play a key role in influencing V toxicity to both animals, and sulphate seems to be a divergent parameter between the two MLR models. The lack of a SO_4 term in the fish model and its presence in the daphnid model could further suggest that the mechanism(s) of uptake for V in daphnids and fish are slightly different. Furthermore, the incorporation of pH and alkalinity into both MLR models suggests that V speciation and competition for binding to the biotic ligand are instrumental in modifying the toxicity of V to both *D. pulex* and *O. mykiss*.

To protect aquatic life and associated ecosystems from being degraded by toxic substances, water quality guidelines are often created and updated (CCME, 2007). However, the water chemistry of aquatic environments can differ widely, complicating the use of generalized CWQG-

PALs. Incorporating the influence of ETMFs into CWQG-PALs allows regulators to apply guidelines with more certainty according to specific site characteristics. In Canada, only a handful of CCME WQG-PALs incorporates the effect of ETMFs on metal toxicity. For instance, Ni, Cu, and Pb guidelines for long-term toxicity are modified according to water hardness within the range of 60-180 mg/L as $CaCO_3$. Water hardness is mainly governed by Ca and Mg, and these cations compete with Ni, Cu, and Pb for the biotic ligand in both invertebrates and fish, reducing their toxicity when the concentrations of Ca and Mg increase (Niyogi and Wood, 2004; Rogers et al., 2003; Rogers and Wood, 2004; Santore et al., 2001). The CCME CWQG for Al is also modified, but in this case, the most relevant ETMF is pH. Similar to V, pH strongly influences Al toxicity to aquatic organisms because it alters the metal's speciation (Helliweli et al., 1983; Leivestad et al., 1987; Poléo, 1995). Canada is not the only country where water quality guidelines (WQG) have incorporated the effects of ETMFs. For instance, in the USA, Cd and Pb both have water quality criteria (WQC) that are modified by water hardness (USEPA, 2019). In Australia and New Zealand, the governments have recommended correcting WQGs for the hardness effect on all hardness-sensitive metals (except copper) when water hardness exceeds 30 mg as $CaCO_3$/L. These two countries have also recognized the need to modify WQGs to account for complexation with dissolved organic carbon (DOC) and the competition between the metals ions and H or HCO_3 (Government of Australia and New Zeland, 2019).

Multiple linear regression has proven to be a useful, easy to use, and precise modelling tool that can be incorporated into CWQG-PALs to account for the influence of ETMFs on metal toxicity to aquatic organisms. For V, it would be interesting to follow the approach of Brix et al. (2017) and try to develop a WQG to protect several species. These authors developed an acute and chronic Cu WQC, accounting for the influence of ETMFs using the stepwise MLR approach

applied to toxicity data from various daphnid species and *P. promelas*. However, in the present work, only *D. pulex* and *O. mykiss* were used as model organisms to understand the influence of ETMFs on V toxicity to aquatic organisms.

An essential step in the development of the MLR model is its validation by comparing the modelled acute LC_{50}s for V with observed acute V-LC_{50}s from peer-reviewed publications where the water chemistry conditions used fell within the ranges tested in this study. To that end, the work presented here was compared with results from previous publications by Schiffer and Liber (2017a, 2017b) and Stendahl and Sprague (1982), who exposed *D. pulex* and *O. mykiss* to V under similar water chemistry conditions. Since *D. dentifera* has a very similar size and physiology to *D. pulex*, it was also included in this validation process (Schiffer and Liber, 2017a). The modelled LC_{50}s for *D. pulex* and *O. mykiss* presented here displayed a two-fold difference from the acute LC_{50}s for V reported by Schiffer and Liber (2017a, 2017b). The difference presented here could be due to genetic differences (different strains) between the two populations. Additionally, Schiffer and Liber performed the toxicity tests with rainbow trout at 12°C rather than 15°C, as was done here. Further research is warranted to investigate the influence of temperature on V toxicity to model freshwater organisms. The modelled LC_{50} for *D. pulex* was similar (only 2% lower) to the one observed for *D. dentifera* (Schiffer and Liber, 2017a), and the modelled LC_{50} for *O. mykiss* was identical to that reported by Stendahl and Sprague (1982).

Additionally, as required for the development of a CCME CWQG-PAL, it is necessary to test the model with a realistic worst-case scenario, accounting for the most influential ETMFs. A worst-case scenario for the acute toxicity of V to *D. pulex* will be that where pH is approximately 9, alkalinity is 70 mg/L as $CaCO_3$, and sulphate is 54 mg/L (Table 4). Under such conditions, the calculated acute LC_{50} for *D. pulex* using the MLR approach was 0.62 mg V/L, which is reasonably

similar to the one obtained from the acute toxicity tests performed by Gillio Meina et al. (2019), where the acute LC_{50} for *D. pulex* was 0.64 mg V/L when the test water pH was 7.99, alkalinity was 75 mg/L as $CaCO_3$, and sulphate was 54 mg/L. The worst-case acute toxicity scenario modelled for *O. mykiss* was only 22% different from the observed LC_{50} when alkalinity was 71 mg/L as $CaCO_3$ and pH was 7.8. Since the alkalinity was similar, the 22% difference is likely due to the higher pH (pH was set at 9) in the proposed worst-case scenario compared to that of the toxicity test (pH 7.8), since the toxicity of V increases as pH increases (Gillio Meina et al., 2019; Stendahl and Sprague, 1982).

Overall, the estimations of the MLR model seem to be accurate enough to be incorporated in interim V CWQG-PAL. However, because models need to be tested, it is recommended that these models be further validated by other laboratories using the same range of water chemistry variables and species employed in this study. This corroboration would confirm whether the MLR models developed here can successfully predict V toxicity to these aquatic organisms. Other algae, invertebrate and fish species should also be incorporated into the analysis to develop a MLR model with site-specific water chemistry that can protect a larger number of aquatic species, through the development of SSDs corrected by the effect of ETMFs. The differences observed between the MLR-predicted LC_{50}s and those obtained by Schiffer and Liber (2017a, 2017b) for *D. pulex* and *O. mykiss* could be indicating that the acute hazardous concentration to the most sensitive fifth percentile of the species tested (HC_5) obtained by these authors, may need to be revised to derive a more site-specific acute water quality benchmark for the Alberta oil sands region (Step 5 from CCME protocol (CCME, 2007). This correction should lead to a more protective CWQG-PAL model for V accounting for different, site-specific water quality characteristics.

CHAPTER 4: INVESTIGATING THE MECHANISM OF VANADIUM TOXICITY IN FRESHWATER ORGANISMS

Preface

This chapter investigates the potential mechanisms of toxicity of vanadium (V) to *Daphnia magna* and *Oncorhynchus mykiss*. According to previous publications, V may cause toxicity to freshwater organisms by disrupting the sodium and calcium balance and possibly by oxidative stress. To determine if V disrupts ion homeostasis, the effect of V on whole body sodium, whole body calcium and unidirectional sodium influx rate were evaluated. Oxidative stress was assessed in *O. mykiss* by investigating the effect of V on enzymatic and non-enzymatic antioxidant defense. The results of this chapter will complement the findings of the previous two chapters by providing information on the physiological effects of V on aquatic organisms.

This chapter will be submitted to *Aquatic Toxicology* under joint authorship with Som Niyogi and Karsten Liber (University of Saskatchewan).

4.1. Abstract

Vanadium (V) is a contaminant of emerging concern for the Alberta oil sands region, one that could present a risk for aquatic organisms. An industry pilot project has used petroleum coke (PC) as a sorbent to remove organic toxicants from oil sands process-affected water (OSPW), but it also caused V to leach from PC into the OSPW, reaching concentrations of up to 7 mg V/L (a level known to be toxic to aquatic organisms). To date, no clear understanding has emerged of the mechanism(s) of toxicity of V in aquatic organisms. Vanadium is a transition metal with several

oxidation states, which could potentially elicit its toxicity through either ion imbalance or oxidative stress. This study investigated the effect of V on *Daphnia magna* and *Oncorhynchus mykiss*. *D. magna* and *O. mykiss* were exposed to concentrations of V equal to their respective calculated median lethal concentration (LC_{50}): 3 mg V/L for *D. magna* and 7 mg V/L for *O. mykiss*. For both organisms, the influence of V on sodium flux and whole body sodium was calculated. Its effect on whole body calcium and the oxidative defence system in *O. mykiss* at the gill and liver levels was also studied. Results suggested that 3.1 mg V/L for *D. magna* and 6.8 mg V/L for *O. mykiss* caused an overall increase in sodium influx in both the daphnids and rainbow trout. However, concentrations of V ranging between 0.2 and 4 mg V/L for *D. magna* and 1.8 and 6 mg V/L for *O. mykiss* reduced whole body sodium in both organisms and whole-body calcium in *O. mykiss*. Concentrations above 3.6 mg V/L caused significant lipid peroxidation in the gills and liver of rainbow trout. In contrast, 1.9 mg/L produced a substantial decrease in the fish gill GSH:GSSG ratio, but no change in the ratio between these thiols in the liver. Concentrations of 6.62 mg V/L sharply increased catalase activity in the liver but not in the gills. Neither liver nor gill superoxide dismutase was altered by V. Overall, results suggest that both ion imbalance and oxidative stress are part of the mechanism of toxicity of V in *D. pulex* and *O. mykiss* and that further research is warranted to fully elucidate the mechanism(s) of V toxicity in aquatic organisms.

4.2. Introduction

The Alberta-Saskatchewan oil sands are one of the largest and most important oil reservoirs in Canada. Oil is present in the form of bitumen and its extraction, refinement and by-products can release metals like molybdenum (Mo), nickel (Ni), sulphate (S), and vanadium (V) into the environment (Bejarano et al., 2001; Di Panfilo and Egiebor, 1995; Furimsky, 1998; Gosselin et

al., 2010; Scott and Fedorak, 2004). One of the steps in bitumen upgrade is coking, a process in which petroleum coke (PC), a charcoal-like by-product, is formed and enriched with metals like V and Ni (Puttaswamy et al., 2010). An on-going pilot research project ran by Syncrude Ltd. at their oil sands site (Fort McMurray, AB) is testing the use of PC to remove organic toxicants from the oil sands process affected water (OSPW), another bitumen extraction by-product (Zubot et al., 2012). Zubot et al. (2012) found that within the first 100 days of treatment, V was released into the OSPW, reaching concentrations close to 7 mg/L. Recent publications have indicated that concentrations of V equal or below 7 mg/L are acutely toxic to aquatic organisms from different trophic levels (i.e., algae, daphnids, and fish) Schiffer and Liber, 2017a, 2017b).

The toxicity of metals is governed by their physico-chemical characteristics like electronegativity, ion size, geometry, and oxidation state, all of which determine how metals interact with targets in the bodies of animals, potentially affecting their overall homeostasis (Crans et al., 2004; Reeder et al., 2006; Wood, 2012). Previous research has shown that metals elicit toxicity through various mechanisms, one of which is the disruption of ion homeostasis (Wood, 2012). For instance, when Pane et al. (2003) exposed *Daphnia magna* to 1,068 µg Ni/L for 48 h and 131 µg Ni/L for 14 d, Ni decreased the uptake as well as the whole body concentration of magnesium (Mg). Zimmer et al. (2014) found that 50 µg copper (Cu)/L inhibited sodium (Na) influx in *Oncorhynchus mykiss* (rainbow trout) larvae, both 3-day post-hatch (dph) and 25 dph, and reduced ammonia excretion in 25 dph larvae. Lead was found to inhibit waterborne calcium (Ca) and Na uptake in *D. magna*, and Na and chloride (Cl) influx in rainbow trout, eventually disrupting the Na/Cl balance (Rogers et al., 2003; Roy, 2009). When adult brook trout were exposed to a combination of low pH and either 111 or 333 µg/L aluminum (Al) for 48 h, there was a marked loss of both Na and Cl from the body (Wood and McDonald, 1987).

Another major mechanism of toxicity of metals at the gill level and interrelated with ion imbalance is the inhibition of key enzymes that regulate the uptake of ions. For example, chromium (0.35 µg Cr/L) and lead (0.63 µg Pb/L) inhibited Na/K ATPase (NAK) and the Mg-ATPase activity in *Oreochromis niloticus* (tilapia) held at 2 or 8 ppt of salinity (Baysoy et al., 2013). Similarly, 0.1 µg/L of cadmium (Cd) and 1.5 µg/L of silver (Ag) inhibited the gill Mg-ATPase, NAK, and Ca-ATPase activity in tilapia exposed for 96 h (Atli and Canli, 2011). Oxidative stress, another important mechanism of metal toxicity, can harm aquatic animals by weakening their antioxidant defences, with a consequential increase in lipid peroxidation, protein carbonylation, DNA damage, or cell function disruption (Wood, 2012). For instance, when *Brycon amazonicus* were exposed to 0.15 mg $HgCl_2$/L, there was an increase in the expression of the principal antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), and glutathione reductase (GR)) (Amaral Monteiro et al., 2010), which depending on the intensity and length of exposure could elicit oxidative stress. It has also been reported that exposure to Cu (20 µg Cu/L) in *D. magna* substantially increased CAT, GPx, and GST activities, as well as lipid peroxidation (Barata et al., 2005b). Metal-induced oxidative damage can lead to structural and functional damage to the critical target organ, such as the gill.

Vanadium has five oxidation states of which V(IV) and V(V) are typically the most abundant in aquatic environments, primarily occurring as VO_4^- since V(IV) is rapidly oxidized to V(V) (Rehder, 1991; Zhao et al., 2006). Inside the animal, V is reduced from V(V) to V(IV) and V(III) by biological reductants like glutathione and ascorbic acid, but it has been postulated that the reduction of excessively high concentrations of V could lead to oxidative stress (Valko et al., 2005). There is still debate about the mechanism of V toxicity in aquatic organisms. The mechanism of toxicity of V inside the fish gills is thought to occur mainly through the inhibition

of NKA and thereby disrupting Na uptake and homeostasis. For example, concentrations of orthovanadate ranging from $1.84x10^{-3}$ to 183.91 mg V/L inhibited NKA in microsomal preparations of *Anguilla anguilla* (eel) gill tissue (Bell and Sargent, 1979). Additionally, NKA activity in freshwater hydroids (*Cordylophora caspia*) was reduced when they were exposed to metavanadate concentrations ranging from $1.36x10^{-3}$ to 136 mg of V/L (Ringelband and Karbe, 1996). Giles et al. (1979) reported that adult rainbow trout exposed for 96 h to V ranging from 3 to 22.5 mg V/L displayed gill hyperplasia (manifested as thickening of the secondary lamellae). Besides, these authors showed that juvenile rainbow trout acutely exposed for 96 h to 5.3 and 16.8 mg V/L (pH ranging from 6 to 9) had cloudy swelling, hydropic degeneration, and hyaline degeneration of the liver.

Another possible mechanism of toxicity could relate to the inhibition of indispensable enzymes because the chemical structure of V resembles that of phosphate (Nechay, 1984). These enzymes include phosphohydrolase, ribonuclease, adenylate kinase, phosphofructokinase, squalene synthetase, glyceraldehydes-3-phosphate dehydrogenase, glucose-6-phosphatase, and phosphotyrosyl-protein phosphatase (Nechay, 1984).

Depending on the global economy over the next ten years, the production of bitumen could double or triple (CAPP, 2017), which could increase V release and the potential for aquatic organisms to be exposed to this metal, especially if PC is used to treat OPSW. Gillio Meina et al. (2019) and Chapter 3 of this thesis showed that an increase of pH from 6 to 9 increased V toxicity to *D. pulex* and rainbow trout; conversely, an increase in sulphate from 100 to 300 mg SO_4^{2-}/L and in alkalinity from 70 to 300 as $CaCO_3$, respectively, decreased V toxicity to *D. pulex* and rainbow trout. These results suggest that V oxyanions may compete with sulphate and/or bicarbonate for the same anionic carriers, reducing the uptake of V. The objective of this work is to understand

the mechanism of V toxicity to aquatic organisms, which will help to better understand the ecological risks of V contamination and formulate possible mitigation strategies. More specifically, the objectives are to test if V disrupts the ion homeostasis and oxidative stress in model freshwater organisms.

4.3. Materials and methods

4.3.1. Chemicals

Sodium metavanadate ($NaVO_3$, anhydrous, min. purity 96%) was purchased from Strem Chemicals Inc. (Newburyport, MA, USA). Radiolabelled ^{22}Na was purchased from PerkinElmer Inc. (Woodbridge, ON, Canada). Ethyl 3-aminobenzoate methanesulfonate (MS-222), N-ethylmaleimide (NEMD), O-phthaldialdehyde, 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid, N-(2-hydroxyethyl) piperazine-N′-(2-ethanesulfonic acid) (HEPES), ethylene glycol-bis (2-aminoethylether)-*N,N,N′,N′*-tetraacetic acid (EGTA) buffer were purchased from Sigma-Aldrich (Oakville, ON, Canada). The remaining chemicals were obtained from Fisher Scientific (Ottawa, ON, Canada). All the chemicals used were of analytical grade.

4.3.2. Animal culturing and holding

4.3.2.1. Daphnia magna

Daphnia magna was chosen as a surrogate for investigating the mechanism of toxicity of V because there is more peer-review work done with this species than with *D. pulex*, and they are bigger than other daphnids. *Daphnia magna* were obtained from an in-house culture maintained in an environmental chamber at the Toxicology Centre, University of Saskatchewan (Saskatoon, SK). Daphnids were cultured in 2-L glass jars in groups of 30-40 individuals following protocols

from Environment and Climate Change Canada (ECCC) and American Society for Testing and Materials (ASTM) (ASTM, 2004; ECCC 1990a). The temperature in the environmental chamber was constant at 24 ± 1 ºC, with 16 h light: 8 h dark photoperiod (light intensity was above 450 lux). The culture medium consisted of bio-filtered, carbon (C)-filtered municipal Saskatoon city water. The pH in the culture jars was maintained at 7.5 – 8.5. During culturing, the daphnids were fed daily with a mixture of algae (70% *Raphidocelis subcapitata* and 30% *Chlamydomonas spp.*) and 4 mL of YCT (Clifford and McGeer, 2009). Selenium (2 µg/L) and vitamin B_{12} (2 µg/L) were added to the culture jars, as they are essential to the overall health of *D. magna* (ECCC 1990b).

4.3.2.2. Oncorhynchus mykiss

Oncorhynchus mykiss (rainbow trout) eyed-eggs were obtained from TroutLodge Inc. (Bonney Lake, WA, USA) and reared in the Aquatic Toxicology Research Facility (ATRF) at the Toxicology Centre, University of Saskatchewan. After hatching, larvae were transferred to 20-L tanks to an environmental chamber located at the Toxicology Centre, following ECCC protocols with slight modifications (ECCC 1998, 1990b). The temperature in the environmental chamber was 15 ± 1 ºC, with 16 h light: 8 h dark photoperiod (light intensity was approximately 400 lux). The fish were raised in bio-filtered, C-filtered municipal Saskatoon city water. Test and culture water was prepared in 50-L plastic carboys and aerated to remove chlorine for at least 24 h before it was used. The pH in the culture tanks was maintained at 7.5 – 8.5. Once the fish reached the swim-up stage, they were fed *ad-libitum* daily with commercial pellets (Ewos Canada Ltd., Surrey, BC). The day before a toxicity test began, V stock solutions were prepared in 50-L plastic carboys and then diluted with culture water to reach the desired V serial dilution (a factor of 2 was used). Fish holding and testing followed Animal Use Protocol #20140036 (University of Saskatchewan).

4.3.3. Experimental design

4.3.3.1. Preliminary experiments

A preliminary toxicity test was conducted to determine the acute (48-h) median lethal concentration (LC_{50}) of V to *D. magna* following ECCC protocols (ECCC 1990a). The calculated 48-h LC_{50} of 3 mg V/L was used as a reference concentration for the design of all the tests on daphnids. The concentrations of V for the tests with rainbow trout were chosen based on toxicity tests done for Chapter 3. In these tests, it was found that the 96-h LC_{50} for swim-up rainbow trout larvae was 7.1 mg V/L when pH, alkalinity, water hardness, and sulphate were approximately 8.00, 100 mg/L as $CaCO_3$, 100 mg/L as $CaCO_3$, and 54 mg/L, respectively.

4.3.3.2. Measurement of rate of unidirectional sodium influx

Juvenile *D. magna* (4-5 d old) were exposed in ten replicates of 10 daphnids each to either control water or 3 mg V/L (calculated LC_{50}) for 1, 2, 6, 12, and 24 h, following Rogers and Wood (2004) and Zimmer et al. (2014). At each sampling interval, daphnids were transferred to 250-mL disposable polyethylene beakers containing 80 mL of either control or V-treated water spiked with radiolabelled sodium (^{22}Na) at a concentration of 0.015 μCi/mL and the influx of Na was run for 2 h. After being removed from the polyethylene beakers, the daphnids were placed for 10 s in new disposable beakers containing a 1 M NaCl solution to remove any ^{22}Na attached to the carapace; they were subsequently rinsed with ultrapure water and blotted dry. Dried daphnids were placed in 7-mL pre-weighed scintillation vials, and internal ^{22}Na concentrations were measured with a 1480 Wizard-3 gamma counter (PerkinElmer, USA). Water samples (5 mL) were collected in duplicate at the beginning and the end of the flux experiment to determine the mean ^{22}Na activity in the exposure water during the exposure period.

Juvenile *O. mykiss* (approximately 2 g wet weight) were exposed to the calculated 96-h LC_{50} and half of the LC_{50} (7 and 3.5 mg V/ L, respectively) for 3, 6, and 12 h and replicated five times per treatment (one individual per replicate). Individual fish were collected at each sampling time and transferred to 250-mL disposable polyethylene beakers for further sodium flux tests. The procedure for the sodium influx tests conducted with the rainbow trout was similar to that used with the daphnids, except that fish were exposed to radiolabeled Na for 1.5 h. At the end of the exposure, fish were humanely euthanized using a lethal dose of MS-222 and counted for ^{22}Na activity along with the respective water samples.

The unidirectional Na influx rate was calculated using the following equation (Eq. 4.1; Glover and Wood, 2005):

$$Jin = \frac{cpm}{(SA*m*t)} \qquad \text{(Eq. 4.1)}$$

where,

cpm = counts per minute in whole body samples

SA = specific activity of the exposure water (cpm of ^{22}Na per µM of total sodium)

m = wet mass of the animal(s) (g)

t = time (h)

4.3.3.3. Whole body sodium and calcium measurements

The experimental design for the test to measure whole body sodium in *D. magna* followed that described by Bianchini and Wood (2002). The design used for measuring whole body sodium and calcium in *O. mykiss* was previously described by Zimmer et al. (2014).

Groups of 10 4- to 5-d-old daphnids (of individual wet weight around 1 mg) in four replicates were exposed to concentrations of V ranging from 0.24 to 4 mg of V/L. Daphnids were exposed to V for 24 h, collected, and then blotted dry using low-lint wipes to remove any excess water. Each group of daphnia was then transferred to labelled, pre-weighed, 15-mL falcon tubes. Tubes containing daphnids were reweighed to obtain the final wet weight. Once all replicates were weighed, the animal tissues were digested with 2N HNO_3 following a ratio of 100 µL of HNO_3 / mg tissue, in a drying oven at 60 °C for 48 h. Digested samples were analyzed for total Na using a flame atomic absorption spectrometer (Analyst 800, PerkinElmer, USA) using a certified Na standard (Fisher Scientific, (Ottawa, ON, Canada).

Juvenile *O. mykiss* (approximately 3 g wet weight) were exposed to V concentrations between 1.5 and 6 mg/L for 48 and 96 h (four replicate tanks per treatment with five fish per replicate). Following the exposure, fish were humanely euthanized using a lethal concentration of MS-222. For each exposure time, all the fish from each replicate were collected and placed in pre-weighed falcon tubes, reweighed, and digested with 2 N HNO_3 using a ratio of 1:5 of HNO_3 /mg of tissue in a drying oven at 60 °C for 48 h. Digested samples were centrifuged at 10,000 *g*, and the supernatant was used to determine the concentration of Na and Ca in the whole fish. Samples were filtered using a 45 µm polyesthersulfone membrane (VWR International, Mississauga, ON, Canada) and diluted 20 times for further analysis by inductively coupled plasma-optical emission spectrometry (ICP-OES, iCAP 6000 Series, Thermo Scientific, Mississauga, ON). Samples containing only natural water standard reference material (1640a; National Institute of Standard and Technology, Gaithersburg, MD, USA) were placed at the beginning, middle, and the end of each batch of samples to assure analytical accuracy (QA/QC). The recovery percentage of Na in the reference material was 95%.

Whole body sodium and calcium (rainbow trout only) concentrations were calculated using the following equation (Eq. 4.2; Roy, 2009):

$$M=\frac{(A*V*df)}{m} \qquad \text{(Eq. 4.2)}$$

where,

A = sodium or calcium concentration in tissue digest (µg/mL)
V = volume of tissue digest (mL)
df = dilution factor
m = wet mass of the animal(s) (mg)

4.3.3.4. Oxidative stress

The time of exposure for this test was chosen after a thorough literature review, which showed that, in general, reactive metals produce oxidative stress within the first 3-4 h of beginning the test (Bagnyukova et al., 2007; Bhattacharya and Bhattacharya, 2007; Bishayee and Chatterjee, 1994). No previous studies have evaluated the potential induction of oxidative stress in rainbow trout by V. Since most metals elicit oxidative stress in fish within the first 48 h (Amaral Monteiro et al., 2010; Bhattacharya and Bhattacharya, 2007; Cuypers et al., 2010), an exposure period of 48 h was chosen. This exposure time also matches that for the whole body sodium and calcium tests. Additionally, since Giles et al. (1979) have reported that V can damage both the gills and liver, V-induced oxidative stress was measured both in the fish gills and liver. Thus, fish were exposed for 48 h to an increasing range of V concentrations between 1.9 and 6.6 mg V/L in 10-L aquaria in five replicates and euthanized using a lethal concentration of MS-222. The gills and liver were immediately removed and placed on ice, weighed, subsampled, and stored at -80 °C for further analysis.

The endpoints measured in *O. mykiss* in both the gills and liver were: lipid peroxidation (TBARS method), reduced and oxidized glutathione ratio (GSH:GSSG), and the effects of V on catalase (CAT) and superoxide dismutase (SOD) activities. The enzyme activities were determined using Cayman Chemical commercial kits (TBARS – kit # 700870, SOD – kit #706002 and CAT – kit #700910). Lipid peroxidation was expressed as nmol of malondialdehyde (MDA) g^{-1} of wet tissue. Activities of SOD and CAT were expressed as nmol min^{-1} mg^{-1} protein. One unit of SOD (U) was defined as the amount of enzyme needed to cause 50% dismutation of the superoxide radical. One unit of CAT was defined as the amount of enzyme that will cause the formation of 1.0 nmol formaldehyde at 25°C. The effect of V on the GSH:GSSG ratio was determined using a protocol described by Jamwal and Niyogi (2017). Briefly, the concentration of GSH and GSSG and the ratio GSH:GSSG were measured as an estimate of cellular redox balance using a fluorometric method, modified to a 96-well microplate based assay. Fluorescence was measured in a multimode microplate reader (Varioskan Flash, Thermo Fisher Scientific, Finland) at excitation and emission wavelengths of 350 nm and 450 nm, respectively. The samples were calibrated against a standard curve prepared from commercially purified GSH and GSSG, and the linearity of the reaction rate was confirmed. Concentration of GSH and GSSG were expressed as µg mg^{-1} protein.

4.3.4. Chemical analysis

At the beginning and end of all tests, water samples (20 mL) were randomly collected in triplicate from the control and V-exposed vessels for water chemistry analysis and for measuring temperature, dissolved oxygen (DO), pH, alkalinity, and water hardness (Table 4.1). Measurements of pH were taken from test solutions using an ORION PerpHect Log R meter model 370. Temperature and DO concentrations were measured using the ORION DO meter model 835

(ORION Research, Beverly, MA, USA). Alkalinity and hardness were measured using the Hach digital titrator model 16900 (Hach Company, Loveland, CO, USA).

Table 4.1: Mean water chemistry (±SEM) obtained from all tests performed with *Daphnia magna* (*n = 2 tests)* and in *Oncorhynchus mykiss* (*n* = 3 tests).

	pH[a]	DO[b]	Temp.[c]	Cond.[d]	Alka.[e]	Hrns.[e]	Ammo.[f]
D. magna **(*n* = 2)**	8.0 ± 0.1	7.9 ± 0.1	23.6 ± 0.1	496.3 ± 0.8	131 ± 5	167 ± 6	-
O. mykiss **(*n* = 3)**	8.2 ± 0.1	9.4 ± 0.1	15.2 ± 0.3	457.1 ± 44.3	123 ± 14	145 ± 18	0.3 ± 0.1

[a] pH is unit-less; *n* = 24.
[b] DO = dissolved oxygen; concentration as mg/L; *n* = 24.
[c] Temp. = temperature, units are °C; *n* = 24.
[d] Cond. = conductivity, units are µS/cm; *n* = 24.
[e] Alka. = alkalinity, Hrns = hardness; concentration as mg/L as $CaCO_3$; *n* = 24.
[f] Ammo. = ammonia, concentration as mg/L; *n* = 18.
- Ammonia concentration not measured.

Before any major ion and V analysis was done, all water samples were filtered using a syringe filter with a 0.45-µm polyesthersulfone membrane (VWR International, Mississauga, ON, Canada). Additionally, samples for V analysis were collected and acidified to 2% HNO_3 with high purity (Fisher Scientific, Ottawa, ON, Canada). Vanadium tissue concentrations were obtained from fish used for the whole body ions experiment. Samples collected for V analysis were measured in-house using an inductively coupled plasma mass spectrometer (ICP-MS) equipped with collision cell technology (Agilent Technologies 8800 ICP-MS Triple Quad, Mississauga, ON, Canada). The limit of detection for V was 0.05 µg V/L. River-water standard reference material (SLRS-5; National Research Council, Ottawa, ON, Canada), and natural water standard reference material (1640a, National Institute of Standard and Technology, Gaithersburg, MD, USA) were used in QA/QC for ICP-MS measurements. The accuracy of SLRS-5 and 1640a analyses were always $\geq$ 85%, and the precision of duplicates exceeded 90% in all cases.

4.3.5. Statistical analyses and inter-specific comparisons

The acute LC_{50} estimates were calculated with 95% confidence intervals using either the moving average TOXCALC 5.0 (Tidepool Scientific, CA, USA) or the Spearman-Karber method, Version 1.5 (Hamilton et al., 1977). Assumption of normality and homogeneity of variance was checked using Shapiro-Wilk's W test and Levene's test, respectively. The effect of V on whole body sodium in *D. manga*, and on oxidative stress endpoints in rainbow trout were assessed by one-way analysis of variance (ANOVA), followed by parametric Tukey's post-hoc tests. The effect of time of exposure, V concentration and their interaction during the whole body sodium and calcium tests in the rainbow trout were assessed by two-way ANOVA. If an interaction was found between these two factors, parametric Student T-tests were conducted to determine difference between those fish exposed for 48 h from those exposed for 96 h. If there was a significant difference between each V treatment at either 48 h or 96 h, a one-way ANOVA followed by Tukey's post-hoc tests for pairwise comparison was performed. The effect of V on sodium influx was assessed by repeated measures statistics followed by parametric Student T-tests to determine if there was a difference between the V-exposed and the control groups at each time interval. In all cases, an α of 0.5 was used to determine significance.

4.4. Results

4.4.1. Effects of V on sodium and calcium homeostasis in Daphnia magna and Oncorhynchus mykiss

During the 24 h exposure period, the daphnids exposed to 3.1 mg V/L presented an overall higher sodium influx than the control group (p = 0.012) and showed a significant difference between the treated daphnia and the control group at 12 h from the beginning of the test (p = 0.04;

Figure 4.1a). Concentrations of V ≥ 0.46 mg V/L (0.46, 0.97, 1.96, and 3.96 mg V/L) caused a non-significant but marked reduction (approximately 29% change for 0.5 and 2 mg V/L and 16% for 1 and 4 mg V/L) of the whole body Na in *D. magna* ($p = 0.056$; Figure 4.1b).

When rainbow trout were exposed to 3.7 mg V/L, sodium influx in the exposed fish was similar to that of the control group ($p = 0.691$). However, in those fish exposed to 6.8 mg V/L the overall sodium influx was higher than that of the control group ($p = 0.028$) (Figure 4.2a and 4.2b). In addition, there was a significant difference between the fish exposed for 12 h to 6.8 mg V/L and the control group, but not in the fish exposed to 3.7 mg V/L ($p = 0.008$; Figure 4.2b). Whole body sodium was affected by a combination of exposure duration and V concentration ($p = 0.019$), and it was due to a difference between the fish exposed for 48 and 96 h to 3.19 mg V/L ($p = 0.009$; Figure 4.2c,d). When the fish were exposed to 6.03 mg V/L, the whole body Na of the fish was substantially reduced by 22% at 48 h ($p = 0.002$). When exposure time increased to 96 h, fish whole body Na concentrations were reduced by 29% at 3.19 mg V/L ($p = 0.003$) and by 40% at 6.03 mg V/L ($p < 0.001$; Figure 4.2c and 4.2d). There was no interaction between exposure time and V effect on whole body calcium ($p = 0.464$). Whole body Ca concentrations only showed a significant reduction of 16% when the fish were exposed for 96 h to 6.03 mg V/L ($p = 0.038$; Figure 4.2f). In these fish, V tissue concentration significantly increased with a concomitant increase in aqueous V concentration, when fish were exposed for 48 and 96 h ($p < 0.001$, Figure C1). Additionally, there was a significant difference between the fish exposed to V for 48 h and 96 h at the concentrations of 1.8 and 3.19 mg V/L ($p = 0.025$ and $p = 0.019$, respectively, Figure C1).

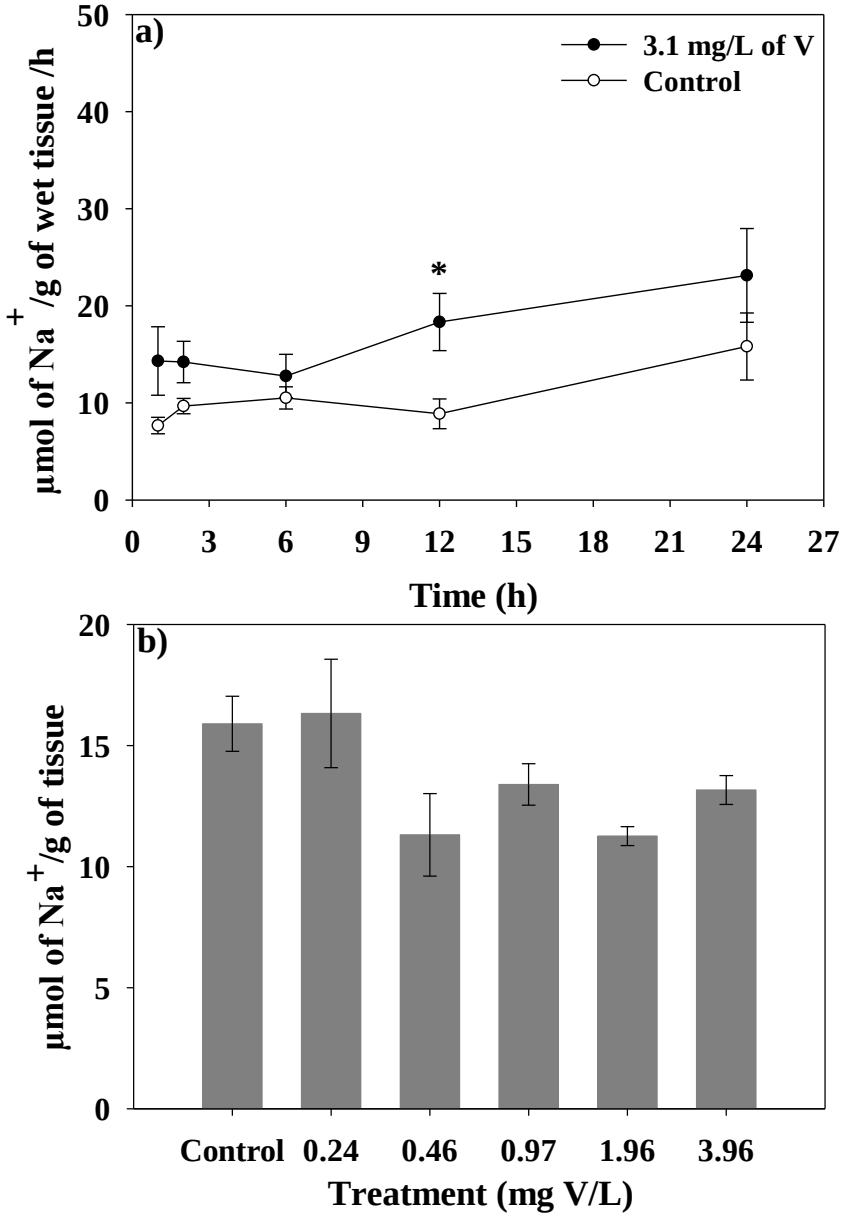

Figure 4.1: a) Sodium influx in *Daphnia magna* exposed to 3.1 mg V/L for 24 h (n = 10; asterisk denotes a significant difference between the control and the treated group at each sampling time). b) Effect of V concentrations ranging between 0.24 and 3.96 mg/L on whole body sodium in *Daphnia magna* (n = 6). In all the cases $\alpha = 0.05$.

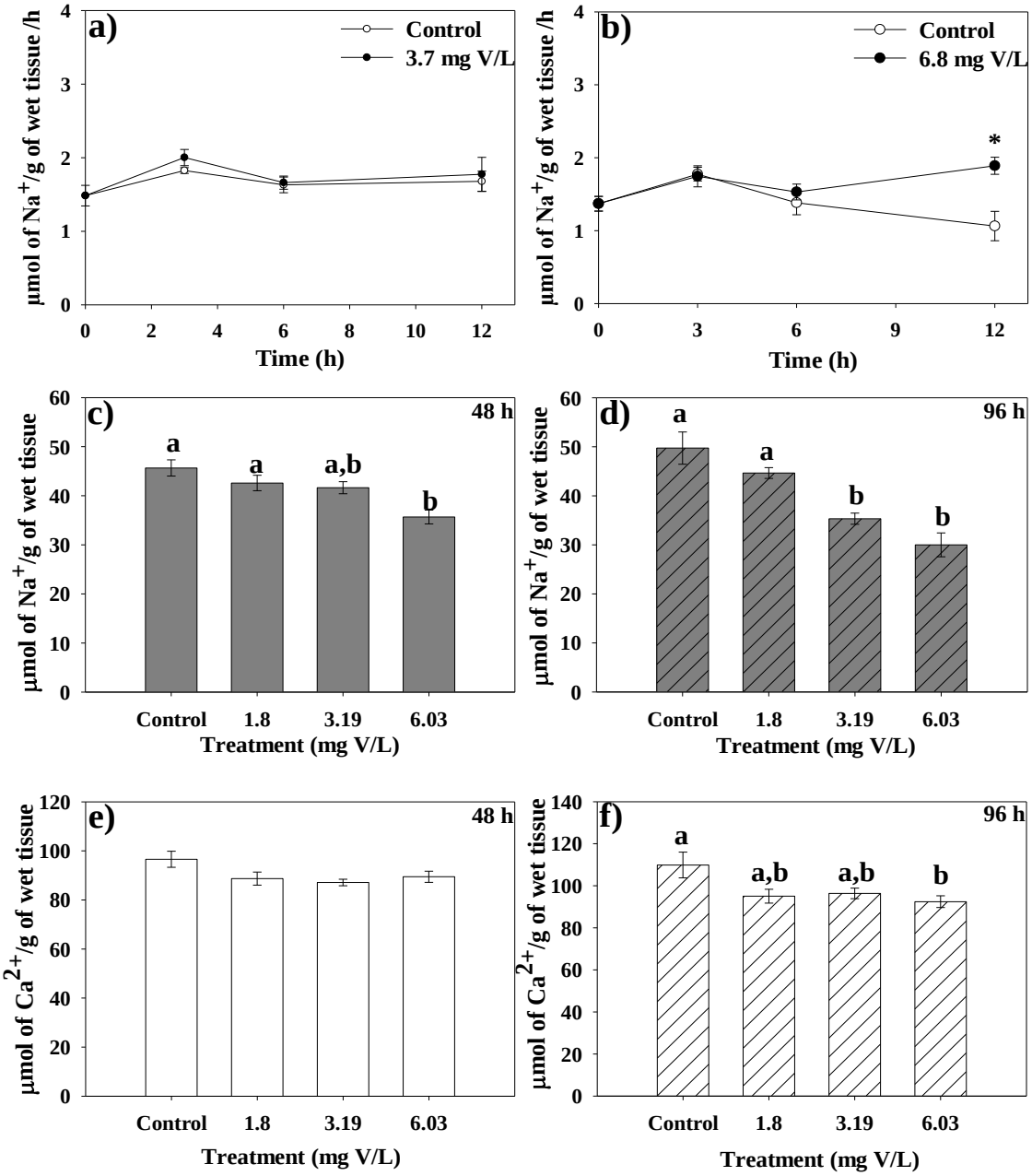

Figure 4.2: Sodium influx in *Oncorhynchus mykiss* exposed to: a) 3.7 and b) 6.8 mg V/L for 12 h (n = 5). Asterisk represents a significant difference between the control and the treated group. Effects of V concentrations ranging between 1.80 and 6.03 mg/L on whole body sodium concentrations exposed for: c) 48 and d) 96 h, and whole-body calcium concentrations exposed for: e) 48 and f) 96 h in *Oncorhynchus mykiss* (n = 4). Letters represent a significant difference among the treatment groups ($\alpha = 0.05$).

4.4.2. Effects of V on redox homeostasis in Oncorhynchus mykiss

Vanadium also caused oxidative stress in the gills and liver of *O. mykiss* (Figure 4.3 and 4.4). For instance, 3.58 and 6.62 mg V/L, respectively, caused a significant increase of 97% (p = 0.04) and 110% (p = 0.002) in lipid peroxidation in rainbow trout gills (Figure 4.3a). Additionally, V significantly decreased the gill GSH:GSSG ratio by an average of 32% when the fish were exposed to all three V concentrations tested (1.92, 3.58, and 6.62 mg V/L; p = 0.004, 0.003, and 0.002, respectively; Figure 4.3b). There was no effect of V on GSH (p = 0.124), but 1.92 and 3.58 mg V/L caused a significant increase (63%, p = 0.012; and 65%, p = 0.009, respectively, Figure 4.3b) in gill GSSG. Neither gill CAT nor SOD activity was affected by V (p = 0.138 and 0.473, respectively; Figure 4.3c and 4.3d). When the fish were exposed to 3.19 mg V/L, there was a significant increase (47%; p = 0.041) in lipid peroxidation in the liver (Figure 4.4a). When fish were exposed to 6.62 mg V/L, there was a significant increase of 49% (p = 0.051) in lipid peroxidation in the liver (Figure 4.4a). The overall liver GSH:GSSG ratio was not altered by V (p = 0.336; Figure 4.4b), but there was a marked reduction of the ratio with all of the exposure concentrations of V tested here. Vanadium had neither effect on liver GSH (p = 0.773), nor on GSSG levels (p = 0.501). When the fish were exposed to 6.62 mg V/L, liver CAT activity significantly increased by 55% (p = 0.014), but liver SOD activity remained unchanged (p = 0.896).

Overall, results indicated that V caused ion imbalance in both *D. magna* and *O. mykiss* and oxidative stress in *O. mykiss* (Table 4.2).

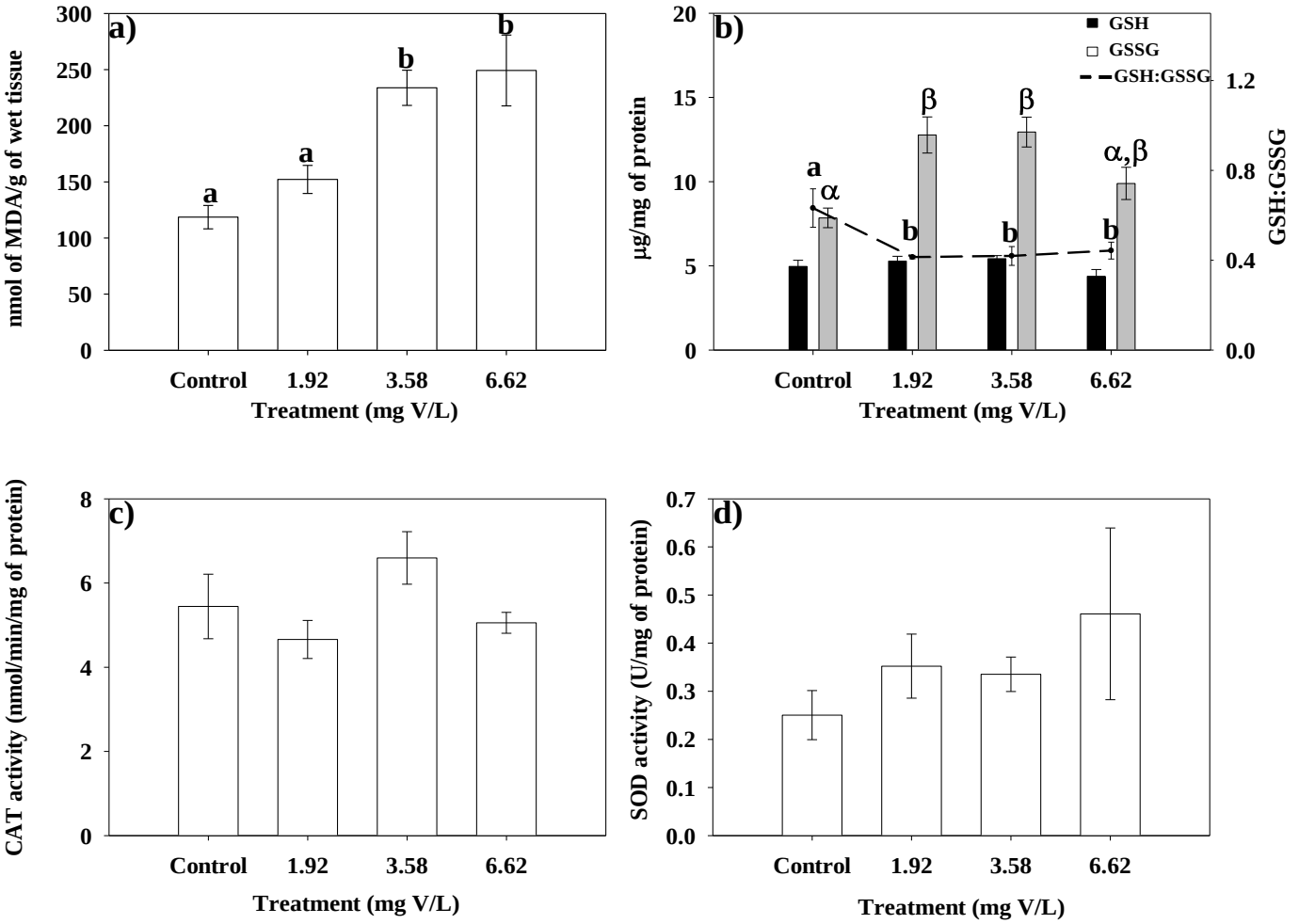

Figure 4.3: Effects of V concentrations in the gills ranging between 1.92 and 6.62 mg/ L *Oncorhynchus mykiss* on: a) lipid peroxidation levels measured as malondialdehyde (MDA) equivalents; b) reduced and oxidized glutathione (GSH and GSSG) and the GSH:GSSG ratio (dashed line); c) catalase (CAT); and d) superoxide dismutase (SOD) activities (n = 5). Letters indicate a significant difference among treatments. For GSH:GSSG ratio small letters indicate a significant difference between GSH:GSSG ratio, and Greek letters indicate a significant difference among GSSG treatments ($\alpha = 0.05$).

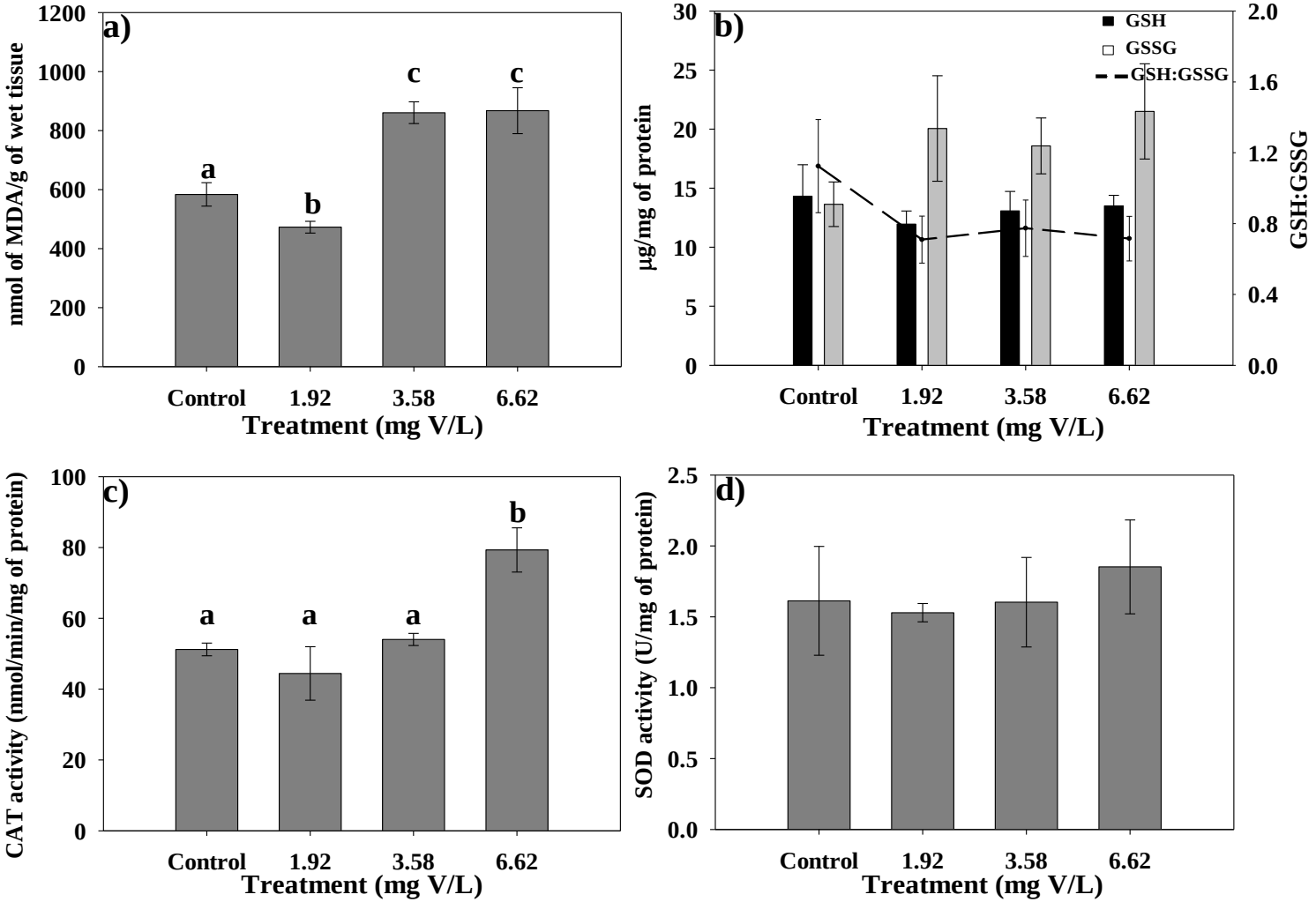

Figure 4.4: Effects of V concentrations in the liver ranging between 1.92 and 6.62 mg/ L of *Oncorhynchus mykiss* on: a) lipid peroxidation levels measured as malondialdehyde (MDA) equivalents; b) reduced and oxidized glutathione (GSH and GSSG) and the GSH:GSSG ratio (dashed line); c) catalase (CAT); and d) superoxide dismutase (SOD) activities (n = 5). Letters indicate a significant difference among treatments ($\alpha = 0.05$).

Table 4.2: Overall effects of V on sodium homeostasis in *Daphnia magna* and *Oncorhynchus mykiss*, and on calcium homeostasis and antioxidant defence in *Oncorhynchus mykiss*.

Test /Variable	Overall effect	
D. magna		
Whole body sodium	↓	
Sodium flux	**?**	
O. mykiss		
Whole body sodium	↓	
Sodium influx	**?**	
Whole body calcium	↓	
O. mykiss	**Gill**	**Liver**
Lipid peroxidation	↑	↑
GSH:GSSG ratio	↓	=
CAT	=	↑
SOD	=	=

= Indicates no effect of V on the endpoint measured; ↑ denotes that V increased and ↓ that V decreased the endpoint measured. **?** indicates no clear effect.
GSH and GSSG = reduced and oxidized glutathione respectively; GSH:GSSG denotes the ratio between these thiol compounds.
CAT = catalase activity.
SOD = superoxide dismutase activity.

4.5. Discussion

The present results suggest that V could potentially elicit its toxicity in aquatic organisms by altering Na and Ca homeostasis, and/or by generating oxidative stress. These two mechanisms of toxicity could coexist and consequently lead to damage to the structure of the gills or other organs where V accumulates, as suggested by Giles et al. (1979). In the present work, both daphnids and rainbow trout exposed to concentrations of V similar to their respective LC_{50}s showed an increase in Na influx between 6 h and 12 h, with a significant difference between the

control and the exposed group at 12 h. Additionally, those *D. magna* and rainbow trout exposed to V for 24 and 12 h, respectively, showed an overall higher influx in V than their respective control groups. During the time of exposure, the control and V-exposed trends were similar (Figure 4.1 and 4.2) with a change in the slopes between 6 and 12 h. However, due to the short exposure period, it is difficult to ascertain that V influenced sodium influx in these species. This uncertainty could be resolved by extending the time of exposure and reducing the concentration of V to half the LC_{50}s to prevent mortalities in those organisms exposed to the highest V concentrations. Conversely, other findings suggest that the mechanism of toxicity of V in hydroids is through the inhibition of the NAK pump (Rehder, 1991; Ringelband, 2001; Ringelband and Karbe, 1996). This pump is responsible for incorporating sodium into the blood, and, if it is inhibited, the influx of Na should decrease, not increase. Furthermore, in both daphnids and rainbow trout, whole body Na was reduced in fish after 48 h of exposure to 6 mg V/L, and after 96 h of exposure to 3.2 mg V/L; and substantially reduced after 96 h of exposure to 6 mg V/L. Additionally, 6 mg V/L significantly reduced whole body Ca in rainbow trout after 96 h of exposure, suggesting that V also disrupts Ca homeostasis. One possible explanation for the apparent discrepancy in Na influx and whole body Na results could be linked to the physiology of freshwater animals. An α of 0.05 was chosen for all the tests performed here. This decision resulted in slightly conflicting results between the effects of V on whole body sodium in *D. magna* and rainbow trout when V caused a non-significant 29% reduction of whole body sodium in the daphnids when exposed to 0.5 and 2 mg V/L ($p = 0.056$), and a significant 29% reduction in fish exposed to 3.19 mg V/L ($p = 0.003$). Had the α value been set at 0.1, that would have turned both relationships statistically significant. However, the results presented here, even though they were statistically non-significant, showed a noteworthy biological effect of V on whole body sodium in both species. This discrepancy likely

resulted from the lower statistical power in the test with daphnids (P = 0.61) than the test with fish (P = 0.74).

The internal environment of freshwater animals is normally hypertonic with respect to the ambient water. As a result, they continually lose ions (e.g., Na, Ca) from their body via the gills/skin by passive diffusion, and they compensate for the loss of ions by actively taking ions from the water across the gill epithelium. It is possible that the V-induced oxidative stress might have resulted in histopathological damage to the gill epithelium, exacerbating the net loss of ions from the body. On the other hand, the increase in Na uptake rate in both *D. magna* and rainbow trout might have occurred as a compensatory response to cope with the increasing loss of ions from the body. Giles et al. (1979) found that after 96 h of exposure to 3.0 to 22.5 mg V/L in pH ranges similar to the ones recorded here (between 6 and 9), hyperplasia was observed in the exposed juvenile rainbow trout. Hyperplasia of the gill epithelial cells results in shortening, rounding, and fusing of the secondary and primary lamellae, thus modifying the normal structure and function of the gill. This phenomenon could consequently contribute to ion imbalance in aquatic organisms. Therefore, lethality in *D. magna* and rainbow trout after acute V exposure could be due to a loss of functionality of the gills, leading to an ion imbalance. This hypothesis is supported by Lin and Randall (1993), who exposed rainbow trout gills to 18.39 mg/L of V, and found a reduction of the activity of H-ATPase by 60%. Previous peer-reviewed work on fish physiology and molecular biology support this hypothesis (Beyenbach and Wieczorek, 2006; Glover and Wood, 2005; Marshall, 2002; Perry et al., 2003). These authors showed that H-ATPase is expressed in the gills of fish and daphnids; therefore, they reasoned, if gill function is impaired or the gill structure is damaged, then the activity of H-ATPase and the H flux will be reduced. Other research has shown that when lobster hepatopancreatic endoplasmic reticulum was exposed

to 10 mM of V, there was significant inhibition of Ca influx into the reticulum (Mandal et al., 2005). The Ca-ATPase is expressed in the basolateral membrane of the gill epithelia. If V damages the gill structure (for example, through lipid peroxidation), then the Ca-ATPase could exhibit the same inhibitory fate as of H-ATPase when freshwater organisms are exposed to V (Ahearn et al., 2004; Mandal et al., 2005). The results presented here showed that whole body Ca concentration in *O. mykiss* was reduced by V after 96 h of exposure. These results could be suggesting that V may be inhibiting the Ca-ATPase, reducing the Ca influx, and eventually lowering the whole body Ca concentrations in the animal. Thus, further research is needed to determine if V affects NAK or Ca-ATPase activities or expressions.

Similar to V, Al can cause ion imbalance and oxidative stress in aquatic organisms. For example, in rainbow trout that were held at pH around 4.8 and increasing concentrations of Al ranging from 111 to 333 µg/L, generated an overall reduction of plasma Na (Wood and McDonald, 1987). The authors suggested Al caused the reduction of the plasma Na by promoting the Na efflux within the first 12 h of exposure through the opening of paracellular channels in the branchial epithelium, and the blockage of Na influx probably due to the inhibition of the NAK (Wood and McDonald, 1987). Within the first 12-96 h, similar concentrations of Al also generated oxidative stress in fish, jeopardizing the structure and functionality of the gill, and potentially exacerbating the ion imbalance. Abdalla et al. (2018); Fernández-Dávila et al. (2012); Galindo et al. (2010); García-Medina et al. (2013) reported that concentrations around and higher than 100 µg/L of Al produced oxidative stress in various fish species at the gill, liver and brain. Therefore, it is reasonable to postulate that when V like Al reaches a toxic threshold at the fish gill, it could cause both ion imbalance and oxidative stress, possibly damaging the gill structure and functionality, and eventually leading to lethality.

Vanadium has five oxidation states, but V(VI) and (V) are the most abundant in natural well-oxygenated water (pH between 6 to 9). According to Rehder (1991), once inside an organism, V(VI) and (V) are rapidly reduced to V(III), and the reduced V can cause oxidative stress. This research intended to determine if V causes oxidative stress in both daphnids and fish, but, due to technical difficulties, only the effect of V on fish redox capacity is reported here. Similar to other transition metals (Farombi et al., 2007), V caused lipid peroxidation in both the gills and liver, as well as a significant reduction in the GSH:GSSG ratio at the gill. Lipid peroxidation leads to the damage of the lipid bilayer of the cellular membrane. The results obtained in the current work supports previous findings of Giles et al. (1979), who showed that V caused structural and functional damage to the gills. We also found an increase in catalase activity in the liver, but not in the gills. This discrepancy could be explained by the fact that the liver has a higher concentration of anti-oxidative stress enzymes than gills since it is the primary site of detoxification. Overall, the results obtained in the oxidative stress experiments indicate that V may act as a redox inactive metal, depleting GSH, and probably binding to sulfhydryl groups of GSH, which impairs its functionality and the cellular redox balance. Previous findings support the interaction between V and GSH, which could confirm that depletion of GSH is causing oxidative stress (Costa Pessoa et al., 2002, 2001; Ferrer et al., 1993). Additionally, V generated reactive oxygen species (ROS) in lung cell lines (A549) such as hydroxyl radicals ($^{\cdot}OH$), hydrogen peroxide H_2O_2 and superoxide anion $O_2^{\cdot -}$ through a mechanism that involves the flavoenzyme-containing NADPH complex and the mi- tochondria electron transport chain (Zhao et al., 2006). Recently, Rehder (2015) indicated that V is reduced in ascidians by Vanabin 2 (a low molecular mass protein, that presents high levels of cysteines that form nine disulfide bonds). Vanabin2 is connected with the GSH:GSSG

and $NADPH_2$: NADP antioxidant systems. Therefore, it could be possible that the depletion of the GSH:GSSG ratio could be the mechanism of oxidative stress of V.

Recent data indicate that mortality is the main outcome of chronic V exposure in invertebrates and fish when a species-specific threshold concentration is reached (Schiffer and Liber, 2017a,b; Gillio Meina et al., 2019). Consequently, the resultant mortality observed in the above-tested fish species could be explained by the cumulative effects of oxidative damage and the loss of ion homeostasis, as observed in the present study. These two mechanisms of toxicity could affect the overall respiratory and cardiovascular physiology of invertebrates and fish, eventually leading to their death.

The present work on the mechanism(s) of V toxicity in aquatic organisms is not exhaustive, and further research should be done to fully understand the underlying mechanism(s). For instance, V, in the form of orthovanadate, resembles the chemical structure of phosphate. Thus, V could inhibit several enzymes where phosphate plays a key role (e.g., phosphorylase-b, Soman et al., 1983). Nechay (1984) also suggested that V could inhibit other enzymes, which could potentially affect several metabolic pathways. Examples of these enzymes could be: the ATP phosphohydrolases, the ribonucleases, the adenylate kinase, the phosphofructokinase, the squalene synthetases, the glyceraldehydes-3-phosphate dehydrogenases, the glucose-6-phosphatases, and the phosphotyrosyl-protein phosphatase, Rehder, 2015, 2003, 1991).

Finally, this work did not focus on understanding the mechanism of V uptake in aquatic organisms. Metals are mostly hydrophilic and use specific transporters to enter aquatic animals. For instance, arsenic mimics phosphate, whereas chromium and molybdenum mimic sulphate (Wood, 2012). Chromium can enter cells through active transport channels that transfer anions with a similar structure and electrical charges such as sulphate (SO_4) and phosphate (HPO_4).

Vanadium may be using transporters that are intended for bicarbonate carbonate (HCO_3, CO_3), or SO_4 (Valko et al., 2005). This hypothesis is at least partially supported by recent findings by Gillio Meina et al. (2019) and Chapter 3 of this thesis, where it was found that both increasing sulphate and increasing alkalinity ameliorated V toxicity to *Daphnia pulex* and rainbow trout. Experiments using sulphate and bicarbonate channel inhibitors could potentially help to determine the role of these transporters in the uptake of V in aquatic organisms.

Taken together, the results presented here suggest that the length of exposure can influence V toxicity and that after 12 h of exposure to V at concentrations near the 96-h LC_{50}, V induces ion imbalance in the body and oxidative stress in critical target organs such as the gills and the liver of fish. These effects could translate into damage of the gill, which could explain why after 48 h and 96 h of exposure to V, there was a reduction of whole body sodium and calcium. Overall, the present work had two significant findings: (i) V caused ion imbalance in both invertebrates and fish following a similar pattern, and (ii) V induced oxidative stress in the gills and liver of rainbow trout. At least in fish, it is hypothesized that there may be a linkage between the effect of V-induced oxidative damage and loss of ion homeostasis, with the latter being the consequence of the former. However, further research is needed to validate this hypothesis.

CHAPTER 5: GENERAL DISCUSSION

5. Discussion

5.1. Project rationale and goals

In aquatic environments (both freshwater and saltwater), vanadium (V) concentrations are usually low (0.5 – 3 µg/L of V, (ECCC and HC, 2010; Hirayama et al., 1992; Rehder, 2015). High concentrations of V in water bodies could indicate that an anthropogenic source has inadvertently introduced V into the aquatic environment. Vanadium is commercially exploited in several countries (Chile, Finland, Namibia, Norway, South Africa, the United States, Russia, and China), and the metallurgical and oil industries are the major sources of V to the environment (ECCC and HC, 2010; WHO, 2001). Alberta's non-conventional oil sands bitumen extraction and refinement produce a solid by-product known as petroleum coke (PC) that is enriched with V and a liquid waste, known as oil sands process affected water (OSPW), containing inorganic and organic contaminants. As a result, federal and provincial regulations require that oil companies store these waste products on-site until they can be treated appropriately. In 2017, Alberta's oil sands coke generation surpassed 10 million tonnes, and OSPW accumulation in tailings ponds reached approximately 976 billion L (AER, 2017; White, 2017). Consequently, the industry is investigating feasible ways to treat OSPW and PC and, eventually, to reclaim the tailings ponds and incorporate them into the surrounding landscape (Gosselin et al., 2010). Syncrude Ltd. has proposed using PC to treat OSPW to reduce organic toxicants based on previous findings that activated charcoal can be used to reduce the toxicity of organic acids (the main organic compounds responsible for OSPW toxicity). However, Zubot et al. (2012) determined that when PC is in contact with OSPW, V

leaches from PC into OPSW, reaching concentrations as high as 7 mg V/L within the first 100 d. The final concentration of V in treated and aged OSPW is therefore likely more elevated than the median background V concentration (0.5 – 3 µg/L of V) found in freshwater environments (ECCC and HC, 2010; Hirayama et al., 1992; Rehder, 2015). Limited aquatic toxicity data for V are available, hindering the development of robust water quality guidelines (WQGs). Recent research has shown that when the V concentration is equal to or even slightly below 7 mg V/L, it is toxic to model and regionally-relevant aquatic organisms (Gillio Meina et al., 2019; Schiffer and Liber, 2017a, 2017b).

In May 2016, Environment and Climate Change Canada (ECCC) released a federal environmental quality guideline for V by applying a species sensitivity distribution (SSD) and determined a chronic guideline of 0.12 mg V/L for freshwater environments. This guideline was created with only eight toxicity values, those available up to 2010. Recently, Schiffer and Liber (2017b) have expanded this database and developed SSD models for the acute and chronic toxicity of V using a larger suite of aquatic organisms, including many representative species of the Alberta oil sands region. These authors calculated an acute hazardous concentration endangering only 5% of the species (HC_5) of 0.64 mg V/L, and a chronic HC_5 of 0.05 mg V/L. However, when obtaining the acute and chronic HC_5s, Schiffer and Liber (2017b), did not consider the influence of exposure and toxicity modifying factors (ETMFs) on V toxicity to aquatic organisms.

Many other studies have shown that metals are influenced by site-specific water quality characteristics (Cusimano et al., 1986; Dietrich and Schlatter, 1989; Soucek and Kennedy, 2005). For instance, in one study an increase in pH from 4.7 to 7.0 decreased 96-h LC_{50}s of zinc (Zn), cadmium (Cd), and copper (Cu) for *Oncorhynchus mykiss* from 671 to 66, 28 to <0.5, and 66 to 2.8 µg/L, respectively (Cusimano et al., 1986). In addition, the influence of ETMFs on toxicity has

been incorporated into some WQGs. In 1987, the Canadian Council of Ministers of Environment (CCME) released long-term Canadian WQGs for Nickel (Ni), Cu and lead (Pb) for the protection of aquatic life that incorporated the influence of water hardness (between 60 and 180 mg/L as $CaCO_3$) on the toxicity of these metals through a mathematical relationship (CCME 2019).

The current thesis advances the knowledge generated by Environment Canada (2016) and Schiffer and Liber (2017b) by investigating how ETMFs representative of the Alberta oil sands region influenced the acute and chronic toxicity of V to two model species: *Daphnia pulex* (water flea) and *O. mykiss* (rainbow trout). This analysis is warranted to develop more accurate and site-specific WQGs that will provide more realistic protection for aquatic life (CCME, 2007). This research also investigated the mechanism controlling V toxicity in model aquatic organisms to help understand how this metal affects the overall physiology of aquatic organisms and to determine the role variation in water chemistry plays in the mechanism of V toxicity.

The overall goals of this thesis were (1) to better understand how ETMFs representative of the Alberta oil sands region alter V toxicity to model aquatic organisms under both short- and long-term exposure conditions, (2) to develop predictive relationships between the most influential water quality variables and V toxicity to model aquatic organisms, and (3) to elucidate the mechanism(s) of V toxicity in the species studied.

5.2. Project summary and results integration

The following section summarizes and integrates the results obtained to provide the Alberta oil sands industry and Canadian regulatory agencies with relevant knowledge that will aid them in the development of a site-specific water quality guidelines for V.

5.2.1. Comparison between short- and long-term vanadium exposure to *Daphnia pulex*

(Chapter 2 of this thesis) demonstrated that for *D. pulex* variation in water chemistry influences acute V toxicity more than chronic V toxicity. Acute and chronic metal toxicity generally occur through different mechanisms. In essence, acute toxicity often results from ionoregulatory or respiratory disorders, whereas chronic toxicity could be derived from a combination of disturbances that gradually affects the physiology of an animal. The chronic toxic effects are diverse and are often related to the over-consumption of energy required to: repair the damages caused by metals, synthesize detoxification tools (like metallothionein or glutathione (GSH)), trigger immune suppression, reduce the ability to produce a corticosteroid stress response, disrupte sensory functions, and generate oxidative stress. Such disturbances could decrease survival, growth, or reproduction (Wood, 2012).

This research project found that when *D. pulex* were chronically (21 d) exposed to V, the adults receiving the highest concentration (1 mg V/L) showed a marked reduction in the production of neonates. This reduced yield was not as a result of a direct reproductive impairment but was due to an increased mortality rate in adults. Similarly, Schiffer and Liber (2017 a) found that *D. pulex* exposed for 21 d presented an LC_{50} of 0.46 mg V/L, but no reproduction effect was manifested with concentrations of $V \leq 1$mg V/L. These continuous high concentrations of V (e.g., levels of V found in PC-treated OSPW) would eliminate the reproductively active adult daphnia, which will negatively impact the daphnia population and possibly the food web in the aquatic environments of the Athabasca region receiving PC-treated OSPW.

5.2.2. The influence of water chemistry on vanadium toxicity to *Daphnia pulex* and *Oncorhynchus mykiss*

The OSPW and the Athabasca River (AR) water chemistry profiles present similarities and differences. In this thesis, the water quality variables that differed the most between OSPW and AR (Allen, 2008b; MacKinnon et al., 2005) were chosen to investigate their influence on V toxicity to model aquatic organisms. The variables chosen were pH, sulphate (SO_4), alkalinity, sodium (Na), water hardness, bicarbonate (HCO_3), and chloride (Cl). These variables could influence V toxicity to aquatic organisms by competing with V for biological uptake, or by changing the chemical structure or form of V (Evans and Brusewitz, 1994; Gillio Meina et al., 2019; Stendahl and Sprague, 1982; Wehrli and Stumm, 1989). The results presented here indicated that only a variation in pH, SO_4, alkalinity, and Na (within the ranges tested) had marked influences on V acute toxicity to *D. pulex* and *O. mykiss*, and that pH and alkalinity were the most influential ETMFs (Chapters 2 and 3). The acute models obtained for daphnids and fish were compared in Chapter 3, and the regression models between V and pH, or alkalinity, or Na described similar patterns for *D. pulex* and *O. mykiss*. However, the model developed for the influence of SO_4 on V acute toxicity to *O. mykiss* and *D. pulex* differed markedly when the concentration of SO_4 was above 2500 mg/L (Chapter 3). In Chapter 2, it was also shown that these ETMFs did not influence V chronic toxicity to *D. pulex*, which suggests that future Canadian water quality guidelines for the protection of aquatic life (CWQG-PAL) for V should only account for the influence of ETMFs on V's acute toxicity to aquatic organisms.

In Chapter 3, it was also determined by using the method of stepwise multiple linear regression (MLR) analysis that both pH and alkalinity played a key role in modifying V acute toxicity in daphnids and fish, whereas SO_4 was only influential in daphnids. The difference

between the models could be due to the disparity in the mechanisms of V uptake, or merely due to an increase in physiological stress experienced by *O. mykiss* above 360 mg SO_4/L. To determine if the influence of SO_4 on V acute toxicity should be considered in the development of a CWQG-PAL, more studies similar to those presented here should be conducted using other invertebrate and fish species. Schiffer and Liber (2017a,b) found that *D. pulex* is more sensitive to V than many other invertebrates and several regional and model fish species, including *O. mykiss*. Therefore, it would be reasonable to propose either developing an interim CWQG-PAL incorporating the MLR model presented here for *D. pulex*, or developing one model for daphnids and one for fish. If the daphnid model is used, regulators should be aware that the extrapolation of this model to other species may be overprotective.

Dissolved organic carbon (DOC) is another relevant water chemistry variable that often influences metal toxicity to aquatic organisms. Cationic metals and cationic forms of metals form complexes with DOC reducing their availability. Within a pH range of 6 to 9, oxyanions are the predominant V species (both HVO_4^{-2} and $H_2VO_4^-$ coexist and dominate). The negative charges and chemical structures of the V oxyanions hinder their ability to complex with DOC because, within the above-mentioned pH range, humic and fulvic acids also have negative charges (Rehder, 1991; Wilson and Weber, 1979). To complex with DOC, V needs to switch to vanadyl (VO_2^+). This change in V speciation only occurs when the water pH drops below 3 (Wilson and Weber, 1979). Such an acidic pH is unlikely to occur in most Canadian freshwater bodies, where pH generally ranges between 6 and 9. This pH range is also common in natural surface waters of the oil sands region (Allen, 2008a; Dubé et al., 2013; Mackenzie River Basin Board, 2003b; Squires et al., 2009). Therefore, DOC should not be considered as a water quality parameter required to develop a CWQG-PAL for V.

5.2.2.1. The influence of water pH on vanadium speciation and toxicity to aquatic organisms

As mentioned, pH was the ETMF that most influenced V acute toxicity to *D. pulex* and *O. mykiss*. Interestingly, the descriptive models for V and pH for daphnids and rainbow trout were very similar (Chapter 3, Figure 3.1a). These similarities were possibly seen because the influence of pH likely occurred before V reached the aquatic organism. Water pH plays a significant role in V speciation, modifying the chemical structure of V (Chapter 2), thus altering the ability of V to compete with other ions with similar chemical structures (HCO_3, hydrogen sulphate (HSO_4), and SO_4, Chapter 3). Changes in chemical structure could also hinder the ability of anion carriers to introduce V into aquatic organisms, consequently reducing V toxicity to these organisms. Speciation modelling with Visual MINTEQ (Chapter 2) showed that the oxyanions HVO_4^{-2} and $H_2VO_4^-$ were both present within the pH range of 6 to 9 (the range tested here), and the proportions of HVO_4^{-2} and $H_2VO_4^-$ changed as pH increased from 6.2 to 9, showing a 50% reduction in the percentage of $H_2VO_4^-$ and an almost 50% increase of HVO_4^{-2}, reaching a 1:1 ratio at pH 9 (Figure 2.3, Chapter 2). With this information available, Chapter 2 proposed that both V species were responsible for V acute toxicity because it was not possible to rule one of them out. New ICP-MS technology allows for the discrimination of metal species in the chemical analyses of water samples, which could validate the speciation modelling proposed here. Knowing which species of V is present in the test water would open several new research avenues, especially in the area of mechanistic toxicology. Thus, it is recommended to further investigate if speciation plays a role in mechanisms like uptake, toxicity and depuration in aquatic organisms.

Changes in water pH could also affect the micro-environment of fish gills. Playle (1998) and Playle and Wood (1989) showed that the micro-environment of fish gills presents a slightly

different pH than the water in which the fish live. In the micro-environment of the gills, ammonia (NH_3) and carbon dioxide (CO_2) play important roles in modifying metal speciation and solubility. When the inspired water pH and buffering capacity of the water change, fish adjust the release rate of NH_3 and CO_2 from their gills to the gill micro-environment (Playle and Wood, 1989). If more NH_3 is released, then more H is consumed, transforming NH_3 into ammonium (NH_4), thus turning the micro-environment more alkaline. The higher release of CO_2 to the gill microenvironment induces more disassociation into HCO_3, acidifying this environment. These micro changes to the gill micro-environment could make V and other metals more or less toxic to aquatic organisms (Playle, 1998; Playle and Wood, 1989). Therefore, the descriptive models developed here could in the future be corrected with measurements of V concentrations at the gill level, similar to what has been proposed for the development of the biotic ligand model (BLM) for cationic metals (Di Toro et al., 2000; Meyer et al., 1999; Niyogi and Wood, 2004).

5.2.2.2. Amelioration of V toxicity to aquatic organisms by alkalinity and sulphate

This thesis demonstrated that increasing alkalinity and concentrations of SO_4 within a range representative of the OSPW and the AR (Table 2.1, Chapter 2) ameliorated V acute toxicity to *D. pulex* (Figure 2.1b, c Chapter 2), and partially to *O. mykiss* (only when SO_4 was below 250 mg/L, Figure 3.1 Chapter 3). Sulphate and HCO_3 may compete with the V oxyanions for the anionic carriers of the aquatic organisms (Evans and Brusewitz, 1994; Rehder, 1991). One way to confirm if there is competition between V and HCO_3, HSO_4, or SO_4 would be to investigate how daphnids or fish take up V as SO_4 and alkalinity changes. Several researchers (e.g., Bell et al. 1980) have suggested that V is incorporated through the gills, where the competition for the biotic ligand may occur. A novel and innovative method using micro-X ray fluorescence and absorption was applied

by Saibu et al. (2018) to study the uptake, distribution, and speciation of Zn in *O. mykiss* gills. These authors successfully demonstrated that Zn was introduced through the gills and predominantly accumulated in the primary lamellae. These authors also showed that Zn and Cd competed for the same uptake route. Thus, it would be interesting to apply micro-X ray fluorescence and absorption techniques to confirm that V is introduced through the gills and that the uptake of V by the gills is reduced when alkalinity or SO_4 concentrations increase. The results of such a study could confirm that V oxyanions compete with HCO_3, HSO_4, or SO_4 for the same anionic carriers.

Chapter 2 proposed that V could be introduced into daphnids and fish through permeases and excreted through the antiporter SLC26. The permeases have not yet been identified in the gills of daphnids or fish, but SLC26 has been found in fish gills (Bayaa et al., 2009; Griffith, 2017; Piłsyk and Paszewski, 2009). The use of inhibitors for anion carriers like 4, 40-diisothiocyanatostilbene-2, 20-disulfonic acid (DIDS), and radioactive $^{35}SO_4$ in unionid *Toxolasma texasiense* has shown that SO_4 is introduced by anion carriers (Griffith, 2017). Therefore, this method could be applied to confirm that the amelioration of V toxicity caused by alkalinity and/or SO_4 is due to competition for the same anionic carrier.

5.2.3. Incorporation of the influence of water chemistry on vanadium water quality guidelines

Aquatic environments across Canada present diversity, depending on the type of sediment, water flow, local geology and geomorphology composition of the riparian area, and human impact, one water body, for example, the Athabasca River (AB), can present different water chemistry conditions from another (i.e., the Grand River, ON). Since ETMFs like pH, alkalinity, DOC, and

water hardness can influence the toxicity of a chemical or element, regulators need to account for the influence of ETMFs when developing WQGs, so that guidelines are accurate for a specific site. Canadian water quality guidelines for the protection of aquatic life are intended to protect aquatic organisms from anthropogenic stressors (CCME, 1999). Incorporating the influence of ETMFs representative of a particular site into CWQGs-PAL enables regulators to apply these guidelines with more accuracy, thus improving the protection of aquatic organisms in the area. The 2007 CCME protocol for the development of a CWQG-PAL presents seven steps that should be followed to develop such guidelines (Figure 5.1). The first three steps of the 2007 CCME protocol for the derivation of a CWQG-PAL were investigated and evaluated for V in the first three chapters of this thesis (including the General Introduction). In the third step, CCME recommends the application of the BLM to quantify the influence of ETMFs on metal toxicity. The BLM model has been successfully developed for cation metals like Cu, silver (Ag), Zn, Ni, Cd, Pb, and cobalt (Co), but (to our knowledge) there is still no BLM model for oxyanions like V (Niyogi and Wood, 2004). The BLM is based on the hypotheses that metals that try to reach the biotic ligand (e.g. fish gill) compete with naturally occurring ions (Ca, sodium (Na), magnesium (Mg), proton (H), chloride (Cl), SO_4, and HCO_3), that abiotic ligands (e.g., DOC) form complexes with these metals and that toxicity occurs when the concentration of a metal at the biotic ligand exceed a threshold concentration (Di Toro et al., 2001, 2000; Niyogi and Wood, 2004). There are two approaches to developing a BLM model: one is based on measuring the concentrations of a metal accumulated in the gills for each scenario of a modified water chemistry condition; and the second is a mathematical derivation of the stability constants from known toxicity data and a broad range of water chemistry measurements (de Schamphelaere et al., 2005; de Schamphelaere and Janssen, 2002; Niyogi and Wood, 2004). These two methods require either a substantial number of toxicity

tests or a complex calculation. Thus, there is an overall perception among some regulators that the BLM is a complicated tool to implement, requiring measurements of several water quality parameters. This perception has hindered BLM's use in WQG in the USA and probably in Canada (Brix et al., 2017; CCME, 2019).

Just recently, ECCC released a federal environmental water quality guideline for Cu-based on the BLM approach (ECCC, 2018), but the application of BLM to CWQG-PAL is still in its infancy. Recently, MLR was successfully applied in the U.S.A. for Cu, Al, and Pb (Brix et al., 2017; DeForest et al., 2018; Esbaugh et al., 2011), and MLR was able to predict Cu toxicity to several species under a wide range of water chemistries as accurately as BLM. The strong resemblance in outcomes between the BLM and MLR modelling has allowed the application of MLR in the development of water quality criteria (WQC) for Cu using the species sensitivity distribution method (SSD), the preferred method by CCME for the development of CWQG-PAL, (Brix et al., 2017; CCME, 2007). The application of SSDs for V was recently studied and presented by Schiffer and Liber (2017b), who obtained an acute HC_5 of 0.64 and a chronic HC_5 of 0.05 mg V/L. The studies described in Chapters 2 and 3 of this thesis advanced the findings of Schiffer and Liber (2017b) by developing an MLR model that accounts for the effects of the most relevant ETMFs influencing V acute toxicity to *D. pulex* and *O. mykiss*. The best-fitted MLR model for *D. pulex* included both on the interaction between pH and alkalinity and that of alkalinity and SO_4, whereas the MLR model obtained for *O. mykiss* incorporated only the individual effects of pH and alkalinity. Thus, pH and alkalinity seem to have key influences on V acute toxicity in both species, and SO_4 is likely the divergent parameter between both MLR models. According to the results presented here and in Schiffer and Liber (2017a), *D. pulex* is more sensitive to V than is *O. mykiss*.

To complete the fourth step of the CCME protocol, it is necessary to test the model with a realistic worst-case scenario, accounting for the influence of ETMFs. For instance, a worst-case scenario for the acute toxicity of V to *D. pulex* will be that where pH is approximately 9, alkalinity is 70 mg/L as $CaCO_3$, and SO_4 is 54 mg/L (Table C1). Under such conditions, the calculated acute LC_{50} for *D. pulex* using the MLR approach is 0.62 mg V/L. This modelled concentration is reasonably similar to the one obtained from the acute toxicity tests performed here, where the acute LC_{50} for *D. pulex* equalled 0.64 mg V/L when pH was 7.99, alkalinity was 75 mg/L as $CaCO_3$, and SO_4 was 54 mg/L. To develop the acute SSD for V, Schiffer and Liber (2017b) used an acute LC_{50} for *D. pulex* of 2.17 mg V/L obtained from a single toxicity test, with a reported water pH of 8.3, alkalinity of 98 mg/L as $CaCO_3$ and a SO_4 concentration of 33.1 mg/L (Schiffer and Liber, 2017a). When these reported water chemistry values are applied to the *D. pulex* MLR model, the predicted acute LC_{50} for V is 0.88 mg V/L. This predicted acute LC_{50} is similar to that of 0.99 mg V/L obtained in a toxicity test from Chapter 2 when the test water pH was 8.08, alkalinity was 105 mg/L as $CaCO_3$, and SO_4 was 54 mg/L, but it is 2.46 times lower than the acute LC_{50} (2.17 mg V/L) used to develop the SSD model (Schiffer and Liber, 2017b). A similar difference was found between the V LC_{50} for *O. mykiss* reported by these authors and the modelled LC_{50} by using MLR. Since the LC_{50}s predicted by MLR for *D. pulex* and *O. mykiss* models are substantially lower than that obtained from a single test conducted by Schiffer and Liber (2017a,b), it is recommended to validate these MLR models by repeating toxicity tests in other reputable laboratories, using the same range of water chemistry variables and daphnia species used in this thesis. However, the differences found between the MLR-predicted LC_{50}s and those obtained by these authors could indicate that a future correction to the acute HC_5 value should be considered to derive a more site-specific guideline for the Alberta oil sands region (Step 5 from CCME protocol). According to

Newman et al. (2000), to derive a guideline value using an accurate SSD, a sample size between 15 to 55 toxicity data obtained from taxonomically diverse species is required. Thus, it is also recommended that additional toxicity tests be performed with regional and model freshwater organisms from other sensitive and unrepresented taxa (Schiffer and Liber, 2017b), modifying the pH, alkalinity, and SO_4 of the tests water within the parameters used here to obtain more site-specific LC_{50}s.

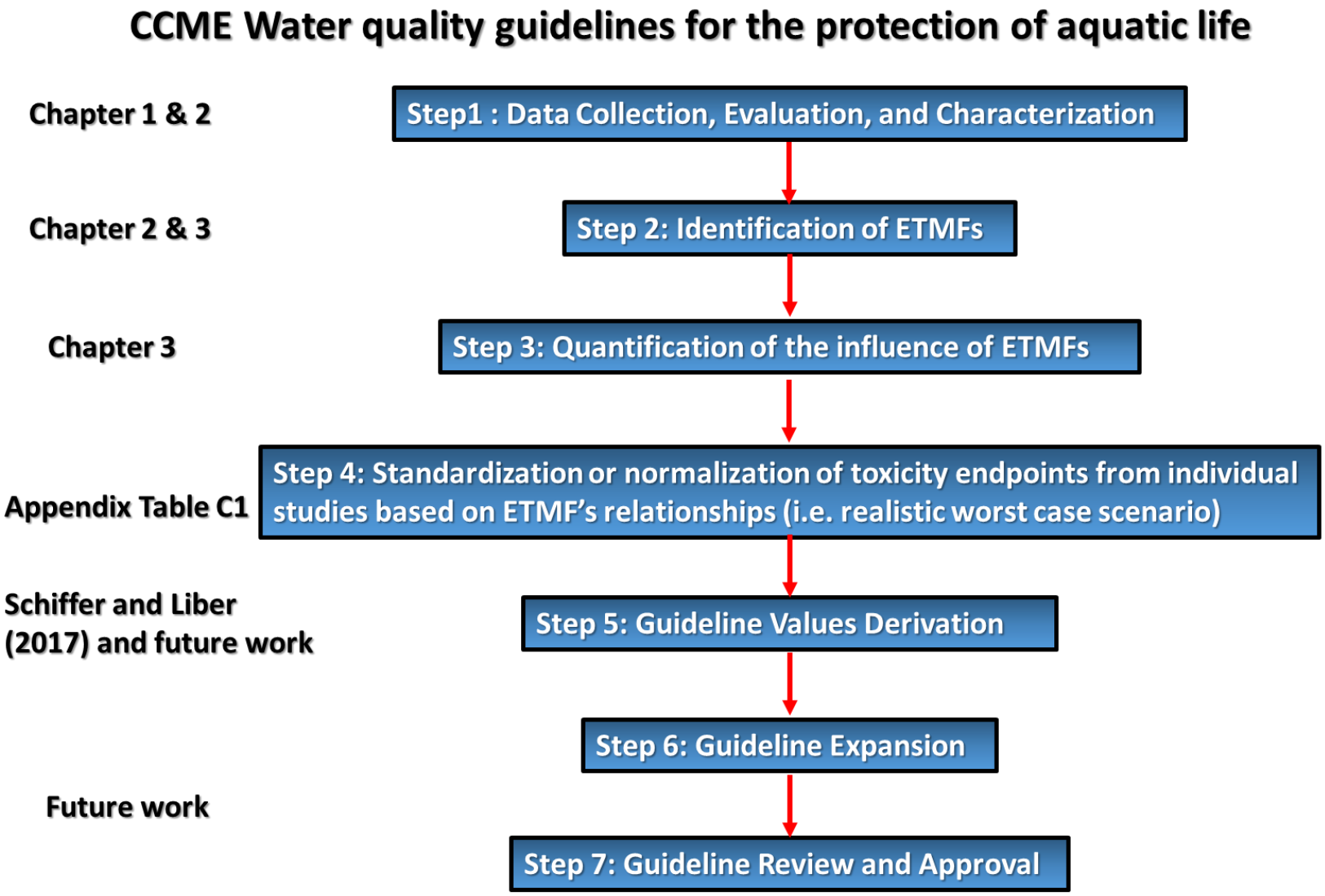

Figure 5.1: Steps for developing a Canadian water quality guideline for the protection of aquatic life as proposed by the Canadian Council of Ministers of the Environment (CCME). Left-side column indicates the chapter(s)/section(s) in this thesis, or in other publications, where each step from the CCME protocol was addressed for V. Modified from (CCME, 2007).

5.2.4. Potential mechanism of vanadium toxicity

Freshwater cladocerans and fish constantly struggle against their hypo-osmotic environment, actively pumping ions to maintain ionic homeostasis (Aladin and Potts, 1995; Bianchini and Wood, 2008; Evans, 1987; Marshall, 2002). These animals drink little water, so they incorporate and excrete ions mainly through their gills, structures that also provide a key route for the uptake of metals (Wood, 2012). The uptake of ions in daphnids and fish, however, is slightly different. In adult daphnids, ions enter through ion-transporting cells located in the epipodites in the thoracic appendages, whereas in fish ions are taken up by mitochondria-rich chloride cells in the gills (Aladin and Potts, 1995). Sodium enters daphnids through the activation of an electrogenic 2Na/H transporter located in the apical membrane, and then through a sodium-potassium ATPase (NAK) located in the basolateral membrane (Bianchini and Wood, 2008). In fish, it is believed that Na is introduced by a Na/H apical channel or an H/NH_4 transporter and then incorporated into the circulatory system by NAK (Marshall, 2002). In both fish and daphnids, Ca is incorporated by an apical channel and then pumped into the blood or hemolymph by a basolateral Ca-ATPase (Marshall, 2002). In daphnids, Ca is quickly sequestered and combined with HCO_3 to form calcium bicarbonate ($CaCO_3$), which builds up the exoskeleton (Roy, 2009). These enzymes can be inhibited by metals like Cu and Ag, decreasing the influx of these ions (Wood, 2012). Researchers have proposed that V could cause Na and Ca imbalance in invertebrates and fish by inhibiting NAK and/or the Ca-ATPase (Ahearn et al., 2004; Bell et al., 1980; Bell and Sargent, 1979; Cantley et al., 1977; Ringelband, 2001; Ringelband and Karbe, 1996). It has also been suggested that V could be introduced through the gills (Bell et al., 1980) of aquatic organisms, and that, if so, it is reasonable to hypothesize that a potential mechanism of toxicity could be Na and Ca imbalance. As mentioned in Chapters 2 and 3, V uptake at the gill may be altered by competition with HCO_3

or SO_4, or changes in speciation due to variations in water pH. The effects of these variables could reduce V uptake, and its subsequent toxicity to aquatic organisms. Confirming that V is introduced through the gills will promote studies that could identify which specific anion carriers are used to get V into the animals. Unitl now, scientists did not identify the specific transporter for V. By knowing the specific transporters used for V uptake, researchers and regulators could easily discern which anions are competing with V, and thus determine the role these anions may play in a MLR model for V. Having identified the anion transporters allows also to determine if there is commonality among different aquatic organisms, due to the presence of the aforementioned transporters. The aquatic species that present anion trasnporters with similar affinity for V should respond similarly to its exposure, and therefore these species should be included in a more accurate site-specific MLR-based CWQG-PAL for V.

According to peer-reviewed publications, V may not only elicit toxicity to aquatic organisms through the disruption of ion homeostasis in the gills but also through oxidative stress (Valko et al., 2006, 2005). Various enzymes and non-enzymatic compounds maintain the antioxidant balance. Certain enzymes—catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD)—play crucial roles in protecting animals against chemically-induced oxidative stress (Kelly et al., 1998). The first two are responsible for reducing H_2O_2 to non-toxic H_2O and O_2 (Chelikani et al., 2004; Mills, 1957), whereas SOD dismutates $O_2^{\cdot}$ into H_2O and H_2O_2 (Kelly et al., 1998). In addition to having an enzymatic army, aquatic organisms have non-enzymatic antioxidants such as glutathione, ascorbate, and metallothionein that collaborate to reduce the oxidative insults (Kelly et al., 1998). The tripeptide GSH is the principal non-enzymatic antioxidant defendant, and when oxidized, it reacts with another oxidized glutathione, forming glutathione disulphide (GSSG; Sies, 1999). In this process, electrons are donated to neutralize

reactive oxygen species (ROS; Wu et al., 2004). Because GSH and GSSG are interrelated, a reduction in the ratio of GSH:GSSG could be used to indicate oxidative stress in the target organ (Jamwal et al., 2016).

A key component of this thesis was to investigate the mechanisms that contribute to the toxicity of V in model aquatic organisms. Chapter 4 of this thesis described that V toxicity was attributed to two processes: the disruption of ion homeostasis (Na and possibly Ca) in the gills, and oxidative stress in the gills and liver. These mechanisms of toxicity could coexist and cause structural and/or functional damage to the gills, releasing essential ions into the aquatic environment. According to the results obtained, V reduces whole body concentrations of Na in *D. magna* and *O. mykiss*, and also of Ca in *O. mykiss*. Different from what was expected, unidirectional Na influx increased when exposed to the highest concentrations of V in both daphnids and fish. Vanadium also caused lipid peroxidation in the liver and gills of *O. mykiss* and reduced the GSH:GSSG ratio in the gills and the liver of fish. The activity of CAT only increased in the liver, and SOD showed no change. Altogether, these results indicate that V (at concentrations above 1 mg/L) could damage the gills, potentially increasing ion leakage and reducing whole-body Na and Ca. In their weakened condition, the daphnids and fish could try to cope by increasing the influx of these ions (at least of Na). Another potential effect of oxidative stress not tested here could be DNA damage by ROS, which could cause cell death (Sharma and Talukder, 1987; Soares et al., 2008; Valko et al., 2005).

The original objective of Chapter 4 was to investigate the mechanisms of V toxicity in *D. magna* and *O. mykiss* and to compare them. However, some difficulties were encountered when the effect of V on the redox capacity of *D. magna* was being investigated, which forced this chapter to mainly focus on how V altered the redox capacity in *O. mykiss*. The difficulties were related to

variability generated among the samples of daphnids, inaccuracy in measuring the response, and incompatibility of commercial kits with the samples collected from the tests with *D. magna*. Several options were investigated to overcome these difficulties, such as pooling daphnids to increase the overall mass of the animal sample, trying different methods to measure antioxidant enzymes activity (Barata et al., 2005a, 2005b), and comparing V's outcome with chemicals known to cause oxidative stress in *D. magna* (Aguirre-Martínez et al., 2015). Even though technical difficulties were encountered, it was still possible to obtain relevant information about the mechanisms of toxicity of V to invertebrates and fish.

The mechanisms of V toxicity investigated here are not exhaustive. For instance, V presents a similar chemical structure to phosphate, and thus V could potentially inhibit phosphohydrolases, ribonucleases, adenylate kinase, phosphofructokinase, squalene synthetase, glyceraldehydes-3-phosphate dehydrogenases, glucose-6-phosphatase, and phosphotyrosyl-protein phosphatases by forming a complex with these enzymes (Nechay, 1984). Therefore, further research should be conducted to fully understand the mechanisms responsible for triggering V's toxicity in aquatic organisms.

5.2.5. Applicability of the knowledge generated by this research project

Overall, the results presented here will assist Canadian regulators and the Alberta oil sands industry to develop more accurate V guidelines for the protection of aquatic life. It was shown here that pH, alkalinity and SO_4 influenced V acute toxicity to both *D. pulex* (one of the most sensitive invertebrate species) and *O. mykiss* (a less sensitive species, but highly valuable for commercial and recreational fishing). Water pH proved to be the most influential ETMF, but because variations in pH, alkalinity and sulphate could significantly influence V acute toxicity to aquatic organisms

at the same time, it is recommended that a future CWQG-PAL for V incorporates the effect of all these variables together in one model. According to the results presented here, MLR modelling is a useful and accurate tool that successfully predicted the effects of pH, alkalinity, and sulphate on V acute toxicity to daphnids and fish. Thus, MLR should be applied to correct the acute HC_5 presented by Schiffer and Liber (2017b) to account for the influence of the above mentioned ETMFs, once more studies with organisms from other sensitive taxa are completed. Furthermore, the results presented here indicated that within the range of ETMFs studied, V did not influence chronic toxicity to *D. pulex*. Since most of the acute V toxicity models developed in *O. mykiss* were similar to those of *D. pulex* (Chapter 3), it was hypothesized that V chronic toxicity to *O. mykiss* should also not be influenced by the ETMFs tested in this thesis. Therefore, it may not be necessary to apply a correction to the chronic toxicity of V to daphnids and fish to account for the influence of ETMFs. However, this hypothesis should be tested. Thus, at least temporarily, the HC_5 of 0.05 mg V/L calculated by Schiffer and Liber (2017b) should be used as a chronic CWQG-PAL for V.

5.3. Future directions

The research presented here demonstrates the need to consider the influence of ETMFs on metal toxicity in the development of site-specific CWQG-PALs. In particular, this research has shown that pH, alkalinity, and possibly SO_4 play key roles in V acute toxicity to *D. pulex* and *O. mykiss*. This thesis developed predictive models that can be applied to a new CWQG-PAL for V and found that V likely elicits its toxicity by disrupting Na and Ca homeostasis in *D. magna* and *O. mykiss*, and by altering the antioxidant capacity in fish. However, to better understand the

potential risks of V to aquatic systems, further research is required. Recommendations for future work include the topics discussed below.

5.3.1. Incorporation of more aquatic organisms into the MLR model

The research completed in this thesis has provided the basis for the development of a future CWQG-PAL specific for the Alberta oil sands region by developing predictive relationships between key ETMFs and V acute toxicity to two model aquatic organisms (*D. pulex* and *O. mykiss*). However, the CCME protocol for the development of CWQGs-PAL suggests using a SSD to obtain a guideline. Newman et al. (2000) indicated that above 15 taxonomically different species should be used to develop an accurate SSD estimation to protect different trophic levels and obtain an HC_5 that protects 95% of these species. For this reason, it is recommended that the tests conducted in Chapters 2 and 3 be repeated with other sensitive invertebrates like *Ceriodaphnia quadrangular*, *Ceriodaphnia dubia*, *Daphnia dentifera*, *D. magna* and *Hyalella azteca*, and vertebrate species like *Pimephales promelas* and *Salvelinus fontinalis*, which according to Schiffer and Liber (2017b), will face lethality within the concentrations of V found in treated OPSW. This recommendation is principally based on recent research by Schiffer and Liber (2017b) who found that, for example, *P. promelas*, a fish species that is widespread in North American and representative of the Alberta oil sands region, was almost four times more sensitive to V than was *O. mykiss*. Thus, it would be interesting to determine if a predictive model between pH, alkalinity, and SO_4 and V acute toxicity for *P. promelas* would also be similar to the ones presented here for *D. pulex* and *O. mykiss*.

In Chapter 2 of this thesis, it was shown that the ETMFs investigated here did not influence V chronic toxicity to *D. pulex*. So, it was hypothesized that ETMFs should not influence V chronic

toxicity to *O. mykiss* or other freshwater species. However, this hypothesis should be tested in the future to ascertain whether there is no need to apply MLR modelling to correct a chronic CWQG-PAL for V toxicity to sensitive fish species.

5.3.2. Applicability of the predicted models to other regions in Canada

One of the last steps (step 6) in developing a CWQG-PAL, as proposed by CCME (2007), is to expand the guideline to improve its applicability to other environmental conditions. The research presented here tested several water quality characteristics representative of the AR and OPSW that present a notable difference between the natural environment and the industrial by-product. Results showed that of the ETMFs investigated, only pH, alkalinity, and SO_4 significantly influenced V acute toxicity to *D. pulex* and *O. mykiss* within ranges representative of water conditions of this region (Table 2.1, Chapter 2). Sodium could be considered a fourth variable of interest, but V was only influenced by Na when its concentration surpassed 300 mg/L, which is rarely found in natural environments of the Boreal ecoregion. The predictive models between pH, alkalinity, SO_4 or Na, and V acute toxicity developed for *D. pulex* and *O. mykiss* (Chapters 2 and 3), including the MLR models developed in Chapter 3, could potentially be applied to other sites presenting similar ranges of water quality characteristics. However, if they were used in sites with different water chemistries than those in the models (i.e., sites with alkalinity lower than 70 mg/L as $CaCO_3$), they would probably predict an inaccurate acute LC_{50} for the organisms of interest. Therefore, future research should expand the limits of water quality characteristics used in these models to allow for a broader spectrum of aquatic environments where the models could be applied.

5.3.3. The influence of multiple water quality variables on V toxicity to aquatic organisms

In Chapter 2, the influence of mixtures of regional representative ranges of SO_4 and Na, and of alkalinity and SO_4, on V toxicity to *D. pulex* were tested to evaluate if the presence of more than one ETMF would modify their individual influence on V acute toxicity to *D. pulex*. The results revealed that only a combination of increasing Na and increasing SO_4 resulted in a different response, in this case, a slightly higher amelioration of V acute toxicity than that caused by the individual ETMFs (Figure 2.2a). However, only a combination of two ETMFs was tested, and aquatic environments present multiple ETMFs (pH, alkalinity, water hardness, DOC, and other ions), many of which have the potential to influence metal toxicity simultaneously. The MLR models were useful in accounting for the effect of multiple simultaneous water quality variables with high accuracy, so the application of these models should be considered for the development of an acute CWQG-PAL for V.

5.3.4. Mechanism of toxicity of vanadium in aquatic organisms

Chapter 4 represented a key part of this thesis, as it focused on investigating how V elicits its toxicity in freshwater organisms. The results showed that V caused Na imbalance in *D. magna*, and Na and Ca imbalance, as well as oxidative stress, in *O. mykiss*. In both species, unidirectional Na influx was slightly increased with increasing concentrations of V (Chapter 4). The increase in the Na unidirectional influx rate contradicts what Bell and Sargent (1979) and Ringelband and Karbe (1996) found in eels and hydroids, possibly indicating that NAK is not the main target of V. However, in the present thesis the inhibition of NAK in daphnids or fish was not investigated. Future research should corroborate if concentrations within the range tested here inhibit NAK,

which can be done using the classical NAK inhibitor Ouabain as a positive control (Bell and Sargent, 1979; Lin and Randall, 1993; Piermarini and Evans, 2000).

Several researchers have suggested that because V shares a similar chemical structure with phosphate (Evans and Brusewitz, 1994), it can inhibit various phosphate-dependent enzymes by binding to their active site and forming a transition state (Willsky, 1990). For instance, V has been shown to inhibit the alkaline phosphatase in the human liver, intestines, and kidneys (Seargeant and Stinson, 1979), the Ca, Mg ATPase in the cardiac sarcoplasmic reticulum of pigs (Hagenmeyer et al., 1980), and the Dynein ATPase in sea urchin sperm cells (Gibbons et al., 1978). Therefore, V could inhibit phosphatase in daphnids and fish in addition to causing ion imbalance and oxidative stress. Consequently, further mechanist research is recommended in this area.

A key step in the expression of toxicity is the uptake of the toxicant because variations in the uptake rate could reduce or enhance internal exposure to the toxicant, thus influencing biochemical and physiological responses. From Chapters 2 and 3, we could infer that V uptake may be altered by the competition between V and HCO_3 and SO_4 for the anion carriers, and changes in V speciation due to variations in pH. It was proposed that V is taken up through the gills by anion carriers (Bell et al., 1980; Gillio Meina et al., 2019). As previously discussed, micro-X ray fluorescence and absorption was applied by Saibu et al. (2018) to study the uptake, distribution, and speciation of Zn in *O. mykiss* gills. This methodology could be useful in future research seeking to corroborate if the uptake of V occurs through the gills.

Understanding the mechanisms of uptake and toxicity of V will help to identify and explain differences in sensitivity of freshwater organisms to waterborne V, which will assist researchers in choosing the most sensitive species to derive more accurate site-specific CWQG-PAL (CCME,

2007). Besides, understanding these mechanisms could also aid in identifying additional ETMFs that should be considered for the development of a CWQG-PAL for V.

5.4. Conclusion

This research has demonstrated that pH, alkalinity and SO_4, within a range of surface water conditions representative of the Alberta oil sands region, influence V acute toxicity and that the effect of these ETMFs should be incorporated into the development of a site-specific CWQG-PAL for V for the region. This research also contributes predictive models that may assist regulators in calculating the toxicity of V to representative freshwater organisms (*D. pulex* and *O. mykiss*), according to the specific water chemistry of Northern Alberta. The research has demonstrated that multiple linear regression modelling is a promising tool that could ease the incorporation of the influence of these and other ETMFs into the calculation of metal toxicity to CWQGs-PAL for metals. The developed models can be applied not only to the Alberta oil sands region but also to any aquatic environment with water chemistry within the ranges used to develop the MLR models. Additionally, this thesis has shown that V toxicity is due, at least partially, to Na and Ca imbalance in daphnids and fish, and to oxidative stress in fish within the range of V concentrations found in treated OPSW (1 to 7 mg V/L). There are different approaches for how to incorporate the treated OSPW to the final landscape in the region. On the one hand, treated OSPW could be contained in reclaimed lakes. However, V and several ions could remain at very high concentrations, impeding the establishment of a healthy ecosystem. On the other hand, if treated OSPW is released to the Athabasca River, the concentration of V within the discharge zone could increase from around 3 µg/L (concentration normally found in freshwater) to near 1-7 mg/L, thus potentially causing toxicity to freshwater invertebrate and fish species. Additionally, the dilution

of treated OSPW into the Athabasca River will cause a reduction of alkalinity from around 600 to 70 mg/L as $CaCO_3$ and of sulphate concertation from 400 mg/L to 50 mg/L. The decrease in these two water chemistry variables will decrease the protective effects that these two ETMFs have on V toxicity. Therefore, V should be reduced or removed from the treated OSPW before it is either incorporated into reclaimed lakes or discharged into the Athabasca River.

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APPENDIX

The tables and figures included in this appendix are separated by chapter.

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Table A1: Water chemistry profile of the prepared Athabasca River water (ARW) obtained by dilution of carbon-filtered, bio-filtered City of Saskatoon municipal water with 30% deionized water and used as base water for the preparation of tests solutions.

Variable	Na [c]	HCO_3 [c]	Cl [c]	SO_4 [c]	Alkalinity [d]	Hardness [d]
Geo Mean [a] (*n*=10)	18	80	8	54	95	119
SD [b]	2	8	1	13	8	22

[a] Geo Mean = Geometric mean
[b] SD = Standard deviation
[c] Concentration as mg/L
[d] Concentration as mg/L as $CaCO_3$

Table A2: Reagent added (mg) or volume in mL of deionized water (DI) water added to prepare Athabasca River water to make up 1-L of test water with the desired alkalinity or hardness used for acute and chronic tests for *Daphnia pulex* or *Oncorhynchus mykiss*.

Variable & Nominal Concentration [a]	$CaSO_4 \cdot 2H_2O$	$MgSO_4$	$NaHCO_3$	DI
Alkalinity				
70	-	-	-	300
100	-	-	-	-
150	-	-	229.6	-
200	-	-	285.7	-
300	-	-	459.2	-
600	-	-	857.2	-
Hardness				
70	-	-	-	190
100	-	-	-	-
140	31.5	31.5	-	-

[a] Concentration in mg/L as $CaCO_3$

Table A3: Reagent Added (mg) to 1 L of Athabasca River water to make up 1-L solutions of a desired ionic concentration for use as test solutions in acute and chronic vanadium toxicity tests for *Daphnia pulex* or *Oncorhynchus mykiss*.

Variable & Nominal Concentration [a]	CHO_2Na	KCl	$KHCO_3$	$MgSO_4$
Na				
10	-	-	-	-
35	59.2	-	-	-
120	310.6	-	-	-
220	591.7	-	-	-
320	887.6	-	-	-
420	1,198.2	-	-	-
Cl				
10	-	-	-	-
35	-	58.4	-	-
120	-	237.1	-	-
250	-	510.5	-	-
HCO_3				
70	-	-	-	-
160	-	-	91.7	-
250	-	-	246.1	-
410	-	-	508.6	-
SO_4				
50	-	-	-	-
100	-	-	-	87.7
200	-	-	-	206.8
300	-	-	-	332.1
340	-	-	-	350.8
380	-	-	-	400.9

[a] Concentration as mg/L

Table A4: Measured water chemistry variables in acute vanadium toxicity tests for *Daphnia pulex*. Values shown are means ± SD.

Variable & Nominal Concentration [a]	pH	DO [b]	Temperature [c]	Conductivity [d]	Alkalinity [e]	Hardness [e]
pH						
6	6.3 ± 0.0	8.0 ± 0.2	23.1 ± 0.6	303.6 ± 6.0	70 ± 3	96 ± 3
6.5	6.5 ± 0.1	7.6 ± 0.5	24.4 ± 0.4	367.9 ± 3.6	84 ± 4	109 ± 3
7	7.2 ± 0.0	8.1 ± 0.3	23.0 ± 0.7	295.4 ± 5.9	84 ± 3	96 ± 3
8	7.9 ± 0.0	8.4 ± 0.2	22.7 ± 0.6	294.1 ± 2.2	105 ± 3	104 ± 4
9	8.8 ± 0.1	8.6 ± 0.2	22.3 ± 3.9	301.8 ± 6.7	105 ± 5	109 ± 4
Alkalinity						
70	8.0 ± 0.4	7.7 ± 0.3	22.9 ± 1.1	310.6 ± 2.9	75 ± 3	111 ± 1
100	8.1 ± 0.0	8.4 ± 0.2	22.7 ± 0.6	294.1 ± 2.2	105 ± 3	104 ± 4
150	8.2 ± 0.0	8.4 ± 0.3	22.8 ± 0.7	365.9 ± 2.1	154 ± 4	105 ± 3
200	8.1 ± 0.1	7.9 ± 0.5	24.0 ± 0.5	514.2 ± 2.8	221 ± 15	110 ± 4
300	8.4 ± 0.1	8.6 ± 0.4	23.5 ± 0.4	617.3 ± 4.7	306 ± 3	92 ± 10
600	8.3 ± 0.1	8.3 ± 0.2	22.9 ± 0.6	1,050.7 ± 31.4	541 ± 4	116 ± 5
Na						
18	8.1 ± 0.0	8.4 ± 0.2	22.7 ± 0.6	294.1 ± 2.2	105 ± 4	105 ± 4
35	7.9 ± 0.1	7.2 ± 0.8	24.4 ± 0.6	411.3 ± 6.8	105 ± 5	106 ± 3
120	7.9 ± 0.1	7.8 ± 0.7	24.2 ± 0.4	752.0 ± 5.5	125 ± 23	105 ± 4
220	8.1 ± 0.1	7.5 ± 0.6	23.5 ± 0.5	1,112.9 ± 6.5	102 ± 3	94 ± 2
320	8.2 ± 0.1	7.3 ± 0.6	23.6 ± 0.5	1,542.2 ± 9.9	123 ± 5	107 ± 4
420	7.9 ± 0.1	7.3 ± 0.9	24.7 ± 0.8	1,929.8 ± 3.2	169 ± 20	107 ± 3
SO_4						
55	8.1 ± 0.0	8.4 ± 0.2	22.7 ± 0.6	294.0 ± 2.2	105 ± 4	105 ± 4
100	8.0 ± 0.0	8.1 ± 0.2	23.3 ± 0.5	350.3 ± 4.4	98 ± 5	144 ± 8
200	7.8 ± 0.1	8.0 ± 0.2	24.2 ± 0.4	593.6 ± 10.6	80 ± 3	253 ± 3
300	8.1 ± 0.0	7.6 ± 0.3	23.7 ± 1.2	772.6 ± 2.9	93 ± 3	370 ± 13
340	8.1 ± 0.0	8.1 ± 0.3	23.2 ± 0.9	808.9 ± 2.9	98 ± 3	407 ± 25
380	8.0 ± 0.0	7.8 ± 0.4	24.3 ± 0.6	887.4 ± 2.0	94 ± 3	464 ± 25

[a] All the concentrations are in mg/L except for pH, which is unit-less
[b] DO = dissolved oxygen; concentration as mg/L; $n = 36$
[c] Units are °C; $n = 36$
[d] Units are µS/cm; $n = 36$
[e] Concentration are mg/L as $CaCO_3$; $n = 24$

Table A5: Measured water chemistry variables in chronic vanadium toxicity tests for *Daphnia pulex*. Values shown correspond to means ± SD.

Variable & Nominal Concentration [a]	pH	DO [b]	Temperature [c]	Conductivity [d]	Alkalinity [e]	Hardness [e]
Alkalinity						
70	7.9 ± 0.2	7.6 ± 0.3	24.5 ± 0.7	308.0 ± 11.5	74 ± 3	102 ± 6
100	8.1 ± 0.1	7.5 ± 0.6	23.8 ± 0.6	310.8 ± 10.1	99 ± 6	104 ± 6
200	8.3 ± 0.1	8.1 ± 0.8	23.8 ± 0.8	539.3 ± 12.2	216 ± 11	102 ± 5
pH						
7	7.2 ± 0.1	7.6 ± 0.9	23.6 ± 0.3	374.5 ± 13.9	92 ± 3	116 ± 6
8	8.1 ± 0.1	7.5 ± 0.6	23.8 ± 0.6	310.8 ± 10.1	99 ± 6	104 ± 6
9	9.1 ± 0.1	8.3 ± 0.5	23.5 ± 0.8	371.3 ± 6.4	94 ± 3	108 ± 5
SO_4						
100	8.0 ± 0.1	7.1 ± 0.7	24.9 ± 0.5	483.7 ± 6.0	94 ± 3	181 ± 6
200	8.0 ± 0.1	7.7 ± 0.6	24.3 ± 0.5	628.6 ± 8.0	100 ± 4	278 ± 7
450	8.1 ± 0.2	8.3 ± 1.3	24.1 ± 0.4	1,072.2 ± 17.7	96 ± 6	545 ± 26

[a] All the concentrations are in mg/L except for pH, which is unit-less
[b] DO = dissolved oxygen; concentration as mg/L; $n = 36$
[c] Units are °C; $n = 36$
[d] Units are µS/cm; $n = 36$
[e] Concentration are mg/L as $CaCO_3$; $n = 24$

Table A6: Measured water chemistry variables in mixture tests for *Daphnia pulex*. Values shown correspond to means ± SD.

Variable & Nominal Concentration [a]	pH	DO [b]	Temperature [c]	Conductivity [d]	Alkalinity [e]	Hardness [e]
Na/SO_4						
18/55	8.1 ± 0.0	8.4 ± 0.2	22.7 ± 0.6	294.1 ± 2.2	105 ± 3	104 ± 4
120/55	7.9 ± 0.1	7.8 ± 0.7	24.2 ± 0.4	752.0 ± 5.5	125 ± 23	105 ± 4
220/100	8.1 ± 0.3	7.5 ± 0.2	23.8 ± 0.6	1,185.8 ± 22.0	144 ± 27	126 ± 6
339/153	8.0 ± 0.1	7.5 ± 0.3	24.1 ± 0.4	1,586.4 ± 18.5	164 ± 68	140 ± 53
420/192	8.0 ± 0.3	7.5 ± 0.3	23.9 ± 0.6	1,976.4 ± 31.2	135 ± 42	115 ± 6
540/246	7.9 ± 0.1	7.3 ± 0.3	24.1 ± 0.6	2,511.2 ± 16.2	180 ± 20	114 ± 8
Na/SO_4						
120/146	8.1 ± 0.3	7.4 ± 0.6	23.7 ± 0.9	961.9 ± 14.1	125 ± 3	219 ± 6
120/192	7.7 ± 0.5	7.3 ± 1.1	23.1 ± 0.7	1,086.3 ± 32.0	105 ± 4	235 ± 3
120/247	8.1 ± 0.3	7.2 ± 0.6	23.6 ± 0.4	973.5 ± 11.4	104 ± 6	299 ± 7
Alkalinity/SO_4						
80/87	7.8 ± 0.1	7.7 ± 0.2	24.2 ± 0.3	382.23 ± 51.5	74 ± 15	144 ± 26
186/209	8.2 ± 0.1	8.0 ± 0.3	23.5 ± 0.7	813.6 ± 6.6	186 ± 8	251 ± 5
200/173	8.2 ± 0.1	8.2 ± 0.4	23.1 ± 0.9	1,079.3 ± 11.4	311 ± 13	333 ± 13

[a] Concentrations as mg/L
[b] DO = dissolved oxygen; $n = 36$
[c] Units are °C; $n = 36$
[d] Units are µS/cm; $n = 36$
[e] Concentration are mg/L as $CaCO_3$; $n = 24$

Table A7: Effective vanadium concentrations (EC) causing 10%, 20% and 50% reproductive impairment and the median lethal concentration (LC_{50}) for *Daphnia pulex* exposed for 21 d. EC_{10}, EC_{20}, EC_{50} and LC_{50} are presented with 95% confidence intervals (CI) (shown in parentheses).

Variable/ Nominal Concentration [a]	EC_{10} (95%CI)	EC_{20} (95%CI)	EC_{50} (95%CI)	LC_{50} (95%CI)
pH				
7	0.2 (0.1-0.4)	0.3 (0.1-0.6)	0.6 (0.4-0.8)	0.3 (0.2-0.4)
8	0.2 (0.0-0.6)	0.5 (0.1-0.6)	0.8 (N/A)	0.2 (0.2-0.3)
9	0.2 (0.1-0.5)	0.5 (0.1-0.6)	0.8 (N/A)	0.3 (0.2-0.7)
Alkalinity				
70	N/A	N/A	N/A	0.4 (0.3-0.5)
100	0.2 (0.0-0.6)	0.5 (0.1-0.6)	0.8 (N/A)	0.2 (0.2-0.3)
200	0.5 (0.0-0.6)	0.6 (0.1-0.8)	1.0 (N/A)	0.6 (0.5-0.7)
SO_4				
100	0.6 (0.1-0.7)	0.7 (0.1-0.8)	0.9 (N/A)	0.6 (0.4-0.8)
200	0.4 (0.1-0.6)	0.5 (0.2-0.6)	0.7 (0.5-0.9)	0.5 (0.4-0.6)
300	0.3 (0.1-0.5)	0.5 (0.2-0.7)	0.7 (0.5-1.0)	0.4 (0.3-0.5)

[a] All the concentrations are in mg/L except for pH, which is unit-less

N/A = Indicates that no reliable EC or CI could be calculated due to a steep concentration-response curve

Table B1: Measured water chemistry variables – acute exposures for *Oncorhynchus mykiss*. Values shown correspond to means ± SD.

Variable & Nominal Concentration [a]	pH	DO [b]	Temperature [c]	Conductivity [d]	Alkalinity [e]	Hardness [e]	Ammonia [e]
pH							
6	6.2 ± 0.1	8.8 ± 0.5	16.3 ± 0.3	386.0 ± 48.2	88 ± 3	116 ± 4	0.1 ± 0.0
7	7.1 ± 0.1	7.5 ± 2.1	16.8 ± 0.4	376.1 ± 15.5	96 ± 6	114 ± 5	0.1 ± 0.0
8	8.0 ± 0.1	9.1 ± 0.1	16.4 ± 0.4	387.3 ± 15.7	99 ± 2	116 ± 5	0.1 ± 0.1
9	9.0 ± 0.1	8.6 ± 0.7	16.5 ± 0.4	395.5 ± 30.2	112 ± 9	112 ± 7	0.1 ± 0.0
Alkalinity							
70	7.8 ± 0.1	9.2 ± 0.1	16.1 ± 0.5	258.9 ± 22.4	71 ± 2	93 ± 5	0.1 ± 0.0
100	8.0 ± 0.1	9.1 ± 0.1	16.4 ± 0.4	387.3 ± 16.7	99 ± 2	116 ± 5	0.1 ± 0.1
200	8.3 ± 0.1	9.4 ± 0.1	15.4 ± 0.6	613.2 ± 23.4	217 ± 8	110 ± 4	0.1 ± 0.1
300	8.6 ± 0.1	9.4 ± 0.2	15.4 ± 0.8	845.9 ± 28.9	330 ± 13	106 ± 12	0.1 ± 0.0
Na							
100	8.0 ± 0.1	9.3 ± 0.2	16.0 ± 0.4	722.6 ± 12.1	108 ± 5	115 ± 2	0.1 ± 0.0
200	8.0 ± 0.1	9.3 ± 0.1	15.8 ± 0.2	1,054.1 ± 23.4	115 ± 6	116 ± 5	0.1 ± 0.0
300	8.0 ± 0.1	9.4 ± 0.2	15.3 ± 0.4	1,383.6 ± 25.1	117 ± 5	118 ± 3	0.1 ± 0.0
400	7.9 ± 0.1	9.1 ± 0.1	16.3 ± 0.4	1,605.6 ± 123.9	115 ± 3	117 ± 2	0.1 ± 0.0
SO_4							
100	8.0 ± 0.0	9.3 ± 0.1	16.0 ± 0.6	537.7 ± 18.6	96 ± 3	142 ± 13	0.2 ± 0.0
200	8.0 ± 0.3	9.4 ± 0.1	15.7 ± 0.5	686.4 ± 20.7	100 ± 3	243 ± 20	0.2 ± 0.1
300	8.1 ± 0.1	9.3 ± 0.2	15.9 ± 0.6	820.9 ± 21.5	95 ± 3	308 ± 9	0.2 ± 0.1
380	8.0 ± 0.1	9.2 ± 0.2	15.7 ± 0.4	922.1 ± 12.1	95 ± 2	212 ± 6	0.2 ± 0.0

[a] All the concentrations are in mg/L except for pH, which is unit-less.
[b] DO = dissolved oxygen;; $n = 36$.
[c] Units are °C; $n = 36$.
[d] Units are µS/cm; $n = 36$.
[e] Units are mg/L as $CaCO_3$; $n = 24$.

Table B2: Stepwise multiple linear regression coefficients and statistical results for *Daphnia pulex* and *Oncorhynchus mykiss* models.

MLR model coefficient	No interaction *D. pulex*	Interaction *D. pulex*	No interaction *O. mykiss*	Interaction *O. mykiss*
Intercept	-0.17	-0.01	3.19	3.53
pH	-0.30	-	-0.36	-0.29
ln (alkalinity)	0.43	-	0.35	0.16
ln [SO_4]	0.13	-	-	-
pH x ln(alkalinity)	-	-	-	0.34
(pH-7.88*) x(ln(alkalinity)-4.77*)	-	0.65	-	-
(ln (alkalinity)-4.77) x (ln [SO_4]-4.43*)	-	-0.40	-	-
(pH-7.88*) x (ln [SO_4]-4.43*)	-	-	-	-
n	17	17	12	12
r^2	0.647	0.651	0.764	0.781
Adjusted r^2	0.565	0.601	0.711	0.698
p	0.003	0.001	0.002	0.005
AIC	61.81	54.48	48.07	50.81
BIC	64.31	56.14	49.04	52.26

AIC = Akaike Information Criterion.
BIC = Bayesian Information Criterion.
- = variables or interactions between variables that were excluded from the MLR model.
* = Constants represent the corrections to the model by mean centering method.

Table B3: Variance inflation factors for the main effects from the best-fitting multiple linear regression (MLR) models for *Daphnia pulex* and *Oncorhynchus mykiss*.

VIF	***D. pulex***	***O. mykiss***
(pH-7.88*)	1.37	-
pH	-	1.27
ln (alkalinity)	-	1.27
ln (alkalinity)-4.77*	1.46	-
ln [SO_4]-4.33*	1.19	-

VIF = Variance inflation factors.
* = Constants represent the corrections to the model by mean centering method.

Table C1: Proposed water chemistry for a worst-case scenario based on the acute toxicity tests performed for *Daphnia pulex* in Chapter 2. Values shown correspond to nominal levels or concentration. pH is unit-less and concentrations of alkalinity are in mg/L as CaCO3 and sulphate in mg/L.

Variable & Nominal Concentration [a]	**pH**	**Alkalinity**	**SO_4**
Worst-case scenario	9	70	54

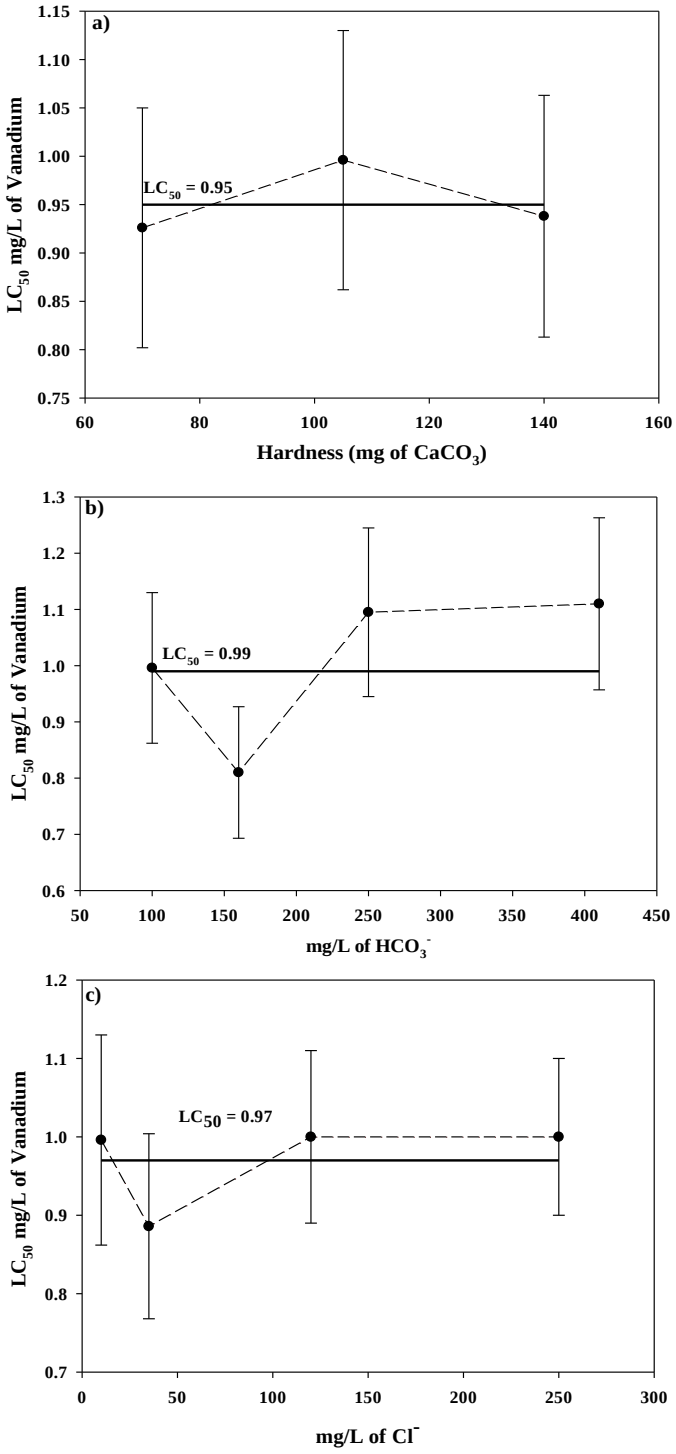
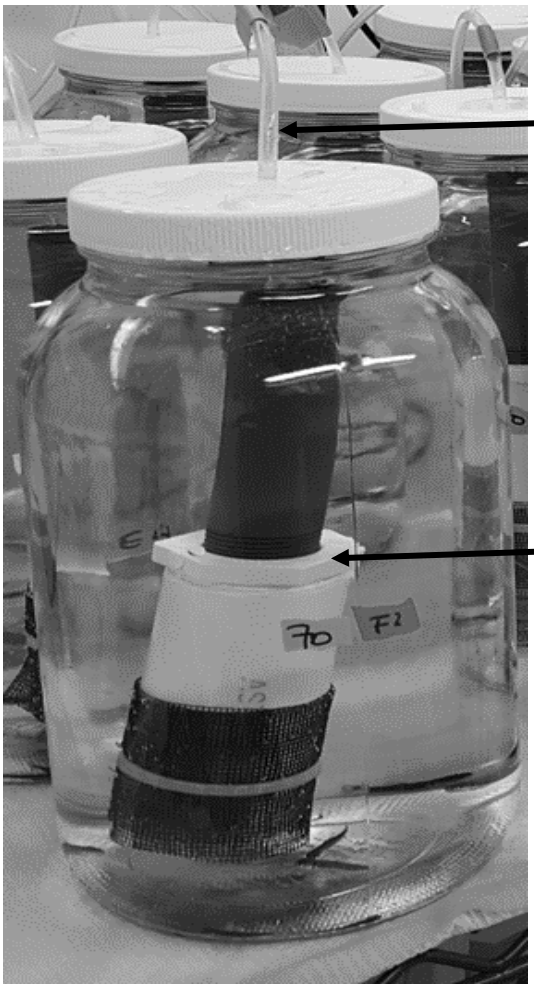


Figure A1: Descriptive models for the acute effects of increasing: a) hardness; b) bicarbonate; and c) chloride, representative of Athabasca River water and OSPW on vanadium toxicity to *Daphnia pulex*. Error bars represent the 95% confidence intervals of the LC_{50} estimates. Thick solid line describes either the statistical model or the geometric mean obtained from the experimental LC_{50} values (mg/L).



Air tubing with an air-stone at the end (air-stone located inside the biofilter)

Biofilter filled with cotton batten pre-soaked with Stability® water conditioner (Seachem Laboratories, Madison, GA, USA)

Figure C1Air tubing with an air-stone at the end (air-stone located inside the biofilter)

Figure B1: 4-L glass jar used in the acute toxicity tests for *Oncorhynchus mykiss* larvae. Image shows the homemade biofilter inside the jar.

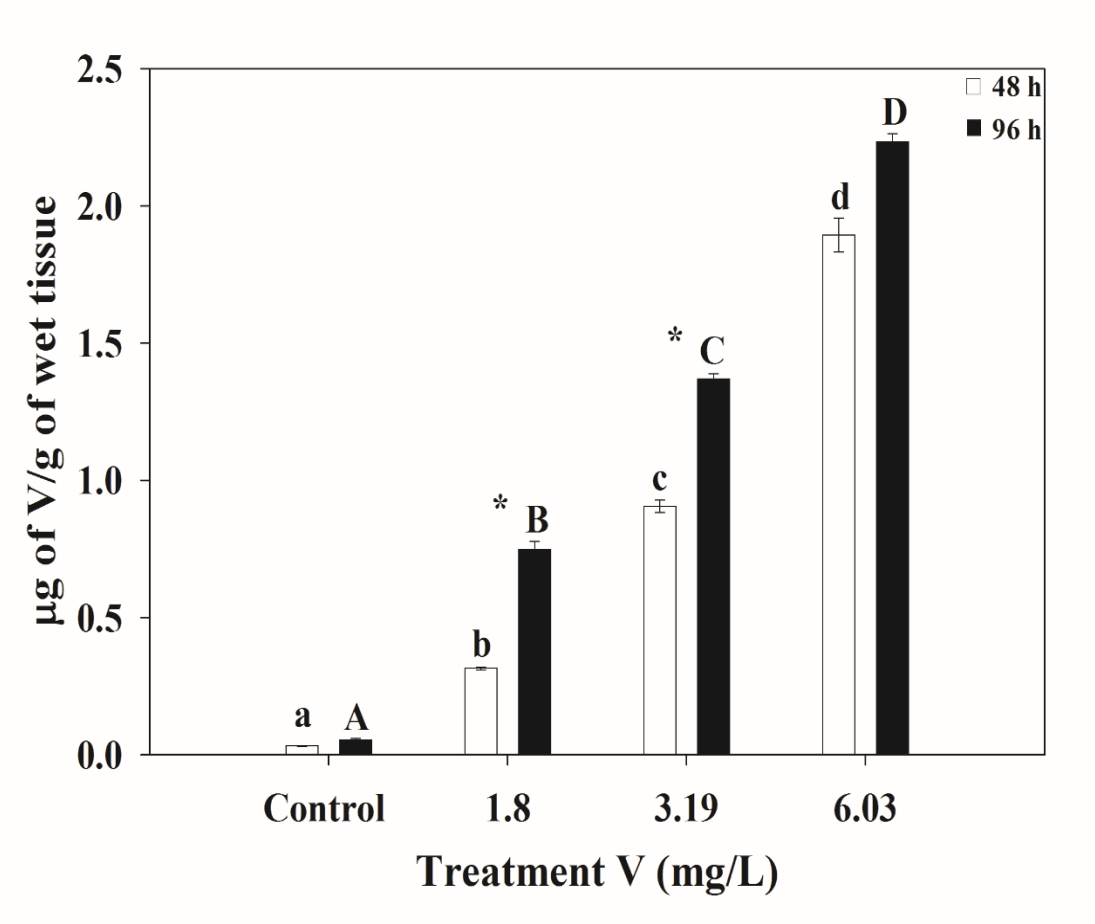


Figure C1: Tissue concentration of V in *Oncorhynchus mykiss* exposed for 48 and 96 h to V concentrations ranging between 1.8 and 6.03 mg/L (n = 5). Significant difference among V treatment for fish exposed for 48 h is shown in small letters and for 96 h in capital letters. Asterisk represents a significant difference between exposure times within a same V exposure concentration. In all the cases $\alpha = 0.05$.