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Metal mixtures, assessing speciation models to predict uptake in Brown Trout (*Salmo trutta*)

Denis Napast

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Preface

I would like to thank Sara Marazuela Reca for all her love, time, patience, help and inspiration. You call out the best in me, the future is ours.

I would like to thank my parents Metka and Miroslav Napast for giving me the opportunity to learn and grow, for all the love and time. I will always be thankful for everything.

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To Amadej and Marcel; find joy in little things as they will make your life complete. Find what you love to do in life and never let it go, you will never work a day in your life.

Amadej and Marcel I wish you the best in the future, learn, study and explore. Make a mark on the world and make it a better place for everyone.

You are here only for a short visit. Don't hurry don't worry. And be sure to smell the flowers along the way.

Walter Hagen

Summary

Alum shale is an organic-rich rock which contains high concentrations of U, Ni, V, Mo, Co, Cu, Zn, As and other metals. These metals are released into the environment by human activities and rock weathering. Many different metals can be found in water as a mixture that can be taken up by aquatic organisms and cause adverse effects and harm.

Uptake of metal mixtures by fish is a complex process that depends on several factors such as pH, temperature, water hardness, element species, the presence of dissolved organic carbon and others. To predict the uptake of metals by fish, computer models like WHAM 7 and Visual Minteq 3.1 are used. These computer models attempt to describe the complex behavior of metals in water, while considering their chemical speciation, charge, amount and complexation with organic matter. The simulated amount of bounded metals to the fish gill was compared to other prediction methods such as free-ion concentration and total metal concentration.

In this project WHAM 7 and Visual Minteq 3.1, developed to predict metal binding to fulvic acid and humic acid, were used to predict metal binding to a fish gill from the assumption that a gill has similar metal binding properties as humic acid. The input data used in both models was obtained from leached alum shale samples taken from a tunnel and road construction site in Norway as part of the NORWAT research.

This master thesis has focused on U, Sr, Cd, Ni and V. From the results we see that the metal mixture approach from these models adjusts better to reality than the free-ion concentration or the total metal concentration approaches. WHAM predicted better for Cd, Ni and V, meanwhile Minteq gave better results for U and Sr. Both WHAM and Minteq predicted more than 50% lower ratios, compared to the free-ion concentration and the total metal concentration. Further model development and model adjustments are needed to improve the predicted metal uptake by fish. Both models performed good, considering that they were not designed as an estimation tool for metal uptake to aquatic organisms.

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Abbreviations

BC - base cations

POC - particulate organic matter

SPM - solid particulate matter

DOC - dissolved organic carbon

TOC - total organic carbon

HS - humic substances

HA - humic acid

FA - fulvic acid

LMM - low molecular mass species

HMM - high molecular mass species

Da - dalton

WHAM - Windermere Aqueous Model

SHM - Stockholm humic model

BSM - basic stern model

Tab.- table

Fig.- figure

Conc.- concentration

Proxy gill - 1µg/l humic acid

1. Introduction

Alum shale is an organic-rich rock which was created in the sea from high deposition of organic materials without oxygen. Alum shale contains high concentrations of uranium (U), nickel (Ni), vanadium (V), molybdenum (Mo), cobalt (Co), copper (Cu), zinc (Zn), arsenic (As) and other metals (Hjulstad, 2015; Jeng, 1992; Lecomte et al., 2017) and easily weathers when exposed to air, releasing metals and generating acidity (Jeng, 1992).

Metals are defined by chemists as elements that have a shining appearance, good electric conductivity and usually enter a chemical reaction as cations. Metals become pollutants commonly through human activities such as mining, smelting and other industrial activities (Walker et al., 2012).

In the environment metals are generally present as mixtures of metals and can interact with organisms separately or as a mixture (Nordberg et al., 2015). In water they can be found as particles, colloids, organic complexes, inorganic complexes and as free ions (Zhao et al., 2016). The amount of free ions in water correlates good with the uptake to organisms (Parker & Pedler, 1997). It is generally agreed that free-ion concentration offer the best prediction for metal uptake and toxicity to aquatic organisms, but there are instances where the free-ion concentration failed to predict correctly (Parker & Pedler, 1997; Zhao et al., 2016).

Speciation of trace elements and radionuclides is defined as the distribution of an element amongst a defined chemical species in a system (Templeton et al., 2000). Equilibrium speciation modelling represent an essential tool for estimating the water chemical composition, bioavailability, effects and the potential risk of metal contaminants. Speciation models provide a deeper understanding of the underlying processes with the possibility to predict metal behavior beyond the limitations of experimental studies in the laboratory and field measurements. Modeling is indispensable when analytical tools for the quantification of chemical species are not available (Di Bonito et al., 2018; Ge et al., 2005).

Computer speciation models such as WHAM 7 (Tipping, 1994; Tipping, 1998; Tipping et al., 2011) and Visual Minteq 3.1 (Gustafsson, 2001) consist of a humic complexation model, a variable charge model and a permanent charge model (Di Bonito et al., 2018). Metals bounded to humic acid bind to different ligand sites from which some are

considered to relate to toxicity. The speciation models take into account the different metal interactions and competitions for the binding sites, providing a better understanding of metal binding to humic acid and uptake to organisms (Stockdale et al., 2010).

The purpose of the thesis is:

- predict metal mixture chemical speciation in natural waters,
- predict metal mixture binding to fish,
- determine the model accuracy of WHAM 7 and Visual Minteq 3.1.

Hypothesis:

- WHAM 7 and Visual Minteq 3.1 can predict metal mixture binding to the fish gill.
- There are no significant differences in predicting metal binding to the fish gill with WHAM 7 and Visual Minteq 3.1, as they use very similar metal to humic substances complexation models.

2. Memory

2.1. Metal speciation

Metals are discharged into the environment because of natural processes or human activities and are distributed between the aqueous phase and the sediments. Most metals undergo hydrolysis and coprecipitation and are deposited in sediments, while only a small fraction stays in solution as free metal ions that can be taken up by organisms (Hou et al., 2013).

According to the IUPAC recommendations, the chemical speciation is defined as “the specific form of an element defined as to isotopic composition, electronic or oxidation state, and/or complex of molecular structure”(Templeton et al., 2000). The concept of chemical speciation was developed during the acid rain research period in the 1970s and 1980s. In acid waters, toxic low molecular Al species with a positive charge were attributed to the large decline of fish population. The total amount of Al measured in the water provided little information about its bioavailability, therefore new speciation analysis methods were developed (Salbu & Skipperud, 2009).

Radionuclide speciation is the distribution of radionuclide chemical species in a system, where radionuclide species are defined based on their physicochemical properties as size, density, oxidation state, charge properties and structure (Salbu, 2006; Salbu, 2009). The species distribution depends on the physiochemical conditions e.g. redox, microbial activity, pH and other factors (Salbu, 2006).

For a long term impact assessment of radionuclides on ecosystems, information about radionuclide speciation, mobility and biological uptake is needed (Salbu & Skipperud, 2009). The characterization of species is crucial for the correct assessment of their bioavailability and mobility in soil, sediments and waters. The interaction of radionuclides and metals with humic substance and clay minerals will change the initial chemical speciation distribution in water, soil and sediments. It is assumed that low molecular mass species (LMM) e.g. molecules and ions, are the most mobile and bioavailable, while high molecular mass species (HMM) e.g. particles, colloids and pseudo-colloids (Fig.1) are not bioavailable. Sorption of LMM species may be irreversible, while HMM radionuclides sorbed to radioactive particles can be released

during weathering (Salbu, 2006).

Diameter	1 nm	10 nm	0.1 μm	0.45 μm	1 μm	10 μm
Molecular mass	$\times 10^2$	$\times 10^4$	$\times 10^6$		$\times 10^8$	
Category	simple compounds	hydrolyzates/colloids	polymers / pseudocolloids		suspended particles	
Examples of species	inorganic, organic ions, complexes, molecules etc.	nanoparticles polyhydroxo complexes polysilicates fulvic acids fatty acids	metal hydroxides clay minerals humic acids proteins		inorganic mineral particles organic particles microorganisms	
Processes influencing Specie distribution		← viruses →		← bacteria →		
Fractionation techniques		Dialysis Ultrafiltration Ultracentrifugation	Density centrifugation		Filtration	Sedimentation
	Ion exchange chromatography		DGT			
	Electrochemical methods					

Figure 1: Association of radionuclides with compounds at different sizes, with fractionation techniques (Salbu, 2009).

Natural radionuclides (particles, colloids, LMM species) present in ground water are released by rock weathering, desorption and radioactive decay. When radionuclides appear in macro concentrations in water, the chemistry of the colloidal system dominates chemical reactions (Salbu, 2006). Aquatic environments are dynamic, rapidly changing systems, that can alter the original distribution of species-radionuclides. Process such as complexation, hydrolysis, aggregation increase the mass of LMM species and reduce their mobility. High molecular mass species undergo dissolution, desorption, dispersion e.g. weathering of radioactive particles, which mobilizes LMM species (Salbu et al., 2004).

Metal species in water can be divided into five fractions (Zerbe et al., 1999):

- Exchangeable metals (metals absorbed to the surface of sediments, the most accessible and extractable metal species, easily migrate to the water column when changes to the ionic composition of water occurs).
- Metals bound to carbonates (decreased pH can release the bound metals and radionuclides).
- Metals bound to Fe and Mn oxides (this fraction is thermodynamically unstable

at anoxic conditions and sensitive to changing redox potentials).

- Metals bound to organic matter (temporary inaccessible for biological uptake when bound to organic matter, released with aerobic or anaerobic decomposition).
- Metals in other forms (metals bound to minerals become inaccessible under normal environmental conditions to living organisms) (Zerbe et al., 1999).

2.2. Metal bioavailability, bioaccumulation, bioconcentration and toxicity

The bioavailable fraction of chemicals is defined as the fraction of the total amount of a chemical present in a specific environment and is available for uptake or can be made available for uptake by microorganisms (Diamond et al., 2010).

In natural waters metals are present as free hydrate ions or complexed with organic and inorganic ligands that can be sorbed on particles or trapped in them. The uptake to organisms appears to be limited to free ions which can cross the exchange surfaces of organisms e.g. fish gills, while other species are not accumulated. Total metal concentrations do not provide a good estimate of the bioavailable species to fish and other aquatic organisms. A better method to predict bioavailability is to determine free ion concentrations (Chowdhury & Blust, 2002). The accumulation of metals in aquatic organisms is affected by water hardness, carbon dioxide, magnesium salts, chelating agents, temperature and other environmental factors (Wang, 1987)

Metals at low concentrations in the environment play an essential role in biochemical and physiological functions in microorganisms, plants and animals. High metal concentrations can cause direct toxicity or reproductive effects on organisms. The toxic effect in organisms is related to the uptake of metal ions (Adamo et al., 2018; Nagajyoti et al., 2010).

Toxicity is defined as “the state of being poisonous or the capacity to cause injury in living organism” (Gupta, 2016). Generally, toxicity is quantified as an LC50 value which is the concentration of the toxicant that kills 50% of the test organisms in a given time. Toxicity depends on the metal species in the water, where dissolved metals are defined as an 0.45 µm filtrate. The speciation of the metals is crucial e.g. Ag is more toxic than AgNO₃. In freshwater, the speciation of elements varies greatly, in general the lower is the pH the higher are the free-ion concentrations. Toxicity is

decreased by ligands which compete for binding sites on organisms (Wood, 2011).

The total concentration of an element is an unreliable indicator of the element toxicity, because of the above-mentioned reasons. Therefore free metal ions are a much better indication of the availability and toxicity of the element (Peijnenburg & Jager, 2003). In freshwater systems metals are distributed between water, sediment particles, pore water and the biota. Sediments represent a long-term storage for contaminants and also provide habitat for benthic organisms. Bioavailability of metals is governed by environmental factors such as pH, water hardness, alkalinity, organic carbon content, ligands (e.g. Cl^- , HCO_3^-), dissolved oxygen and sulfides (Fig.2). The bioavailability of metals in freshwater systems is controlled by the competition of metal cations with other cations (e.g. Ca^{2+}) for metal binding sites. Metals can also compete for binding sites if they appear as colloids or metal organic complexes. Sediments contain precipitated metals which can be released with changing water conditions e.g. a lower pH will release certain metal cations back into the aquatic

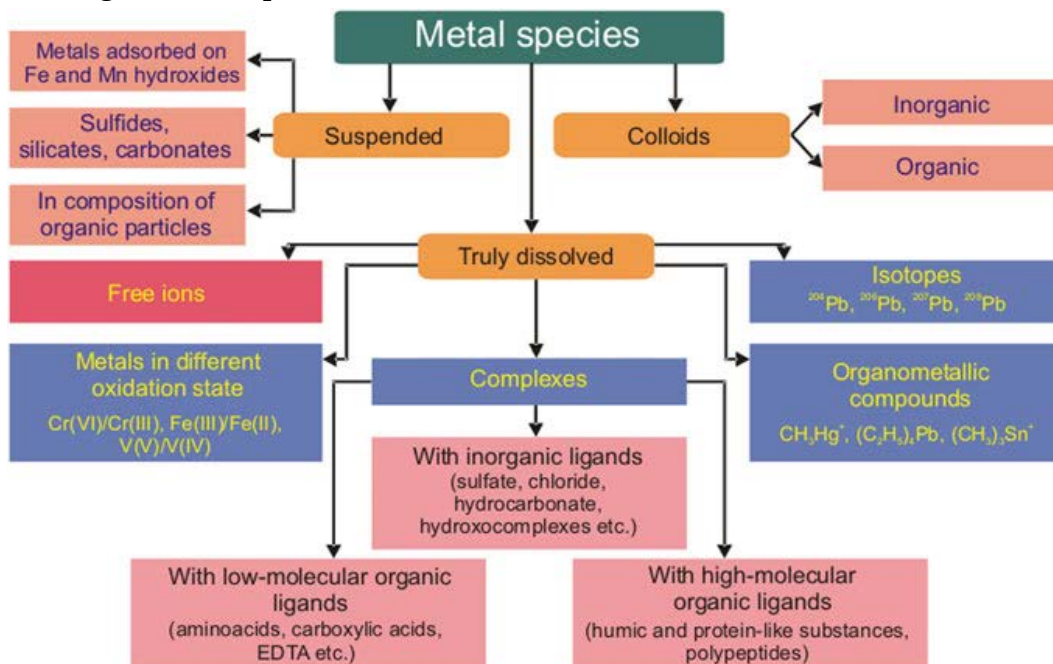


Figure 2: Different forms of metals found in the environment (Ashraf et al., 2016).

environment (Peijnenburg & Jager, 2003; Väänänen et al., 2018). Metals interact with sediments where they associate with carbonates, Fe-Mn oxides, sulfites, silicates and organic matter (Hou et al., 2013).

LMM species can penetrate the biological membranes of organisms (active uptake), while HMM species are inert but can be accumulated in a passive manner e.g. uptake

by filtering organisms (Salbu et al., 2004).

2.3. Metal mixtures

Organisms are often exposed to metals, metalloids and their compounds as mixtures. It is important to consider the combined action of these elements, their impact mechanisms and their risk. Metals can interact with organism independently, where two metals interact separately of each other and combined i.e. mixture, where two metals impact the same site in an organism without influencing each other's effects. (Nordberg et al., 2015). Most often metal mixtures are the cause of environmental pollution, where single metal pollution occurs seldomly (Väänänen et al., 2018).

Metals found in aquatic environments are in form of hydrated free ions, organic complexes e.g. organic matter or organic molecules, of natural or anthropogenic origin and inorganic complexes e.g. ligands such as Cl^- , OH^- and CO_3^{2-} . Most metals in natural waters are present as colloids and complexes, where their concentrations are often higher than the free metal ions (Zhao et al., 2016).

In aquatic environments metals such as Fe, Zn, Mn can occur simultaneously. High concentrations of these metals are commonly a consequence of industrial discharges and mining. Oliveira et al. 2018, exposed fish (*P. lineatus*) for 96 h to a mixture of only Zn (1 mg/l), Mn (0.5 mg/l) and Zn-Mn mixture with and without Fe (5 mg/l). When exposed to only Zn and Mn the fish accumulated Zn and Mn in their gills and kidney. When exposed to the Zn-Mn-Fe mixture the concentration of Fe increased in the muscles and gill, Zn concentrations increased in the gills and liver while Mn concentrations increased in blood cells and the kidney. When Fe was removed from the mixture a drop of Fe was detected in the brain but no in the gills, while Zn and Mn levels remained high in the blood, liver and kidney (Oliveira et al., 2018).

Hamilton and Buhl 1977, exposed fish larva from flannelmouth sucker (*Catostomus latipinnis*) to a metal mixture of As, B, Cu, Mo, Se, U, V and Zn. When the 13 days old larvae were exposed to a mixture of B, Cu, Se, V and Zn the toxicity increased with time (24-96 h). When larvae were exposed to a mixture of As, Mo, Se and U, the predicted toxicity did not increase with time. It was concluded that the metal mixture posed a threat to the fish even when the concentration of the single element in the mixture was below the threshold. It was demonstrated that the toxicity of the metals

was additive in all mixtures tested (Hamilton & Buhl, 1997).

It is necessary to further investigate the uptake and the effects of metal mixtures to fish. There is limited literature and research on the exposure of fish in natural waters to metal mixtures that include U, Cd, Sr, Ni and V.

2.4. Alum shale

Metal rich black shales stretch from Norway to Estonia and across the Baltic sea. Alum shale is an organic-rich rock that contains high concentrations of Ni, Mo, Co, Cu, U, V, Zn, As, Au and other elements. (Lecomte et al., 2017). Alum shale consist of various silicates, sulfide minerals, kerogen and bituminous limestone (Falk et al., 2006). Alum shale was formed in oceans, under anaerobic conditions with high concentrations of organic and inorganic materials. The absence of oxygen prevented the oxidation of organic materials and high sulfide concentrations precipitated metals into sulfides such as pyrite (FeS_2). The binding of Zn, Pb, Cu with sulfides from the surrounding environment lead to the precipitation of these metals (Falk et al., 2006; Jeng, 1992).

When alum shale becomes exposed to air it easily weathers. Pyrite and other sulfides react with air and generate acidity which dissolve silicates and releases Al, Fe, Mn and other metals. In general, under aquatic acidic conditions metals are found in the form of free metal ions (Jeng, 1992). Acids produced in the weathering of alum shale may be buffered by calcium carbonates which precipitate metal ions as metal hydroxides. If the buffering capacity is exceeded the decrease in pH can cause further release of metal free ions and the formation of unstable secondary minerals (Jeng, 1992).

Jeng 1992, tested different alum shale samples in the Oslo area and determined that the concentrations of pyrite, metals and carbonates varied. Three out of four rock samples contained high concentrations of Zn, Cd and Pb, where the slightly weathered sample contained lower concentrations. When alum shale was acidified, the low pH was accompanied with a high release of metals to the surrounding solution, while some of the alum shales showed good acid buffering capacities. Moderate mobile metals were Zn, Mn, Cu and Cd in the acid solution. Their mobility was limited by the amount present in the rock sample. The study shows the importance of acids entering the environment, considering that southern Norway

receives precipitation with a mean pH of 4.5 or below (Jeng, 1992).

Hjulstad 2015, tested different alum shale samples from the wider Oslo area, Norway. Alum shale samples collected from a road and tunnel construction between Jaren and Gran were leached in synthetic water for five weeks. The leached water contained high concentrations of U, Cd, Al, Ni, Mn, Fe, V and Mo. As shales do not contain only one metal but rather a wide range of different metals in high concentrations. When fish were exposed to these high concentrations, the uptake of U, Cd, Ni and other metals was reported. The metals accumulated extensively in the gill and liver of the fish (Hjulstad, 2015; Skipperud et al., 2016).

2.4.1. Uranium

Uranium (U) was discovered in pitchblende, a mineral which consists of mostly uraninite oxide (UO_2) and U_3O_8 (Grenthe et al., 2011). The Earth's crust contains approximately 0.0004 % of the naturally occurring U. Uranium is found as a mixture of three isotopes ^{238}U , ^{235}U and ^{234}U . The natural abundance of U isotopes is 99.27%, 0.72 % and 0.006 % respectively (Bea, 1998; Grenthe et al., 2011; Gubel et al., 2013). In minerals U appears with the oxidation states 4+ and 6+, while 5+ is very unusual and 3+ has not been found (Bea, 1998). Uranium ore deposits formed from slow cooling magma about 1.7-2.5 billion years ago and are mainly found in granitic rock bodies. There are 60 known minerals that contain U (Bea, 1998).

Approximately 1.7 billion years ago, the atmosphere became filled with oxygen. The rain seeped into the rocks and began dissolving U as an anionic complex. Most likely U associated with sulfate and carbonates e.g. $\text{UO}_2(\text{CO}_3)$ (Choppin et al., 2013). Oxide uraninite (pitchblende) is the most abundant U bearing mineral. In oxidizing environmental conditions U is oxidized to the uranyl ion (UO_2^{2+}), which can be easily mobilized and taken up by organisms. Other minerals containing U are uranospherite $[(\text{BiO})(\text{UO}_2)(\text{OH})_3]$ and betafite $[(\text{U,Ca})(\text{Nb,Ta,Ti})_3\text{O}_9 \cdot n\text{H}_2\text{O}]$. Most uranium 6+ bearing minerals contain silicates, carbonates, phosphates, sulfates, molybdates, vanadates, tantalates and titanates (Bea, 1998).

Uranium has two dominant aqueous redox states U (IV) and U (VI). Hydroxyl and carbonate ions significantly influence the U aqueous speciation. At pH below 4.5 the free uranyl ion (UO_2^{2+}) is the dominant species, while at higher pH carbonate and hydroxy species dominate. Inorganic ligands such as sulfate or fluoride will form complexes with UO_2^{2+} only at low pH, as their binding affinity is low at higher pH (Goulet et al., 2011).

The speciation of U is highly dependent on the sorption to clay minerals below pH 5, while at higher pH to biotic surfaces, Fe and Al oxides. Uranium sorption to insoluble organic molecules or other organic matter can reduce the mobility of U. In general, with an increase in pH, U sorption increases until a threshold is reached (Markich, 2002). The threshold depends on the competing ions e.g. carbonates, the ionic strength and chelating agents (Choppin, 1999). The solubility of U (VI) at pH 6-8 is at its minimum while the sorption to humic substances is near its maximum (Choppin, 1992; Markich, 2002).

Organisms do not require U for any known role in their biochemical reactions. Evidence suggests that U is taken up by organisms unintendedly as it is confused for Ca by the organism, which play an essential role in biochemical reactions (Chao & Lin-shiau, 1995). Uranium species in oxygenated surface waters appear as UO_2^{2+} and UO_2OH^+ and form stable complexes with sulfates, phosphate, carbonates and organic carbon. The presence of organic carbon influences the uptake of U to aquatic organisms (Goulet et al., 2011; Wall & Krumholz, 2006). Organisms located lower on the food chain accumulate typically more U compared to organisms at the top of the food chain (Goulet et al., 2011).

2.4.2. Cadmium

Cadmium (Cd) is a transition metal and a member of group IIB of the Periodic Table. The most common radioactive Cd isotopes have the mass number 104, 105, 107, 109, 111, 113, 115 and 117 (Thornton, 1986). High concentrations of Cd can be found with marine black shales and is regularly found in Zn, Pb, Cu sulfide ore deposits. In oxygenated waters Cd can be found as Cd^{2+} at pH 8 and above. At pH > 8 CdCO_3 becomes the predominant species, when sulfides are present in the solution the predominant species becomes CdSO_4 . In natural waters Cd hydroxides appear only at high pH > 10. The weathering of phosphate rocks, volcanic activity, aerosols, industry (Ni-Cd battery industry), phosphate fertilizers and others, contribute to

elevated Cd concentrations in freshwater and in the sea (McGeer et al., 2011). Cadmium concentrations in natural waters are control by sorption reactions. When Cd appears in reducing conditions, in the presence of sulfur it precipitates (Smith, 1998). It is considered that Cd^{2+} is the most organism relevant species for interactions and uptake (Xue & Sigg, 1998).

Organic ligands found in waters such as humic (FA) and fluvic (HA) acids can bind with Cd and affect speciation (McGeer et al., 2011; Smith et al., 2002). Bacteria and minerals can potentially become binding sites for Cd, while metal-ligand-iron oxides present in a system can reduce or increase Cd adsorption, depending on the water conditions (McGeer et al., 2011; Song et al., 2009).

Freshwater fish exposed to Cd accumulate the metal first at their fish gill, where it competes with Ca^{2+} for high affinity binding sites. Once taken up, Cd prevents further uptake of Ca^{2+} by the fish gill. The presence of different ions in water e.g. Ca^{2+} , Mg^{2+} , Na^+ and pH (H^+) may compete with Cd^{2+} for binding sites on the fish gill and alter its toxicity. The toxicity of Cd decreases with increasing water hardness as one of the mayor components is Ca^{2+} (Niyogi et al., 2008).

Fish in lakes with pH 6.6 - 6 or less accumulate more Cd and other metals compared to fish in higher pH lakes. This is partly because of the abundance of biologically available Cd^{2+} and other metals at low pH. At low pH, hydrogen ions compete for binding sites on the fish gill. When taken up, H^+ depletes the fish accumulated Ca concentrations, promoting uptake of elements from the surrounding environment. This can cause a higher uptake of Cd. The presence of dissolved organic carbon (DOC) may not reduce the bioavailability of Cd^{2+} to a high extend (Spry & Wiener, 1991).

Sediments containing Cd in Europe can be mainly found in rivers, lakes and estuaries between the sea and rivers. The source of Cd in sediments comes from industrial activities e.g. Ni-Cd battery production. The rapid adsorption of Cd with particles leads to a high deposition of Cd into the sediment layer. The adsorbed Cd is not permeant bound and can be release with changing aquatic conditions e.g. pH or water hardness, back to the water column (Thornton, 1986).

2.4.3. Strontium

Strontium (Sr) is an alkaline earth element located in group IIa on the Periodic Table, with similar properties as Ca. When exposed to air Sr oxidizes rapidly to a yellowish

colored oxide. There are four stable Sr isotopes ^{84}Sr , ^{86}Sr , ^{87}Sr and ^{88}Sr . The natural abundance of Sr isotopes is 0.56%, 8.86%, 7.00% and 82.58% respectively (Chowdhury & Blust, 2002; Hellmann et al., 1998). The most common minerals that contain Sr are celestite (SrSO_4) and strontianite (SrCO_3). The accumulation of Sr in sediments highly depends on the presence of clay minerals (high sorption) and the substitution of Sr^{2+} for Ca^{2+} in carbonate minerals (Hellmann et al., 1998).

In natural freshwaters with pH 6 - 8, 95% of the Sr is present as a free metal ion Sr^{2+} and 5% as a carbonate complex. Strontium complexes in natural waters with Ca and humic materials, suggesting a high potential for bioavailability (Smith et al., 2009). Calcium like Sr is accumulated in fish in bony tissues, where Sr is preferred by the uptake mechanism. The greater accumulation of Sr is related to organisms living in soft water (low Ca concentrations) (Chowdhury & Blust, 2011).

2.4.4. Nickel

Nickel (Ni) belongs to the transition elements and lies in the group IIB. Nickel has five naturally occurring isotopes, ^{58}Ni , ^{60}Ni , ^{61}Ni , ^{62}Ni , and ^{64}Ni . The two most abundant naturally occurring isotopes are ^{58}Ni (67.88 %) and ^{60}Ni (26.23 %). Nickel can exist in several oxidation states 0, -1, +1, +3, +4 and +2 which dominates natural aquatic systems. Nickel ores are made up of sulfides, oxide and silicates. Nickel can be easily mobilized during rock weathering and often precipitates with Mn and Fe oxide (Galoisy, 1998; Pyle & Couture, 2011).

Mn oxides are more important in controlling Ni speciation compared to Fe hydroxides e.g. $\text{Fe}(\text{OH})_3$, because Mn oxides are less impacted by the change in pH. Nickel also readily binds with humic acid and CaCO_3 . At high pH Ni binds strongly to Fe oxides because of the negatively charged surfaces on the oxide. Sulfides in anoxic waters regulate Ni speciation by forming insoluble Ni sulfides. In waters with pH 5 - 9 without the presence of DOC, Ni^{2+} becomes the dominant species. Nickel can form inorganic complexes with OH^- , Cl^- and NH_3 (Green-Pedersen et al., 1997; Pyle & Couture, 2011). Free Ni^{2+} concentrations in water can be completely complexed with DOC, when concentrations are high e.g. in lakes. In water systems where DOC concentrations are low e.g. underground aquifers, free Ni^{2+} concentrations are higher because of little to no complexation with DOC. The distribution of Ni in natural waters may not reach equilibrium because of the constant and slow exchanges of

various ligands (Pyle & Couture, 2011; Xue et al., 2001).

The exposure of rainbow trout to high concentrations of waterborne Ni caused damage to the gill and reduced the amount of taken in oxygen. When the fish were infused with Ni directly into their bloodstream, the metal predominantly accumulated in the kidney and not in the fish gill cells (Pane et al., 2004; Pyle & Couture, 2011).

2.4.5. Vanadium

Vanadium (V) is a transition element that lies in the periodic table in group V and is the lightest element in the group. Vanadium found in terrestrial minerals is typically in the valence state 3+, but it can occur as 2+, 3+,4+ and 5+. Vanadium has two naturally occurring isotopes, ^{50}V and ^{51}V . The most abundant stable isotope is ^{51}V accounting for 99.75% of the total V. Typically V can be found as a trace element in minerals, but it can play an important role in the structure of minerals such as vanadate [$\text{Pb}_5(\text{VO}_4)_3\text{Cl}$] and carnotite [$\text{K}_2(\text{OU}_2)_2(\text{VO}_4)_2 \cdot 3\text{H}_2\text{O}$]. (Snyder, 1998)

The solubility of V depends on the pH, temperature and V concentration in the aqueous solution, where V(V) i.e. HVO_4^{2-} represents the most soluble oxide. Acidic solutions generally benefit the release of V, at pH 3.1 - 10 the rate of V(V) release from the substrate is greater than the V(III) and V(IV) (Hu et al., 2017; Pourret et al., 2012). Fan et al., have shown that the majority of vanadium exists as V(V) in a Chinese lake at pH 7.5 (Fan et al., 2005). In natural waters V is commonly present as H_2VO_4^- , HVO_4^{2-} or as VO_2^+ (Cumberland et al., 2016).

Vanadium speciation is impacted by the sorption to Fe oxides, clay minerals, particulate matter (Peacock & Sherman, 2004; Wehrli & Stumm, 1989), organic matter, Mn oxyhydroxides and association with humic material (Gardner et al., 2017; Wehrli & Stumm, 1989). The presence of organic and inorganic species in aqueous solutions may increase the stability of V(IV) which is not thermodynamically stable at pH > 7 (Pourret et al., 2012).

2.5. Dissolved organic carbon

In freshwater organic matter exist as particles, colloids and molecules. Carbon in freshwater is classified as inorganic or organic carbon, additionally organic carbon is classified based on its volatility or particle size. Dissolved organic matter ranges in molecular weight from a few daltons (Da) to 100,000 Da (colloidal range). There are

many different fractions of carbon that can be quantified (e.g. POC-particulate organic carbon), the most common analytical parameters are dissolved organic carbon (DOC) and total organic carbon (TOC). TOC encloses all organic carbon species ranging from small molecules to macro-molecular e.g. proteins and ligands. DOC is characterized as organic compounds that can pass the 0.45 μm filter, while any particles that cannot pass the filter are designated particulate organic carbon (POC) and usually represent around 10 % of TOC. In freshwater dissolved organic matter (DOM) is a complex mixture of organic compounds, with different elemental compositions, molecular weights, chemical and physical properties. Aspects of metal complexation, redox and acid based chemistry of DOM are manifestations of hydrogen-carbon and oxygen-carbon ratios (Leenheer & Croué, 2003; Pagano et al., 2014; Perdue & Ritchie, 2003). Concentrations of DOC in water depend on the natural watershed, POC inputs, algae blooms, runoff and seasonal variation. Groundwater DOC concentrations range from 0.1 mg/ L⁻¹ and up to 50 mg/ L⁻¹ in bogs (Leenheer & Croué, 2003).

The DOC composition can be divided into humic and non-humic fractions. These humic substances (HS) are divided based on their solubility into humic acids (HA), fulvic acids (FA) and humin. In humic materials aromatic and aliphatic compounds with amide, carboxyl, keton and other functional groups are found (Leenheer & Croué, 2003; Pagano et al., 2014). The non-humic fraction consists of known biomolecular compounds e.g. lipids, carbohydrates, polysaccharides, amino acids, waxes and proteins (McDonald et al., 2004). HS are transformed from biomolecules or created from the interaction between small organic compounds, released from the metabolic processes of natural macromolecules. Therefore any formed humic structures should reflect structures that occur in plants, microorganisms or their degradation products (McDonald et al., 2004).

Fulvic acid is soluble in alkaline acid and water. It is assumed that FA is a colloidal polymer with varying sizes and yellow to brown-black color. Its molecular size ranges from 175 to 3570 Da. Humic acid is insoluble in water and in acidic conditions. Although it is soluble in some basic solvents. Generally, HA is characterized by dark brown to black color and is assumed to be hydrophilic and acidic by nature. The size of HA can vary from several Da to several thousand of Da (Tan, 2014). Humin is not soluble at any pH in water (L. Malcolm, 1990; McDonald et al., 2004).

Ion interactions with humic substances influence the bioavailability of radionuclides

and metals, impact the acid-base balance and the solubility of humic materials in sediments, water and soils (Dwane & Tipping, 1998). Not all DOC has the same ability to complex with metal cations. Sources of DOC can be considered to be allochthonous (sources of organic carbon from the land i.e. soil and plant organic matter) and autochthonous (sources of organic carbon produced in the aquatic environment i.e. algae) (Thurman, 1985; Wood et al., 2011). Autochthonous DOC tends to be more optically lighter and consists of smaller molecules with a lower amount of aromatic ring structures compared to allochthonous DOC which tends to be optically darker, contains larger molecules with more aromatic rings i.e phenolic groups (Wood et al., 2011).

2.6. Modelling chemical speciation and computer simulation

The advancement of computers has enabled the study of chemical speciation, their distribution and transformation processes using mathematical models. While chemical speciation is often determined with analytical methods, models have become viable alternatives in recent decades. Modelling provides an insight into processes such as bioavailability, toxicity of metals in waters, transport and complexation of radionuclides with humic colloids, organic matter and more (Lumsdon & Evans, 2007).

Since the 1970s several computer models were developed, and some attempts were made to summarize different models. Some models that were developed are still being improved and actively used e.g. Windermere Aqueous Model (WHAM), Visual MINTEQ, Biotic Ligand Model (BLM), CHEAQS Next and others (Di Bonito et al., 2018).

Environmental studies on soil and sediment water systems can greatly benefit from the modeling of solute speciation. By determining the chemical form of metals in water systems, it is possible to determine their uptake, accumulation and toxicity, as the interactions with intercellular compartments are dependent on the metal speciation. Some species are able to bind with external proteins on organisms, some adsorb to the cell walls and others diffuse through the membrane and enter organisms (Di Bonito et al., 2018). Speciation binding to organisms can be influenced by a number of processes which can change the water chemistry e.g. surface exchange hydrolysis, monovalent metal ion dispersion, precipitation of heavy metals by hydroxylation, oxidation and hydrolysis reactions of inorganics and organics, physical

adsorption, chemical metalloids reactions and soil dissolution reactions (Evangelou, 1998).

For modelling proposes the number of variables is reduced to those which control mobility and solubility e.g. pH, alkalinity, redox potential and ligands. The main properties that characterize the soils are the capacity for organic chelation, microbes (influence on pH and redox potential), soil and sediment hydrology, adsorption and exchange capacities. These factors further depend and are influenced by the reactive particle surfaces, binding constants for the various complexes, other thermodynamic and physicochemical variables, such as temperature, ionic strength, fugacity. Most speciation models assume that there is a thermodynamic equilibrium between the phases (Di Bonito et al., 2018).

Computer software such as WHAM and Visual Minteq consider the chemical speciation of elements, their complexation with ligands and can also predict the binding of elements to organic compounds e.g. humic matter. Computer software like WHAM and Minteq are constructed of a model to describe the binding to humic substances, variable charge, permanent charge and consider a system in equilibrium (Di Bonito et al., 2018).

2.6.1. Visual Minteq 3.1 and WHAM 7

Both Visual Minteq 3.1 and WHAM 7 use a very similar humic ion binding model described by Tipping, 1998. Therefore in the next section, the description of the models has been combined, and the differences have been examined.

Minteq uses the Stockholm Humic Model to describe the interaction of humic substances with metals and protons, where WHAM uses the humic ion binding model VII, which describes the binding of protons and metals by humic substances (HA and FA). Humic substances are represented as rigid spheres of uniform size, with proton dissociating groups on the surface that can bind with metal ions (Tipping, 1998; Tipping et al., 2011). In Stockholm humic model (SHM) it is assumed that humic substances form gels, separate from the water phase. They are treated as impermeable spheres and their electrostatic interaction is model using the basic stern model (BSM) (Gustafsson, 2001).

2.6.2. Mathematical description of the models for humic substances

It is assumed that there are four strong binding sites on humic substances, referred as type A sites (number 1, ..., 4) and four weak binding sites, type B sites (numbers 5, ..., 8). The type A sites are mainly represented by strong carboxylic acid groups, where B sites are represented by weak acids groups such as phenolic acid groups. The sum of all binding sites is n , n_A for type A sites and n_B is the sum of all type B sites, all expressed in mol/g (Eq. 1) (Gustafsson, 2001; Tipping et al., 2011).

$$\begin{cases} n_A = n_1 + n_2 + n_3 + n_4 & \text{and} & n_1 = n_2 = n_3 = n_4 \\ n_B = n_5 + n_6 + n_7 + n_8 & \text{and} & n_5 = n_6 = n_7 = n_8 \end{cases} \quad (1)$$

For model VII, used in WHAM, the total amount of type B sites is 50 % of the total amount of type A sites for both HA and FA (Eq. 2). In SHM, used by Minteq, the relation of type A sites and type B sites is the same as in model VII but for FA the total amount is 30 % of the amount of the type A sites (Eq. 3) (Tipping, 1998).

$$WHAM: \quad \begin{cases} HA \\ FA \end{cases} n_B = 50 \% n_A \quad (2)$$

$$Minteq: \quad \begin{cases} HA: n_B = 50 \% n_A \\ FA: n_B = 30 \% n_A \end{cases} \quad (3)$$

The following explanation is true for both WHAM and Minteq.

The proton dissociation reaction between the humic molecules and protons is



where R represent the humic molecule, and $pK(i)$ is the intrinsic dissociation constant. There are eight RH sites, therefore there are eight $pK(i)$ values. This is a mathematical construction that does not describe physically present discrete sites on the humic substances but enables a mathematical description of proton binding sites for humic substances.

Here we present the mathematical construction for group A. Analog explanation for group B will follow. For group A sites, the intrinsic equilibrium constant for proton

dissociation is defined as

$$pK_A(i) = pK_A + \frac{(2i - 5)}{6} \Delta pK_A \quad \text{for } i = 1, \dots, 4. \quad (5)$$

The $pK(i)$ values are described using a median term pK_A and ΔpK_A which defines the distribution of the value. Both are constants fitted from experimental data (Tipping, 1998).

The metal binding reaction with humic substances can be written as



where M represents the metal bound to the humic substance R.

For group A, this reaction can be expressed by the metal binding intrinsic equilibrium constant:

$$\log K_{MA}(i) = \log K_{MA} = \log K_B - \frac{(2i - 5)}{6} \Delta LK_1 \quad \text{for } i = 1, \dots, 4; \quad (7)$$

where $\log K_{MA}(i)$ is the equilibrium constant and ΔLK_1 is the distribution term, both constants estimated from data fitting.

In general, the metal-proton exchange reaction is



This means that the metal-proton exchange depends on the amount of bounded protons which are deduced from the amount of bound metal ions.

$$pK_{MH}(i) = pK(i) - \log K_M(i) \quad (9)$$

For group A:

$$pK_{MHA}(i) = pK_A(i) - \log K_{MA}(i) \quad (10)$$

Substituting Eq. 5 and Eq. 7 in Eq. 10, we obtain the metal-proton exchange constant

$pK_{MHA}(i)$ for group A as:

$$\begin{aligned}
pK_{MHA}(i) &= pk_A + \frac{(2i-5)}{6} \Delta pK_A - \log K_{MA} - \frac{2i-5}{6} \Delta LK_{A1} \\
&= pk_A - \log K_{MA} + \frac{2i-5}{6} (\Delta pK_A - \Delta LK_{A1}) = \\
&= pK_{MHA} + \frac{2i-5}{6} (\Delta pK_A - \Delta LK_{A1}) \quad \text{for } i = 1, \dots, 4
\end{aligned} \tag{11}$$

Analogically for type B sites:

$$pK_B(i) = pk_B + \frac{(2i-13)}{6} \Delta pK_B \quad \text{for } i = 5, \dots, 8 \tag{12}$$

$$\log K_{MB}(i) = \log K_{MB} = \log k_B - \frac{(2i-13)}{6} \Delta LK1 \quad \text{for } i = 5, \dots, 8 \tag{13}$$

$$\log K_{MHB}(i) = pK_B(i) - \log K_{MB}(i) \tag{14}$$

Substituting Eq. 12 and Eq. 13 in Eq. 14, we obtain the metal-proton exchange constant $pK_{MHB}(i)$ for group B as:

$$\begin{aligned}
pK_{MHB}(i) &= pk_B + \frac{(2i-13)}{6} \Delta pK_B - \log K_{MB} - \frac{2i-13}{6} \Delta Lk_{B1} \\
&= pk_B - \log K_{MB} + \frac{2i-13}{6} (\Delta pK_B - \Delta Lk_{B1}) = \\
&= pK_{MHB} + \frac{2i-13}{6} (\Delta pK_B - \Delta Lk_{B1}) \quad \text{for } i = 5, \dots, 8
\end{aligned} \tag{15}$$

In WHAM 7 the values for ΔLK_{A1} (Eq. 11) and ΔLK_{B1} (Eq. 15) are obtained from literature (0.6) and are $\ll \Delta pK_A$ and ΔpK_B , therefore ΔLK_{A1} and ΔLK_{B1} can be considered 0 with no major impact. This approximation results in

$$WHAM: \quad pK_{MH}(i) = \begin{cases} pK_{MHA} + \frac{2i-5}{6} \Delta pK_A & \text{for } i = 1, \dots, 4, \\ pK_{MHB} + \frac{2i-13}{6} \Delta pK_B & \text{for } i = 5, \dots, 8. \end{cases} \tag{16}$$

In SHM, it is considered that all the $pK_{MH}(i)$ are the same and equal to pK_{MH} inside of a group, what mathematically can be expressed by forcing ΔpK_A to be equal to ΔLK_{A1} , and ΔpK_B equal to ΔLK_{B1} in Eq. 15. To adjust the model to the empiric constants, the variable i has been introduced for $i = 0, \dots, 3$ together with the distribution term that modifies the strength of complexation sites, ΔLK_2 , constant

fitted from experimental data (Tipping, 1998).

$$\text{Minteq: } pK_{MH}(i) = \begin{cases} pK_{MHA} + i \cdot \Delta LK_2 \\ pK_{MHB} + i \cdot \Delta LK_2 \end{cases} \quad \text{for } i = 0, \dots, 3. \quad (17)$$

That the mathematical equations are different doesn't have any impact, as the constants used are different and calculated to fit experimental results in both cases. Differences in how precisely these constants have been calculated to fit does have an impact on the results. As the method to calculate these constants is not known, we cannot determine which of the models is more accurate based on these equations.

Both WHAM and Minteq can predict bidentate binding with WHAM having the possibility to predict also tridentate binding (Gustafsson, 2001; Tipping, 1998; Tipping et al., 2011).

The differences between WHAM and Minteq in their models are shortly summarized in Tab. 1.

Table 1: Differences in models between WHAM and Minteq (Di Bonito et al., 2018).

Model name	Humic complexation model	Variable charge model	Permanent charge model
WHAM 7	Model VII	SCAMP	Gaines-Thomas
Visual Minteq 3.1	SHM	GD-MSIC/ GTLM	Donnan

3. Materials and methods

3.1. Data of use

As a part of the NORWAT project (Skipperud et al., 2016) leaching experiments with alum shale were performed before exposure experiments with fish. The measured concentrations of leached metals from alum shale are presented in Tab. 2 at different dilutions in mol/l.

Table 2: Measured water composition, leached from alum shale at different dilutions in mol/l. TOC is presented in µg/l.

	Dilution											
	Control (AB)		93.75%		87.5%		75%		50%		0%	
°C	8.89	±0.4	9.2	±0.1	9.14	±0.14	9.29	±0.16	9.13	±0.33	9.23	±0.14
pH	7.33	±0.06	7.28	±0.04	7.28	±0.05	7.29	±0.05	7.35	±0.05	7.38	±0.07
TOC (µg/l)	1775	±389	1650	±70.7	1600	<0.01	1750	±212	1850	±212	2150	±212
F	2.11	±NA	2.11	±NA	2.11	±NA	2.11	±NA	3.90	±1.19	6.90	±2.90
	E-06		E-06		E-06		E-06		E-06	E-06	E-06	E-06
Cl	8.89	±3.99	7.76	±1.99	6.91	±1.99	6.21	±NA	5.08	±NA	2.44	±1.99
	E-05	E-06	E-05	E-06	E-05	E-06	E-05		E-05		E-05	E-07
NO ₃ ⁻	7.34	±1.49	1.14	±1.14	1.61	±NA	2.42	±NA	3.95	±1.14	7.10	±2.28
	E-07	E-07	E-06	E-08	E-06		E-06		E-06	E-07	E-06	E-07
SO ₄ ²⁻	1.20	±4.29	1.22	±NA	1.20	±7.36	1.16	±7.36	1.11	±NA	1.04	±2.21
	E-03	E-05	E-03		E-03	E-06	E-03	E-06	E-03		E-03	E-05
NH ₄ ⁺	2.22	±7.84	2.22	±NA	2.11	±NA	2.61	±NA	3.83	±NA	5.45	±1.78
	E-05	E-07	E-05		E-05		E-05		E-05		E-05	E-06
Base cations												
Na	3.27	±6.62	3.40	±5.63	3.36	±2.11	3.35	±1.83	3.27	±1.06	3.20	±6.86
	E-04	E-06	E-04	E-06	E-04	E-07	E-04	E-06	E-04	E-06	E-04	E-07
Mg	1.07	±8.83	1.06	±2.60	1.06	±9.95	1.06	±3.02	1.02	±8.26	9.79	±1.99
	E-04	E-06	E-04	E-06	E-04	E-07	E-04	E-07	E-04	E-07	E-05	E-06
K	7.60	±4.74	6.47	±1.75	6.53	±8.43	6.67	±7.84	6.86	±1.15	7.33	±2.26
	E-05	E-06	E-05	E-06	E-05	E-07	E-05	E-07	E-05	E-06	E-05	E-06
Ca	7.98	±4.46	8.19	±2.13	8.19	±2.42	8.22	±1.43	7.94	±2.55	7.86	±4.03
	E-04	E-05	E-04	E-05	E-04	E-05	E-04	E-05	E-04	E-05	E-04	E-05
Metals of no interest												
Th	1.60	±3.46	9.84	±1.04	3.14	±1.74	1.26	±3.36	6.58	±7.49	1.88	±8.33
	E-11	E-11	E-12	E-11	E-12	E-12	E-12	E-14	E-12	E-12	E-12	E-13
Mn	2.57	±1.96	5.10	±1.06	1.30	±1.58	2.44	±8.08	4.67	±4.40	9.14	±6.36
	E-07	E-07	E-07	E-08	E-06	E-08	E-06	E-09	E-06	E-08	E-06	E-08
Fe	1.13	±6.99	1.03	±3.37	7.95	±3.63	7.92	±6.33	8.05	±7.08	8.74	±2.61
	E-07	E-08	E-07	E-08	E-08	E-09	E-08	E-09	E-08	E-09	E-08	E-08
Cu	1.92	±2.40	9.90	±6.57	9.35	±6.47	9.58	±1.45	8.95	±3.58	9.59	±2.52
	E-07	E-07	E-08	E-09	E-08	E-09	E-08	E-10	E-08	E-09	E-08	E-09
Zn	5.82	±1.47	8.18	±2.16	1.37	±6.02	2.15	±3.05	3.73	±3.87	6.60	±1.56
	E-08	E-08	E-08	E-09	E-07	E-09	E-07	E-09	E-07	E-09	E-07	E-08
As	1.42	±3.08	2.04	±1.21	5.87	±7.85	1.17	±1.22	2.19	±1.60	4.34	±3.76
	E-09	E-09	E-09	E-10	E-09	E-10	E-08	E-10	E-08	E-10	E-08	E-10
Al	6.06	±NA	8.08	±NA	9.78	±NA	1.29	±NA	2.10	±NA	3.47	±NA
	E-08		E-08		E-08		E-07		E-07		E-07	
Mo	2.76	±4.95	1.73	±1.12	5.99	±1.10	1.19	±4.25	2.32	±1.36	4.60	±1.51
	E-09	E-09	E-06	E-08	E-06	E-07	E-05	E-08	E-05	E-07	E-05	E-07

Dilution												
	Control (AB)		93.75%		87.5%		75%		50%		0%	
Metals of interest												
U	2.87 E-10	±7.19 E-10	3.09 E-07	±3.95 E-09	1.06 E-06	±4.26 E-09	2.10 E-06	±1.26 E-07	3.98 E-06	±4.94 E-07	7.69 E-06	±1.01 E-06
Sr	1.42 E-07	±NA	3.82 E-07	±1.15 E-08	9.35 E-07	±2.86 E-08	1.72 E-06	±2.55 E-08	3.25 E-06	±1.49 E-09	6.26 E-06	±8.14 E-08
Cd	8.47 E-11	±2.00 E-11	6.70 E-10	±3.84 E-11	2.34 E-09	±1.16 E-10	4.63 E-09	±1.58 E-10	9.19 E-09	±8.44 E-11	1.77 E-08	±5.80 E-10
Ni	1.31 E-08	±9.51 E-09	1.83 E-07	±1.46 E-09	6.14 E-07	±7.70 E-09	1.21 E-06	±2.67 E-09	2.40 E-06	±2.26 E-08	4.73 E-06	±1.51 E-08
V	7.41 E-07	±4.02 E-07	2.44 E-06	±2.11 E-07	7.85 E-06	±8.06 E-07	1.48 E-05	±3.23 E-07	2.85 E-05	±7.72 E-08	5.69 E-05	±3.47 E-07

In Tab.2 the dilution 0% means no dilution (the leached alum shale water is not diluted with synthetic water), 50% diluted (1:1 dilution), 75% (3:4 dilution), 93.75% (67.5:2.5 dilution). Control (AB) is the average element concentration of the control samples A and B. The measured water concentrations are presented in µg/l in Appx. 1, as they were used as the input data for WHAM and Minteq.

The elements of interest (U, Sr, Cd, Ni and V) are presented in Tab. 2 and Tab. 3. Their interest lies on the fact that their concentration changes with different dilutions, they accumulate in fish and the binding can be simulated with WHAM and Minteq.

The alum shale samples were collected from a road and tunnel construction site between Jaren and Gran (Oppland, Norway) (Skipperud et al., 2016). The rocks were crushed with a jaw crusher. The crushed rocks (19.26 kg) were placed on cone-shaped holders fitted inside the container to ensure water circulation and filled with 200 liters of synthetic rain water. The rocks were leached for five weeks. After the leaching period, the water was filtered through a 0.45 µm filter and stored in different plastic containers (200 l). The concentration of different trace elements and common ions (Na, K, Mg, Ca, Cl, NO₃⁻, SO₄²⁻) were determined by an ICP-MS and used to create a control water. The control water contained only the above-mentioned elements without any added metals e.g. Fe, U, Sr. One week before the exposure experiment the supplied fish were put into a 400 l incubation container with the control water at 10 °C for acclimation (Hjulstad, 2015). Five containers were filled with 200 l of 0%, 50%, 75%, 87.55% and 93.75% diluted water. Seven fish were incubated for 264 hours in each container. After the incubation period the fish were dissected (gills, liver, kidney, olfactory and brain) and analyzed for metals (Hjulstad, 2015; Skipperud et al., 2016). The metal concentration measured in the gills is used in this thesis (Tab

3).

Table 3: The average measured metal content in the fish gill (mol/g dry weight).

	Diluted 93.75 %	Diluted 87.5 %	Diluted 75%	Diluted 50%	Diluted 0%	Control AB
U	2.26E-11 ±1.19E-11	4.67E-11 ±1.06E-11	8.64E-11 ±2.46E-11	1.10E-10 ±4.35E-11	1.77E-10 ±4.09E-11	1.09E-12 ±2.21E-10
Sr	3.00E-09 ±4.67E-10	3.09E-09 ±2.43E-10	3.24E-09 ±2.89E-10	3.41E-09 ±3.43E-10	3.28E-09 ±5.33E-10	3.19E-09 ±5.73E-07
Cd	3.79E-11 ±2.90E-12	4.26E-11 ±8.31E-12	4.89E-11 ±1.25E-11	6.97E-11 ±1.67E-11	1.05E-10 ±1.05E-10	3.48E-11 ±3.48E-08
Ni	5.05E-10 ±9.80E-11	5.57E-10 ±8.48E-11	5.66E-10 ±1.20E-10	6.68E-10 ±1.14E-10	8.20E-10 ±2.66E-10	4.37E-10 ±6.74E-08
V	2.41E-11 ±9.45E-12	2.31E-11 ±8.18E-12	2.42E-11 ±8.05E-12	2.46E-11 ±8.38E-12	2.92E-11 ±8.79E-12	2.42E-11 ±1.08E-08

3.1.1. Concept of simulated metal binding to the gill

The modeled binding to the gill (proxy gill), assumes that aquatic organisms bind protons and metals to non-specific ligands. The binding sites consist of monodentate, bidentate and tridentate sites. Minteq considers mono- and bidentate binding sites, while WHAM considers all, mono-, bi- and tridentate binding sites. It is assumed that the bonded metals and protons concentrations correspond directly to metabolically available cations for the organisms. The bounded metals and protons do not directly relate to toxicity but rather provide an estimate of metal-proton bioavailability. It was assumed that all DOC in the water was FA (approximately 99.9%), therefore HA could be used as a proxy gill (1µg HA) for simulating metal-proton binding. To the simulations 1µg/l of HA was added, as it was considered a low enough concentration to not influence the overall chemical speciation or to compete directly with FA for metal ions and protons. All simulations assume equilibrium between the water phase and the binding sites (Stockdale et al., 2010).

3.2. WHAM 7 and Visual Minteq 3.1

The proxy gill approach assumes that the binding properties of HA are reassembling the binding properties of a fish gills for metals. Both WHAM and Minteq allow to separate DOC into HA and FA while assigning different concentrations for both acids.

The calculated binding to HA is used to calculate a ratio in between the simulated binding to HA at different dilutions and the average control sample AB. It is assumed that all TOC is DOC as it passed the 0.45 µm filter and in both models it is represented by FA. It is assumed that 100% of the DOC is active for metal-proton binding. The measured concentration of elements presented in Tab. 2 was used in all

simulations and no changes were made.

3.2.1. How to compare predicted binding to proxy gill and real gill

From both models, the binding to the proxy gill was used to calculate the uptake ratio, which was compared to the real gill ratio:

- metal concentration in the gills of the exposed fish divided by metal concentration in the gills of the control,
- modeled metal binding to the proxy gill (HA) from the selected water exposure scenario divided by the control.

The measured concentrations in water were converted from $\mu\text{g/l}$ to mol/l and the measured metal uptake in the fish gill was converted from mg/kg to mol/g dry weight.

3.2.2. General setting in Visual Minteq 3.1

In Minteq the pH was set to fixed and the ionic strength to be calculated. For the input, the concentration was in $\mu\text{g/l}$, the activity correction was set to Davies and the temperature was set to $^{\circ}\text{C}$. The DOC was added as DOC (SHM) which was further modified in the available submenu to assume that all DOC used was dissolved HA and FA with no solid particles. The counter-ion accumulation option in the DOC settings was turned off for both HA and FA. This was chosen to prevent free-ions that are bound to HA or FA acid to be changed by ion-exchange reactions. The partial pressure of CO_2 was set to 0.00038 atm, to simulate an equilibrium with the atmosphere.

The surface complexation reactions were turned off and no redox couples were added, since the water was oxic. Under “Parameters, specify pe and Eh” the setting “Do not perform any redox calculation” was selected, to prevent the model to assume a fixed pe value of 0, which would not represent oxygenated water conditions.

For each of the elements, their chemical species were selected based on a literature review and the model limitations e.g. the model does not always contain the desired chemical species. If the species determined in the literature review were not available in Minteq, the model available species were selected. If the literature review was inconclusive about the most likely environmental occurring chemical species, the more toxic species were selected for the model. For more detail about the species used

see section 3.3, Tab.4.

3.2.3. Settings in WHAM 7

In WHAM 7 under “Charge balancing options” the option “Do not balance the charge” was selected and the activity correction was set to Deby-Huckel. Under “Precipitation options” all selected parameters were disabled. The default unit for solid particles matter (SPM) was set to mg/l, pCO₂ to atm, default solute unit to µg/l, the temperature was set to °C, the default phase unit was set to mg/l and the default alkalinity unit was set to E/l.

DOC was added to the model as “Colloidal Humic acid” and “Colloidal Fulvic acid”. The partial pressure of CO₂ was assumed to be 0.00038 atm to simulate an equilibrium with the atmosphere. The chemical species for each element were selected based on a literature review conducted and the model limitations, see section 3.3, Tab.4. If the species determined in the literature review were not available in WHAM, the model available species were selected. If the literature review was inconclusive about the most likely environmental occurring chemical species, the more toxic species were selected for the model. Element settings were set under “Chose Species type” to “Total species” for all simulations.

3.2.4. Metal mixture binding to the gill with WHAM and Minteq

To simulate the binding of metal mixtures to the gill, 1µg of HA was added to both models as a proxy gill. The element concentrations were added following the real measured data, shown in Tab. 2. The binding to HA was sorted from the predicted data and compared using the ratio.

3.2.5. Comparing different approaches to assess metal uptake to fish

There are different approaches to assess metal uptake to fish. In the present study we compared the ratio between the total metal concentration and the simulated free-ion concentrations after binding to DOC and the simulated binding to the proxy gill to assess metal uptake to fish.

3.2.6. Change in metal binding to the gill by the addition of metals

The aim of this simulation is to compare how WHAM predicts the binding to the proxy gill when only one metal e.g. U, is present in water with DOC, F, Cl, NO₃, SO₄, NH₄,

Na, Mg, K and Ca. Then one by one other metals are introduced into the simulation and the change in binding for the metal of interest e.g. U, is observed. The order in which the metals were simulated was based upon the concentration of the element in water, its affinity and demonstrational purposes.

The same settings were used as in the initial simulation.

3.2.7. Single metal binding and comparison to mixture binding with WHAM

To simulate single metal binding the same settings were used as in previous simulations for both models. There was a change only in the simulated water composition. Metals were removed from the simulation leaving only the measured concentration of DOC, F, Cl, NO₃, SO₄, NH₄, Na, Mg, K, Ca and the metal of interest.

3.3. Chemical speciation in WHAM and Visual Minteq

The chemical speciation that can be selected in WHAM and Minteq is presented in Tab.4. In WHAM the user chooses the element and for some elements the oxidation state. In Minteq the user chooses the elements and in some instances the specific oxide e.g. MoO₄²⁻. The elements are presented in the same format as in the models.

Table 4: Selected chemical species for modeling in Minteq and WHAM (as can be selected in the model).

Element	Chemical species (Minteq)	Chemical species WHAM
Uranium	U (VI)	UO2
Strontium	Sr ⁺²	Sr
Thorium	Th ⁺⁴	Th
Aluminum	Al ⁺³	Al
Molybdenum	MoO ₄ ⁻²	/
Cadmium	Cd ⁺²	Cd
Vanadium	VO ⁺²	VO
Arsenic	As (III)	/
Iron	Fe ⁺³	Fe (III)
Nickel	Ni ⁺²	Ni
Copper	Cu ⁺²	Cu
Zinc	Zn ⁺²	Zn
Manganese	Mn ⁺²	Mn
Magnesium	Mg ⁺²	Mg
Potassium	K ⁺¹	K
Calcium	Ca ⁺²	Ca
Fluoride	F ⁻¹	F
Chloride	Cl ⁻¹	Cl
Nitrate	NO ₃ ⁻³	NO ₃
Sulfate	SO ₄ ⁻²	SO ₄
Ammonium	NH ₄	NH ₄
Sodium	Na ⁺¹	Na

3.4. Statistical methods and data handling

All the data analysis and calculations were performed in Excel 2016. All the figures were constructed using Matlab R2018a.

All regression lines in the thesis are second degree polynomial lines. This type of regression line was selected because of its good fit with the data and the flexibility a second-degree polynomial line offers. It can be used for subjectively looking linear and non-linear data points. A first-degree polynomial line could have been selected, producing a straight line, this would introduce a high variance in the results, compared to a second-degree polynomial line.

The *gill ratio* is calculated by using the gill measured metal concentration from the fish exposed to the different dilution ratios and dividing it by the gill measured metal

concentration from the fish exposed to the control water.

$$\text{gill ratio} = \frac{\text{measured concentration of metal in the gill}}{\text{measured concentration of metal in the control gill}}$$

The *proxy gill ratio* is calculated by simulating the binding of metals to the proxy gill at different dilution rates and dividing it by the simulated metal binding to the proxy gill from the control water.

$$\text{proxy gill ratio} = \frac{\text{simulated concentration of metal in the proxy gill}}{\text{simulated concentration of metal in the control proxy gill}}$$

The *total metal concentration ratio* is calculated using the measured metal concentrations in water from the leached alum shale and the measured metal concentrations from the control water.

$$\text{total metal ratio} = \frac{\text{measured metal concentration in the leached water}}{\text{measured metal concentration in the control water}}$$

The *free-ion ratio* is calculated using the simulated free-ions by WHAM and Minteq in the presence of DOC in the leached alum shale water. The free-metal ions left after the binding to the DOC in the leached water are divided by the number of free-ions left after the binding to DOC in the control water

$$\text{free - ion ratio} = \frac{\text{free ions left after binding to DOC in the leached water}}{\text{free ions left after binding to DOC in the control water}}$$

Biological concentration factor (BCF) is calculated using the measured metal uptake in the fish gill and dividing it with the measured metal concentration in water.

$$\text{BCF} = \frac{\text{metal concentration in the gill}}{\text{metal concentration in the water}}$$

4. Results and discussion

4.1. Measured data

The measured metals of interest concentrated in the fish gill are presented in Fig. 3. In undiluted water, the highest concentration of metals in fish gills was measured for $V > U > Sr > Ni > Cd$, when the control values were not subtracted. The general trend in the figure shows a decrease of metals bound to the fish gill with an increase in dilution i.e. lower metal concentration.

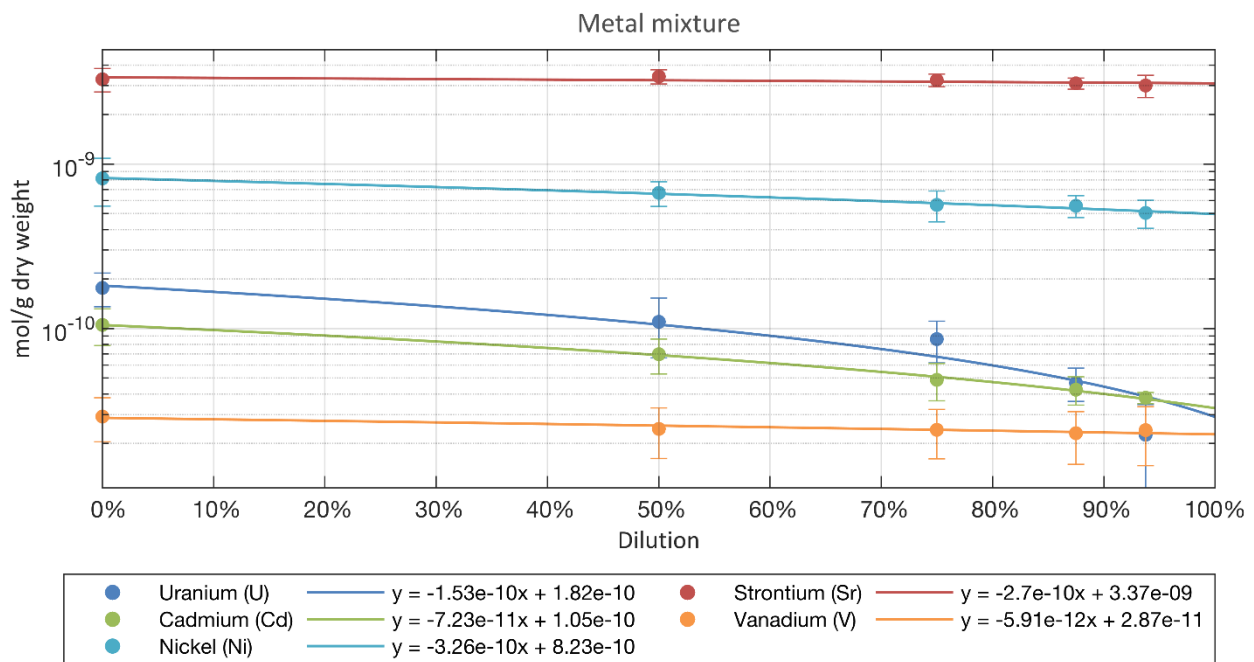


Figure 3: The average measured metal content in the fish gill (mol/g dry weight) when in undiluted water.

The decreasing trend is expected for all the metals as with a decrease in concentration less can be accumulated by the fish gill. One of the more noticeable decreases with increased dilution is for U and Cd. The decrease in uptake can be explained by complexation with DOC, the formation of metal complexes with other compounds and the competition for binding sites on the fish gill with other metals.

The concentration of measured V in the experimental water was very high compared with any other element of interest (Tab. 2), however a relatively low binding to the gill was measured. It is reasonable to assume that the amount of bioavailable V was very low, and it was therefore not accumulated in large amounts as shown in Fig 3 and Fig. 4.

The average metal concentration in the fish gill after subtracting the metal uptake from the control is presented in Fig. 4

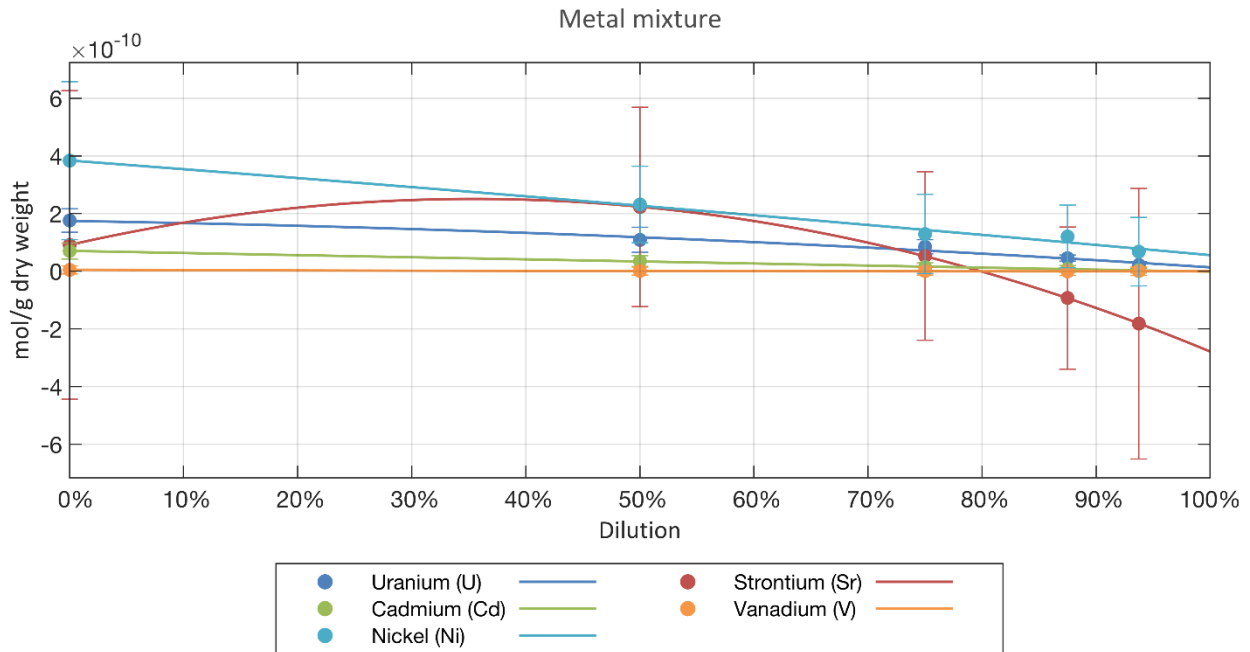


Figure 4: The average measured metal concentration in the fish gill (mol/g dry weight), when exposed to undiluted leached water, with subtracted control.

When comparing the trendlines of Cd, Ni, V and U, the decline can be considered linear, where $Ni > V > U > Cd$ accumulation decreases fastest with higher dilution. The highest calculated concentration of Sr is 2.5×10^{-10} l/g dry weight at 35% dilution. After the highest uptake at 35% dilution the concentration decreases. The strong uptake of Sr at 35% can be explained by less competition for binding sites from other elements, as its concentration is still high in relation to other elements concentrations e.g. Cd, Ni. When the dilution increases, less Sr is taken up as its lower concentration and its lower binding affinity for HA cannot compete with other metals for binding sites. Any negative values do not have physical meaning but indicate that Sr is not being accumulated compared to the control sample.

All elements of interest have high uptake ratios to the fish gill when in undiluted waters (Fig. 5). The highest calculated uptake ratio in undiluted waters is for U at 179, followed by Cd 3.34, Ni 2.80, V 1.34 and Sr 1.14. At higher dilution (93.75%, 87.5%, 75%, 50%) the calculated uptake ratios decrease significantly. The decrease of uptake is expected, because of the lower metal concentrations while DOC concentration is stable at all dilutions. The binding of metals with DOC is relatively

larger at high dilution compared to low dilution. Additionally, the complexation of metals with base cations (BC) such as Ca, K, Mg and other ligands in the water reduces the free metal ions that can be taken up by the fish gill.

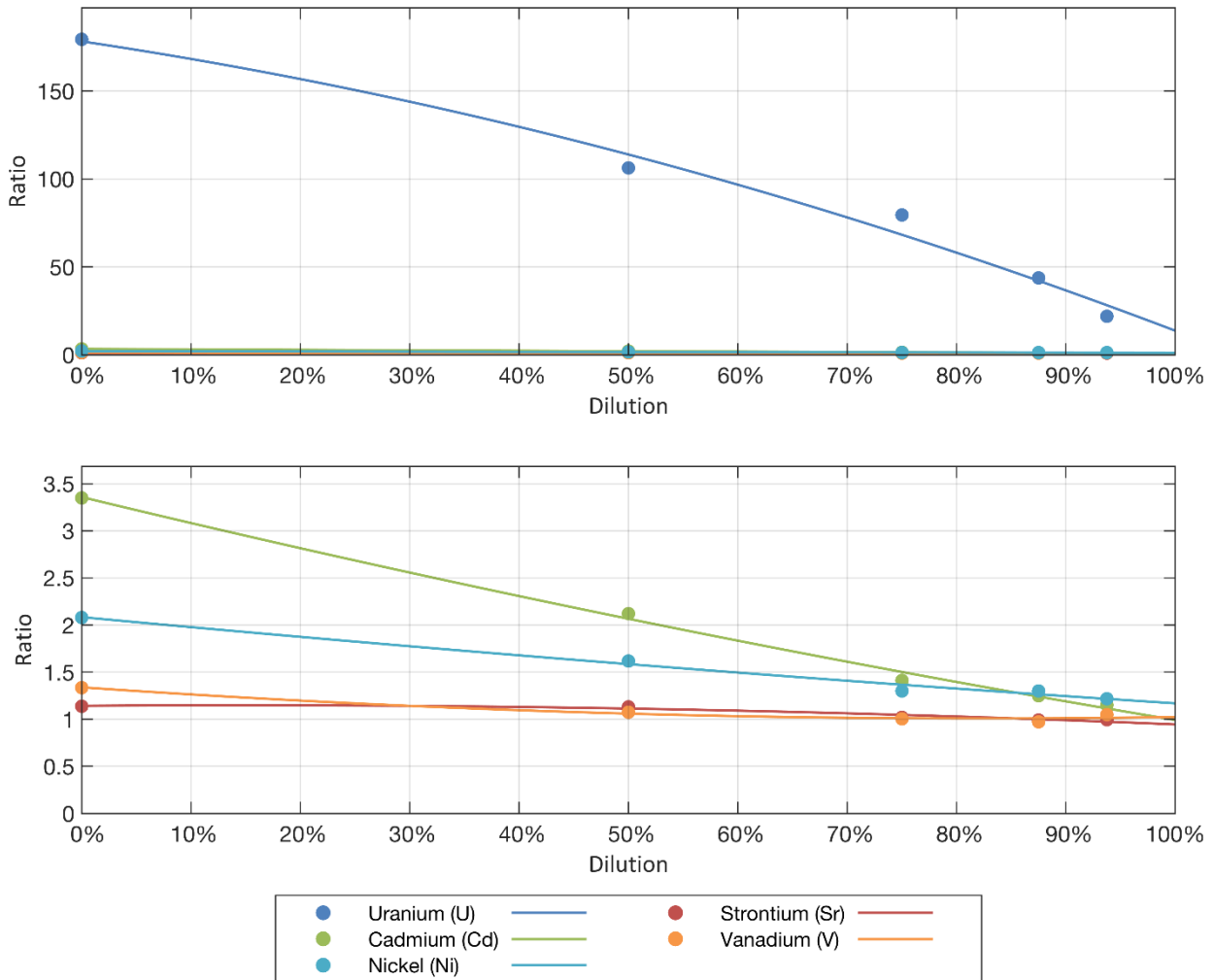


Figure 5: Gill uptake ratio U, Cd, Sr, Ni and V.

The calculated bioconcentration factors (BCF) with subtracted control values are presented in Fig. 6. Results show an increase in reactivity of the metals by increasing dilution. Based on the BCF, Cd, U and Ni are more reactive at high dilution compared low dilution. Strontium is more reactive at 50% dilution compared to any other dilution and is not reactive at dilutions above 75%. When at 50% dilution Sr is more reactive than U but less reactive than Ni. Negative values for Sr and V BCFs are not shown. They are the mathematical result of the control being higher than the measured concentration. When comparing Sr and V, which are the least reactive

elements at 75% dilution, Sr is still magnitudes more reactive compared to V.

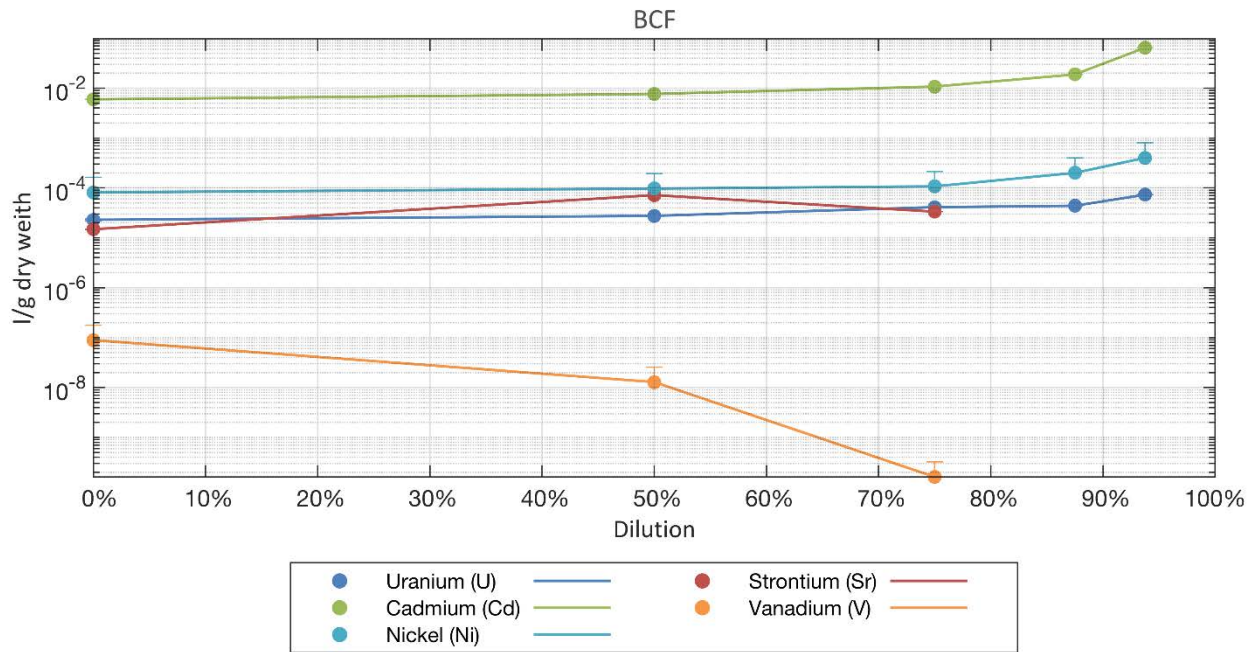


Figure 6: Calculated BCF for metals of interest, relative compared to the control.

4.2. Uranium prediction

4.2.1. Uranium mixture binding to the gill with WHAM and Minteq

The simulated U binding to the proxy gill is presented in Fig.7.

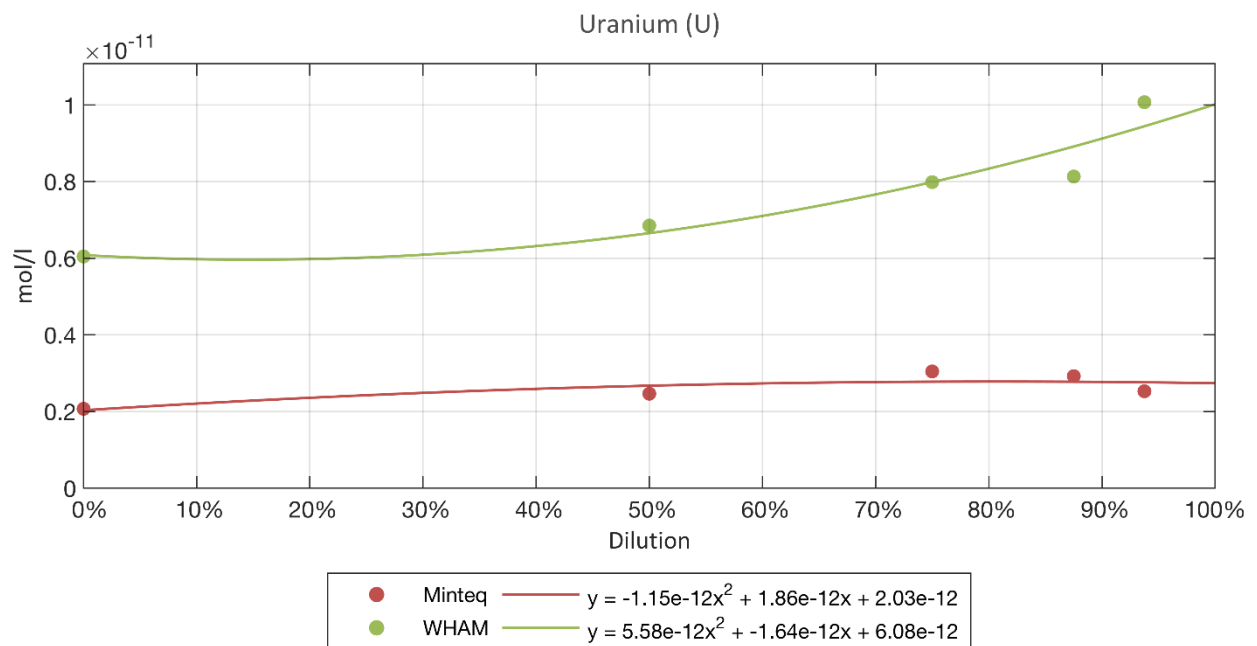


Figure 7: WHAM and Minteq simulated U binding to the proxy gill.

WHAM predicted higher binding of U to the proxy gill at all dilutions, compared to Minteq. This trend can be explained by a high concentration of the test metals e.g. V which interferes with other metals in the solution, allowing U to be taken up to the proxy gill while preventing other metals access to the binding sites. Minteq predicted an increase in bound U until 75% dilution. While at dilutions above 75% it predicted a lowering of U binding to the proxy gill. The raw output data from Minteq (Appx. 2), shows a formation and binding of other species (OU^2OH^+) to the proxy gill with increasing dilution while the biggest increase in U binding to the gill originates from UO_2^{2+} . The differences in the binding of UO_2^{2+} and other species for WHAM and Minteq can be explained by the different humic complexation models (section 2.6).

The simulated free-ion concentration left after the binding to DOC are presented in Fig. 8.

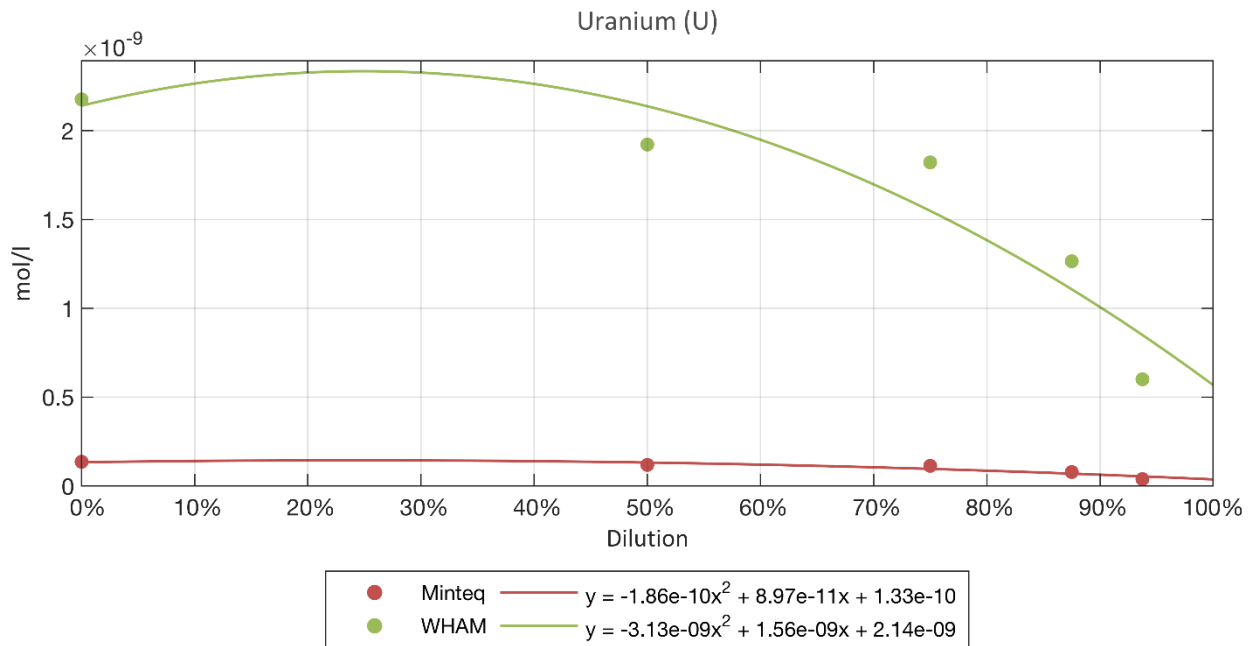


Figure 8: Simulated U free-ion concentration (mol/l) left after binding to DOC.

WHAM simulated free ions left after the binding show the opposite trend as in Fig. 7. The WHAM trendline in Fig.8 shows an expected decline of U free ions as with less U in the water there would be less U free ions. Minteq predicted that U free ions are decreasing with dilution. The lower free-ion concentration predicted by Minteq can be explained by the higher complexation of U with other elements and the initially lower estimation of U free ions in the water. Additionally, the difference between models used in WHAM and Minteq can cause differences in predicting the initial concentration of free ions and their complexation with other elements and ligands.

This theory has not been researched in this thesis.

Both WHAM and Minteq predict that from the total metal concentration only a small fraction is present in the water as free metal ions. No complexed species are presented in this thesis as the main focus is on free metal ions bound to HA.

4.2.2. Comparing different approaches to assess U uptake to fish

The comparison of different ratio approaches for assessing U uptake to fish gill is presented in Fig. 9.

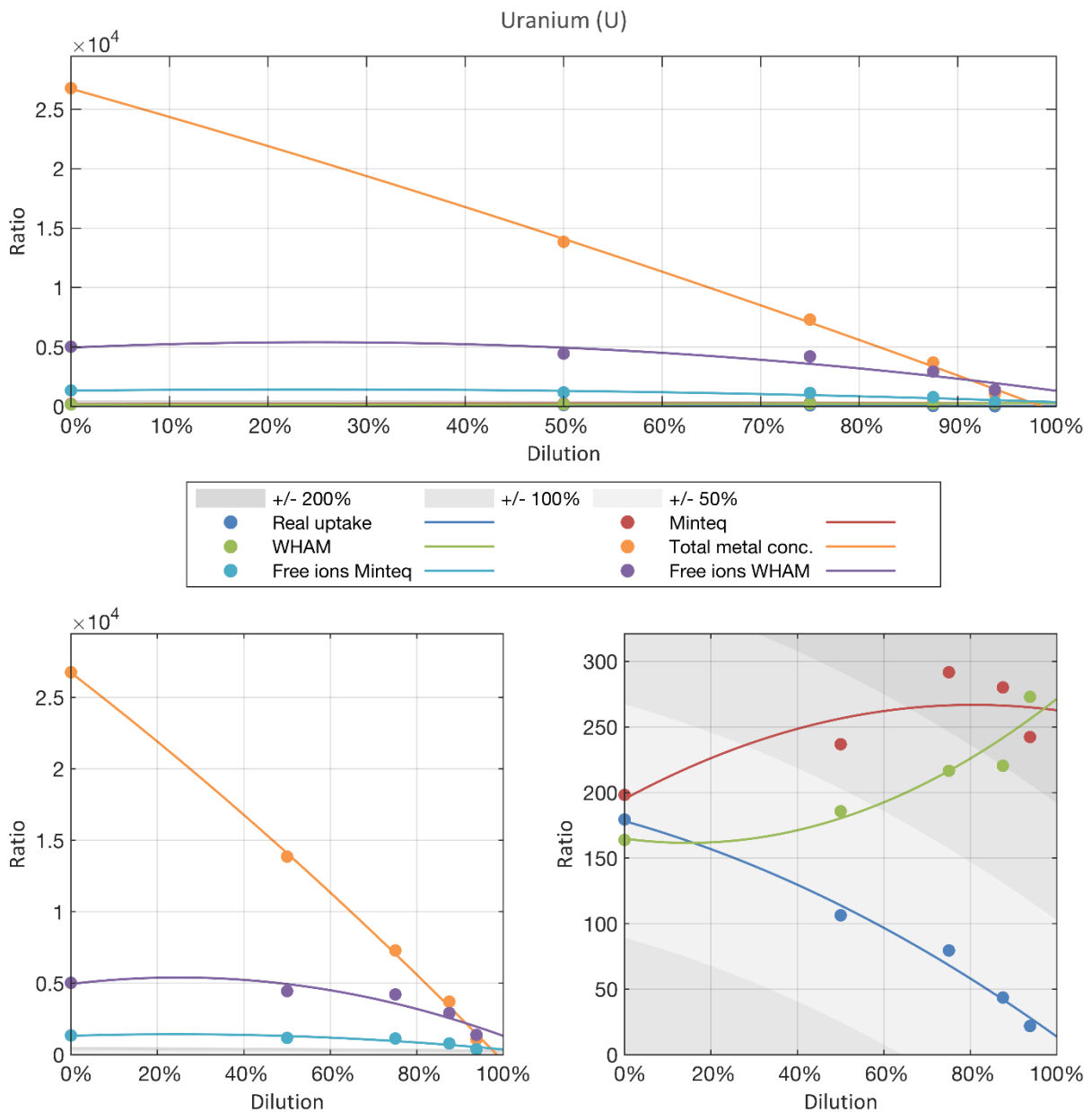


Figure 9: Comparing real gill uptake with total metal concentration and modeled free-ion concentration using the ratio approach.

The grey areas in Fig. 9 show a deviation in percentage compared to the trend line of the metal binding to the real gill ($\pm 50\%$, $\pm 150\%$ and $\pm 200\%$). If the model exceeds $\pm 50\%$ it is considered unreliable, as it does not mirror the reality close enough. The addition of the other grey areas is to give reference to the reader.

The total U concentration ratio predicts the highest uptake compared to any other ratio assessment. The estimated total metal ratio is 26763 when in undiluted waters and 1075 when in highly diluted waters. This is because the total measured U concentration approach does not consider any complexations with ligands or other elements, while it is considered that 100% of the U can be taken up by the organism. Because of this, the total U concentration should not be used as a predictor of U uptake to the fish gill.

The simulated free-ion concentration ratio is a better predictor of U fish uptake, compared to the total metal concentration. It is assumed that only free U ions can be taken up by fish, while it does not consider the U complexation with other elements. The predicted free-ion ratio for WHAM is 5031 when the water is undiluted and 1386 when the water is at high dilutions. The predicted Minteq U free-ion ratio is 1346 when the water is undiluted and 380 when the water is diluted the most. The best model for predicting free-ion ratios is Minteq as it estimates a lower ratio compared to WHAM. The free-ion ratio approach is a better predictor of U uptake compared to the total metal concentration, because it estimates less U uptake compared to the real gill.

The binding ratio predicted by WHAM and Minteq provides the closest estimation of binding to the real gill, compared to any other ratio approach. WHAM predicts a ratio of 168 when the water is undiluted and a ratio of 272 when the water is diluted the most. Minteq predicts a ratio of 198 when the water is undiluted and a ratio of 242 when the water was diluted the most. The increase in U binding ratio by increasing dilution in WHAM can be explained by the V interaction with other metals, enabling more U to bind to the proxy gill. Compared to the real gill uptake, the WHAM ratio predicts U binding to the gill the closest to reality and is reliable up to 58%, while Minteq predicted a higher U binding ratio to the gill and is reliable up to 28%. The simulated binding of metals to the gill with both models is the best approach to estimate U uptake to fish.

In Fig. 7 WHAM predicts higher binding to the gill compared to Minteq, while in Fig.

9 the ratio shows that WHAM predicted a lower binding to the gill. This is a disadvantage of using the ratio approach, as it skews the data. The ratio approach uses the simulated binding to the proxy gill (HA) with metal concentrations from the control water and the metals in the exposure water (metal mixture). When the binding is simulated with the control water in WHAM, the model predicts greater binding to the gill. When the bound metal concentration from the control is used to calculate the ratio, the relatively large amounts of bound metals cause the ratio to be lower for WHAM. The opposite happens for Minteq, where lower concentrations of metals bind to the gill from the control, therefore producing a higher ratio. This trend has been observed for U, Sr and V. Considering that the ratio approach is not always a valid comparison it still provides a relatively good method to compare different uptake results directly.

The differences between WHAM and Minteq have to be further studied to determine why there is more U binding to the gill, with decreasing concentrations of U in the water.

4.2.3. Change in U binding to the gill by addition of metals

The simulated change in U binding to the gill with the addition of other elements, shows that U binds most when alone in the solution (Fig.10).

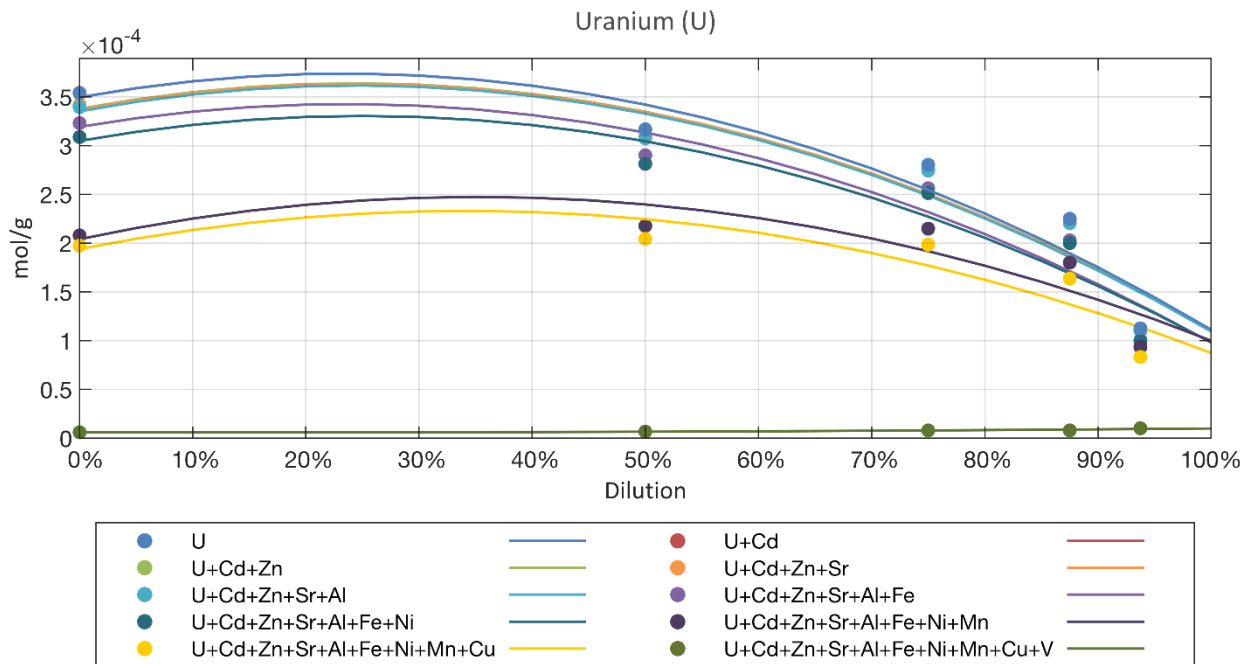


Figure 10: Change in binding of U with the addition of other elements to the proxy gill.

The lowest binding of U to the gill is when all elements are added (U, Cd, Zn, Sr, Al,

Fe, Ni, Mn, Cu and V). When more elements are competing with each other on the gill, the less U can be taken up. The complexation of U with other metals reduces its binding to the gill further. The competition for the binding sites on the gill depends on the concentration of the specific element and its affinity to HA (proxy gill). The presence of Cd decreases the binding of U to the gill. The study conducted by Teien et al. 2010, showed that when Atlantic salmon is exposed to a mixture of Cd and U, the presence of Cd does not increase the uptake of U (Gilbin et al., 2010). This confirms that U uptake to the gill does not increase with Cd.

When Mn was added to U, Cd, Zn, Sr, Al, Fe and Ni, the uptake of U was reduced at all dilutions. To test if only Mn caused the decline or the combination of all the element to U binding, only U and Mn were simulated using the same concentrations as in all experiments. Mn did cause a similar decline as can be seen in the Fig. 10, therefore Mn alone has a big impact on the binding of U. This can be explained by relatively high Mn concentrations in the water compared to other metals e.g. Cd, Al, which are at much lower concentrations and a high enough binding affinity for HA to reduce U binding.

The presence of V in Fig. 10 causes WHAM to predict higher binding of U with increasing dilution. In undiluted water high V concentrations compared to U, prevent U to bind to the gill, therefore the low uptake at 0% dilution in Fig. 10. As the dilution increases i.e. less U and other metals in the water, U starts binding more to the proxy gill as lower concentrations of other metals and V have a lower impact on its binding. This trend continuous with increasing dilution, until U is taken up the most at the highest dilution where other metals and V cannot outcompete U efficiently as in undiluted waters.

Vanadium appears to be the only element that triggers the model to predict more U binding when there is less U in the water, as no other element tested caused such U predictions. This theory has not been tested in this thesis. In WHAM and Minteq V(IV) was chosen, over V(V) which would be the more correct species in natural waters. Because WHAM does not enable the user to use V(V) in the model, V(IV) had to be used in both models so that the binding results could be compared directly for WHAM and Minteq. It is plausible that if both models would use V(V) instead of V(IV) the uptake results for U and V binding would be different.

It appears that Ni, Mn and V have the highest effects on the binding of U at all

dilutions. This is mainly because Ni and V are present in relatively high concentrations in the water compared to any other metal, coupled with high binding affinity for HA. This simulation confirms the results of simulating metal mixture binding to the gill with WHAM, as it shows an augmentation of U binding with lower U concentrations in the water.

4.2.4. Single U binding and comparison to mixture binding

The Fig.11 shows the comparison of only U bound to the proxy gill without any other metals (single metal) and U binding in a mixture of metals (metal mixture).

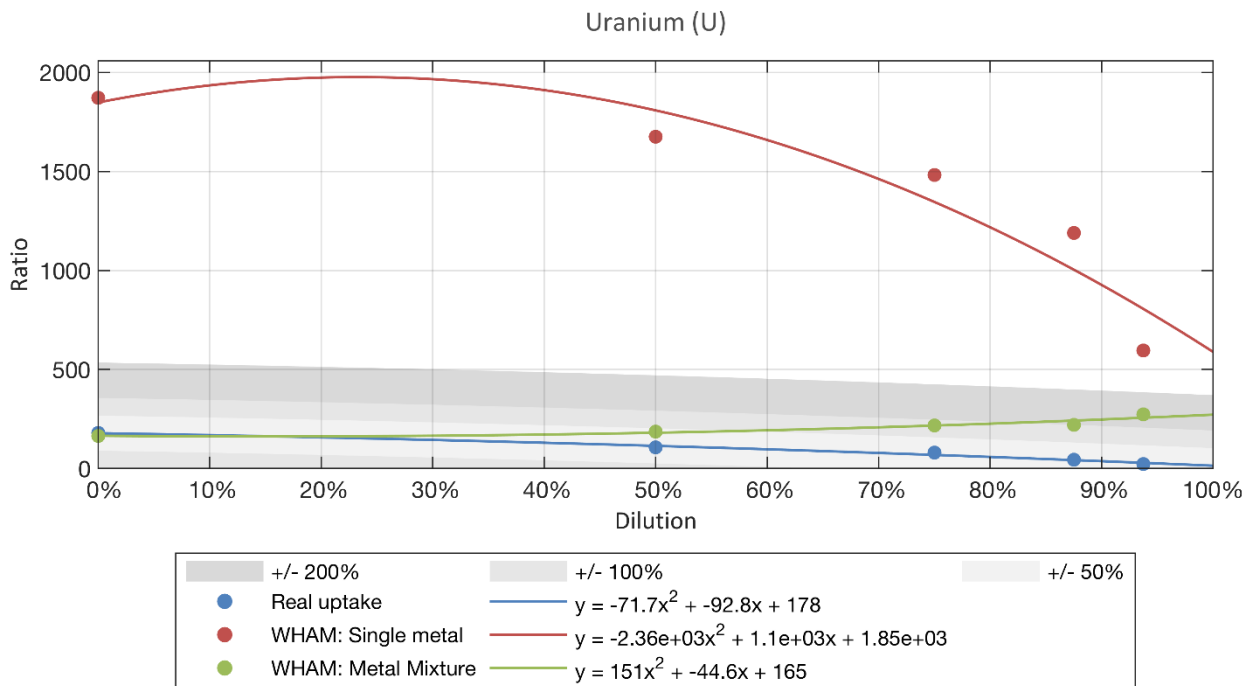


Figure 11: Comparison of single metal U binding, real gill and metal mixture binding.

The data used for constructing of the trendline metal mixture comes from Fig.10 where all elements are considered.

The modeled single U binding ratio is 1871 when the water is undiluted (Fig. 11). These results demonstrate the importance of considering metal mixtures when predicting uptake to the gill. The complexation reactions that occur in metal mixtures reduce the amount of U free ions that can bind to the gill. This significantly reduces the uptake and allows for a better binding prediction for U. Results from section 4.2.3 show that V significantly changes the binding of U. The competition of other metals for the same binding sites on the HA (proxy gill) additionally influences U free ion binding.

The modeled U uptake without any other metals (single metal) cannot consider the competition of other metals for the same binding sites with U. This causes U overestimation as the model predicts that a majority of free U ions will bind directly to the proxy gill with minimal binding competition from metal cations or DOC (FA).

4.3. Cadmium prediction

4.3.1. Cadmium binding to the gill with WHAM and Minteq

The simulated Cd binding to the proxy gill with WHAM and Minteq is presented in Fig. 12.

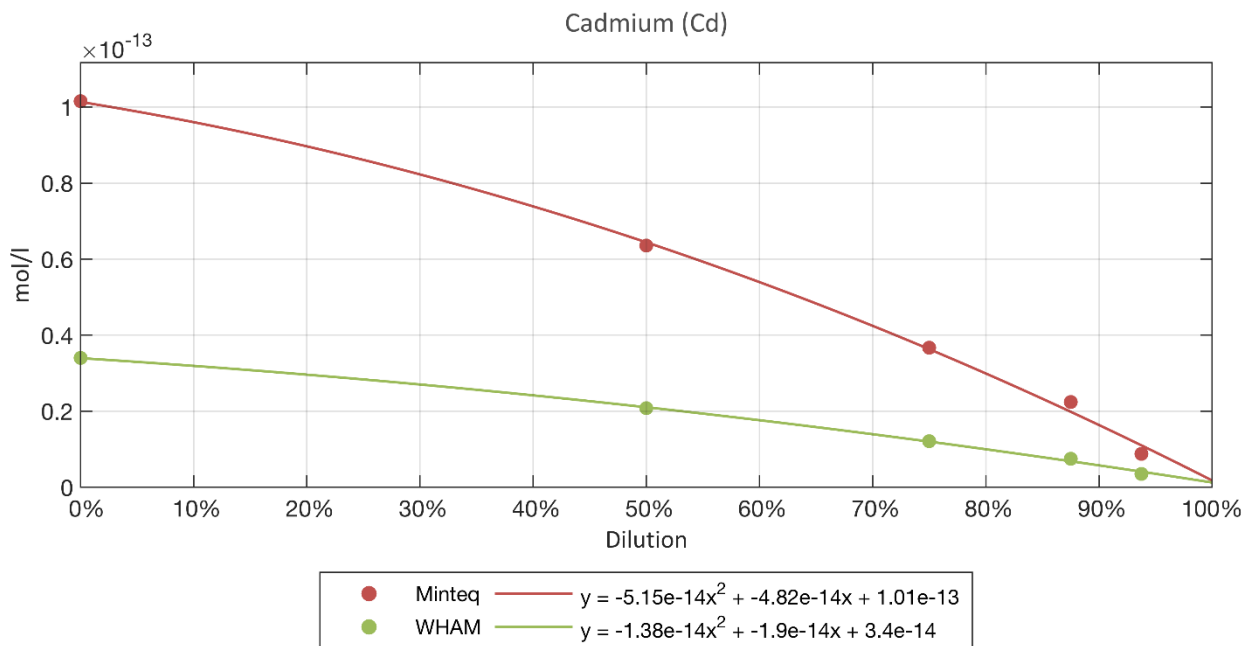


Figure 12: WHAM and Minteq simulated Cd binding to the proxy gill.

WHAM predicts lower binding to the gill at all dilutions compared to Minteq. Both WHAM and Minteq trendlines show a decline in binding when there is less Cd present in the water i.e. higher dilution. There is a difference between predicted WHAM and Minteq uptake that can be explained by the different humic complexation models. However, the main reason is assumed to be the difference in models that predict free ion Cd concentrations in the solution (section 2.6, Fig. 13).

Compared to U binding in Fig. 7 where the concentration of U bounded to the proxy gill increases with dilution, Cd follows a predicted decline in binding to the gill with increasing dilution. The decreasing trend of Cd for both WHAM and Minteq is almost linear. The simulated free-ion concentration is presented in Fig. 13 after the binding

to DOC.

WHAM also predicts lower free-ion concentration left after the binding to DOC, compared to Minteq. The overall difference is that the prediction of Cd complexation to other elements is higher in WHAM therefore reducing the prediction of free-ion concentration and influencing the predicted binding of Cd to the proxy gill. The differences between WHAM and Minteq ion prediction models have not been analyzed in this thesis.

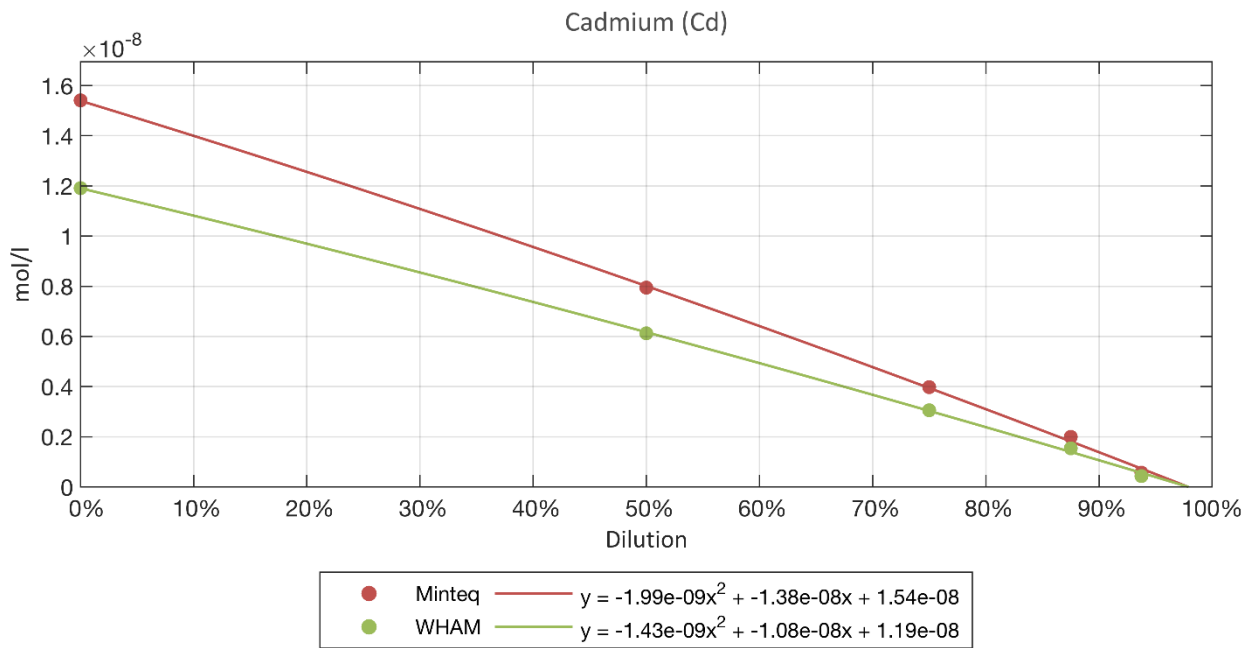


Figure 13: Simulated Cd free-ion concentration after the binding to DOC and the proxy gill.

Compared to U free ions left after the binding to DOC in Fig. 8 for both WHAM and Minteq, the simulated Cd free ions left after the binding to DOC follow a steeper decline with increasing dilution.

4.3.2. Comparing different approaches to assess Cd uptake to fish

Using the ratio approach, results show that both the total metal ratio and the free-ion ratio predict a higher ratio than the real uptake ratio. The predicted uptake ratio when the water is undiluted is 208 and 218 respectively, and when in highly diluted

water the ratio is 7.9 and 8 for the total and free ion ratio, respectively.

The highest ratio for Cd was predicted by WHAM and Minteq free-ion ratios (Fig. 14). The estimated ratio is 218 when in undiluted water and 8 when highly diluted. The overestimation by both models can be explained by the used mathematical models that predict the free ion concentrations.

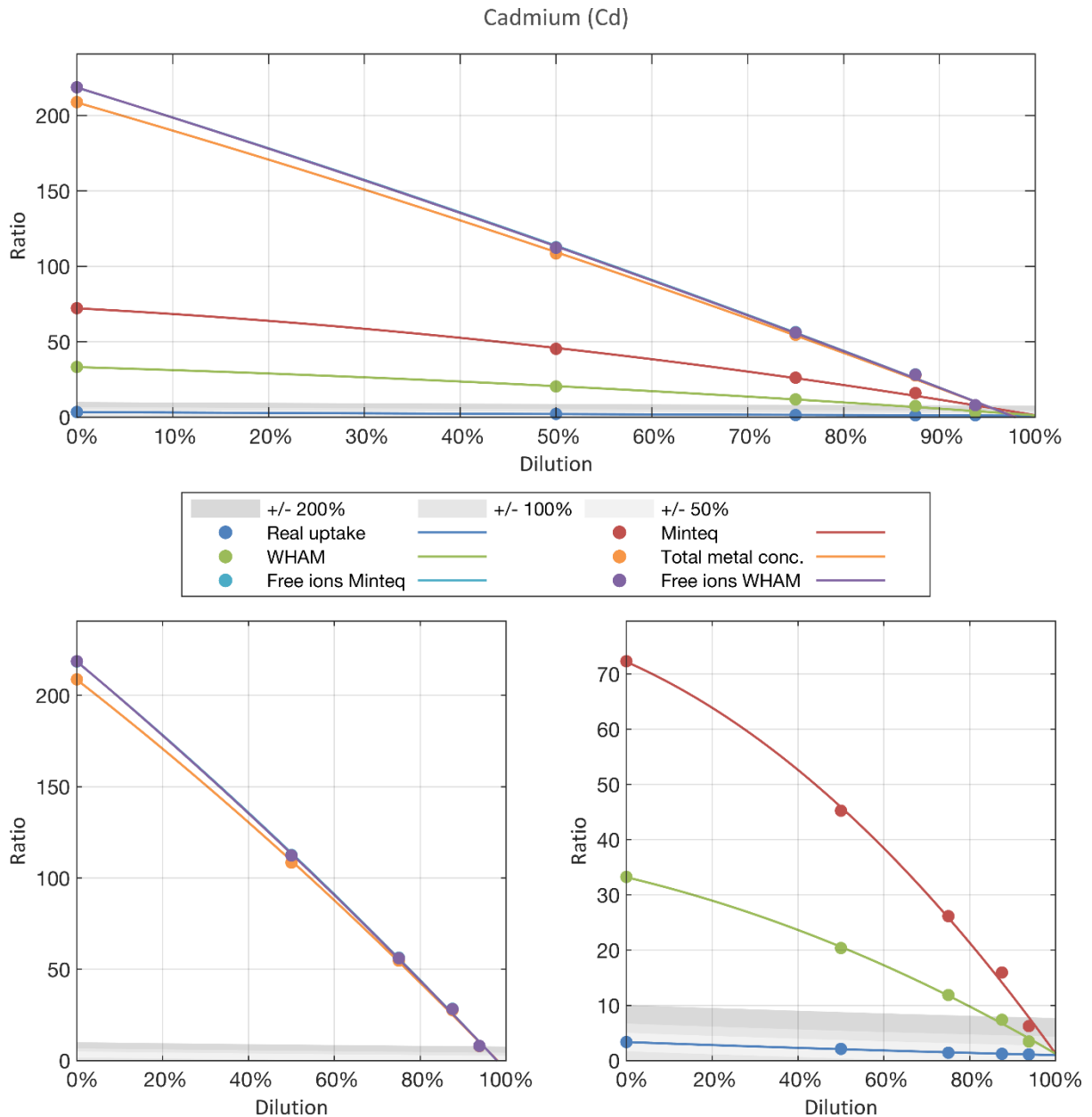


Figure 14: Comparing real gill uptake with total metal concentration and modeled Cd free-ion concentrations using the ratio approach.

The simulated binding ratio predicted by WHAM and Minteq provides the best estimation of Cd binding to the real gill, compared to any other ratio approach. WHAM predicted in undiluted waters a ratio of 33 and a ratio of 3 when diluted the

most. Minteq predicted a ratio of 72 when the water is undiluted and a ratio of 6 when the water is highly diluted. The changes are considered to be caused by higher predictions of metal interactions causing lower concentrations of free ions and therefore a lower binding to the humic compounds. However, the lower predicted uptake by WHAM can also be explained by the different humic complexation models which predicts the binding of metals and protons to the simulated fish gill (section 2.6). As both WHAM and Minteq predicted similar free-ion ratios while the uptake ratio for WHAM is lower. It is evident that the humic complexation model plays the most important role. Based on the trendlines WHAM reliably predicts the uptake from 96% to 100 % and Minteq reliably predicts the uptake from 98 to 100%. The simulated binding of metals to the gill with both models is the best approach to estimate Cd uptake to fish.

The differences between WHAM and Minteq have to be further studies to determine why Minteq binds more Cd to the gill at all dilutions, compared to WHAM.

4.3.3. Change in Cd binding to the gill by addition of metals

The simulated change in Cd binding with the addition of other metals, shows that Cd binds most when alone in the solution (Fig.15).

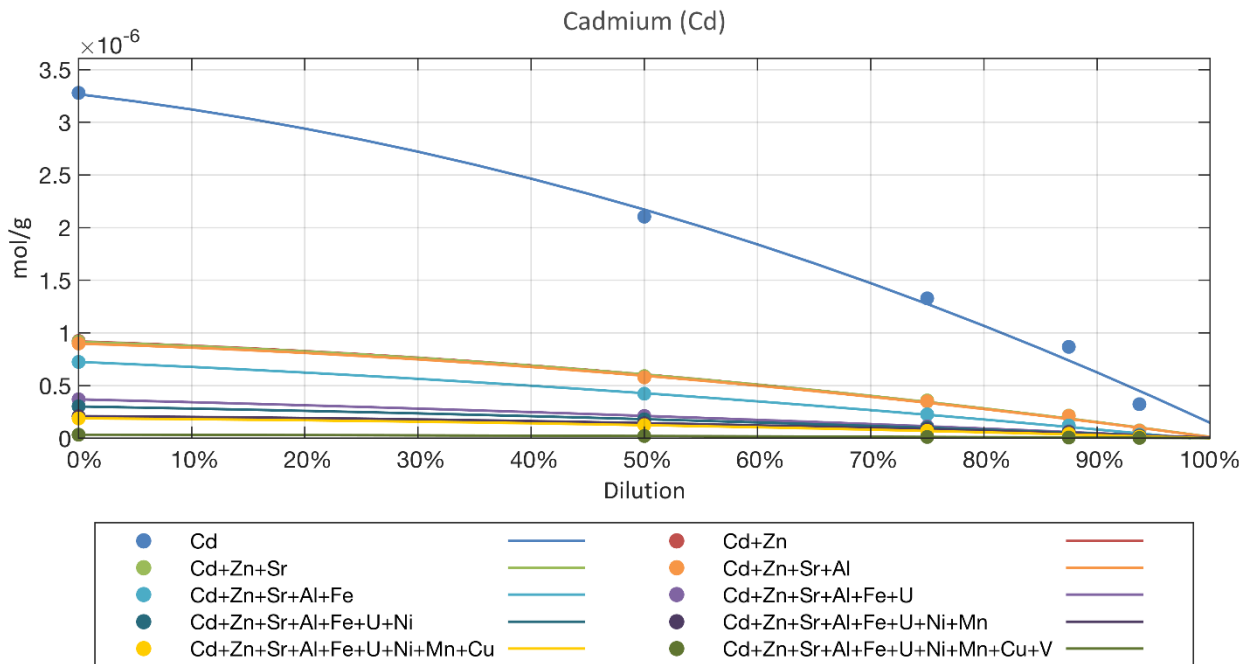


Figure 15: Change in binding of U with the addition of other elements to the proxy gill.

The lowest binding of Cd to the gill is when all elements were added (Cd, Zn, Sr, Al, Fe, U, Ni, Mn, Cu and V). When more elements are competing for binding sites on the gill, less Cd can be taken up. Cadmium will complex with metals and ligands, changing its speciation, instead of binding to the gill. The direct competition of other metals for binding sites directly influences the uptake of Cd. The competition for the binding sites on the gill depends on the concentration of the specific element and its affinity to HA (gill). The interaction between elements also influences the speciation, which translates into decreased free-ion concentrations in the water and therefore lower binding to the gill. As demonstrated with the predicted binding this seems to have an effect. However as demonstrated with the ratio approach this seems not to be the main effect.

The presence of Zn decreases the binding of Cd to the gill the most. The concentration of Zn is higher than the concentration of Cd. The decrease in binding is attributed to the amount of Zn in the water and its higher binding affinity for HA. Saibu et.al. 2018, exposed rainbow trouts to only Zn, Zn+Cd and Zn+Cu to assess the uptake of the metals. When the fish were exposed to Zn only, there was an increase in uptake to the gill, while exposure to Zn+Cd significantly reduced the uptake to the gill. When exposed to Zn+Cu the uptake of Zn was not reduced. This shows a competitive interaction between Zn and Cd across the fish gill. Zinc and Cu are taken up by the fish gills via different routes, therefore Cu influenced the uptake of Zn only slightly. It was shown that Zn was mainly found in the gills complexed with phosphates and amino acids (cysteine, histidine) (Saibu et al., 2018).

The addition of U to Cd, Zn, Sr, Al, Fe, decreased the binding further in the model. The concentration of U at all dilutions is very high which reduces the binding of Cd. The amount of added metal to the simulation plays a greater role in reducing the uptake of Cd compared to any other factor. When all metals were added, Cd uptake was the lowest, as all the metals compete for binding sites.

4.3.4. Single Cd binding and comparison to mixture binding

The data to calculate the metal mixture ratio is taken from Fig. 15 which includes all metals of interest. To the Fig. 16 the single metal line was added, which represents the simulated Cd binding to the proxy gill when there are no other metal present.

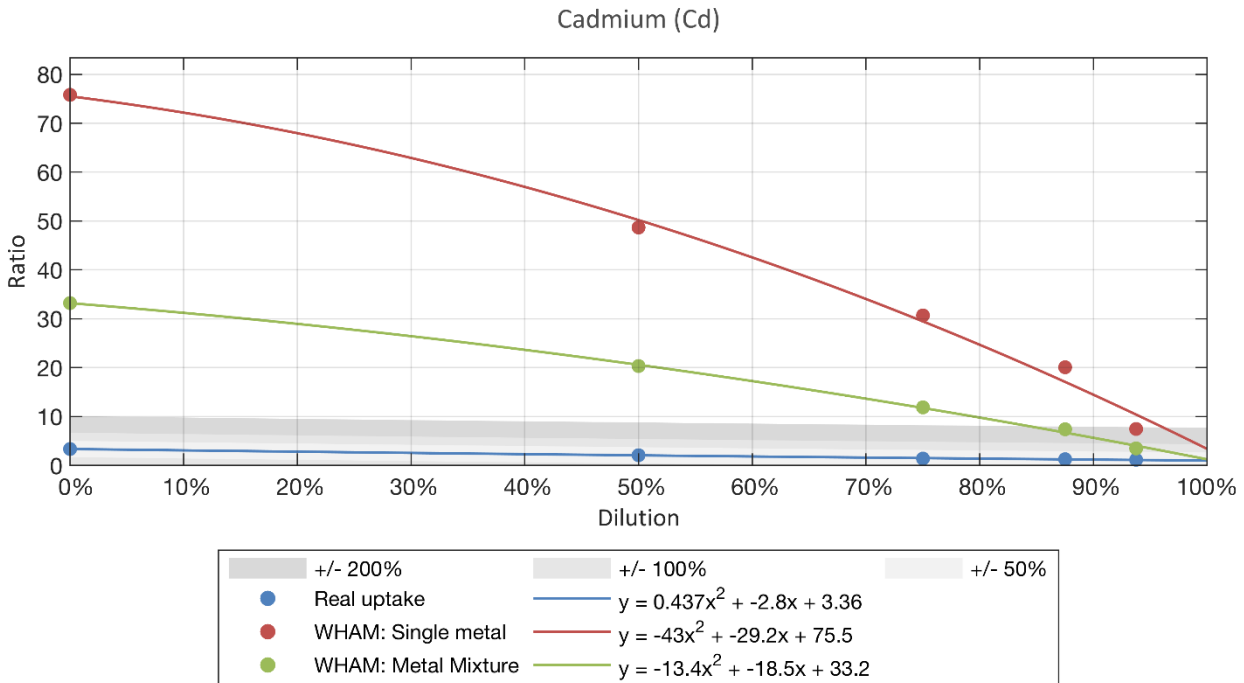


Figure 16: Comparison of single metal Cd binding, real gill and mixture binding.

The modeled Cd single metal ratio is 76 when in undiluted water and 7 when the water is diluted the most. This result shows the importance of considering metal mixtures when predicting metal mixture uptake to the gill. The complexation reactions that occur in metal mixtures reduce the amount of free ion Cd that can bind to the gill. This significantly reduces the uptake and allows for a better binding prediction for Cd. The competition of other metals for the same binding sites on the HA (proxy gill) additionally influence Cd free ion binding.

4.4. Strontium prediction

4.4.1. Strontium mixture binding to the gill with WHAM and Minteq

The simulated Sr binding to the proxy gill with WHAM and Minteq is presented in Fig. 17.

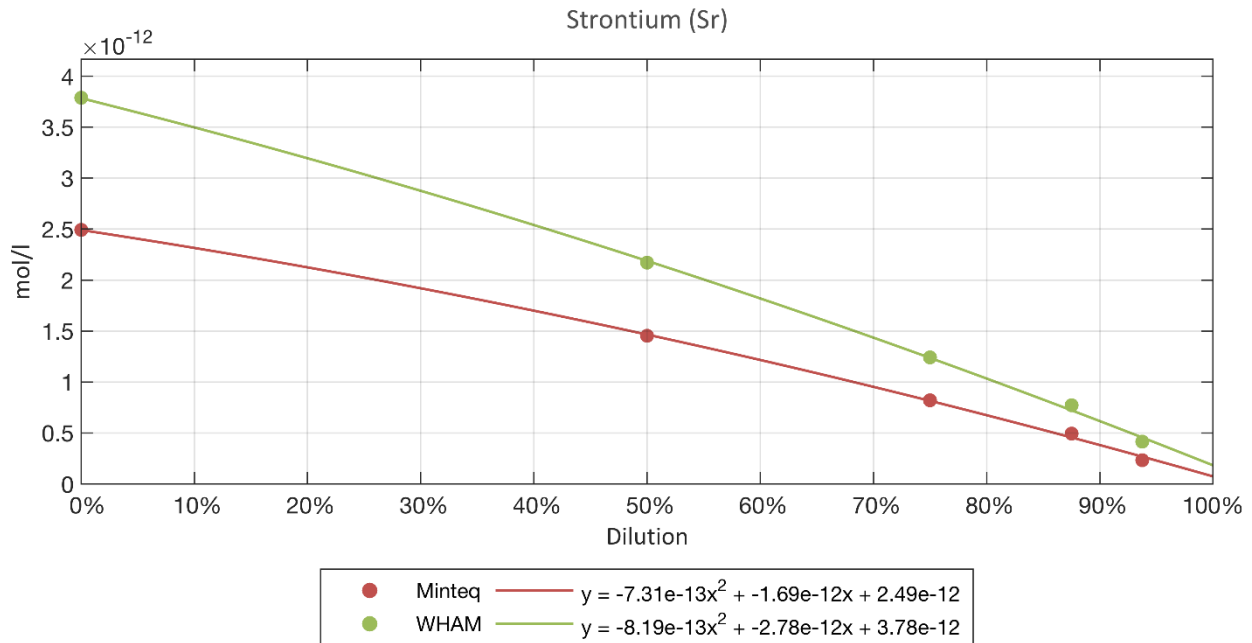


Figure 17: WHAM and Minteq simulated binding to the proxy gill.

A similar declining trend with increasing dilution is observed for Sr as for Cd and can be explained by the difference in free ion prediction models and difference in humic complexation models, see section 4.3.1. The difference in between Sr and Cd is that WHAM predicted more binding for Sr compared to Minteq.

Compared to Cd in Fig. 12, the bounded Sr in Fig.17 shows a much steeper decline with increasing dilution. This shows that Sr is quickly losing its ability to bind to the gill. This hypothesis is further supported by Fig.6 where Sr stops being accumulated in the real fish gill at dilutions higher than 75%.

The simulated free ion concentrations left after the binding to DOC are presented in Fig. 18.

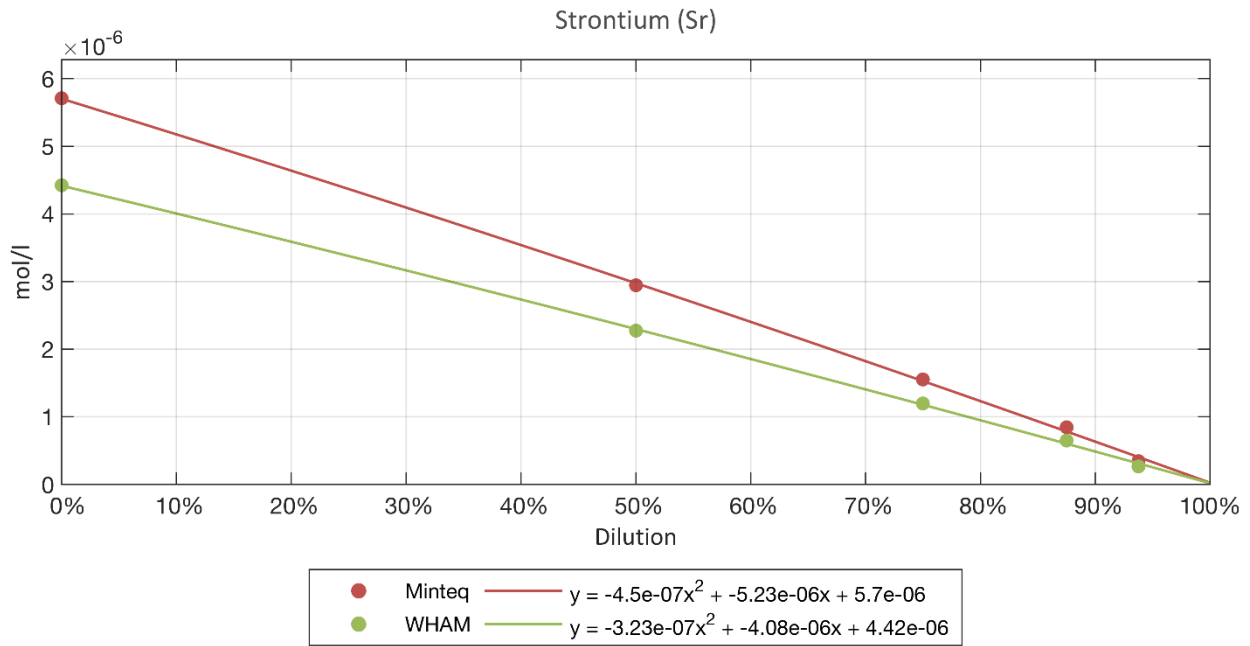


Figure 18: Simulated Sr free ion concentrations left after the binding to DOC and the proxy gill.

WHAM predicted lower concentrations of free ions left after the binding compared to Minteq. This confirms Fig. 17, where WHAM predicted more binding to the gill than Minteq. When there are less free ions left after the binding the more was bound to the gill. The difference between WHAM and Minteq simulated free ions are connected to the different humic substances complexations models and the free ion predictions models. The differences between the humic substances complexation models are described in section 2.6.

4.4.2. Comparing different approaches to assess Sr uptake to fish

The comparison of different approaches to assess Sr uptake to the fish is presented in Fig. 19.

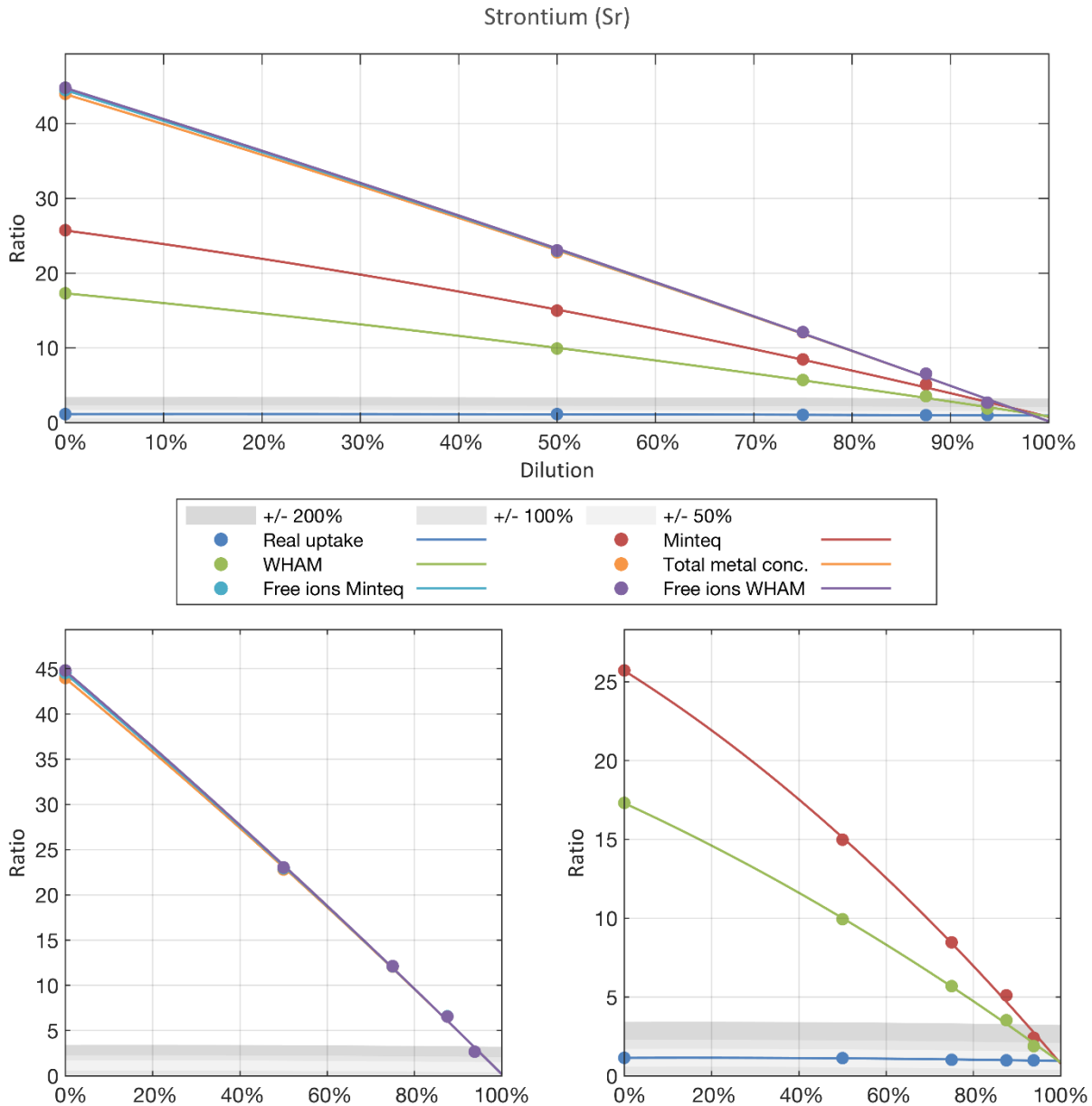


Figure 19: Comparing Sr real gill uptake with total metal concentration and modeled free ion concentrations using the ratio approach.

The highest ratio is predicted by WHAM free ion ration and Minteq free ion and the total metal ratio. This can be explained by the overestimation of free-ion concentrations by the models used in WHAM and Minteq. Both WHAM and Minteq ratio approaches predict the closest ratio to the real gill uptake. Using the ratio approach Minteq predicted higher uptake to the gill than WHAM, however in Fig. 17 WHAM predicted more binding to the gill than Minteq. This is a drawback of using

the ratio approach to predict Sr uptake, as the ratio depends on the binding of Sr uptake from the control water. A similar drawback occurs when using U, see section 4.2.2.

4.4.3. Change in Sr binding to the gill by addition of metals

The simulated change in binding of Sr with the addition of other metals to the proxy gill is presented in Fig. 20.

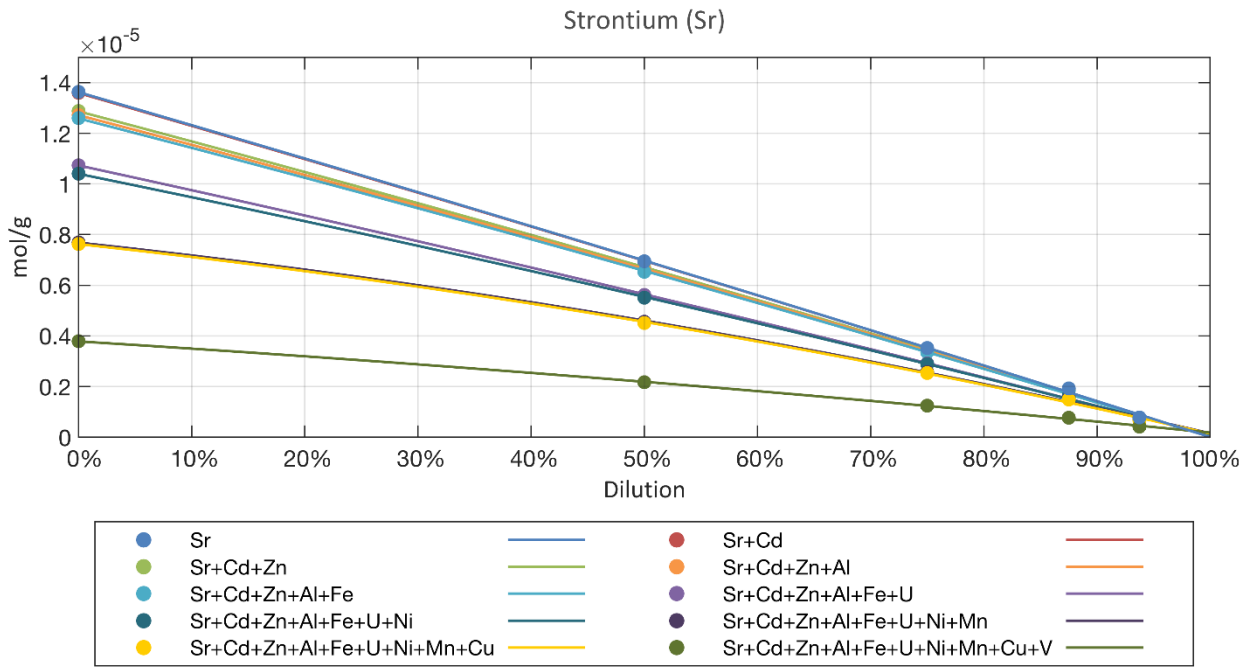


Figure 20: Change in binding of Sr with the addition of other elements to the proxy gill.

Strontium binds the most to the gill when alone in the simulation. The addition of U impacts the binding of Sr to the gill significantly, as the U concentration in the water is almost four times higher compared to Sr. The addition of metals in high concentrations impacts the binding of Sr to the proxy gill significantly. With each added metal with a concentration higher or the same as Sr a decline in bound Sr is observed. This is evident the most when U and V are added to the model.

It is known that Ca competes with Sr for uptake in fish. The uptake and accumulation of Sr^{2+} by the common carp (*Cyprinus caprio*) depends on the competition of Ca^{2+} free-ions, which can block its uptake (Chowdhury & Blust, 2002; Smith et al., 2009).

4.4.4. Single Sr binding and comparison to mixture binding

The comparison of single metal Sr binding, real gill and metal mixture binding is presented in Fig. 21.

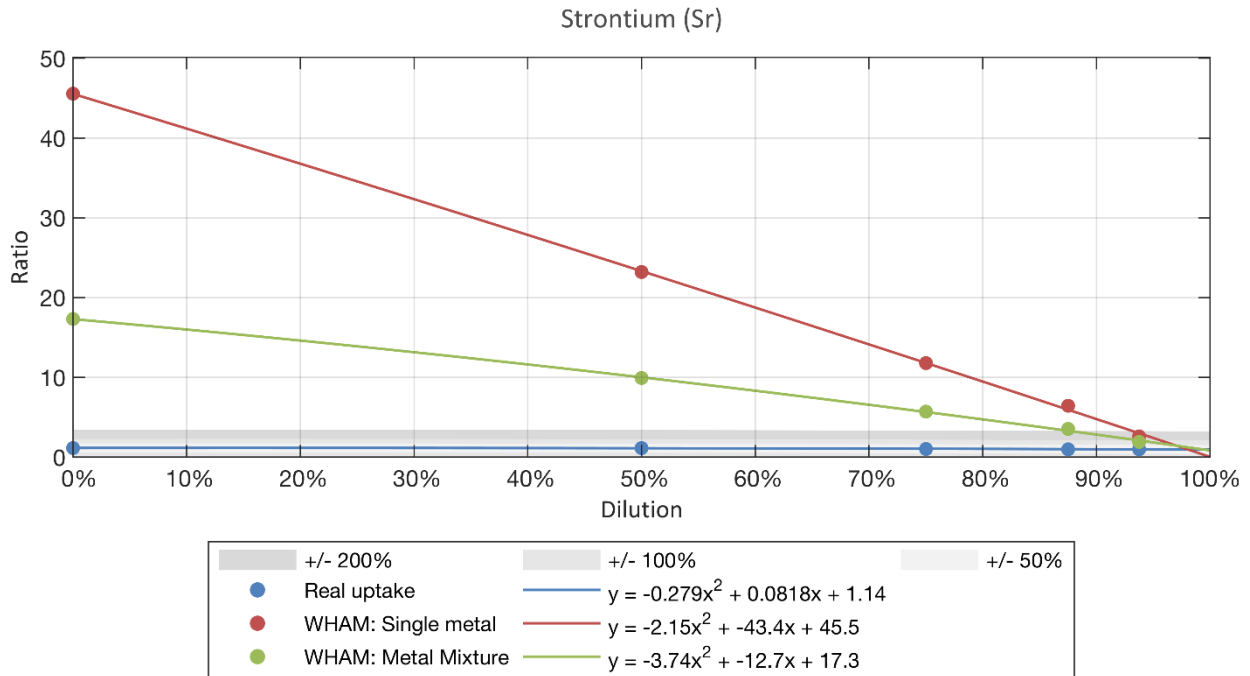


Figure 21: Comparison of single metal Sr binding, real gill and metal mixture binding.

The single metal binding predicts more than double the binding compared to the metal mixture. The single metal approach overestimates Sr binding to the gill as it does not consider any complexation reactions with other metals and experiences little to no competition on the binding sites of the proxy gill. A similar trend is observed for Cd in the section 4.3.4.

4.5. Nickel prediction

4.5.1. Nickel mixture binding to the gill with WHAM and Minteq

The simulated Ni binding to the proxy gill with WHAM and Minteq for Ni is presented in Fig. 22.

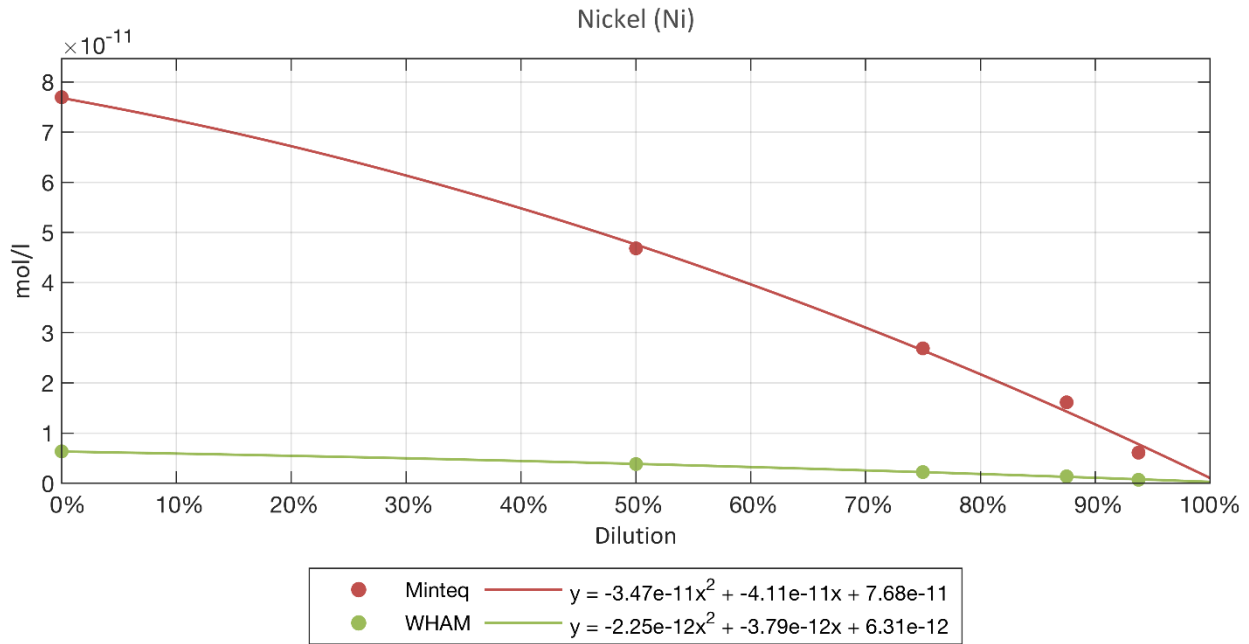


Figure 22: WHAM and Minteq simulated Ni binding to the proxy gill.

WHAM predicted lower binding of Ni to the gill, compared to Minteq which predicts higher concentrations. This can be explained by the difference in humic complexation models in WHAM and Minteq and the different free ion prediction models. As both WHAM and Minteq use different affinities for estimating binding to the proxy gill for Ni and other metals. The differences in the humic complexation models are presented in section 2.6.

Compare to Cd in Fig. 12 the predicted trendline for Ni in Fig. 22 follows a similar declining trend which is steeper. This show that Ni loses its ability to bind to the proxy gill faster compared to Cd.

The simulated free ion concentrations left after the binding to DOC and the proxy gill are presented in figure 23.

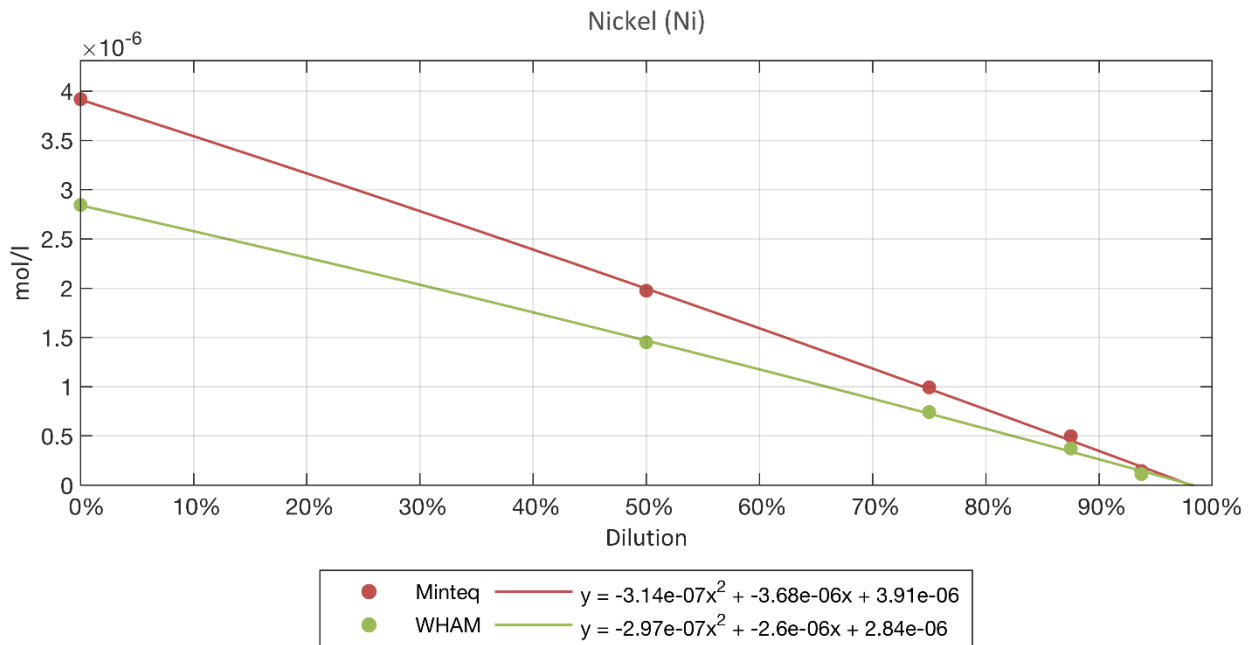


Figure 23: Simulated Ni free ion concentrations left after the binding to DOC and the proxy gill.

WHAM predicts lower free ion concentration after the binding compared to Minteq at all dilutions, although Minteq also predicts significantly higher binding to the proxy gill. The changes can be attributed to the lower prediction of interactions and complexation with other elements by Minteq than WHAM. A similar decreasing trend is observed for Cd, where with higher dilution the amount of free ion Cd decreases. In the case of Cd and Ni, WHAM predicted lower concentrations of binding to the proxy gill and lower concentrations of free ions left after the binding to DOC (section 4.3.1).

4.5.2. Comparing different approaches to assess Ni uptake to fish

The comparison of different approaches to assess Ni uptake to the gill is presented in Fig. 24.

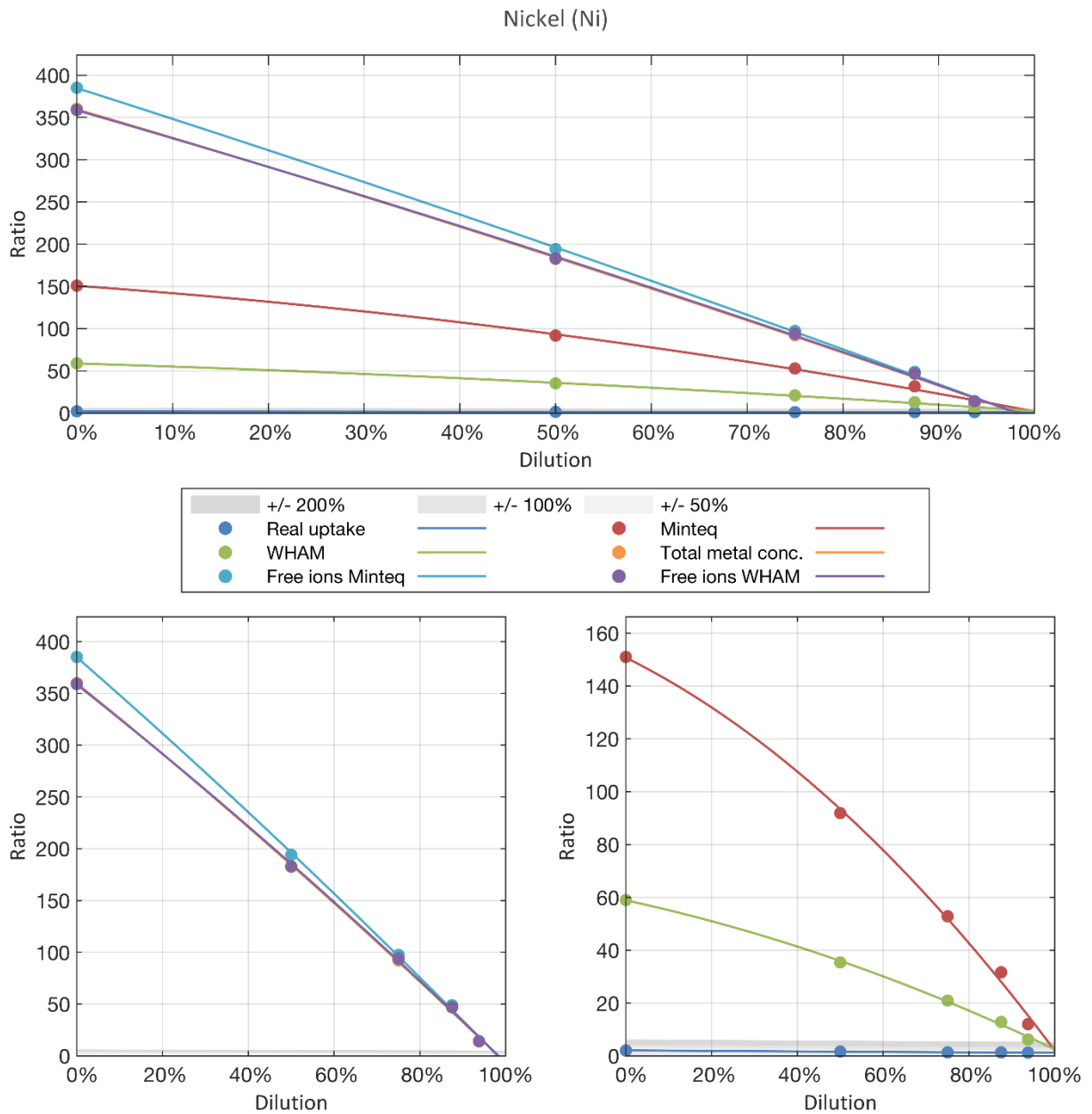


Figure 24: Comparing real gill Ni uptake with total metal concentration and modeled free-ion concentrations using the ratio approach.

The Minteq free ion ratio predicted the highest uptake at all dilutions, followed by the total metal ratio and WHAM simulated free ions. The best fit compared to the real gill, provide WHAM and Minteq with their simulated binding to the gill. WHAM predicts a lower ratio at all dilutions, while Minteq predicts more than double the ratio at low dilutions. This is probably due to the higher binding prediction to the

proxy gill by Minteq than by WHAM as demonstrated in 4.3.1.

4.5.3. Change in Ni binding to the gill by addition of metals

The simulated change in Ni binding with the addition of other elements to the proxy gill is presented in Fig. 25.

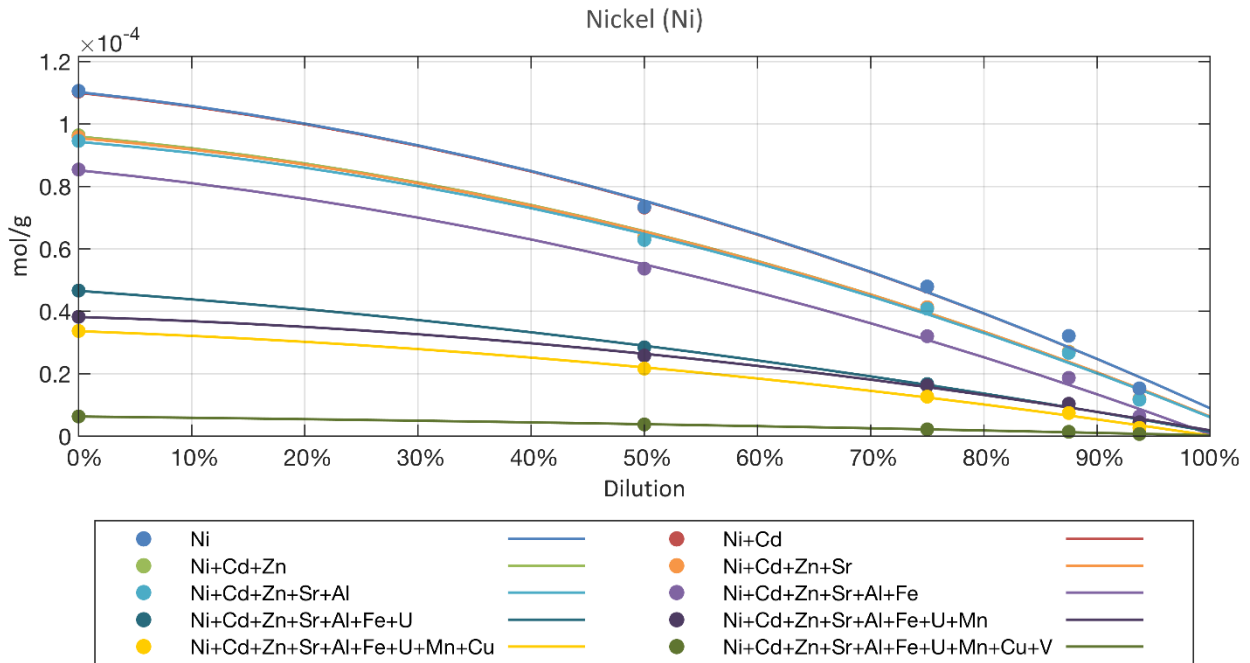


Figure 25: Change in binding of Ni with the addition of other elements to the proxy gill.

Nickel binds to the proxy gill the strongest when no other metals are in the water. The addition of Zn to the water reduces the Ni binding, this occurs because of the higher concentrations of Zn in the water and its higher binding affinity. When U is added the model predict a steep decline in binding of Ni to the gill, as U concentrations are much higher the competition for binding sites increases and less Ni is taken up. When all metals are added to the water there is the least binding to the gill, as more metals compete for the binding sites and outcompete Ni. A similar trend is observed for Cd in section 4.3.3.

4.5.4. Single Ni binding and comparison to mixture binding

The comparison between single Ni binding, real gill and metal mixture binding is presented in Fig. 26. The data for the construction of the ratio trendline metal mixture is the same as is in Fig. 25 and includes all elements.

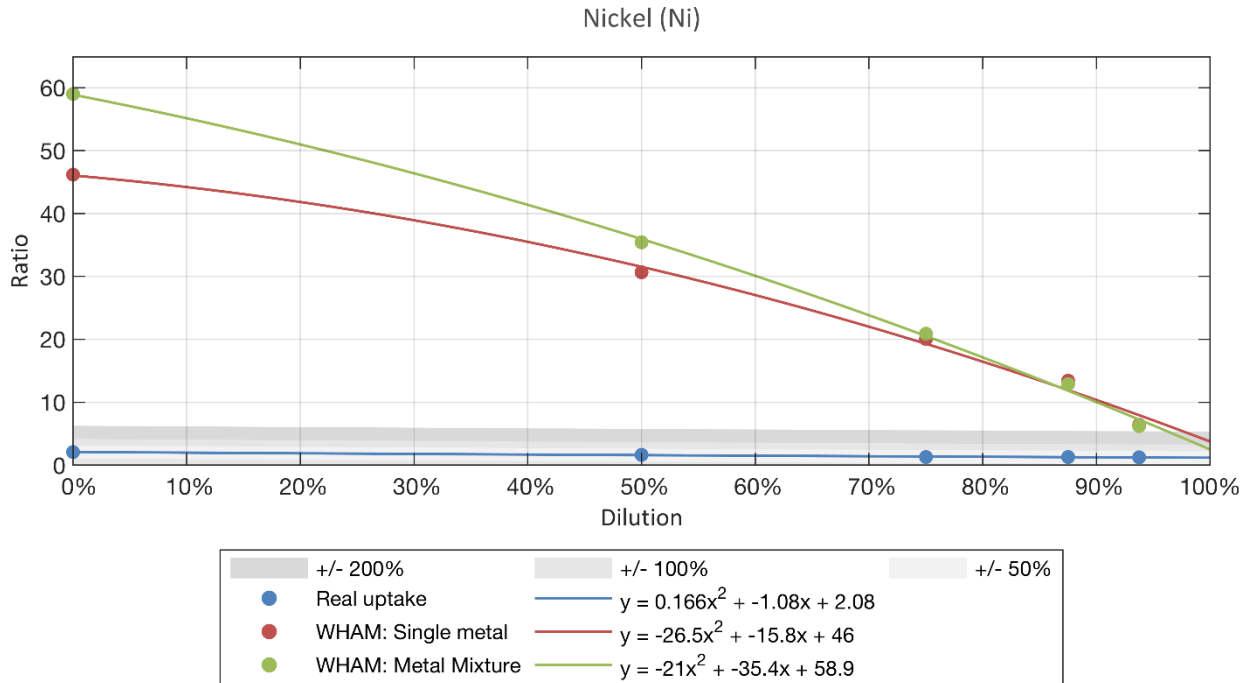


Figure 26: Comparison of single metal Ni binding, real gill and metal mixture binding.

WHAM predicts that the single metal ratio is a better predictor of Ni binding compared to WHAM until 75% dilution. This shows that the speciation model in WHAM does not consider well the competition of Ni with other metal cations, as it estimates a higher binding at undiluted waters for metal mixtures.

In the case of Ni, the single metal uptake predicts a lower ratio at low dilutions compared to the metal mixture which predicts lower ratios at higher dilutions.

4.6. Vanadium prediction

4.6.1. Vanadium mixture binding to the gill with WHAM and Minteq

The simulated binding of V to the proxy gill with WHAM and Minteq is presented in Fig. 27.

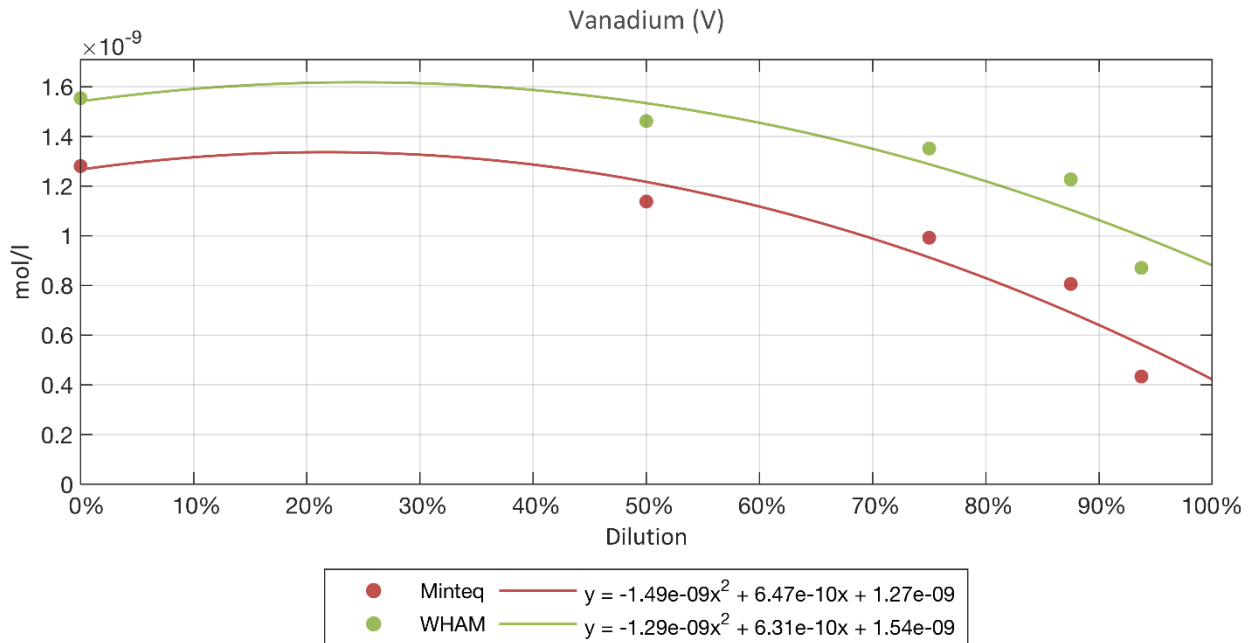


Figure 27: WHAM and Minteq simulated V binding to the proxy gill.

WHAM predicts higher binding compared to Minteq at all dilutions. The reason lies in the different complexation models and HA binding models. Minteq predicts a higher complexation of free ions and lower concentrations of free ions before the binding to DOC compared to WHAM, therefore the lower binding to the proxy gill. A similar trend is observed for Cd, where WHAM predicts less binding than Minteq. The difference between the models lies in the humic complexation models (section 2.6 and 4.3.1).

The simulated free ion concentrations left after the binding to the DOC and the proxy gill are presented in Fig. 28.

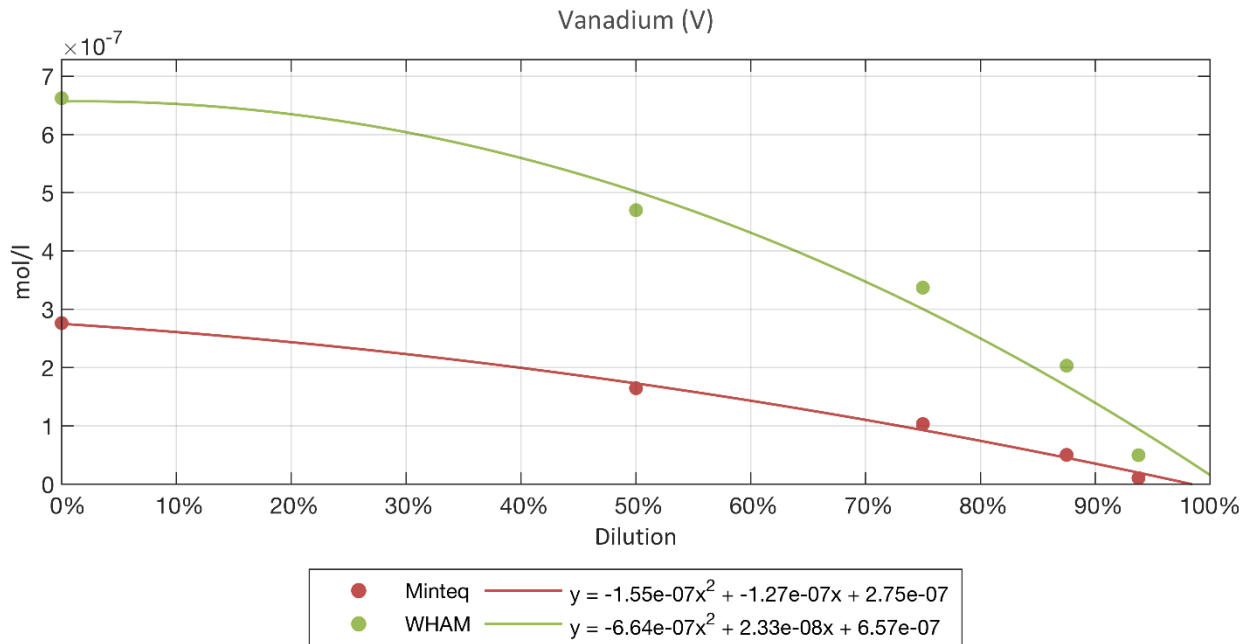


Figure 28: Simulated V free-ion concentration left after binding to DOC.

WHAM predicts higher free ion concentrations after the binding compared to Minteq. The free ion concentrations predicted by Minteq before the binding to DOC were also lower compared to WHAM. This can be explained by the different HA complexation models, the different metal mixture complexation and the different binding affinity for HA by V in WHAM and Minteq.

4.6.2. Comparing different approaches to assess V uptake to fish

The comparison between the real gill, total metal concentration, modeled free-ions using the ratio approach is presented in Fig. 29.

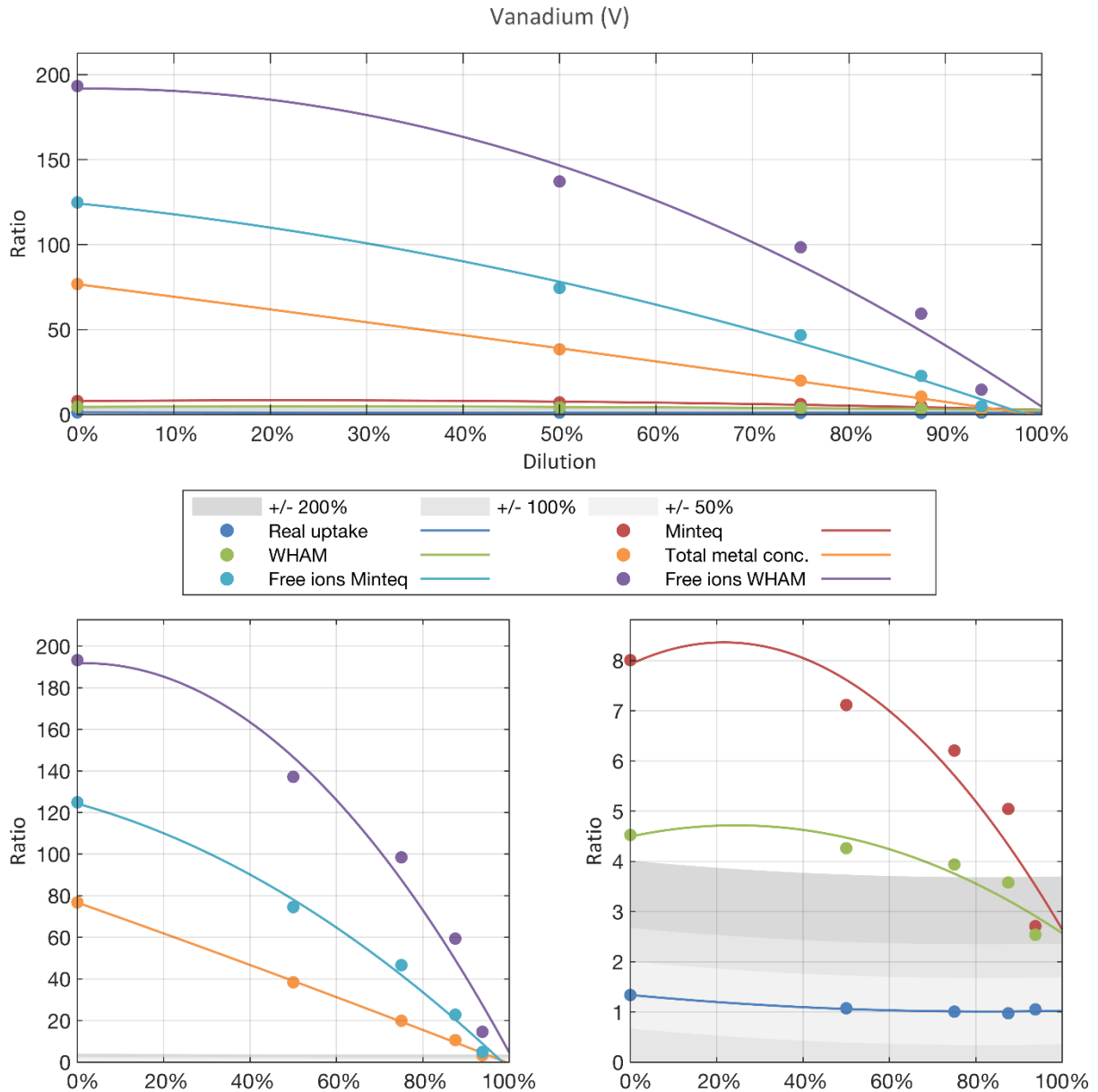


Figure 29: Comparing real gill V uptake with total metal concentration and modeled free-ion concentrations using the ratio approach.

The highest uptake ratio is predicted by WHAM free-ions, followed by Minteq free-ion ratio. The total metal concentration is a better predictor of V uptake than the simulated free-ion concentrations by both models. This overestimation can be explained by the free-ion prediction models which poorly describe V ion formation and complexation in metal mixtures. This theory was not tested in this thesis and the

free-ion prediction models were not examined. The predicted ratio approach provides the lowest estimation of V binding to the gill as it considers metal complexation reactions. In Fig. 27 WHAM predicted more binding to the gill compared to Minteq, while in Fig. 29 the ratio approach with WHAM predicts a lower ratio compared to Minteq. This is a drawback of using the ratio approach as is also shown for U in section 4.2.2.

4.6.3. Change in V binding to the gill by addition of metals

The simulated change in binding of V with the addition of other metal is presented in Fig. 31.

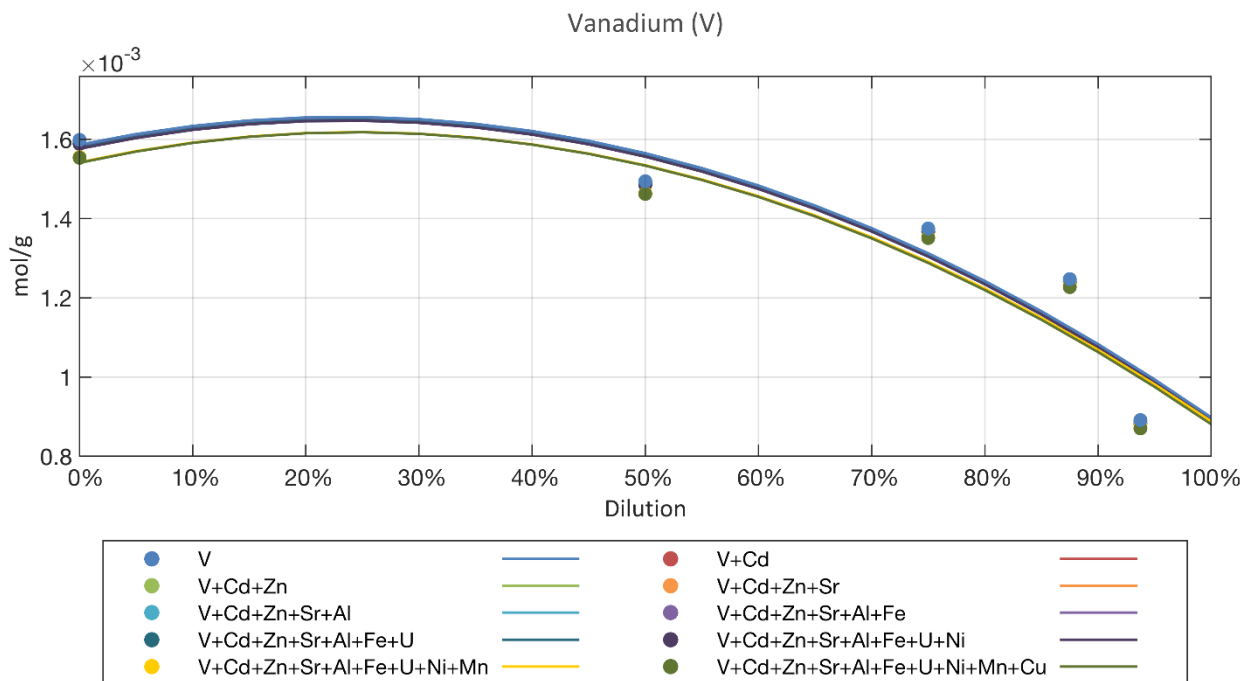


Figure 30: Change in binding of V with the addition of other metals to the proxy gill.

The binding of V is the highest when there are no other metals present in the water. When V, Cd, Zn, Sr, Al, Fe, U and Ni are in the water the binding of V decreases. Only slightly. The biggest impact on V binding is when all metals are. The combined competition for binding sites reduces the binding of V. The addition of U to the mixture seems not to have a mayor effect on the binding of V, as observed for the impact of V on U binding. This can be explained by the high V concentrations in the water, which are almost double the amount of U. The interference from U to V binding is therefore minimal.

4.6.4. Single V binding and comparison to mixture binding

The comparison of single metal V binding to the real gill and metal mixture is presented in Fig. 31.

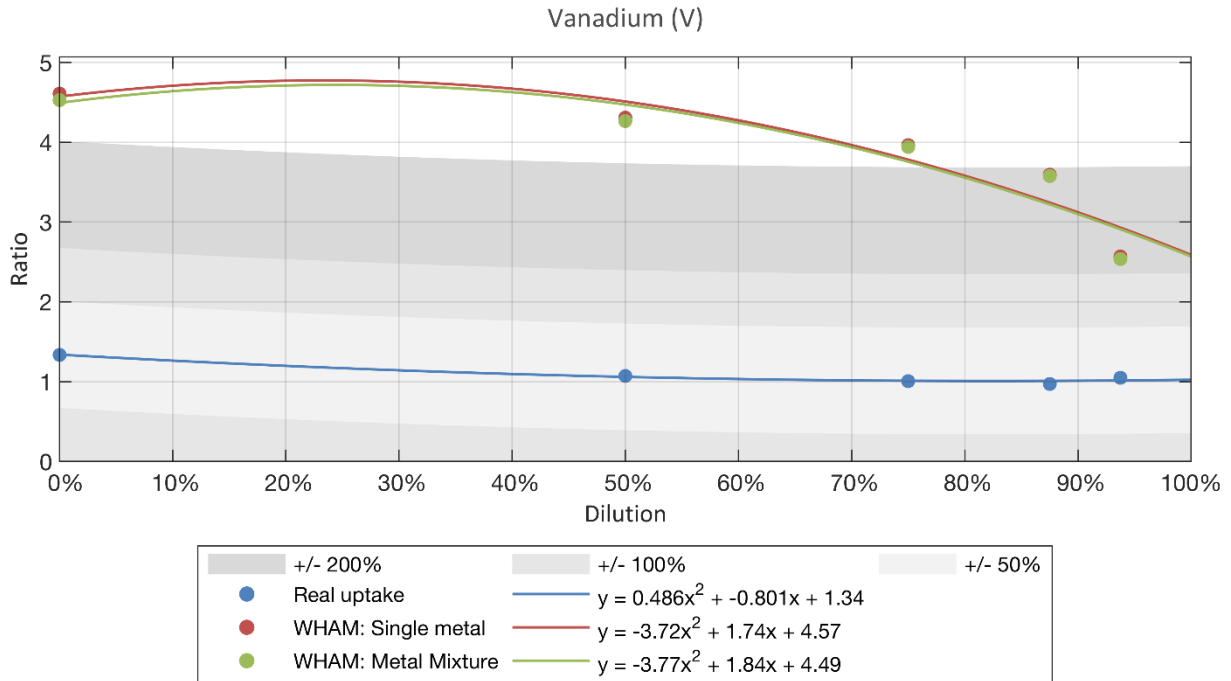


Figure 31: Comparison of single metal V binding, real gill and metal mixture binding.

The simulated V single metal uptake and the mixture produce similar binding ratios. This similarity can be explained by the lack of V complexation with other metals and surface sorption to oxides. This theory has not been tested in this thesis. The metal mixture approach predicts lower uptake concentrations of V and is a slightly better approach to predict V binding to the gill.

4.7. General prediction trends for elements of interest

The summarized results for the elements of interest are presented in Tab. 5. All the elements of interest decrease their binding to the proxy gill with dilution except U which increased. The increase in U binding is caused by V, while for no other metal such an interaction was detected.

Table 5: Summarizing table for U, Sr, Cd, Ni and V considering increasing dilution.

	U	Sr	Cd	Ni	V
Metal binding to the proxy gill	Increasing	Decreasing	Decreasing	Decreasing	Decreasing
Free-ion conc. after exposed to DOC	Decreasing	Decreasing	Decreasing	Decreasing	Decreasing
Best fit compared to real gill	Proxy gill ratio	Proxy gill ratio	Proxy gill ratio	Proxy gill ratio	Proxy gill ratio
Simulated change in binding adding metals one-by-one	Increasing. V causes the increase and has the biggest impact	Decreasing. Mn and U has the highest impact	Decreasing. Zn and U have the highest impact	Decreasing. U has the highest impact	Decreasing. U has the highest impact
Single versus mixture binding compared to the real gill	Metal mixture is a better predictor	Metal mixture is a better predictor	Metal mixture is a better predictor	Metal mixture is a better predictor from 87.5-93.75%	Metal mixture is a better predictor
Binding to the proxy gill (best model)	Minteq	Minteq	WHAM	WHAM	Minteq
Proxy gill ratio (best model)	WHAM	WHAM	WHAM	WHAM	WHAM
Does the ratio skew the data?	Yes	Yes	No	No	Yes

The proxy gill ratio approach provides uptake results that are closer to the real gill uptake, compared to the total metal ratio and to simulated free-ion concentration by WHAM and Minteq. The proxy gill ratio skews the proxy gill binding data for U, Sr and V, where it shows that WHAM predicts a lower binding compared to Minteq and therefore obtains closer ratio results to the real gill. When comparing the binding to the proxy gill without the ratio approach Minteq predicts lower binding for U, Sr and V, while WHAM predicts lower binding concentrations for Cd and Ni. It is important to consider first the simulated amount of metal to the proxy gill by WHAM and

Minteq, before concluding if the proxy gill ratio approach is valid.

The predicted proxy gill binding ratios by both WHAM and Minteq are too high for Cd, Sr, V and Ni, compared to the real gill ratio at all dilutions. The predicted ratios are closer to the real gill at lower dilutions but are still too high. Both models predicted good U binding ratios at low dilutions (0-50%) while at dilutions above 50% overestimated the binding to the proxy gill, compared to the real gill.

The single versus mixture binding simulated with WHAM to the proxy gill, shows that the model considers the interaction of different metals, their complexation and competitive binding to the gill and therefore produces a lower ratio i.e. metal mixture. WHAM predicted for U, Sr, Cd and V a lower binding ratio while other metals are present in the water i.e. metal mixture, compared to the single metal binding ratio. For Ni the metal mixture ratio was only closer to the real gill uptake at high dilutions (above 87.5%) compared to the single metal ratio. This shows that for Ni WHAM does not consider sufficient Ni complexation with other metals at high metal concentrations.

The differences in simulated binding by WHAM and Minteq are most likely because they use different humic complexation models, different metal interaction models and different metal binding affinities for HA (proxy gill).

4.8. Challenges and possible improvements

4.8.1. Metal speciation in natural waters

The metal speciation measured in natural water varies case by case which makes it challenging to decide which metal redox state to choose. Metals do not always appear in water as only one cationic species but rather a ratio between different redox states. It is challenging to find in literature what would be the ratio between an elements redox states in natural waters at pH between 7-8, as many studies use site specific water with a specific water chemistry.

4.8.2. Working with the data set

Working with the experimental dataset from leached alum shale has been a challenge. The data is fragmented between filtered (0.45 μ m) and ultrafiltered water (<10 kDa), therefore for consistency reasons the filtered water concentrations were

used in modelling. Using filtered water values and not ultrafiltered, therefore overestimations can occur. The models assume that a percentage of the input metals are present as cations which complex and bind to the proxy gill. If ultrafiltered metal concentration data would be used for modelling it can be assumed that the simulated binding to the gill would be lower.

4.8.3. The ratio method

The ratio approach can be successfully used to compare Cd and Ni simulated binding to the real gill as it does not skew the simulated data. The ratio approach cannot be used to compare WHAM and Minteq predicted ratios for U, V and Sr. When WHAM predicts more binding to the gill in the exposure water it also predicts more binding from the control water. This generates a lower ratio for WHAM which skews the results and makes the comparison harder. It is important to examine first the simulated binding to the gill (g/mol) for WHAM and Minteq to determine which model predicts higher or lower binding. If the simulated results match with the calculated ratio for the chosen metal, the ratio approach can be used to compare the ratio of WHAM and Minteq directly to any other approach e.g. total metal, real gill uptake ratio.

4.8.4. WHAM and Minteq

When using Minteq to simulate the binding to the proxy gill, the greatest challenges lie in the interpretation of the generated data. The data is exported into an Excel sheet which contains all the complexation reaction with other metals, simulated free ions, bounded metals to HA and FA. Excel functions must be written to sort through the data, as manual checking of metal binding to HA would be too time consuming. Minteq also lacks an easy method to simulate multiple exposure scenarios at the same time. The option menu in Minteq is designed good but the included manual sometimes fails to explain properly what the adjustable parameters are and how to adjust them for the intended use. There is room for improvement in the models help file which is significantly fragmented.

The positive side of Minteq is that the generated data shows directly which elements bind together, to HA and FA and in which amounts. It has a great selection of components that can be chosen e.g. VO^{2+} and the model even supports the addition of some anionic metal forms e.g. MoO_4^{2-} which are not supported in WHAM. The model is complex and offers the user a variety of parameters that can be adjusted after

studying the user manual. The model includes various metal-proton complexation models e.g. SHM, Nica-Donnan and Guassian DOM.

WHAM offers a wide range of elements that can be added to the simulation. It does not include any anionic metal species like Minteq. Therefore, all metals are assumed to be present in the solution as free metal cations. The data generated in WHAM is exported to an Excel file. Its content is not mixed and can be easily sorted by the sort function in Excel. The data does not offer any way of determining which first oxide of a metal binds to HA or FA. It shows only the selected metal species that was chosen in the beginning of the simulation which is bounded to HA as a sum of all its species. WHAM instructions are elaborated and explain in detail the functioning of the software. The simulated free ions in the water are displayed as mol/l, where the bounded metals to the HA and FA are presented in g/mol without the possibility to change it to mol/l.

The main challenges when working with models is determining the cause of different metal interactions. The main interaction factors are the metals concentration, binding affinity to HA and the difference in WHAM and Minteq humic complexation models.

In general WHAM and Minteq predict better results when lower concentrations of metals are present in the water, compared to high concentrations. WHAM predicts results that are closer to the real gill uptake for Cd and Ni, while Minteq predicts results that fit better with the real gill uptake for U, Sr and V.

Stockdale et al., 2010 used WHAM to predict mixture metal binding to the proxy gill (HA) to assess metal toxicity to stream macroinvertebrates. This thesis has followed a similar approach, where a very low concentration of HA was used to assess metal mixture binding and relate it to toxicity (Stockdale et al., 2010). In this thesis the amount of bound metals to HA was used only to assess the amount of bounded metals from a metal mixture and tested for a wider range of metals in a mixture.

It is important to consider that WHAM is a paid software while Minteq is freely available on the internet. Since Minteq provides similar binding results to HA for the tested metals and offers additional setting that may improve its predictions, therefore it is the favored model for any further studies.

4.8.5. Further work

The mathematical description of WHAM is not well explained and fragmented in between many research papers with corrections and different notations. A review of the mathematical description of the humic complexation model VII has to be undertaken to summarize the years of model development. The user interface should be updated and made more attractive. For both WHAM and Minteq different user interfaces can be developed, one for basic users and another one with more detailed options for the advanced user. This could attract more researchers which would help to develop and improve the models further.

In the future fish exposure experiments with multiple mixtures should be undertaken and the results should be used to adjust WHAM and Minteq metal complexation reactions and metal binding to HA and FA. Models are only as good as the data that was used to adjust their bindings and complexations.

This thesis demonstrated the difficulty to model metal mixtures binding in high concentrations to HA and the challenges connected with comparing simulated metal binding with the real metal uptake in the gill. Other approaches can be developed to compare the modeled binding data directly to the uptake in fish.

5. Conclusions

WHAM and Minteq can be used to predict the uptake of metals to fish. Both models consider metal complexation reactions with other elements and DOC. This significantly improves the prediction of metal uptake to fish compared to other methods e.g. total metal concentration and free-ion concentration. WHAM and Minteq were not developed to assess metal uptake to aquatic organisms but are still capable to provide reasonably good estimation without any model adjustments.

Future models can be developed to be specifically adjusted for uptake of metals to aquatic organisms which consider metal complexation reactions and predict results that are in closer agreement with reality. It is important to determine the affinity for all the metals to the fish gill which can be used to better predict the metal uptake.

The used ratio approach to compare uptake data from the real fish gill, the simulated gill and the total metal concentrations must be adjusted or replaced with an approach that can compare the uptake of metals by fish directly without skewing the simulated data as it does for U, Ni and V. For example, the bounded U to the proxy gill (mol/l) shows that Minteq predicts lower concentrations compared to WHAM. When the ratio is calculated, it shows that WHAM predicts a lower ratio compared to Minteq which directly contradicts the bounded U (mol/l) to the proxy gill.

WHAM predicts a better proxy gill ratio for Cd and Ni which is closer to the real gill ratio. Minteq predicts a better proxy gill ratio for U, Sr and V which is closer to the real gill ratio.

The following hypothesis were set in the beginning of the thesis:

- WHAM 7 and Visual Minteq 3.1 can predict metal mixture binding to the fish gill.

This hypothesis is proven to be true as WHAM and Minteq can predict the metal mixture binding to the fish gill with the ratio approach. Their predictions differ according to the metal of interest.

- There are no significant differences in predicting metal binding to the fish gill with WHAM 7 and Visual Minteq 3.1, as they use very similar metal to humic

substances complexation models.

This hypothesis is disproven as there are significant differences between WHAM and Minteq binding predictions which are because of the different mathematical models used. The difference in complexation models plays a significant role in the metal binding predictions and the different metal affinity for HA (proxy gill).

6. References

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6.1. Appendix

Appendix 1: Measured control and leached alum shale water in µg/l, used as input values for WHAM and Minteq.

Element	Control AB		Diluted 93.75 %		Diluted 87.5 %		Diluted 75%		Diluted 50%		Diluted 0%	
	average	SD	average	SD	average	SD	average	SD	average	SD	average	SD
Temp °C	8.89	0.40	9.20	0.10	9.14	0.14	9.29	0.16	9.13	0.33	9.23	0.14
pH	7.33	0.06	7.28	0.04	7.28	0.05	7.29	0.05	7.35	0.05	7.38	0.07
TOC	1775	389	1650	70.7	1600	<0.01	1750	212	1850	212	2150	212
F	<0.04	NA	<0.04	NA	<0.04	NA	<0.04	NA	74	22.6	131	55.2
Cl	3150	70.7	2750	70.7	2450	70.7	2200	<0.01	1800	<0.01	865	7.07
NO3-	45.5	6.36	70.5	0.71	100	<0.01	150	<0.01	245	7.07	440	14.1
SO42-	115500	2828	117000	<0.01	115500	707	111500	707	107000	<0.01	99500	2121
NH4+	400	14.1	400	NA	380	NA	470	NA	690	NA	983	32.1
Base cations												
Na	7513	91.8	7811	129.5	7735	4.86	7696	42	7526	24.3	7353	15.8
Mg	2610	150	2585	63.2	2574	24.2	2571	7.33	2488	20.1	2381	48.4
K	2972	97.2	2529	68.6	2554	33	2609	30.6	2684	44.9	2867	88
Ca	31986	1241	32823	853	32818	969	32937	573	31840	1022	31519	1616
Metals of no interest												
Th	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Mn	14.12	6.30	28	0.58	71.5	0.87	134.2	0.44	256.4	2.42	502.3	3.49
Fe	6.32	3.91	5.78	1.88	4.44	0.20	4.42	0.35	4.50	0.40	4.88	1.46
Cu	12.23	7.77	6.29	0.42	5.94	0.41	6.09	<0.01	5.69	0.23	6.10	0.16
Zn	3.80	0.57	5.35	0.14	8.94	0.39	14	0.20	24.4	0.25	43.1	1.02
As	0.11	0.12	0.15	<0.01	0.44	0.06	0.87	<0.01	1.64	0.01	3.25	0.03
Al	1.63	NA	2.18	NA	2.64	NA	3.47	NA	5.66	NA	9.36	NA
Mo	0.26	0.28	166	1.07	575	10.6	1138	4.08	2227.8	13	4418	14.5
Metals of interest												
U	0.07	0.09	73.6	0.94	253	1.01	498.8	30	947.4	117	1830	239
Sr	12.48	NA	33.5	1.01	82	2.51	151	2.24	284.6	0.13	549	7.13
Cd	<0.01	<0.01	0.08	<0.01	0.26	0.01	0.52	0.02	1.03	<0.01	1.99	0.07
Ni	0.77	0.30	10.73	0.09	36.0	0.45	71	0.16	140.7	1.32	278	0.89
V	37.76	20.5	124	10.8	400	41.1	753	16.47	1449.9	3.93	2901	17.7

Appendix 2: Minteq simulated binding of U to the proxy gill, different U species

Dilution	Bound to HA (mol/L)				
	93.75%	87.50%	75%	50%	0%
UO ₂ +2G(aq)	1.31E-17	2.50E-17	3.34E-17	3.31E-17	3.43E-17
UO ₂ OH+G(aq)	6.20E-18	1.18E-17	1.60E-17	1.74E-17	1.97E-17
UO ₂ ⁺ (aq)	1.66E-12	2.23E-12	2.44E-12	2.03E-12	1.73E-12
SUM	1.66E-12	2.23E-12	2.44E-12	2.03E-12	1.73E-12



Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitenskapelige universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway