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Leaching, Uptake and Effects in Brown Trout (*Salmo trutta*) of Radionuclides and Metals from Black Shales and Sulphur Bearing Gneiss

Utlekking, Opptak og Effekter i Brunørret (*Salmo trutta*) av Radionuklider og Metaller fra Svartskifre og Svovelførende Gneis

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Preface

The presented work is the final thesis included in a two-year Master of Science degree in Environmental Pollution and Ecotoxicology at the Norwegian University of Life Sciences and the Institute for Environmental Sciences (IMV). The research was conducted between 2014 and 2015 at Centre for Environmental Radioactivity (CERAD), in collaboration with the Norwegian Public Road Administration (NPRA) and Bioforsk. The Nordic Road Water (NORWAT) program has been responsible for the funding of the experiments.

Firstly I would like to thank my main supervisor Hans-Christian Teien (IMV/CERAD) and co-supervisors Lindis Skipperud (IMV/CERAD) and Turid Hertel-Aas (NPRA) for all the help and support. Their guidance and the ability to give me a push in the right direction when needed have been of great help. I am especially grateful to Hans-Christian for all the help during the exposure experiment where countless hours were spent at the fish laboratory.

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Finally a big thank you to my family and friends who have given support and encouragement. A special thanks to my parents, Haldor Hjulstad and Guri Tronstad for proofreading and always being a phone call away when needed.

Summary

Sulphur rich rocks can leach high concentrations of radionuclides and metals into the aquatic environment, when exposed to air and moisture and this might lead to uptake and negative effects in aquatic organisms. Thus during construction in areas with these types of rocks, information about possible leaching and critical levels are needed to perform risk analysis. The present work focuses on the transfer of radionuclides and metals from rock to water and further to organism.

Six rock samples, five black shales and one sulphur bearing gneiss, were collected from different road and rehabilitation projects in Norway. Two leaching experiments were conducted to assess the presence and concentrations of radionuclides and metals in the leachate, as well as the pH and concentrations of major ions. The leachates from the crushed alum shale samples (100g rock/L) increased continually during the experimental period of seven weeks and were dominated by high concentrations of uranium (118-602 µg/L), cadmium (0.06-0.98 µg/L), and molybdenum (77-2032 µg/L) in combination with high pH (7.5-8.0) and high concentrations of major ions (48-515 mg Ca/L). The sulphur bearing gneiss leachate was dominated by high concentrations of aluminium (11601 µg/L), copper (535 µg/L), manganese (513 µg/L) and nickel (261 µg/L), in combination with low pH (2.8-4.2) and low concentrations of major ions (3.8 mg Ca/L).

The exposure experiment showed significant uptake of and uranium (0.7 and 0.1 µg/g) in gills and liver, and cadmium (0.5 µg/g) in the gills of the fish exposed to the shale waters, and higher uptake compared to the corresponding control and reference fish. The fish exposed to the sulphur bearing gneiss leachate of uranium at a factor of 350 lower (0.47 µg U/L) showed higher uptake of uranium in the gills (0.9 µg/g) compared fish exposed to the shale waters. Thus, the results indicate the importance to understand the underlying mechanism in transfer of radionuclides and metals in risk assessment. The fish exposed to the sulphur bearing gneiss also showed very high concentrations of aluminium in the gills (up to 1042 µg/g) and at levels associated with mortality. This was therefore assumed to be the main reason for the observed physiological changes determined by increased blood glucose and mortality (14%). Thus, the gneiss leachate diluted 1:100 caused mortality, while no dilution of shale leachate illustrate the potential of toxicity and variation between rocks.

Sammendrag

Svovelrike bergarter kan øke konsentrasjonen av radionuklider og metaller i det akvatiske miljø når de utsettes for oksygen og fuktighet. Dette kan igjen kan føre til opptak og negative effekter i akvatiske organismer. Det er derfor viktig med kunnskap om mulig utlekking fra svovelrike bergarter, samt kartlegging av kritiske nivåer for å kunne gjennomføre risikoanalyser i forkant av anleggsarbeid. Det presenterte arbeidet fokuserer på utlekking av radionuklider og metaller fra stein til vann, og på opptak og effekter av disse i organismer.

Seks steinprøver, fem svartskifre og en svovelførende gneis, ble samlet inn fra ulike veg- og rehabiliteringsprosjekter i Norge. To utlekkingseksperimenter ble gjennomført for å undersøke hvilke radionuklider og metaller som ble mobilisert fra de ulike bergartene, og hvor høye konsentrasjoner. I tillegg ble pH og konsentrasjoner av hoved ioner i utlekkingsvannet undersøkt. Utlekkingsvannet fra alunskiferne (100 g stein/L) var dominert av høye konsentrasjoner av uran (118-602 µg/L), kadmium (0.06-0.98 µg/L) og molybden (77-2032 µg/L). I tillegg hadde vannet høy pH (7.5-8.0) og høye konsentrasjoner av hoved ioner (48-515 mg Ca/L). Utlekkingsvannet fra den svovelførende gneisen var dominert av høye konsentrasjoner av aluminium (11601 µg/L), kobber (535 µg/L), mangan (513 µg/L) og nikkel (261 µg/L), og hadde lav pH og lave konsentrasjoner av hoved ioner (3.8 mg Ca/L).

Eksponeringsforsøket viste signifikant opptak av uran (0.7 og 0.1 µg/g) i gjeller og lever, og kadmium (0.5 µg/g) i gjeller, for fisken eksponert for utlekkingsvannet fra skifrene, samt høyere opptak sammenlignet med kontroll og referansefisk. Fisken som ble eksponert for utlekkingsvann fra gneisen, med uran 350 ganger lavere enn i utlekkingsvannet fra skifrene (0.47 µg U/L) viste høyere opptak av uran i gjellene (0.9 µg/g) sammenlignet med fisken eksponert for skifervannet. Disse resultatene indikerer viktigheten av å forstå de underliggende mekanismene for overføring av radionuklider og metaller, for bruk i risikovurdering. Fisken som ble eksponert for fortynnet (1:100) utlekkingsvann fra svovelførende gneis viste svært høye konsentrasjoner av aluminium i gjellene (opp til 1042 µg/g), og dette er nivåer som er assosiert med dødelighet. Det ble derfor antatt at dette var hovedgrunnen for observerte fysiologiske forandringer, bestemt av økte glukoseverdier i blodet og dødelighet (14%). Fisk eksponert for utlekkingsvannet fra skifrene viste ingen dødelighet eller økt glukoseverdier i blodet.

List of Abbreviations

E18 – E18 Gneiss

DOC – Dissolved Organic Carbon

HBT-AS-NW – Hammersborgtunnelen Alum Shale Non Weathered

HBT-AS-W – Hammersborgtunnelen Alum Shale Weathered

IC – Ion Chromatography

ICP-MS – Inductively Coupled Plasma Mass Spectrometer

IMV – Institute for Environmental Sciences

K34-AS – Kirkegata 34 Alum Shale

KLIF - Klima og Forurensningstilsynet/Climate and pollution control

LC_{50} – Lethal Concentration 50

LMM – Low Molecular Mass

NMBU – Norwegian University for Life Sciences

NORWAT – Nordic Road Water

NPRA – Norwegian Public Roads Administration

PEC – Predicted Environment Concentration

PNEC – Predicted No Effect Concentration

Rv.4 - AS – Rv.4 Alum Shale

Rv.4 GS – Rv.4 Galgeberg Shale

SEM – Scanning Electron Microscopy

WFD – Water Framework Directive

XRD – X-Ray Diffraction

XRF – X-Ray Fluorescence

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1. Introduction and background

Development and construction of infrastructure for the benefit of the society entails interventions in the nature. In Norway the Norwegian Public Road Administration (NPRA) is the leading road constructer and have the responsibility for the environment when planning, building and managing all Europe and state highways (NPRA 2013). When constructing roads a substantial amount of soil and rock are to be excavated, moved and deposited. Interventions of this magnitude will alter the surroundings and conditions of these soils and rocks. The environment may for example change from anoxic to oxic conditions or from dry to moist conditions. These changes to the conditions of the soil and rocks can lead to leaching of different elements from the soil and rocks and some of these elements can be harmful for all living species in the aquatic environment.

Acid rock drainage occurs when sulphur rich rocks and materials excavated from below the earth's surface gets in contact with air and moisture, oxidizes and releases sulphuric acid (Orndorff & Lee Daniels 2002). This problem is not new in road construction in Norway. An example is highway 18 between Grimstad and Kristiansand where the road cuts through sulphide bearing rock, and the deposits near Lillesand for the rocks of this type have lead to toxic conditions for aquatic organisms downstream, due to low pH in combination with moderate to high concentrations of metals (Hindar & Nordstrom 2014). Due to the severity of metal rich acid runoff from sulphide bearing rocks it is important to investigate how this might affect the aquatic organisms.

In 2012 the NPRA started a four-year research and development program called Nordic Road Water (NORWAT) which is an agency program with the purpose of research to make sure that the NPRA plans, build and operate the road network without causing unacceptable harm to the aquatic environment (NRPA 2014). Within funding from agency program a collaborative research project between CERAD/NMBU, Bioforsk and NPRA was set to investigate effects and environmental risk related to road construction in areas with sulphur rich minerals using the state highway 4 (rv.4) construction at Gran, Hadeland as a research and pilot area. This area was chosen for the project due to construction of roads and tunnel in black shale bedrock. Several master theses and experiments has been conducted in this project with regards to source

characterization, particles and weathering, effects on biota, risk and risk management to get an understanding of how construction in sulphide rich minerals can affect the environment (Fjermestad 2013; Helmers 2013; Santos 2014). The Rv.4 project is split into five tasks, and this the research performed in this present thesis is performed under task three. Task three is set to investigate radionuclides and metals in drain off from the tunnel and road construction and the uptake and ecologically relevant effects of these elements in biota.

There are rules and regulations regarding masses that are classified as contaminated soils that have to be followed during road construction. According to the pollution law and regulation *Forskrift om begresning av forurensning, Del 1. Kap. 2,* 2013) all sediment/ground which produces acid or other substances which can entail pollution in contact with water and/or air is classified as contaminated ground. This means that sulphuric rocks needs to be treated as contaminated masses, as these will lead to acid runoff when exposed to air and moisture. At the E18 highway project at Lillesand the deposits created for these contaminated masses were not well enough executed(NIVA 2011). The M20 deposit was built in 2007 to secure runoff from being acidic, with the use of slaked lime and layers with large amounts of shell sand within the deposit. However in winter 2010/spring 2011 there was found a fluffy white precipitate and reduced pH in a downstream river of the deposit (NIVA 2011).

Black shales has potentially a high content of radionuclides, with uranium concentration ranging from 3 to 250 ppm (Swanson 1961). In addition to the uranium, the daughter nuclides of uranium will be present as the decay chain of uranium includes radionuclides like thorium, radon, radium and polonium (Olley et al. 1996). The pollution law (Lovdata 2011) states that masses containing uranium 238 with activity above 1 Bq/g are classified as radioactive waste and need to be treated and deposited in a way to avoid harm for the environment, however knowledge about critical levels especially in mixtures of such elements are highly limited. At the Rv.4 project at Gran approximately 100 000 m^3 of black shale will and have been blasted out of the bedrock for the tunnel. These masses with uranium above the limit for special deposition will be deposited in anoxic conditions in a bog near the building site. However, knowledge about critical levels especially in mixtures of radionuclides and metals is highly limited and needed.

1.1. Hypothesis and objectives

Based on the need for knowledge about leaching of radionuclides and metals, and the uptake and effects of these elements has in fish the following hypothesis was set, to help with the risk assessment of construction work in areas with sulphur rich minerals.

- The mineral composition in the rock samples will affect the pH in the leachate, which radionuclides and metals that leach out and the concentrations of these.
- Radionuclides and metals leached from sulphur bearing rocks and black shale will be present as bioavailable species in water that could be taken up by organisms, depending upon water quality.
- Exposure to radionuclides and metals leached from sulphur bearing rocks in water can cause toxic effects in Brown trout (*Salmo trutta*)

These hypotheses has been investigated by study potential leaching of radionuclides and metals from black shales and sulphur bearing gneiss, speciation in water and uptake and effects of these radionuclides and metals in brown trout. The first objective was to investigate the leaching of these radionuclides and metals from chosen rock samples, identify and quantify the elements and leaching kinetics. This was done by conducting two leaching experiments, one pilot and one large-scale, where the leachate from the large-scale experiment was used in the fish exposure experiment, The second objective was to investigate the uptake and effects of these radionuclides and metals in fish, using brown trout, which was done by conducting a fish exposure experiment.

2. Theory

2.1. Black shale

Black shales are a class of sedimentary rocks composed of mineral grains of clay and silt size and containing sufficient organic mater, iron sulphide, or manganese sulphide to give the rock an overall dark-grey to black color (Swanson 1961). In Norway the location with the highest density of black- and alum shale in the bedrock is the southeast area near Oslo called Oslofeltet. The alum shale in Oslofeltet was created during cambrium and early ordovicium and stretches from Langesund in south to Hamar in the north (Skjeseth 1957). When this area was below the sea surface dark silt with a substantial amount of organic material made sediments on the sea floor and this is the source of the uranium rich shale in Oslofeltet (Ramberg et al. 2007). The main reason why the alum shale in Oslofeltet is so extensively documented is due to the fact that the radioactive radon gas is in the uranium decay chain and this radon gas can give negative health effects, being the second cause of lung cancer in Norway after smoking (NGU & NRPA 2011). Figure 1 shows the distrubution of the alum shale documented in eastern and south-eastern part of Norway. The largest area of the alum shale is found in at the border between the west part of Hedmark and south part of Oppland.

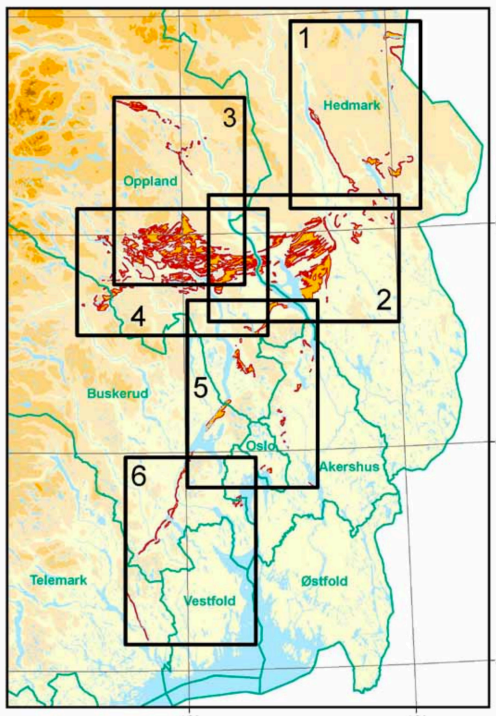

Figure 1 - Map of alum shale in Eastern Norway, showing where the alum shale is occurs as bedrock near the surface. Taken from NGU and NRPA (2011)

2.2. Mobilisation of metals from sulphur rich rocks

Alum shale is a sedimentary rock under "black shales" and consists of a various silicate minerals, sulphide minerals, calcite, stinkstone and kerogen and is easily weathered in conditions with air and moisture(Falk et al. 2006). This type of rock is known to contain high concentrations of uranium as well as other radionuclides and metals. There are two steps to the weathering process, firstly oxygen and water may oxidize the sulphide minerals and make sulphuric acid (H_2SO_4). Secondly the sulphuric acid can destabilize the minerals and kerogen, and therefore release potentially toxic elements such as Cd, U, As, Zn, Ni, Mo (Falk et al. 2006). Because the weathering is dependent on moisture and availability to oxygen, anthropogenic activities such as mining and infrastructure development can increase this release of toxic elements (Lavergren et al. 2009).

Generally speaking, in sulphide rich bedrock the main sulphuric mineral is pyrite (FeS_2), which yields sulphate and sulphuric acid when oxidised. The oxidation of pyrite occurs in several steps and the first is oxidation with atmospheric oxygen. In the second step the ferrous iron will be oxidised to ferric iron when oxygen is present. The ferric iron reacts with water and oxygen and makes a compound of oxides and hydroxides. When the ferric iron is present the oxidation of the pyrite will go faster (Hindar & Iversen 2006).

1) $FeS_{2(s)} + 7/4O_{2(g)} + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+$
2) $Fe^{2+} + 1/4O_{2(g)} + H^+ \rightarrow Fe^{3+} + 1/2\ H_2O$
3) $Fe^{3+} + H_2O \leftrightarrow FeOOH_{(s)} + 3H^+$
4) $FeS_{2(s)} + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+$

Pyrite is not the only sulphuric mineral that is relevant in this project with regards to acidic runoff. Jarosite is a rare mineral containing ferric iron and sulphide ($KFe^{3+}{}_3(OH)_6(SO_4)_2$) and is present in arid environments on earth, as it rapidly decomposes in humid climates (Madden Elwood et al. 2004).

One of the main products from these oxidation reactions is H^+, which decreases the pH, and the low pH in the water might therefore again increase the leaching of other elements from the minerals into the water. The content of pH buffering minerals in the

rocks, such as calcite ($CaCO_3$) are therefore important as these can reduce the decreasing pH in the water and thereby reduce the leaching of metals into water.

2.3. Radionuclides and metals in the environment

At all times there will be a background concentration of radionuclides and metals in the aquatic environment, dependent on factors mobilisation from rocks. To establish if there are increased or abnormally high concentrations of radionuclides and metals in the water it is important to compare these levels with the background concentrations. The Norwegian Pollution Control Authority (SFT) released in 1997 a report (SFT 1997) with classification of environmental quality in water. This report is still the classification guideline being used today. The concentrations are dependent on the harm that can be caused on organisms in water and sediment with classes ranging from one to five. The different classes is a general assessment of the effects these elements can do on the ecological system in the aquatic environment (SFT 1997). Class one is high background concentration based statistical reference material and class five is very strongly contaminated water.

Table 1 - Classification of water based on concentrations of metals in water. Taken from SFT (1997). Class I: Insignificantly contaminated. Class II: Moderately contaminated. Class III: Moderately/bad contaminated. Class IV: Strongly contaminated. Class V: very strongly contaminated.

Metals	I	II	III	IV	V
Zinc **(µg Zn/L)**	<5	5-20	20-50	50-100	>100
Lead **(µg Pb/L)**	<0.5	0.5 – 1.2	1.2 – 2.5	2.5 – 5	>5
Cadmium **(µg Cd/L)**	<0.04	0.04 – 0.1	0.1 – 0.2	0.2 – 0.4	>0.4
Copper **(µg Cu/L)**	<0.6	0.6 – 1.5	1.5 – 3	3 – 6	>6
Chromium **(µg Cr/L)**	<0.2	0.2 – 2.5	2.5 – 10	10 – 50	>50
Nickel **(µg Ni/L)**	<0.5	0.5 – 2.5	2.5 – 5	5 – 10	>10

As seen in Table 1 the insignificant/background concentration of elements such as cadmium, nickel and copper are all below 1 µg/L.

This classification of water is however a bit out-dated, and proposals for updated classification guidelines have been published, where the concentrations have been updated according to new research and science. In 2008 the EU Water Framework Directive (WFD) was put in effect in Norway, as this directive also includes the EØS countries, and the directive set to ensure a good status of surface and ground water by 2021 (NIVA 2015). With the EU WFD in mind KLIF published in 2012 a new draft for environmental quality standards and classification of environmental pollution in water, sediment and biota where both the classification system and concentrations of the environmental pollution has been adjusted and updated (KLIF 2012).

Uranium is always present in the environment, both in soil, water, and air dependant on factors like bedrock and anthropogenic activities like mining. In soils uranium occurs naturally with concentrations around 1-2 mg/kg and in water it can range from 0.01 to 1500 µg/L (Arfsen et al. 2001). In Norway the concentration of uranium in the groundwater, measured in 1996 was between <0.02 and 170 µg/L, with a median in the Oslo area of 3.72 µg/L (WHO 2001). A study conducted by Reinmann et al. (2009) showed that the median concentration of uranium in 39 different surface water in the Oslo area, Norway was 0.59 µg/L, with a minimum of 0.03 µg/L and maximum of 3.50 µg/L.

Cadmium is one of the most toxic elements in the aquatic environment, as it can cause tissue and vertebral deformations, respiratory issues and death at low concentrations (Yesilbudak & Erdrem 2014). The presence of cadmium in the environment is rare and emissions are regulated like by the EU WFD in which it has been identified as a priority hazardous substance (Wood et al. 2012). Cadmium often co-occurs with copper and zinc and is roughly proportional to the relative abundance in rocks (Mebane 2010). In SFT (1997) the background concentration of cadmium is set as <0.04 µg/L, but in the new proposal for classification of environmental pollutants (KLIF 2012) the background concentration has been to 0.03 µg/L for both hard and soft water.

Of metals, aluminium has been of special focus in Norway as aluminium has been leached to critical concentrations in freshwater systems in Norway due to acid rain(Henriksen 1984). The concentration of aluminium in the aquatic environment varies greatly and is dependent on the physiochemical and mineralogical factors in both the water and the geochemical environment. The concentration of dissolved aluminium

in waters with near neutral pH usually ranges between 0.001 and 0.05 mg/L, and increase to 0.5 -1 mg/L in more acidic or waters rich on organic matter (WHO 2003). Speciation of aluminium has high impact upon the uptake and toxicity towards aquatic organisms such as fish (Teien et al. 2005).

2.4. Speciation

Total concentration of an element in a liquid gives an overview of what is present, however this does not give us any information the bioavailability of the element the organisms are exposed to. Elements can be present in a variety of forms, with different sizes, charges. This is dependant on parameters such as pH, temperature, complexing agents, solubility and the presence of organic carbon (Teien et al. 2005). The physio-chemical form of the element is the chemical speciation and speciation is important for the mobility in water, bioassesability, bioavailability and uptake in organisms.

Size is an important factor in speciation of elements, as the size will influence their uptake and therefore effects in organisms. We mainly split the species of elements into two groups, particles and dissolved matter, where particles will sediment due to gravity in a solution and dissolved matter will remain in solution. The dissolved fraction is split into several partitions based on their size; pseudo-colloids, colloids, and single compounds/low molecular mass (Salbu 2009). With regards to biological uptake, the low molecular mass species (defined as <10KDa) are believed to be mobile and bioavailable, as their size lets them pass biological membranes (Salbu 2007). To differentiate between particle matter and dissolved matter a membrane filter with pore size of 0.45 μm is normally as the cut-off between particles and dissolved matter as seen in figure 2. To differentiate between colloids and simple compounds/low molecular mass species e.g., ultrafiltration is used. Charge of the element is also important for the speciation of elements, as these can affect uptake in organisms directly and also complexion binding with other elements. To obtain information about the distribution of cations and anions in water ion chromatography of anion and cation exchange resins can be used (Abelwahab et al. 2013; Wang et al. 2012).

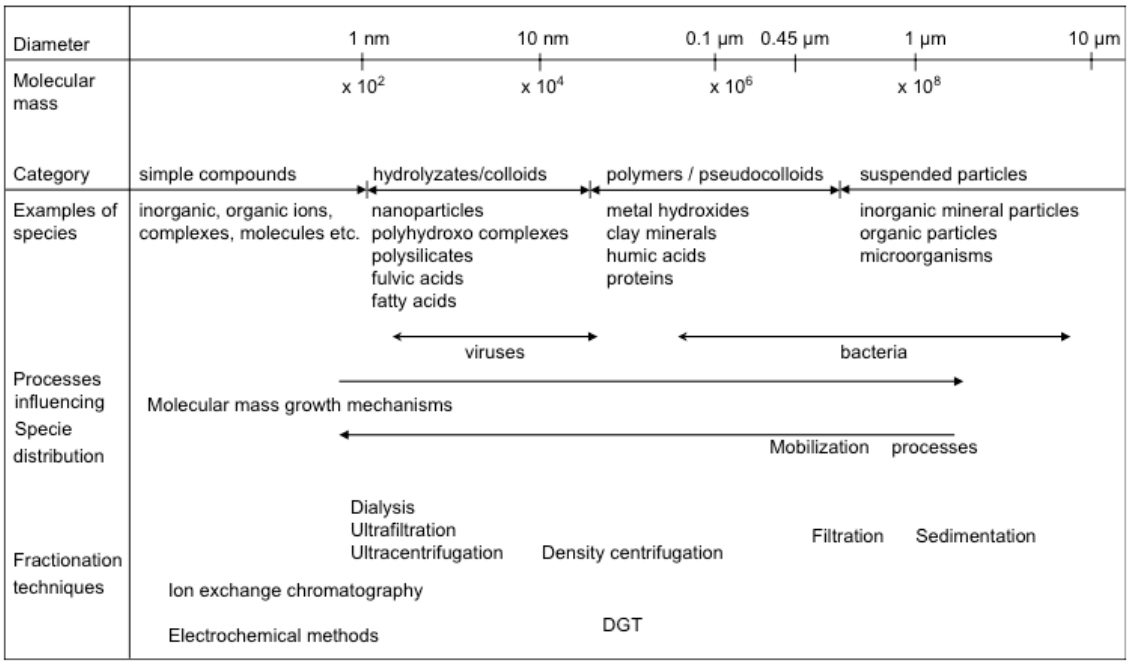

Figure 2 - Association of radionuclide species with compounds in different size ranges. Transformation processes and fractionation techniques are indicated (Salbu 2009).

2.4.1. Uranium

In water uranium can be present as different species such as the uranyl ion, UO_2^+, or other ions dependent upon pH and as both inorganic and organic complexes. It is assumed that UO_2^+ species are the most bioavailable (Markich 2002). Humic acid (fulvic acid, humic acid and humin) plays an important role as complexing agents for uranium in neutral and water with low pH and specially fulvic acid reduces the bioavailabiliy of uranium (Zhao et al. 2009). Teien et al. (2014) found that the uranium toxicity towards juvenile Atlantic salmon (*Salmo salar*) is strongly dependent on the pH, with lower LC_{50} concentrations at low pH. Since UO^{2+} ion is assumed to be one of the most bioavailable species of uranium (Markich 2002), we can assume that the toxic species are more present with lower pH.

2.4.2. Cadmium

The toxicity and bioavailability is dependent on its species and it is the free Cd^{2+} that control Cd-organism interactions (Xue & Sigg 1998). Cadmium can be complexed with both inorganic anions such as Cl^-, H_2S, OH^- and H_2CO_3 and organic ligands like humic

and fulvic acids. In seawater the Cl^- is important but in freshwater we can most of the time ignore this complexing agent (Wood et al. 2012). It is reported that both increased concentration of Ca and organic substances such as humic and fulvic acids highly reduce the toxicity of Cd(Wood et al. 2012). Thus the toxicity of Cd is highest in low ionic strength water with low Ca concentration and with minimum organic content.

2.4.3. Aluminium

Aluminium can be present as many different species, both organic and inorganic, and it is mostly the speciation that determines how harmful the aluminium can be to organisms. Inorganic complexing ligands such as silicate and organic complexing ligands such as fulvic and humic substances, competing ions such as Ca and pH are key factors influencing Al speciation, bioavailability and toxicity. The positively charged aluminium-species are the main toxic species to fish due to the accumulation of Al on fish gills, and as these species (Teien et al. 2005). Figure 3 shows the relationship of dissolved aluminium in water, dependent on pH. At pH below 6 most of the aluminium species is present as cationic species. Thus, the toxicity of Al is highest in low ionic waters with low Ca concentration and with minimum content of organic and inorganic complexing agents such as humic substances and silicate.

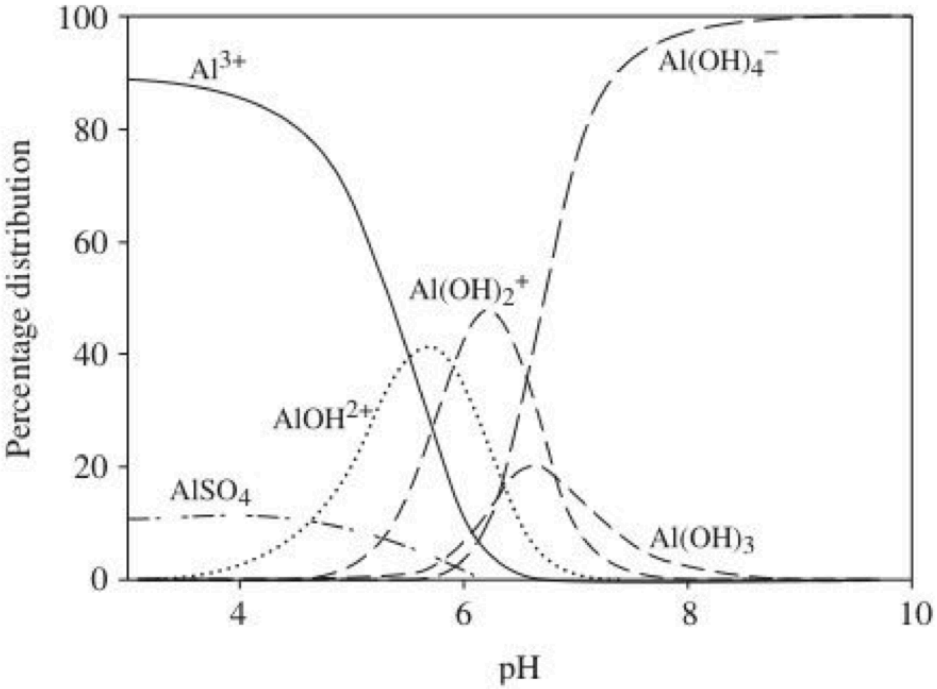

Figure 3 – Speciation modeling for Al in synthetic freshwater over a range of pH from 3 to 10 (Wood et al. 2012)

2.5. Uptake in fish

Heavy metals may enter the fish body in three different ways; through the gills, the digestive tract, and trough the body surface. The gills are regarded as the most important site for uptake of heavy metals directly from water (Amundsen et al. 1997). To be taken up in organisms, the elements will have to pass a biologic membrane, like gill membranes. The gills are a highly complex vasculature surrounded by a high surface area epithelium that provides a thin barrier between the fish's blood and the aquatic environment to ensure effective gas exchange of oxygen from water to blood and also get rid of carbon dioxide from the blood (Evans et al. 2005). The thin barrier of the gills, and the exchange of elements between the blood and the aquatic environment around the fish make the gills susceptible to uptake of eco toxicants in the water. Gills are the main organ for osmoregulation and ensure correct water-ion balance. Special Cl-cells located in the gills transport active essential ions from the water to the blood of fish. Elements mimicking essential ions could influence the ion transport and cause ion regulation problems and acute toxic effects in fish.

When assessing the uptake of radionuclides and metals in fish compared to the concentration of elements in the water there are many factors that influence the process. Elements will compete with each other with regards to uptake in the fish. We can use models like the biotic ligand model (BLM) (Di Toro et al. 2001) to predict the bioavailable metals in water, based on speciation models and key variables including like pH, organic carbon content, competing ions, and metals concentrations. The biotic ligand is the place of uptake on the fish, for example the negative charged mucus on the gills, but for the models sake it is set as a more general site of action so the model is applicable on other organisms, not only fish (USEPA 2007). The metals in the water will interact with both organic and inorganic substances in the water, and create complexes. When these complexes are made, the bioavailable portion of the metals in the water will decrease, as it is the low molecular mass species/free metal ions that are most bioavailable and react with the biotic ligand. The free metal ions will also compete with competing cations, like Ca^{2+}, Na^{+} and H^{+} for the uptake sites in the organism. These interactions are shown in figure 4 taken from USEPA (2007).

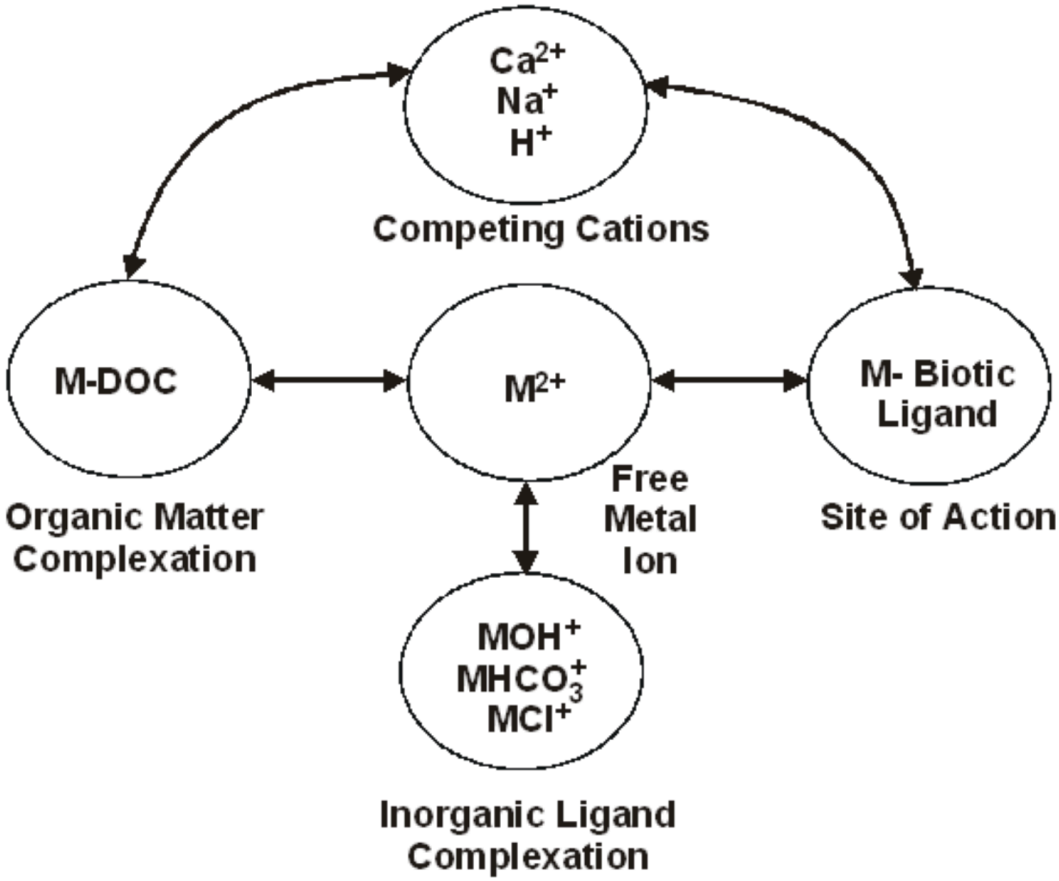

Figure 4 - Schematic diagram of generalized biotic ligand model (BLM) framework for acute toxicity of a divalent cationic metal (USEPA 2007).

The blood is transported from gills, past the stomach and intestines into the liver (Wedemeyer 1996). One of the livers function is to remove and metabolise foreign chemicals from the blood and several metals and organic pollutants tend to accumulate in high concentrations in the liver (Heath 1995).

2.6. Effects in fish

Following acute exposure to toxicants several mechanisms in organisms could be triggered, from molecular responses to physiological changes and mortality as worst outcome. Several types of biomarkers are used in ecotoxicological experiments when trying to assess dose responses. To cope with stressors, the energy demand increases in the fish, and production of glucose provides energy to organs and tissue. According to Iwama et al. (1999), the stress hormones adrenaline and cortisol have been shown to increase glucose production in fish, by glucogenesis and glycogenolysis. Normal

glucose values in blood for fish that is being fed is reported to be below 7 and in non-fed fish the normal glucose values is below 5 (Teien 2015).

The gill epithelium, including mucus layer, on fish has several negative charge sites and the positive charged metal species could therefore interact with these sites. Metal sorption to gills causing increased diffusion distances of gases and or inactivation of enzymes will cause respiratory or ion regulation problems. Increased (in seawater) or decreased (in freshwater) concentration of blood plasma ions (Na^+, Cl^-, and Ca) can indicate ion regulation problems (Rosseland & Staurnes 1994).

For cadmium, fish exposed to environmentally realistic exposure concentrations (1μg/L) will primarily be affected by disruption of ion homeostasis, particularly Ca regulation, where there is a direct interaction of Cd^{2+} with the Ca^{2+} ATPase because the Cd^{2+} have a high affinity for the Ca^{2+} binding sites and will thereby directly compete with the Ca^{2+}. In addition to ion regulation issues, cadmium has been shown to result in production of reactive oxidative species which has the potential for damaging biological molecules (Wood et al. 2012).

Uranium, being both a heavy metal and radionuclide can harm organisms in two ways, as it can be both radiotoxic and metal toxic. The radiotoxic effects from U are the alpha radiation, which can be harmful for biologic tissue if the U gets taken up in the fish. Since uranium have a low specific activity the metal toxicity is regarded as higher than the radiotoxic effect (CCME 2011). For uranium, it is shown that fish is generally tolerant to U, as it is not highly acute toxic to fish, but exposed to lethal concentrations (100μg/L) it was shown that zebrafish experienced gill damage with severe edema and chloride cell hyperplasia (Wood et al. 2012). Teien et al. (2014) found lethal concentration for 50 per cent of the population (LC_{50}) values for Atlantic salmon (*Salmo salar*) ranged from 1.4 at pH 6 or 5.5 to 25 mg U/L at pH 7.9.

2.7. Multiple stressors/mixed toxicity

Numerous experimental studies and research has been done on single elements alone. These experimental scenarios are not realistic compared to the complexity of the nature. At all times organisms in the aquatic environment are exposed to a variety of pollutants,

toxicants, and conditions and these multiple stressors can affect each other with regards to uptake, bioavailability, uptake and effects (Folt et al. 1999; Heugens et al. 2001)

Multiple stressors can affect each other in different ways, additive, antagonistic and synergistic. Additive effects occur when the combined effect of the multiple stressors are equal to the sum of the individual effects. Synergistic effects occur when the combined effect of the multiple stressors is greater than the sum of the effects and antagonistic effects occur when the combined effect of the multiple stressors is less than the sum of the effects (Folt et al. 1999). These interactions are very difficult to predict or assess, as it becomes complicated when there are numerous stressors in the environment. Because there is a potential of many different radionuclides and metals to leach from sulphur bearing rocks, fish living in water with drain off from these rocks will exposed to multiple stressors.

2.8. Ecological risk assessment

Risk assessment is a part of the process of analysing the possible effects on organisms of exposure to substances and other potential hazards. There are three main steps to risk assessment, which together makes up the risk characterisation. Firstly one has to identify the hazards, which can be biological, chemical, and physical. The second step is a dose-response assessment and the third step is the exposure assessment. The dose-response relationship is one between the dose and the proportion of individuals in an exposed group that demonstrates a defined effect (Yassi et al. 2001). This dose-response relationship is most often investigated in exposure experiments with defined doses and set effects measured. The exposure assessment is set to measure the exposure itself in the environment of the organism at risk, investigating the source of the toxicant, the concentration in the environment, the routes of intake and estimation of intake/uptake of the toxicant into the organism.

When calculating and assessing risk it is commonly that Risk = Probability x consequence. If there is both high probability for the exposure to happen and the consequence of it happening is severe, there is a big risk. And if there are minor consequences and/or the probability is low, the risk is low. A more precise way of measuring risk is calculating the risk quotient, or the PEC/PNEC ratio. The risk quotient

is calculated by dividing the predicted environmental concentration (PEC) by the predicted no effect concentration (PNEC). If the ratio is <1 (PNEC>PEC) it is defined as a risk and risk assessment is required (Hampel et al. 2007). The PNEC is calculated using numerous eotoxicity test performed and it is calculated using the $EC_{10,}$ for the most sensitive species, which is the lowest concentration where 10 percent of the population tested shows effects from the toxicant, divided by a safety factor (Hampel et al. 2007). The assessment factor or safety factor of 10 to 1000 dependent on the amounts and types of ecotoxicological exposure test performed with the pollutant/element in question (TGD 2011). Limits and guidelines for risk assessments are based on toxicity data from single element exposure tests. These tests often neglect potential mixture effects which can lead to an underestimation of the risk present for organisms (Beyer et al. 2014).

3. Method and materials

3.1. Bedrock sampling, preparation and analysis

Six rock samples as seen in table 2, taken from four different locations were used in the experiments as described in section 3.1.1 and 3.1.3. A full mineralogical analysis was just performed on the three of the rock samples, the ones used in the large-scale leaching and exposure experiment.

Table 2 - Overview over the rock samples used in both pilot and large-scale leaching experiment.

Sample	Rock type	Pilot experiment	Large-scale experiment
Rv.4-AS	Non-weathered alum shale	X	X
Rv.4-GS	Non-weathered galgeberg shale	X	
HBT-AS-NW	Non-weathered alum shale	X	
HBT-AS-W	30 year old weathered alum shale	X	
K34-AS	Non-weathered alum shale	X	X
E18-G	Weathered sulphur bearing gneiss	X	X

3.1.1. Sampling sites and bedrocks

Kirkegata 34

Sample one was taken from Kirkegata 34 (K34-AS), that is located downtown Oslo (figure 5). This sample is a non-weathered alum shale. The sample location is a building site where the foundation of the apartment building at Kirkegata 34 is being renovated. The apartment building is built on bedrock containing alum shale and this shale, due to water and air, has begun to swell(Endre 2014). The foundation of the apartment building is therefore being renovated. The sample was already hatched out of the bedrock so the samples were picked up and put in thick plastic bags and delivered to the IMV CERAD Isotope Laboratory and stored in room temperature.

Figure 5 - Map of Kirkegata 34 (Norgeskart 2015)

Hammersborgtunnelen

Two rock samples were collected from Hammersborgtunnelen in down town Oslo: HBT-AS-NW and HBT-AS-W. They were from the same area, but one was weathered for 30 years (HBT-AS-W) and the other was un-weathered (HBT-AS-NW). The Hammersborg tunnel and some parts of the Government Quarter are built in and on top of shale (indicated with light blue color in figure 6) and it is from this area between the tunnel and the Government Quarter the rock samples were gathered. The samples were collected in a thick plastic bag and delivered to the CERAD Isotope laboratory at and stored in room temperature.

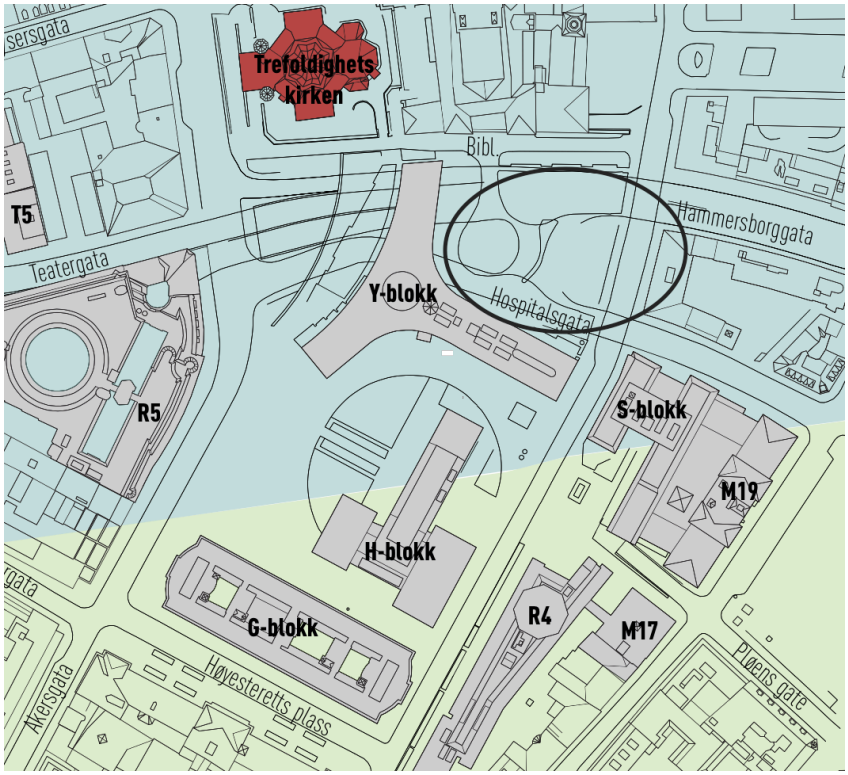

Figure 6 –Map of the location where the shales from Hammersborgtunnelen was gathered. (KVU & B06)

State Highway 4

State highway 4 (rv. 4) from Roa to Lygna is currently being upgraded and 21 km new road is being built and current roads are being restored. On this stretch of road there is a new tunnel being built (dotted line in fig 7) to place the road outside a populated area at Gran. In the bedrock where the tunnel is constructed there are areas of two types black shale, galgeberg shale and alum shale. To limit the environmental impact it has been established a depot for the shales containing higher specific radioactivity above 1 Bq/g (Lovdata 2011). To assess the concentration of uranium in the rocks x-ray fluorescence (XRF) measurements is used to determine if the total specific radioactivity is above 1 Bq/g, by measuring the concentration of U in the rocks. If the concentration is above 100 mg/kg, the total specific radioactivity is above the limit. Approximately 25 kg of the alum shale sample (Rv.4-AS) was collected from blasted rocks in connection with the construction. Approximately the same amount of the galgeberg shale sample (Rv.4 GS) was hatched out from inside the tunnel. The rock samples were delivered to the CERAD Isotope Laboratory and stored in room temperature.

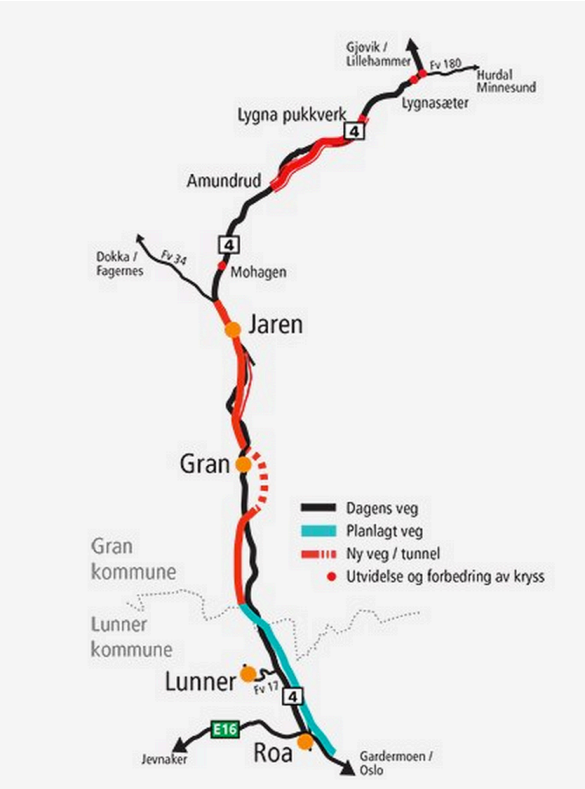

Figure 7 – Map of the planned and occuring road construction at Gran, Hadeland. (NPRA 2015)

Europe road 18 Lillesand

In Aust Agder there was opened a 38.3 kilometer four lane road between Grimstad and Kristiansand in 2009, which were to decrease traveling time and increase safety. However, Europe road 18 goes through sulphuric and acid producing bedrock near Lillesand, which has lead to extensive environmental problems, with acid drain off and heavy metal pollution in nearby aquatic environments. The masses that were excavated and moved from the road construction was placed in several depots to try to avoid these problems, but these depots show acidic runoff and metal pollution in spite of efforts to avoid it. For this study the rock samples of the gneiss (E18-G) was gathered from a road cut near the M20 depot, which lies in Urdalen. The rocks were placed in thick plastic bags and delivered to the CERAD Isotope Laboratory and stored in room temperature.

Figure 8 – Map of the new E18 from Grimstad to Kristiansand with the three deposits for the sulphur bearing rocks (Hindar & Nordstrom 2014).

3.1.2. Crushing of the rocks

All the rock samples collected had rocks of different size ranging from 1 cm to 20 cm. To ensure similar size distribution and large leaching surface the rocks were crushed using a jaw crusher (Retch KG type BB1, 220 V, nr. 15365). The only setting on the jaw crusher was a sliding wheel adjusting the space between the two grinding stones. Both the rock samples in the pilot experiment and in the large-scale leaching experiment were crushed using the same rock crusher. Figures 9 to 14 shows the rock samples after being crushed with the jaw crusher.

One of the biggest sources of error in the leaching experiment is the particle distribution of the rocks. Due to the lack of settings on the jaw crusher we cannot be sure that the size distribution of the crushed rocks were consistent. In addition to the inconsistency of the jaw crusher the rocks will react differently to the crushing due to differences in hardness, mineral composition and structure in the rock. This will affect the leaching of radionuclides and metals from the rocks as the smaller particles have a bigger surface area and are therefore more reactive. A grain size analysis could have been performed to see how the distribution of different sized particles was in the samples, but there was no time to perform this analysis. However, this can be done later based on stored fractions of the crushed rocks.

Figure 9 - Rv.4-AS after crushing

Figure 10 - HBT AS-W after crushing

Figure 13 – HBT-AS after crushing

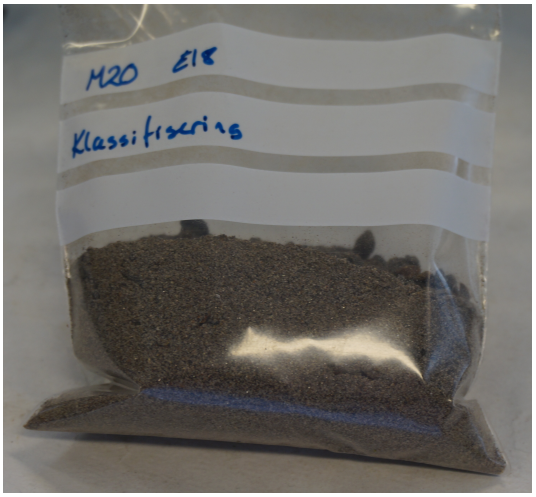

Figure 14 - E18-G after crushing

Figure 12 - K34-AS after crushing

Figure 11 - Rv.4-GS after crushing

3.1.3. Mineralogical analysis

A full mineralogical analysis was done on the alum shales from Kirkegata 34 and rv.4, and on the sulphur bearing gneiss from E18. To get a representative sample of the rock with all size fractions included, the samples were spread out on a sheet and with a clean plastic spoon, randomly taken until the plastic vial used were full. The mineralogical analysis was performed by Per Hagelia (NPRA) and Harald Foldvik (Natural History Museum, Oslo) and all the described analysis results is from Hagelia (2015). The

analysis was performed using a binocular magnifying glass, x-ray diffraction (XRD) and scanning electron microscope (SEM).

The use of the binocular magnifying glass was to investigate the rock samples with regards to taking samples to investigate with the XRD and SEM. The magnifying glass had 10x – 50x magnifying with two separate moveable light sources based on fibre optic.

After representative samples were chosen, a few grams of the grain fraction <0.1mm was finely crushed by hand using an agar mortar with ethanol. Each sample was let to dry in room temperature and placed on a holder with a few drops ethanol. The XRD analysis was performed using a Siemens D 5005 Spectrometer. The x-ray spectrum was run from 2^o to 70^o on 2-theta scale (0.050^o/seconds) with Ni-filtrated CuKα rays with wavelenght of λ=1.54178 Å.

For the SEM analysis both fine and coarser grains within the 0-2 mm fraction was chosen. The samples were attached on carbon tape and placed in the vacuum chamber of the SEM instrument, which was a Hitachi 3600 N with EDS from Thermon Electronic Corporation with an acceleration voltage of 15 kV and 10Pa vacuum.

K34-AS

In the shale from K34 the main minerals were identified, using XRD scanning was quartz, graphite, pyrite and some sandine. The SEM analysis showed also elements of barite and anhydrite. With the SEM it was also found iron sulphide, which corresponds to the pyrite documented with the XRD.

Rv.4-AS

In the Rv. 4 the main minerals, found with XRD scanning, were quartz, calcite, pyrite, chalcopyrite and graphite. The SEM analysis did not find any other minerals than the XRD analysis but confirmed the presence of pyrite. The presence of calcite gives this rock type a buffer capacity, which is higher than the other rock samples in the experiments, as the $CaCO_3$ acts as a buffer against decreasing pH. It was not done a mineralogy analysis on the galgeberg shale from Rv.4.

E18-G

The XRD scanning showed that the main minerals in the E18-G sample were quartz, muscovite (V, Ba), plagioclase and titanite. If was not found iron sulphide in the analysed material with either XRD or SEM but with the SEM jarosite was found in the sample and this mineral might be the main contributor to acid drain off from the deposit.

3.2. Leaching experiment

To investigate the leaching of radionuclides and metals from the rock samples two leaching experiments were designed: one pilot experiment and one large-scale leaching experiment followed by a fish exposure experiment. These experiments were set up in such a way that it was possible to get an understanding of which radionuclides and metals leached out from the different rock samples, the amount leached, and the leaching kinetics. In addition to measuring radionuclides and metals other parameters like pH, conductivity, temperature and major ions was measured.

3.2.1. Synthetic rain water

To mimic natural conditions in the environment in a controlled manner, synthetic rainwater was used in both the leaching experiments. In the pilot experiment, a stock solution that was made previously for another experiments with recipe taken from Overrein et al. (1980) was used. To increase the leaching it was decided to adjust the pH down to 4.3, as this was the pH in the reference used. In the large-scale leaching experiment a new concentrated solution of the rainwater was made using the same recipe for the stock solution in the pilot experiment. The stock solutions were made using the salts and the amount shown in table 3 at the isotope laboratory at NMBU, and the stock solution was diluted 1:100 for the final synthetic rainwater. The concentration of ions in the diluted waters is shown in table 4. The pH in the synthetic rainwater in the large-scale experiment was not adjusted down to 4.3, but kept at the pH in the diluted water, around 4.5, to follow the recipe of (Overrein et al. 1980)

Table 3 – Overview of salts used for the synthetic rainwater according to Overrein et al. (1980) . Including manufacturer, purity and weight of salt added to one litre of distilled water.

Salt	Manufacturer and purity	Weight of salt (mg/L stock solution)
Na_2SO_4	SIGMA ALDRICH ≥99%	42.6
$CaCl_2 \cdot 6H_2O$	VWR International – 98.6%	120.5
$Mg(NO_3)_2 \cdot 6H_2O$	SIGMA ALDRICH 98-100%	25.6
NH_4NO_3	MERCK – 99%	88.0

Table 4 – Nominal concentration of major ions in diluted synthetic rainwater in mg/L (Overrein et al. 1980)

Ions	Concentration mg/L
Na^+	0,136
Ca^{2+}	0,217
Mg^{2+}	0,024
NH_4^+	0,198
Cl-	0,39
NO_3^-	0,806
SO_4^{2-}	0,29

3.2.2. Pilot experiment

The main goal with the pilot experiment was to investigate the leaching of radionuclides and metals in synthetic rainwater from the rock samples described in 2.1.1. It was designed in a way to get an understanding of which elements leached, the concentrations, and the leaching kinetics. The results of the pilot experiment would decide which rock samples that was to be included in the large-scale leaching and exposure experiment

The pilot experiment was performed on all the six rock samples, where 100 grams of crushed rock sample were added to 1 litre of synthetic rainwater in l litre plastic bottles and placed in a temperature-controlled cabinet at 10 C^{o}. The lids of the bottles were off during the entire experiment, except for when the bottles were shaken once a day. Water samples was taken at day 1, 3, 7, 14 21, 28, 35 and 49 and pH and temperature were measured at the same times. Water samples were collected with syringes, using with a syringe filter with 0.45 μm cut-off to prevent particles in the sample and the samples were acid conserved with 5% ultrapure HNO_3 in 15ml tube before being measured on the inductively coupled plasma mass spectrometer (ICP-MS).

At every sampling 9.5 mL was collected for the water samples to be analysed and approximately 7 mL was taken for the pH and temperature measurement. Because the pilot experiment was done on such a small volume of water the percentage of water taken every week would have an impact on the concentration of radionuclides and metals in the water.

3.2.3. Large-scale leaching experiment

Selection of rocks

Based on the results from the pilot experiment, three of the rock samples were selected for the large-scale leaching experiment, followed by the fish exposure experiment. The large-scale leaching experiment was designed with intent on using the leachate in the exposure experiment. It was decided from the results of the pilot experiment, which samples were to be included in the large-scale leaching experiment. The rock samples that were chosen were the Rv.4-AS and K34-AS and the E18-G from the road cutting near the M20 depot by E18. The Rv.4-AS was to be included in the large-scale experiment, because of directly relevance of the funding project, and the results from the exposure experiment is important for understanding the effects that this type of shale can cause to aquatic organisms around the depot near Gran. The alum shale from Kirkegata 34 was chosen because the combination of relatively high leaching of uranium and cadmium, and relative low concentration of protecting base cations like Na^{+}, Mg^{2+} and Ca^{2+}. The combination was assumed to cause the highest uptake of

uranium among the studied types of rock. The E18-G stood out the most compared to the shales, as it would, as this is gneiss and not shale. But this rock sample was included because of its high leaching of aluminium and copper, and low concentration of base ions, in combination with the low pH. In addition the rock type has been known to have acidic and metal rich run off and has caused toxic conditions to aquatic organisms near the depots near Lillesand (Hindar & Nordstrom 2014).

Leaching conditions large-scale experiment

To follow standardised exposure protocols (OECD 1992) and to use the minimum amount of fish needed, 200 litres of water was calculated to be a minimum. Leaching experiment were then designed based on 200 L of synthetic rainwater, and 1kg rocks to 10 L water, as this was the same ratio of rocks to water as used in the pilot experiment. For the shales 19.26 kg of Rv.4-AS to 195 L of water and 20.06 K34 to 200 L of water was used. Of the E18-G 9.99 kg to 100 L of water was used. The reason why it was only used half the amount of rocks and water in the leaching of the E18-G was because measurements from the pilot experiment showed that the concentrations of especially aluminium and copper was too high for fish to survive in it, so the leachate was diluted 1:100 with the same water used for the E18-G control water.

The synthetic rainwater was pumped into 200 litre barrels with a plastic bag lining, meant for food supplies, to avoid contamination from previous experiments and contamination from the barrels. To get circulation of water and oxygen around the rocks for maximum leaching the crushed rocks were placed in cone shaped containers fitted above the barrels. The cone shaped containers had pumps attached to the bottom and water could then be pumped from below and up through the rocks and before it drained down into the barrel. This setup remained turned on for the duration of the leaching experiment, which was five weeks.

Every week water samples was collected and pH, temperature, and conductivity in the waters were measured by taking out approximately 20 mL for measurements. At each time three water samples were collected, one to measure metals, radionuclides, and main base ions, one for dissolved organic carbon content (DOC) and one for the ion chromatography (IC) analysis to measure chloride, nitrate, sulphate and fluoride. The water sample were taken with a syringe, using a syringe filter with 0.45 μm cut-off to

prevent particles and the samples were conserved with 5% ultrapure HNO_3 in 15ml tubes before being measured on the ICP-MS.

3.3. Exposure experiment uptake

The exposure experiment was designed to investigate uptake of radionuclides and metals in fish and the effects from these radionuclides and metals. This was done by exposing the individual fish to the mixtures of radionuclides and metals in the leachate produced during 5 the weeks leaching from the large-scale leaching experiment. Before the fish were added to the water the rocks were removed and the water was pumped over in a new barrel and filtrated using a 0.45 μm pump filter. Three different exposure waters/leachates were established with three corresponding control waters. The control waters were made by adding specific amount of $NaCO_3$, $CaSO_3$, KCl, $MgSO_3$ and $CaCO_3$ (table 6) to mimic the same ion concentration as in the individual leachate. The minimum exposure time was six hours and the maximum was 264 hours. Individual fish were taken out and dissected with intervals between these times, to collect tissue and study the uptake over time. To measure the effects on the fish, blood variables were collected and analysed. Thus, the bioavailability of the leached elements and following effects from the different leach out were compared by regression analysis.

Control waters

It was decided to make a control for each of the three leachates. With reverse osmosis 600L of water were produced and pumped into three barrels with plastic lining, holding 200L each. During the leaching phase the concentration of Na, K, Mg, Ca, Cl, NO_3^-, SO_4^{2-} in the water samples were measured (table 13) and using these measurements as the basis to make water with the same ion concentration (table 5), but without the radionuclides and metals. Stock solutions, using salts was made in the laboratory (table 6), and diluted in the control water barrels. From measurements in the pilot experiment and early measurements of metals in the leaching experiment it was needed to dilute the E18-G water due to lethal high concentrations of aluminium and copper. The E18-G water was diluted 1:100 in water made from the same stock concentration as the E18-G control water to get the same concentration of major ions in both exposure and control water.

Table 5 - Suggestion for concentrations of major ions and pH in the three control waters

Ion	E18-G mg/L	K34-AS mg/L	Rv.4-AS mg/L
Na^+	1.4	1.8	3.2
K^+	6.0	11.5	3.6
Mg^{2+}	10.0	9.0	2.5
Ca^{2+}	5.6	82.0	42.0
Cl^-	3.3	3.5	2.5
NO_3^-	0.1	0.0	0.1
SO_4^-	180.0	200.0	65.0
pH	5.3	7.5	7.5

Table 6 - Overview of the salts used to make stock solutions for the control water. Including manufacturer and purity

Salt used	Manufacturer	Purity
$NaHCO_3$	VWR International	100%
KCl	MERCK	99.5%
$MgSO_4$	SIGMA ALDRICH	≥ 99.5%
$CaSO_4$	SIGMA ALDRICH	≥ 99%
$CaCO_3$	J.T. Baker Chemicals	99.0%

3.3.1. Design

Fish

Oslomarkas Fiskeadministration (OFA) delivered the parr brown trout (*Salmo trutta*) from Sørkedalen in Oslo. (Sørkedalen 914, 0758 Oslo). They use wild fish from Nordmarka Oslo as brood and the fish used in the experiment was hatched in april 2014 so they were approximately 7 months old during the exposure experiment. The weight of the fish used in the exposure experiment varied from 7.1 g to 13.54 g with an average weight of 9.34 g (standard deviation 1.50). The outer length of the fish varied from 9.3 cm to 11.3 cm with an average of 9.9 cm (standard deviation of 0.51).

Acclimation

One week before the exposure experiment started the fish were picked up from Sørkedalen, Oslo and put into a 400L tank with circulating 1000 L water with the same ionic composition as the control water for alum shale Rv.4. This was done so that the fish would acclimate to the water and the conditions in the exposure experiment. The lid on the tank was closed to maintain darkness for the fish and the fish were kept in a room where the temperature was below room temperature. The fish were kept in a room without climate control, but the temperature in the room was approximately 10 C^{o} during the acclimation period.

Because the exposure experiment was performed without feeding it was decided to only give the fish some feed five days until prior to the start of the exposure experiment started. The reason why it was given five days before the experiment started was so that the feed would be digested and their bowels emptied so no faecal matter would be in the exposure waters and affect the uptake of radionuclides and metals. The fish loading during the acclimation period was 1.17 g fish per litre of water.

Reference fish

Before the exposure experiment began, five fish was taken out from the acclimation tank and measured and dissected using the same protocol described below. This was to get a reference of concentrations of radionuclides and metals in the organs of the fish.

Exposure conditions

The exposure experiment was performed in the same barrels as the large-scale leaching experiment. The fish, 21 fish per barrel, were placed inside their respective water within 1 hour of the first fish. The barrels used in the exposure phase were black, with plastic lining to avoid contamination from the barrels. The lids on the barrels were closed during most of the exposure phase, only removed when water and fish was sampled. There was no change of water during the exposure phase so it was a static experiment. During the entire exposure phase aerating stones was attached to a pump, which was supposed to distribute equal amount of air into the six different barrels.

The average fish loading between 0 and 96 hours was 0.8 g/L and 0.23 g/L between 96 and 264 The fish loading was never above 1g/L/week as recommended in the OECD guideline for testing of chemicals (OECD 1992).

After the fish was added to the barrels the pH fluctuated every day, mostly increasing. The pH was therefore measured every day and adjusted to the decided pH using either 1M HCl or Ca(OH).

Sampling

The maximum exposure time was set for 264 hours, from Monday to the next Friday with totally 5 outtakes of fish at different times. Three fish were sampled from each barrel at 6 and 12 h and five fish from each barrel at 24, 96, and 264 hours. When the fish was sampled for dissection they was caught with a net and euthanized with a small blow to the head. The length of the fish was measured twice, both inner and outer tail fin, and the fish was weighed. Then blood was taken from the left caudal vein with a syringe and the blood was analysed for glucose concentration using a OneTouch®UltraEasy® (LifeScan INC, Milpitas, USA) and glucose strips (OneThouch® Ultra®). The blood was inserted into the test strip directly after drawing blood from the fish with the syringe and the value given was on the instrument was read and noted. For the 264 h outtake the blood was also analysed on an I-STAT machine with EC8+ cassette to measure a variety of parameters in the blood.

After the blood was taken the fish was dissected and gills, liver, kidney, brain and olfactory taken out. The second gill on the right and one half of the liver was rapidly frozen in liquid nitrogen to look at gene expression changes in the fish. The left gill, other half of the liver, kidney, brain, and olfactory was frozen in a -20 C^o freezer for analysis of radionuclides and metals.

All the samples, except the gills and liver frozen in nitrogen, were put in flat bottom 2.5 ml tubes with lids. If there was any remaining blood, this was put in eppendorf tubes. After the fish was dissected the remaining fish was put in plastic bags and frozen down in case further analysis was to be done on the fish e.g. polonium analysis on the bones. The organs for gene analysis were kept in an 80^{o}C freezer and the organs for metal and radionuclide analysis was kept at -20 C^o until the analysis.

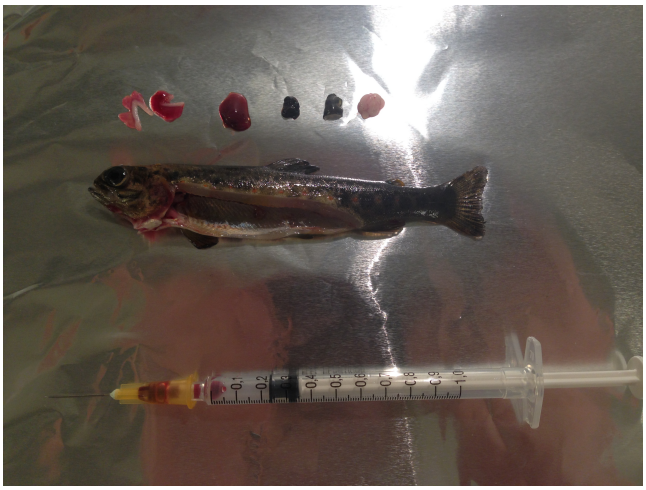

Figure 15 - Picture taken from the dissection showing all tissues sampled from the fish. Blood in the syringe, organs from left: gills, liver, kidney, olfactory and brain.

To make sure that there was no contamination of the fish organs during the fish dissection the protocol from the EMERGE (Rosseland et al. 2001) was followed for the dissection of organs to be analysed in the experiment. All the utensils, like tweezers and scissors were cleaned between the fish and the scalpel blades were changed. To avoid contamination of the organs directly from the aluminium foil used to protect the table, the organs was always placed on the fishes own tissue, that was cut off the fish when opening the abdomen, when taken out of the fish. All organs except the liver, which had

to be cut in two on the fish tissue, were placed directly into the test tube after dissection. The lids on the tubes were closed after each fish to avoid contamination.

3.4. Analysis of water

To characterise the control and exposure waters, samples were taken to measure a variety of parameters.

3.4.1. Water sampling and parameters

All the samples except the samples needed for ultrafiltration and cation exchange filtration was taken with a 50 ml syringe from the barrels. The syringes were reused throughout the experiment, being kept in their original packages between sampling to avoid contamination. The samples for ultrafiltration and the cation exchange filtration were taken with a 1 litre plastic bottle using gloves, and the same bottles were used during the filtrations. The water samples were taken before the fish was added to the barrels, at 0 hours, at 96 hours and 264 hours. Ultra filtration and cation exchange filtration was only performed on the control waters at only at 264 hours.

All water was filtrated through 0.45 μm before start of exposure, thus water samples collected represent dissolved elements and particles formed during experimental time. The concentration of radionuclides and metals in unfiltered samples give an overview of the metals and concentrations in the water, but it does not say much about bioavailability. To avoid particles in the water it was also taken samples that were filtrated with a 0.45 μm syringe filter. To assess the concentration and speciation of low molecular species, ultrafiltration and cation exchange filtration was performed. As in the pilot experiment the pH, conductivity and the temperature was measured at the same time water samples were measured. Samples for ion chromatography (IC) and DOC were taken and these were also filtrated using the same 0.45 μm syringe filter as for the total sample. During the experiment a data logger was placed in the alum shale Rv.4 control water to continuously measure the pH, O_2 concentration, and conductivity. This data logger also measured temperature in the water to make sure that the temperature in the barrel was stable throughout the exposure phase.

3.4.2. Ultrafiltration

To investigate the amount of low molecular mass species in the water, a sample of the water was ultra filtrated using hollow fibre filtration with a cut-off of 10 000 dalton. This was done using hollow fibre ultrafiltration cartridges and a pump, which pumped the water through the cartridges. The hollow fibre cartridges used in the ultrafiltration was hollow fibre modules from PALL Microza with 10KDa cut-off. The cartridges were washed using the hollow fibre washing protocol at the CERAD/Isotope laboratory at IMV before use. Separate cartridges were used for the control water.

3.4.3. Cation exchange resin

To determine the concentration of cationic species in the water a sample of the water was filtrated using cation exchange resin (Chelex ® 100 Resin from Bio Rad). The resin will take up any cations in the water by having ligands that selectively bond with certain types of metal cations. In this case Chelex-100 was used and this is a chelating ion-exchange resin having functional IDA groups in a styrene-divinylbenzene matrix (Gode & Pehlivan 2003). Because the cation exchange resin only bond to cations both anions and neutral ions will pass the resin, however the functional groups in the resin could compete with inorganic ligands in solution and thus overestimate the real amount of cations especially during long time contact. By using this removal of the cations in the water sample, the total concentration of anions and neutral ions in the water can be measured and by subtracting the concentration of LMM anions and neutral ions from the total concentration of LMM, we get the concentration of cations.

3.4.4. Measuring of pH

The pH meter was calibrated every time it was used, using DuraCal buffer solution with pH 4 and pH 7. To get an accurate measurement of the pH measured in the different waters, a sample of the water was taken out, and the pH electrode was kept still in the sample until the reading on the pH meter was stable, and a time limit of approximately 1 minute was set for stabilizing. To avoid any contamination in the water, water was taken out of the water bottles/barrels using a syringe and the pH was measured in a 50ml sarstedt tube.

3.5. Analysis of fish

To restrict the focus of the masterwork it was decided to only analyse the gills and liver from the fish for this thesis. The gills was freeze dried overnight using an Epsilon 2-4 LSC freeze drier to get the moisture out of the gills before decomposition. The livers was taken out of the freezer, thawed and weighed in for decomposition. The reason why the liver was not freeze dried was because it is difficult to get all the liver tissues out of the tubes after freeze drying. Because the liver was not freeze dried results are presented as µg/wet weight of liver, but as µg/dry weight of gills.

3.5.1. Decomposition of fish organs

The decomposition of the organs was done using an UltraClave Milestone on the IMV laboratory, which decomposes samples using high temperature and microwaves in a pressurized chamber. When doing analysis on the ICP-MS the sample have to be on a liquid form and homogenous, and this is what the UltraClave does. Using H_2O_2, H_2O and H_2SO_4 in the load, high temperature and microwaves to digest the organs. The starting pressure in the chamber was 50 bars and the temperature rose from room temperature up to 260^{o}C during the decomposition, staying at max temperature for 25 minutes.

Organs were weighed into the teflon tubes used for the decomposition, and 100 µl internal standard, 1ml HNO_3, and 1 ml of type 2 water was added to the tubes and the lids were placed on the tubes. The reason of using internal standard in the decomposition is that we can correct the data measured by the ICP-MS, in case of inaccurate dilution or loss of the decomposed organs after the decomposition stage. After the decomposition the samples were transferred to 15mL tubes and diluted to 10mL with deionized water and then analysed on the ICP-MS.

After the organ samples was measured on the ICP-MS the concentration was given in µg/L, and had to be multiplied by 0,01 to get µg/ 10mL and then divided by the weight of the organs weighed in to get µg element per gram organ.

3.5.2. Use of reference material

To make sure that all the organs was properly decomposed during the decomposition, and that the measurements of the radionuclides and metals in the organs on the ICP-MS was accurate, two reference materials was used. The certified reference material IAEA-414 was used for the radionuclides and the certified reference material DOLT-4 was used for trace metals. The IAEA-414 reference material is processed fish tissue of mixed species from the Irish Sea and the North Sea. The certified quantity values of the radionuclides, including ^{137}Cs, ^{234}U, ^{235}U, ^{238}U, ^{238}Pu, ^{40}K, in the IAEA-414 were determined after 90 different laboratories, world wide sent in results (Pham et al. 2004). The DOLT-4 reference material is processed dogfish (*Squalus acanthias*) liver with a set concentration of a variety of trace metals, including cadmium, arsenic, iron, nickel, lead and zinc (NRCC 2008).

3.6. Analysis of samples

To measure the concentration of radionuclides and metals in both water samples and organ samples the ICP-MS (Agilent Q8800) was used. All the measurements was performed using the ICP-MS at the radioisotope laboratory at IMV/CERAD, NMBU and was performed by Hans-Christian Teien. From the pilot leaching experiment the 0.45 μm filtrated samples were analysed on the ICP-MS. From the large-scale leaching experiment the 0.45 μm filtrated and ultra filtrated samples were analysed on the ICP-MS. From the exposure experiment the 0.45 μm filtrated, the 10KDa filtrated, the cation exchange filtrated, and the 10KDa cation exchange filtrated samples were analysed on the ICP-MS. The main elements measured were Al, U, Cd, Cu, Mn, V, Fe, Ni, Zn, As, Sr, Mo, Th, Ca, Mg, Na, and K.

The IC and DOC samples were sent to the IMV laboratory and analysed.

3.7. Data processing and statistical methods

3.7.1. Limit of detection and quantification

The limit of detection and the limit of quantification were tested by measuring concentration of radionuclides and metals in blank samples into the ICP-MS. From these blank samples we can calculate the limit of detection and the limit of

quantification by two formulas. The limit of detection are used to describe the lowest possible concentration to be reliably distinguished from the limit of blank which is the highest apparent analyte concentration expected to find in a blank sample with no analyte. The limit of quantification is the lowest concentration which an analyte can be reliably detected and accepted (Armbruster & Pry 2008). From the standard deviation of the values measured in the blank samples we can find both the limit of detection and quantification by using these formulas:

Limit of detection = 3 x Standard Deviation of blank

Limit of Quantification = 10 x Standard Deviation of blank

All measurements that are below the limit of detection will be presented as <LoD.

3.7.2. Principal component analysis

To determine if there are any differences between the rock samples from the pilot experiment a principle component analysis (PCA) was performed using Minitab 17. Principal components analysis is a tool to summarize a big data set with a smaller number of representative variables that explain most of the variability in the original set. This statistical tool is used for making a low-dimensional representation of the data that captures as much information as possible (James et al. 2013), by performing a covariance analysis between factors (Chahouki 2011). This is done by converting a set of observations of possible correlated variables into a set of uncorrelated variables called principal components. The first principal component accounts for as much of the variability in the data as possible, and the second explains as much variability as possible that is uncorrelated with the first principal component (Chahouki 2011). The data set included non-quantitative measurements, as some of the concentrations measured were below the detection limit for the ICP-MS, and these were defined as half the limit of detection. Due to the fact that some of the concentrations measured were very high and others were low, the data was log-transformed before performing the principal component analysis.

3.7.3. Regression analysis

To get an understanding of uptake of the radionuclides and metals in the fish over time, non-linear regression analysis was performed using Microsoft Excel 2011. Regression analysis is a statistical process to model a relationship between two variables and is used to describe a causal effect of one variable upon another (Løvås 2004). From the regression analysis we also get a coefficient of determination (R^2), which is defined as a proportion of variance in the data explained by the regression model, given in percentage, and can be used as a measure of success of the model applied (Nagelkerke 1991).

3.8. Ethics

The exposure experiment was performed on fish, which means that ethics had to be considered. To be allowed to perform the experiment, an application had to be sent to and approved by Norwegian Animal Research Authority (NARA), which is a public organ, set to protect animal welfare with regards to experiments done on animals. The application includes a description of the project and methods, how the animals will be handled before, during and after the experiment, and if there is any alternatives to the use of animals to accomplish the same results in the experiment. The exposure was approved in advance by NARA (NARA ID:4615)

4. Results and discussion

4.1. Quality Analysis

4.1.1. Quality of ICP-MS analysis

The results from the accuracy tests are shown in table 7. It shows the measured values of some trace metals measured by the ICP-MS at three different times, measurement one is from analyse of the water samples from the pilot experiment only. Measurement two is from analysis of water samples from both leaching experiments, and the third measurement is from the exposure experiment.

The percentage error is found using this equation:

$$\%\ error = \frac{Observed\ concentration - Expected\ concentration}{Expected\ concentration} \text{ x } 100$$

Table 7 - Quality analysis for accuracy. Expected and observed concentrations for elements in 1643 in-house standard material. The % error is calculated as the difference between observed and expected concentration divided by the expected concentration. Values marked with green are within the 10% error, and values marked red is above the 10% error.

Element	**Al**	**As**	**Cd**	**Cu**	**Fe**
Expected range	140.2 - 157.4	59.73 - 61.17	6,495 - 6.641	22.45 - 23.07	96.1 - 99.5
Observed	142	58	6	22	95
% Error	4 %	4 %	9 %	5 %	3 %
Observed	148	61	7	24	102
% Error	0 %	1 %	4 %	4 %	4 %
Observed	147	66	8	27	106
% Error	1 %	9 %	14 %	19 %	8 %
Element	**Mn**	**Mo**	**Ni**	**Sr**	**U**
Expected range	38.52 - 39.42	120.1 - 122.7	61.72 - 63.1	319.5 - 326.7	0.96-1.01
Observed	35	115	58	303	0.99
% Error	10 %	5 %	7 %	6 %	1 %
Observed	41	126	63	323	1
% Error	5 %	4 %	1 %	0 %	0 %
Observed	47	126	70	327	0.99
% Error	21 %	4 %	12 %	1 %	1 %

The acceptable range for the values lies within 10% of the expected range. All the measurements from analysis one and two lies within the range of 10% error, three of the measurements from the third measurement are outside the 10% range. This might be

due to systematic errors, e.g., preparing of calibration standard used for this measurement.

4.1.2. Quality of decomposition of organs

Table 8 shows the relevant elements measured in organs with the standard reference materials DOLT-4 (fish-liver) and IAEA-414 (fish flesh). Both the cadmium and the first measurement of uranium were within the 10% error range and are acceptable. The second measurement of uranium was however not within the error range acceptable. Since the in house 1634 reference sample of uranium had 1% error and the DOLT-4 had 3% error it is assumed that the decomposition was fully completed. Inaccurate weighing of the reference material might cause the low concentration measured in the second observed measurement.

Table 8 - Quality analysis for accuracy and decomposition. Expected and observed concentrations for elements in the DOLT-4 and IAEA-414 reference material (NRCC 2008; Pham et al. 2004), The % error is calculated as the difference between observed and expected concentration divided by the expected concentration. Values marked with green are within the 10% error, and values marked red is above the 10% error.

Element	U	Cd
Reference material	IAEA-414	DOLT-4
Expected range	0.086 - 0.094	23.5 - 25.1
Observed	0,084	24
% Error	6 %	3 %
Observed	0,067	-
% Error	25 %	-

4.2. Pilot experiment with all six rock samples

4.2.1. pH and major ions

The pH and the concentrations of major ions in the leachate from each of the rocks are presented in table 9. The initial pH of the synthetic rainwater was 5.3 before the rock samples were added, while after after 7 weeks the pH was changed drastically. The pH in the E18-G leachate was decreased to 3.3 and the pH in the shale waters were all increased to 7.5 or above, with the highest pH of 8.4 measured in the Rv.4 GS.

The amount of major ions leached from the rock samples after seven weeks reflects the results from the pH measurements in the waters, specially considering calcium. HBT-

AS-W stands out as it leached most calcium and magnesium of all the rock samples. The increase in Ca^{2+} due in the leachate of Rv.4-AS was expected, as calcite was present in the sample. The low pH in the leachate from the E18-G water in combination with the high SO_4^{2-} indicates production of sulphuric acid by oxidizing of sulphide minerals, probably jarosite. The concentration of base cations in leachate from the E18-G was very low. Taken into consideration that cations such as Ca^{2+} and Mg^{2+} compete with trace metals for uptake in organisms, further part of the study have focused on focus are rocks with high concentrations of radionuclides/metals and low concentration of Ca^{2+} and Mg^{2+}.

Table 9 – Concentrations of major ions (0.45µm filtrated) in the leachates from different rocks (100g/L water) after seven weeks in the pilot experiment. However, pH value given represent week 4, as no pH was measured after 7 weeks. Numbers marked with * was analysed at the IMV laboratory and no SD/RDS was given.

	Control	HBT-AS-NW	HBT-AS-W	E18-G	K34-AS	Rv.4-AS	Rv.4-GS
pH 4 weeks	4.2	7.6	7.6	3.3	7.5	7.5	8.4
Ca^{2+} (mg/L)	<0.1	194±0.6%	515±1.5%	3.8±25%	58±3.7%	48±8.4%	12±3%
Na^{+} (mg/L)	0.1±2.7%	2.2±1.5%	7.6±4.3%	0.8±4.8%	0.9±2.4%	0.6±4.4%	34±1.6%
Mg^{2+} (mg/L)	<0.1	6±1.82%	126±2.3%	9±4.4%	17±1.9%	2±3.3%	3.3±0.9%
K^{+} (mg/L)	<0.1	9.5±2.5%	1.6±2.9%	11±3.5%	9.9±0.7%	5.4±0.7%	6.2±3.5%
NO_3^{-} * (mg/L)	0.03	<0.020	<0.020	<0,020	<0.020	<0.020	0.3
SO_4^{2-} * (mg/L)	2.2	570	190	200	170	78	31

4.2.2. Leaching of metals

Table 10 shows the concentration of metals and radionuclide leached from the six different rock samples included in the pilot experiment, and is the basis for choosing the rock samples which were to be included in the large-scale leaching- and exposure experiment. The highest uranium concentrations were found in the shales from Hammersborgtunnelen, followed by the K34-AS. Highest concentrations of cadmium were found in the E18-G and K34 leachate. The E18-G dominated the aluminium leaching, and this was also the case for the leaching of copper, manganese, and nickel.

The highest concentration of molybdenum was found in the K34 leachate. Further discussion on the concentrations of radionuclides and metals in the leachates is presented in section 4.3.3.

Table 10 – Dissolved (0.45µm) concentrations of metals and radionuclide after seven weeks in the pilot experiment. Standard deviation given as RSD. Concentrations given in µg/L

	Control	HBT-AS-NW	HBT-AS-W	E18-G	K34-AS	Rv.4-AS	Rv.4-GS
U	0.4 ±2%	602 ±2.4%	531 ±4%	18 ±2.4%	406 ±1.6%	118 ±1.5%	17 ±1%
Cd	<0.02	0.4 ±7.6%	0.06 ±24%	0.94 ±7%	0.98 ±3.5%	0.09 ±6.6%	<0.02
Al	5.1 ±34%	12 ± 9.3%	8.3 ±9.6%	11601 ±3.2%	14 ±9.4%	19 ±4.9%	59 ±5.3%
Cu	1.9 ±1.1%	0.7 ±6.9%	0.6 ±2.3%	535 ±0.7%	0.4 ±20%	0.3 ±7.5%	0.4 ±11%
Ni	0.3 ±14%	207 ±2.6%	12 ±4.4%	261 ±0.8%	172 ±0.7%	20 ±5.4%	0.8 ±45%
Mn	<0.12	141 ±0.7%	2.1 ±5.3%	513 ±0.8%	351 ±1.2%	97 ±1.2%	13 ±2.8%
Mo	<0.3	1041 ±0.9%	78 ±2.1%	4 ±12%	2032 ±4%	555 ±1.2%	35 ±1.3%

4.2.3. Statistical analysis pilot experiment

Figure 16 and 17 shows the results of the principal component analysis performed on the leaching data gathered during the pilot experiment. The PCA was done on the concentration of elements in the leachate after 7 weeks leaching period. The elements included in the analysis were U, Ni, Cd, Mn, Sr, Mo, Zn, Fe, and Cu. The samples that leached similar elements and similar concentrations are clustered together in the diagram, and dissimilar samples are placed far apart. The E18-G sample is the one that clearly stands out with regards to elements and concentration leached from the sample and is dominated by Zn, Fe, Cu and Al leaching. There is a slight overlap between the Rv.4-AS and the K34-AS but they are mostly separated. From the score plot it is also apparent that the Rv.4 GS shale is the shale that is most different from the other shales with regards to radionuclides and metals, and the concentration of these in the leachate.

The loading plot shows correlation between the elements leached. If two elements are close to each other this indicates that there is a correlation between them. From the loading plot the leaching of Al, Zn, Fe and Cu is strongly correlated, and this might be due to the fact that these are the elements that dominated the in the leaching from the

E18-G sample. Ni, Cd, and Mn is grouped up and strongly correlated. The leaching of U seems to correlate mainly with Sr and Mo.

Based on the pilot experiments it was decided to focus on Rv.4-AS, K34-AS and E18-G, and limit the focus to U, Cd, and Al to study the transfer to fish.

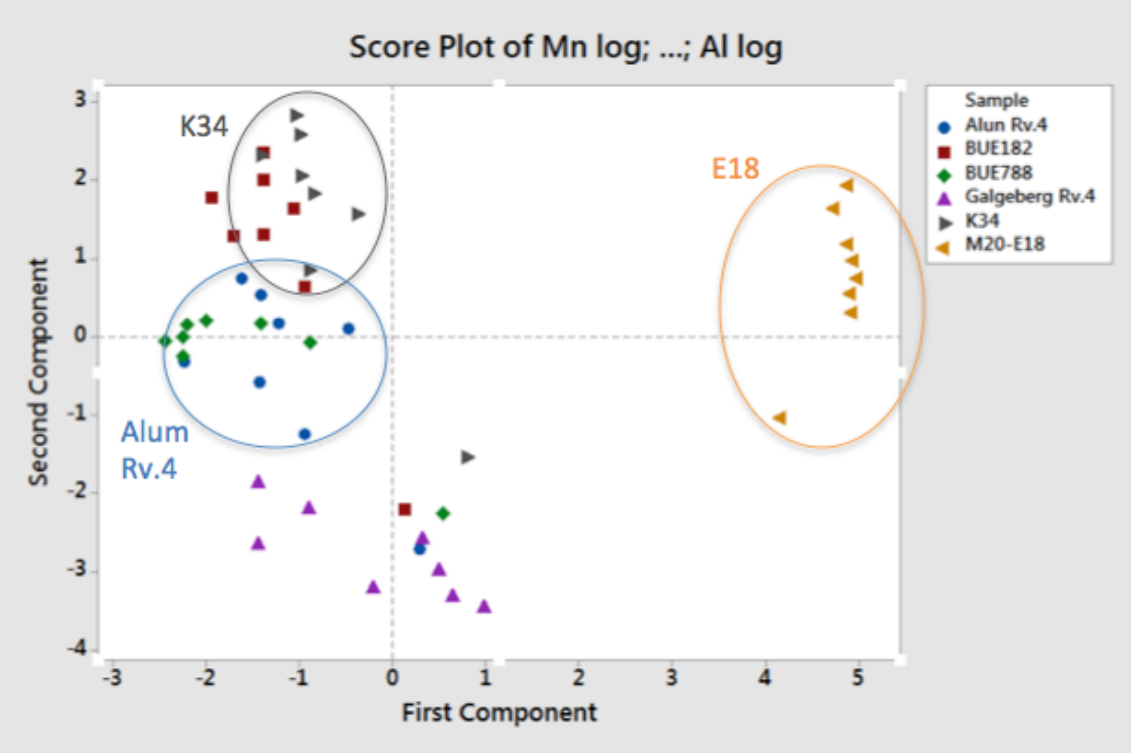

Figure 16 - Score plot from principal component analysis of the concentration leached from the six different rock samples in the pilot experiment after seven weeks. Large distance between samples indicate big variation between the samples.

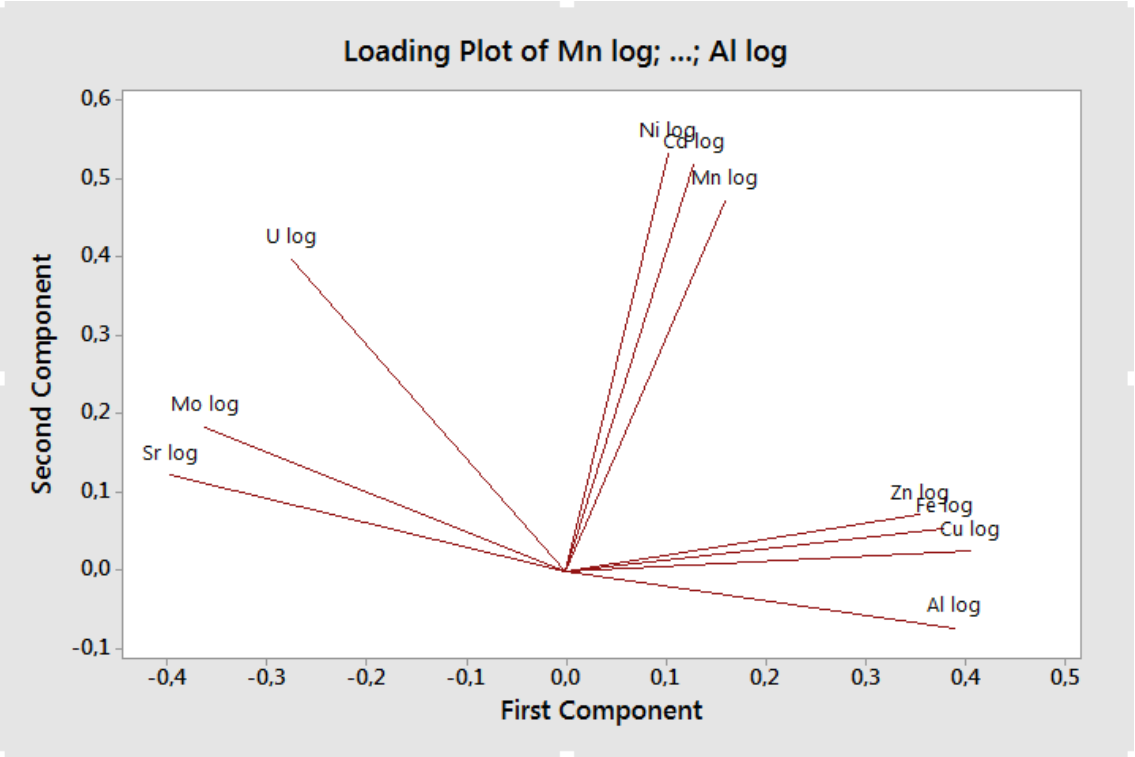

Figure 17 – Loading plot from the principal component analysis of the concentrations leached from the six rock samples. Shows the chemical composition in the leachate from the experiment.

4.3. Leaching over time

4.3.1. Temperature

The temperature in the pilot experiment was supposed to be 10 C^o. There was, however, an error with the refrigerator where the samples was kept, so the temperature changed from an average of 15 C^o to 19 C^o between 24 and 72 hours. But from the measurements taken after week 1 the temperature was stable around 16 C^o throughout the rest of the pilot experiment phase. For the large-scale leaching experiment the temperature was stable throughout the leaching phase, with an average of 10 C^o.

4.3.2. pH and major ions

The pH in the leachate from the E18-G decreased both in the pilot and the large-scale experiment. From the initial pH of the synthetic rainwater to 3.5 after a week, and

further decreasing throughout the leaching phase (table 11). The same pattern as in the pilot experiment was present for the shale rocks, with an increase of pH from 4.5 to around 8 within the first week before staying quite constant throughout the remaining leaching phase.

Table 11 - pH over time from the large-scale leaching experiment.

Week	E18-G	K34-AS	Rv.4-AS
1	3.6	7.9	8.0
2	3.1	8.0	7.8
3	2.8	8.0	7.8
4	2.8	7.8	7.8

The pH in the shale waters might be this high due to dissolution of $CaCO_3$ that acts as a buffer against decreasing pH from sulphuric acid production as seen in table 12. This was also found in Helmers (2013) where alum shale from Rv.4 was added to solutions of different pH, ranging from 2-8, where the pH in the solutions ranged from 5.5-9.0 depending on the pH in the initial solution. In the solution with pH 2, the pH was instantly neutralized when coming into contact with the rock samples, reaching 5.5-7.5, which indicated a buffering capacity most likely explained by high content of carbonate minerals in the sample.

The opposite was found in Falk et al. (2006) where the pH in water, that had surrounded crushed shale samples, decreased straight after being added. However, this experimental setup was performed in such a way that the water ran through the crushed rock samples and was caught and analysed, and not continuously leaching like in the experimental setup in this project. This and different mineralogy in the rock samples might cause the rapid decrease of pH, instead of an increase.

There are some differences in the leaching of major ions in the large-scale leaching experiment compared to the pilot experiment. Especially higher leaching of Ca^{2+} from the K34-AS, and overall higher concentration of major ions leached from the E18-G.

Table 12 – Dissolved (0.45µm) concentrations of major ions in the three large-scale experiment rock samples. STD given in RSD Ions marked with * was measured by the IMV laboratory and no standard deviation or RSD was given.

Element mg/L	E18-G	K34-AS	Rv.4-AS
Ca^{2+}	8.5±1.8%	83±0.5%	39±1.1%
Na^{+}	1.5±0.9%	1.9±1%	3.2±0.5%
Mg^{2+}	10±0.2%	10±0.7%	2.5±0.5%
K^{+}	10±1.7%	11±0.5%	3.2±1.6%
Cl^{-} *	3.4	3.3	3.1
NO_3^{-} *	<0.020	0.03	0.05
SO_4^{2-} *	270	220	61

4.3.3. Leaching kinetics and concentrations of radionuclides and metals

The results from the pilot- and large-scale leaching experiment are presented in figure 18-20, showing leaching of dissolved (0.45µm) uranium, cadmium, and aluminium over time. To limit the focus it was decided to only include uranium, cadmium, and aluminium, as these will be focused on in the fish experiment.

Uranium

The concentration of dissolved uranium in the leachate as a function of time in both from the rock samples in pilot and large-scale experiment are presented in figure 18. The general trend is high leaching of uranium at the beginning of the experiments before the graphs seem to level out. From the figure it seems like the most leaching occurred, for all the three rock samples, within the first week, and then it looks like the graph levels out.

The highest concentrations of uranium leached in both experiments were from both shales from Hammersborgtunnelen. From figure 18, it is clear that there was higher leaching of uranium from the three rock samples in the large-scale experiment compared to the same rock samples in the pilot experiment the first week. However,

from 25 days it looks like the K34-AS rock in the pilot experiment reaches the same concentrations of uranium in its leachate as in the large-scale experiment, if the large-scale experiment had continued further. This indicates a higher rate of leaching at the beginning of the large-scale experiment compared to the pilot experiment.

As the natural concentration of uranium in waters worldwide can range from 0.01 µg/L to 1500 µg/L (Arfsen et al. 2001), even the highest concentration leached from HBT-AS-NW (table 10) was within the norm. But compared to the measured concentrations from Reinmann et al. (2009) all the concentrations except from the E18-G and the Rv.4 GS are above the range of uranium in surface water in the Oslo area.

Leaching experiments have previously been done on the alum shale from Rv.4, using sequential extraction. There it was shown that the concentration of uranium leached in H_2O was low compared to the total concentration of elements in the rock sample, and that the highest leaching of uranium happened by leaching the substance in HNO_3, which is a strong acid and oxidizing agent (Fjermestad 2013). It is also found that uranium leaching from alum shale is pH dependant, as Helmers (2013) found that there was a substantial higher concentration of U leached from alum shale at pH 2 compared to pH 4.6 and 8. In that study it was found that 2 - 36% of the total U in the rock samples leached out into the water at pH 2, compared to 0 - 9 % of the total U leached in water with pH 4, 6, and 8.

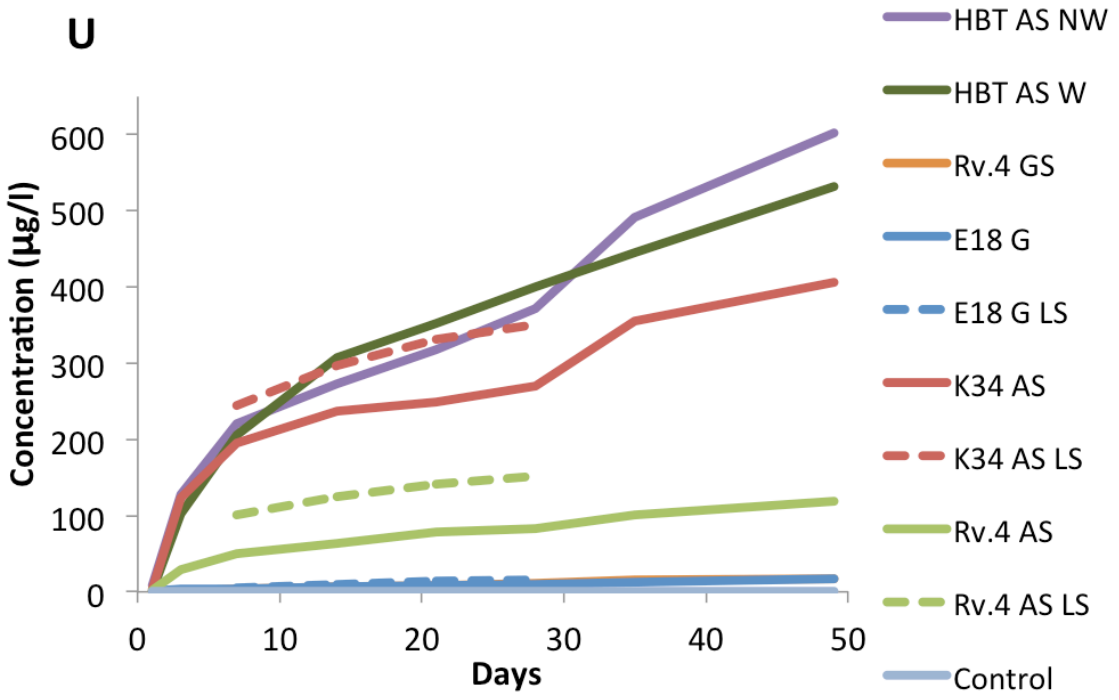

Figure 18 - Leaching of dissolved (0.45µm filtrated) uranium in the leachate as a function of time. Concentration of dissolved uranium in leachates from pilot experiments shown as full lines and concentration of dissolved uranium in leachate from large-scale (LS) experiment shown as dash lines. The rock to water ratio was 100g/1 L water in both experiments. HBT AS NW: non-weathered alum shale from Hammersborgtunnelen, HBT AS W: Weathered alum shale from Hammerborgtunnelen. Rv.4 GS: Galgebergshale from Rv.4. E18 G: weathered gneiss from E18. K34: alum shale from Kirkegata 34. Rv.4 AS: Alum shale from Rv.4.

Cadmium

The concentration of dissolved cadmium in the leachate as a function of time from the rock samples in both pilot and large-scale experiment are presented in figure 19. The general trend in both experiments is high leaching short time after mixing the rock and water. The concentration of cadmium seems to continue to increase in the leachate, as the graphs does not seem to level out at the end the experiments.

The two rock samples that leached the most cadmium in both experiments were the E18-G and the K34-AS. These two samples followed each other in the with regards to leaching in the pilot experiment and after seven weeks the concentrations in the leachates was 0.89 µg/L and 0.98 µg/L respectively. However, the concentration of Cd in the leachate from the E18-G in the large-scale experiment was almost twice as high compared to K34-AS. The leaching was, however significantly higher in large-scale than pilot experiment for all rocks tested. This might be because of the increased

circulation of oxygen in the large-scale experiment led to more oxidising conditions in the E18-G water and leached more cadmium. This is also consistent with the lower pH in the large-scale experiment (table 9) and the higher concentration of SO_4^{2-} in the E18-G leachate (Table 13).

The Rv.4-AS leached <0.02 µg/L after four weeks in the pilot experiment and after four weeks in the large-scale experiment the concentration of cadmium was 0.5 µg/L.

From Hammersborgtunnelen the two shales leached different concentration of cadmium. HBT-AS-NW leached 0.37 µg/L cadmium and the HBT-AS-W leached 0.06 µg/L cadmium from the samples. The cadmium in the weathered shale might have been leached out of the rock if it has been exposed to moist/oxic conditions.

All the rock samples in both pilot- and large-scale experiment leached concentrations higher than the background concentration given in SFT (1997). The concentration of cadmium in both the control and in the Rv.4 GS was below the detection limit of the ICP-MS of <0.02 µg/L in the pilot experiment.

The leaching of cadmium from the rock samples in the large-scale leaching experiment was over all higher than in the pilot experiment. Especially the E18-G gneiss which leached over twice as much as in the pilot experiment. Again, all the concentrations of cadmium in the waters were above the background concentration of <0.04 (SFT 1997).

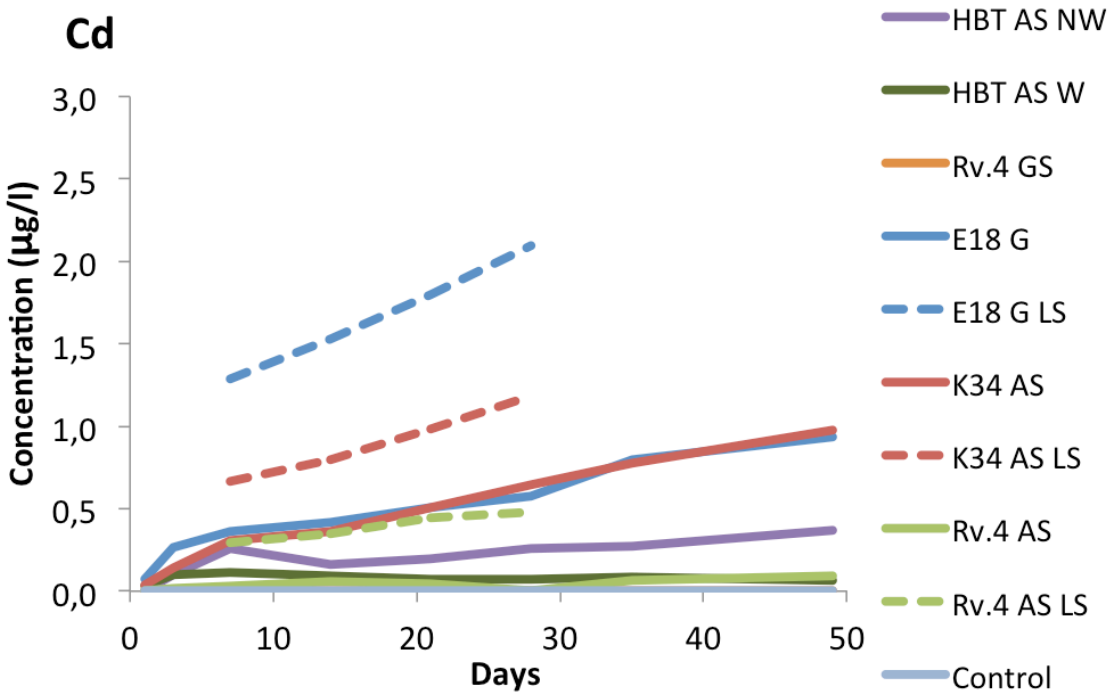

Figure 19 – Leaching of dissolved (0.45µm filtrated) cadmium in the leachate as a function of time. Concentration of dissolved cadmium in leachates from pilot experiments shown as full lines and concentration of dissolved cadmium in leachate from large-scale (LS) experiment shown as dash lines. The rock to water ratio was 100g/1 L water in both pilot experiments. HBT AS NW: non-weathered alum shale from Hammersborgtunnelen, HBT AS W: Weathered alum shale from Hammerborgtunnelen. Rv.4 GS: Galgebergshale from Rv.4.: weathered gneiss from E18. K34: alum shale from Kirkegata 34. Rv.4 AS: Alum shale from Rv.4.

Aluminium

The concentration of dissolved aluminium in the leachate as a function of time from the rock samples in both pilot and large-scale experiment are presented in figure 20. From the first measurement it was clear that one of the rock samples stood out when it came to leaching of aluminium. After four weeks the concentration of Al in the E18-G water was 5219 µg/L and 16673 µg/L, in the pilot and large-scale experiment respectively. These concentrations are extremely high, as *Drikkevannsforskriften* 2002) states that maximum concentration of aluminium allowed in drinking water in Norway is 200 µg/L. From the graph it also looks like the aluminium concentration will continue to increase over time, as the graph does not level out. The difference in the leachate concentration between the two experiments might be related to different leaching conditions and various mineralogical compositions of the rock samples used. This might

lead to the lower pH observed in the large-scale experiment, compared to the pH in the pilot experiment, resulting in higher leaching. Increased circulation of oxygen in the large-scale experiment led to more oxidising conditions in the E18-G water could also be one factor. The low buffering capacity of this rock sample cannot counteract the production of sulphuric acid in the leachate, and the decreased pH leads to mobilization of Al from the rock sample. This was also found in Hindar and Nordstrom (2014) where studies was done on rock samples from the same area as the E18-G was Al concentrations in the water started to increase to high levels (<1 mg/L) at the same time as pH decreased to below 4.8 (deposit M17) and 5.5 (deposit M15/16). This is also found in Lawrence et al. (2007) where increased mobilization of aluminium was strongly correlated to decreasing pH. The concentration of aluminium in the leachate from the E18-G in both experiments was higher than the background concentration of 0.5-1 mg/L in acidic water (WHO 2003).

All of the alum shale samples leached concentrations less than 24 µg/L and within the background range of aluminium (0.001-0.05 mg/L) in near-neutral pH waters (WHO 2003). However, the highest concentration of aluminium in the Rv.4 GS leachate was 400 µg/L and this is above the background concentration.

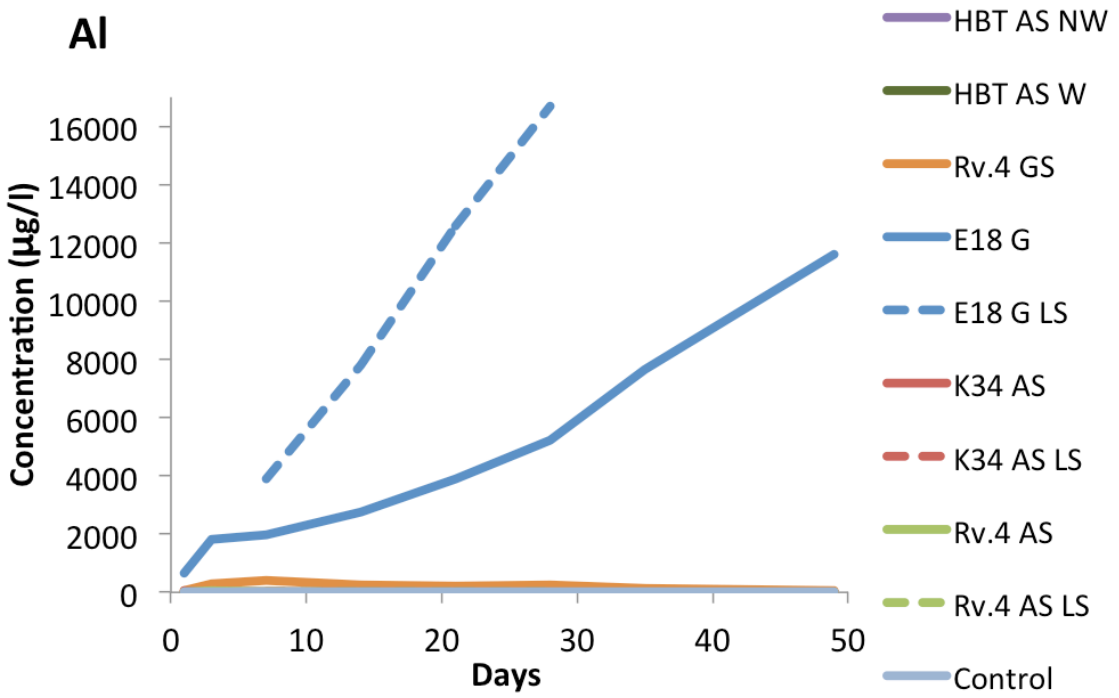

Figure 20 - Leaching of dissolved (0.45µm filtrated) aluminium in the leachate as a function of time. Concentration of dissolved aluminium in leachates from pilot experiments shown as full lines and concentration of dissolved aluminium in leachate from large-scale (LS) experiment shown as dash lines. The rock to water ratio was 100g/ L water in both experiments. HBT AS NW: non-weathered alum shale from Hammersborgtunnelen, HBT AS W: Weathered alum shale from Hammerborgtunnelen. Rv.4 GS: Galgebergshale from Rv.4. E18 G: weathered gneiss from E18. K34: alum shale from Kirkegata 34. Rv.4 AS: Alum shale from Rv.4.

Generally, there were higher concentrations of the elements in the leachate from the rock samples in the large-scale experiment compared to the pilot experiment. This might be due to the fact that there was a continuously circulation of water which was continuously mixed with oxygen around the rock samples, compared to the pilot experiment where the water samples was only shaken once every day and with no air bubbled in. This continuously circulation of water around the rock samples increase the contact of rock to water and might increase the leaching. The continuously mixing of oxygen into the water in the large-scale leaching experiment might increase the leaching of trace elements from the rocks, as it leads to more oxidizing conditions. This is also found in Fjermestad (2013) where the highest concentrations of Cd, U, and Al leached in sequential extractions from alum shale happened in HNO_3.

In addition to differences in the experimental setup it is important to remember that in the pilot experiment, only 100g rock samples was used. With this small volume it is difficult to get a representative sample of the rock. In the large-scale experiment the

rock samples were 20 and 10kg. The mineralogical composition of the rocks in the two experiments can therefore vary a lot, and hence the concentrations in the leachates can vary.

4.4. Speciation of trace elements and presence of major ions in the exposure water

As described in the method and material chapter the water samples from the exposure experiment was both ultra filtrated and cation exchange filtrated to understand the speciation of elements leached out from the rock samples. Because the focus in the exposure experiment was on U, Cd, and Al, the speciation of these elements are presented below. The data presented in this chapter is from the water samples taken at 0 hours, 96 hours and 264 hours in the exposure experiment.

4.4.1. Uranium Speciation

The concentration of uranium after leaching and filtration in the three exposure waters was stable throughout the exposure phase. The concentrations of the different uranium species are shown in table 13.

Table 13 - Average concentration of uranium in the exposure and control waters collected during the exposure period at 0h, 96h and 264h. Numbers marked with * shows single measurements with no standard deviation.

Uranium µg/L	E18-G	E18-G control	K34-AS	K34-AS control	Rv.4-AS	Rv.4-AS control
Dissolved	0.47±0.04	<0.5	359±5.2	0.60±0.3	170±1	0.70±0.3
LMM	0.57±0.04	0.65*	363±2.6	0.90*	175±4.1	1.20*
Anions + neutrals	0.47±0.04	0.40*	172±78	0.40*	129±7.8	0.60*
Cations	0.10±0.07	0.25	191±78	0.50	45±8.85	0.60

The K34-AS water had the highest average concentration of dissolved uranium with 359 ± 5.2 µg/L, and all the uranium was LMM species. The concentration of anions and neutral ions was 172 ± 78 µg/L and the concentration of cations was 191 µg/L, with a distribution of 47% anions and neutral species and 53% cations. The reason why there is such a high standard deviation for the anions and neutral ions concentration is that the

analysis of this parameter showed much lower concentrations of LMM anions at 96 hours, compared to the measurements from 0h and 264h. This low measurement decreased the average concentration of anions and neutral species, and affected the given concentration of cations in the water, as this is calculated from the LMM anion and neutral concentration.

The Rv.4-AS had an average concentration of dissolved uranium of 170 ± 1 µg/L, and all the uranium was LMM species, like in the K34 water. The concentration of anions and neutral ions in the water was 129 ± 7.8 µg/L and the concentration of cations was 45 µg/L, with a distribution of 74 % anions and neutral species and 26% cations. Most of the LMM was present as anions or neutral ions, but since no fractionation was performed to separate the anions and neutral ions we cannot assess the concentration of anions alone.

In the E18-G water the average concentration of uranium was 0.47 ± 0.04 µg/L after 1:100 dilution and most of the uranium was on the LMM anion and neutral ion species with 0.1 µg/L present as cations, with a distribution of 82% anions and neutral species and 18 % cations. At this pH (5.3) we expect uranium to be present as cations or neutral ions according to Krestou and Panias (2004) so we can assume that the anion and neutral fraction mostly consists of neutral uranium species.

4.4.2. Cadmium Speciation

After leaching and filtration of water the concentrations of cadmium in were stable throughout the 264 hours of the exposure experiment with limited changes in speciation or concentrations. The concentrations of the different cadmium species are shown in table 14.

Table 14 - Average concentration of cadmium in the exposure and control waters collected during the exposure period at 0h, 96h and 264h. Numbers marked with * shows single measurements with no standard deviation.

Cadmium µg/L	E18-G	E18-G control	K34-AS	K34-AS control	Rv.4-AS	Rv.4-AS control
Dissolved	0.03±0.01	<0,015	1.29±0.01	0.03±0.003	0.51±0.01	0.02±0.001
LMM	0.03±0.004	0.02 *	1.31±0.003	0.03*	0.52±0.009	0.02*
Anions + neutral	<0,015	0.15*	0.24±0.01	<0.015	0.12±0.02	<0.015
Cations	<0.03	0	1.07±0.01	<0.03	0.40±0.02	<0.02

The K34-AS water had the highest average cadmium concentration (1.29 ± 0.01 µg/L) with most cadmium present as LMM species. The low standard deviation also indicates small or no changes in concentration over time in the exposure phase. The concentration of cations species in the water was 1.07 µg/L while the concentration of anions and neutral ions was 0.24 ± 0.01 µg/L, with a distribution of 18 % anion and neutral species and 82 % cations. This indicates that most of the cadmium present in the water is of the bioavailable cationic species (Wood et al. 2012).

The Rv.4-AS had an average concentration of cadmium of 0.51 µg/L throughout the exposure experiment. The concentration of cations was 0.40 µg/L and for anions and neutral ions the concentration was 0.12 ± 0.02 µg/L, with a distribution of 24 % anions and neutral species and 76 % cations. As with the K34 water most of the cadmium present was on the bioavailable cation species.

In the E18-G water the concentration of cadmium was 0.03 ± 0.01 µg/L after 1:100 dilution in control water. The concentration of anions and neutral ions in the E18-G water was below the detection limit of the ICP-MS.

4.4.3. Aluminium speciation

After leaching and filtration the concentration of aluminium species in the K34-AS and the Rv.4-AS water was stable throughout the 264 hours of the exposure experiment (table 15). However, the concentration of aluminium in the E18-G water was not stable from start to end of the exposure experiment.

Table 15 - Average concentration of aluminium in the exposure and control waters collected during the exposure period at 0h, 96h and 264h. Numbers marked with * shows single measurements with no standard deviation.

Aluminium µg/L	E18-G	E18-G control	K34-AS	K34-AS control	Rv.4-AS	Rv.4-AS control
Dissolved	97±61	7.3±0	19±0.6	11±1.8	19±0.23	14±8.78
LMM	50±28	33*	23±2.9	10±*	25±4.6	9.1*
Anions + neutral	28±19	52*	17±4.2	7.4*	19±1.35	8±3*
Cations	22±34	0	7.1±5.7	2.8	6.5±4.48	1.1

In the K34-AS water the average concentration of dissolved aluminium was 19±0.6 µg/L and most was present as LMM species. Most of the LMM species was present as anions or neutral ions, with a concentration of 17±4.2 µg/L and 7.1 µg/L was present in the water as cations. This gives a distribution of 71 % anion and neutral species and 29% cations. At this pH most of the aluminium species should be present as anions as seen in figure 3.

In the Rv.4-AS water the average concentration of dissolved aluminium was 11±1.8 µg/L and as LMM species. As expected at this pH most of the LMM species was present as anions or neutral ions, with a concentration of 7.4±4.2 µg/L, and 2.8 µg/L present as cations, with a distribution of 74% anion and neutral species and 26 % cations.

The aluminium concentrations decreased from 182 µg/L to 38 µg/L from 0 to 264 hours in presence of fish. This might be because there were some sorption to fish, surface in the tank, and particles. In average the concentration was 97±61 µg/L as 50±28 µg/L present as LMM species. Of these 28±19 µg/L was present as anions or neutral ions and 43 µg/L was present as cations. This gives a distribution of 55 % anions and neutral species and 44 % cations. We cannot differentiate between anions and neutral ions with the use of cation exchange resin, but with this low pH we expect neutral or cation species of aluminium as seen in figure 3.

4.4.4. Major ions present in the water

The concentration of major ions in the water will affect the uptake of metals as these can act as competing ions to the metals for the biotic ligands. The E18-G water was diluted 1:100 in the same water that was used for the E18-G control water. The concentrations of major ions in the control waters were pretty consistent with the concentration of major ions in the exposure waters, with some deviations (table 16).

Table 16 – Concentrations of dissolved ions in the leachate from the large-scale leaching experiment. Determined after filtration (0.45 µm). Shown in mg/L.

Element	E18-G *	E18-G control	K34-AS	K34-AS control	Rv.4-AS	Rv.4-AS control
Ca^{2+}	1.2±2.1%	0.9±3.2%	79±1.8%	67±1.2%	42±1.5%	36±1%
Na^{+}	0.7±0.4%	0.7±1.8%	1.8±0.8%	2.4±1%	3.1±1.7%	3.5±0.6%
Mg^{2+}	1.1±1.4%	0.9±2.4%	9.2±0.4%	8.3±1.4%	2.8±2.3%	2.3±1%
K^{+}	0.7±1.1%	0.6±1.4%	10±0.5%	10±1%	3.3±0.7%	2.5±1%
Cl^{-} *	0.78	1.4	2.7	10	2.3	2.6
NO_3^{-} *	0.06	0.05	<0.02	0.1	0.03	0.06
SO_4^{2-} *	9.1	6.2	200	180	62	79

* E18 G water diluted 1:100 with E18 control water

4.5. Results fish exposure experiment

The results from the fish analysis include concentration of radionuclides and metals in both gills and livers of the fish, as well as blood glucose levels as one effect parameter are included in the results. Only three of the radionuclides and metals measured (U, Cd and Al) will be included in the results and discussion to narrow down the amount of results discussed. The rest of the measurements of concentrations in the organs are included in the appendix as tables 19 and 20.

4.5.1. Reference fish

In table 17 the concentration of the selected radionuclides and metals in both gills and liver for the reference fish are listed and used as background concentrations before start of exposure. The concentration of elements in the different organs of the exposed fish

will be compared to the reference fish, which was sampled after acclimation, but before the fish exposure experiment started (0h).

Table 17 – Average concentration of Al, Cd, and U in the gills (n=5) and liver (n=5) of the reference fish sampled before the exposure experiment. Concentrations in the gills and livers are given as µg/g dw and µg/g ww, respectively.

Reference fish	Al	Cd	U
Gills n=5	7.86 (n=1)*	0.69±0.1	0.09±0.04
Liver n=5	2.63±0.3	0.02±0.003	<0.015

4.5.2. Uptake of radionuclides and metals

Uranium in gills

The uptake of uranium in the gills is presented in figure 21. The average concentration of uranium in the gills of fish exposed to the leachate from K34-AS and the Rv.4-AS leachate was of 0.37 ± 0.03 µg/g dw and 0.20 ± 0.04 µg/g dw respectively after 264 hours. For both the K34-AS and Rv.4-AS it is an increase of concentration of uranium in the gills over time. The logarithmic regression analysis also indicates that the concentration of uranium in the gills reached equilibrium with the concentration of uranium in the water after 264 hours. The standard deviations for both K34-AS and Rv.4-AS are small and this strengthens the regression analysis, but the R^2 is 0.68 and 0.75 so there is still variance in the data that is not explained by the logarithmic regression model. The concentration of uranium in the gills of the fish exposed to the K34-AS leachate was more than a factor of 35 times higher than in the control fish and four times higher than in the reference fish after 264 hours. The concentration of uranium in the gills of the Rv.4-AS fish was more than a factor of 12 times higher than the concentration of uranium in the gills of the control fish and twice as high as in the reference fish after 264 hours. This indicates a difference, even though no statistical performed any to claim a statistical significant difference.

With similar pH in both shales water (7.5-7.7) and approximately the same concentration of DOC (1.1±0,1 mg/L in the K34-AS and 1.1 ±0.8 mg/L in the Rv.4-AS

water), we can assume that the concentration of uranium in the water and the concentration of competing ions are the factors that decides the uptake of uranium on the gills. Song et al. (2013) found that in a dose-response experiment, with uptake of uranium, which no differences in water quality variables suggested that any group-specific differences observed in uptake of uranium was due to the fish being exposed to different concentrations of U. However since there is a difference in competing ions in the two waters, especially the concentration of Ca^{2+} and the Mg^{2+} which competes with UO^{2+}, this can affect the uptake of uranium. The concentration of dissolved uranium in the water for both the K34 and Rv.4 water is higher than the recommended short-term (33 µg/L) and long-term guideline (15 µg/L) concentrations for uranium present in freshwater in Canada (CCME 2011)

The E18-G fish had higher uptake of uranium at the beginning of the exposure experiment than the fish exposed to the shale samples even though the E18 water had much lower concentration of uranium in the water compared to the shales. The highest uptake of uranium was between 6 and 12 hours and after this the uptake decreased. There is a high standard deviation for the concentrations at 12, 24 and 96 hours, indicating that there are large differences in the amount of uranium taken up on the gills on the fish at these times. The change of uptake of U in the gills could not be explained by changes in water as the concentrations of major ions and pH was relatively stable.

The fish exposed to the E18-G water had much higher uptake compared to the fish exposed to the leachate from the shales. The reason why the uptake was higher could be a result of higher bioavailability or reduced competing effects from base cations. The concentration of competing ions in the E18-G water was lower than in the other two waters, however competing ions have been reported to have minimum effects. The pH in the E18-G water was low compared to the two shale waters, and at levels associated with high bioavailability (Teien et al. 2014). This results shows, however that the uptake of uranium in gills are extremely dependent upon water quality, as 0.5 µg U/L causing higher uptake than 360 µg U/L in this study.

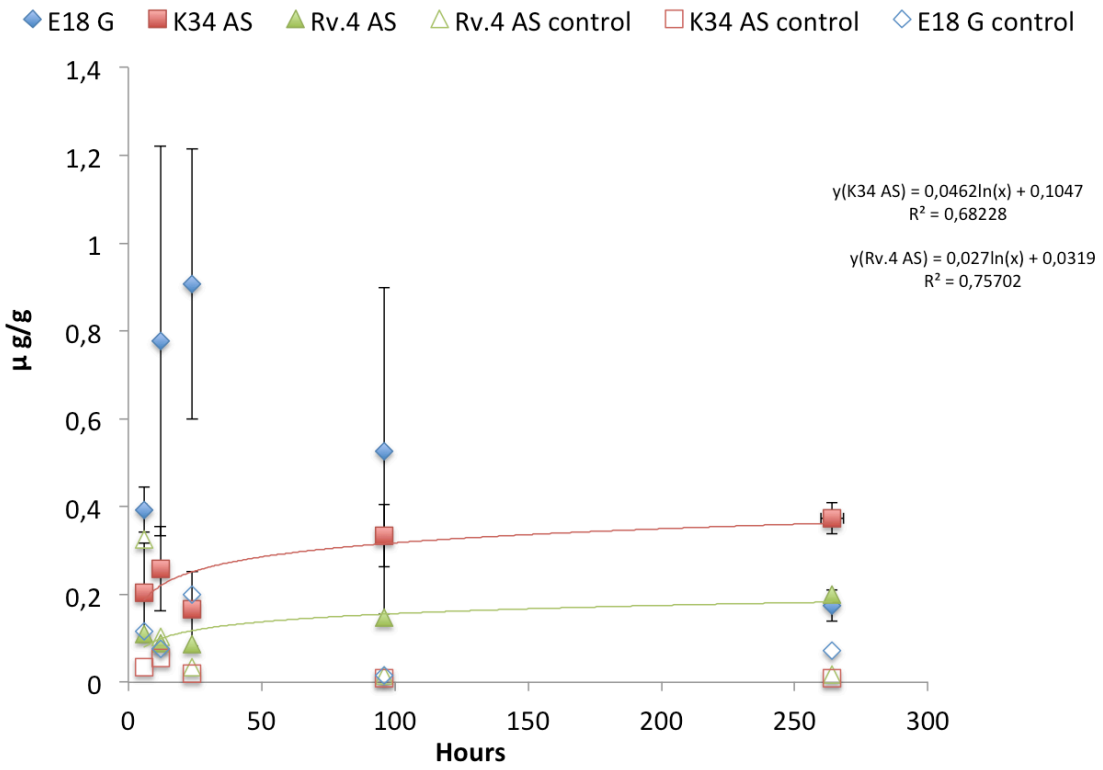

Figure 21 – The average concentrations of uranium in gills (µg/g dw) in control and fish exposed to leachate from the different rocks. The lines were fitted to the data from K34 AS and Rv.4 AS using logarithmic regression analysis. The standard deviations given are the standard deviation of the average concentration of uranium in the gills.

Uranium in liver

The concentrations of uranium in the livers of fish exposed to the different leachates follow trend as the concentration of uranium in the waters (table 14) as seen in figure 22, with highest concentrations of U in the livers of the fish exposed to the highest concentrations of U. In average the livers collected from the fish in the K34 water had highest concentration of uranium, whereas the lowest concentrations were observed in the livers from the fish exposed to the E18-G leachate.

Fish exposed to the K34-AS exposure water shows the highest concentration of uranium in the liver reaching an average of 0.015 ± 0.003 µg/g ww after 264 hours of exposure. The regression analysis indicates a high uptake at the start of the exposure phase and it looks like the trend line has not level out after 264, indicating that there will still be an uptake of uranium in the liver if the exposure experiment continued further. However, the standard deviations are so high for the average concentration we cannot say this for

sure. The concentration of uranium in the liver of the K34-AS control and in the reference fish was below the detection limit of the analysis for all the samples taken, indicating that there is an increased uptake of uranium in the livers of fish exposed to K34-AS water compared to the control and reference fish.

The concentration of uranium in the livers exposed to the Rv.4-AS leachate reached an average of 0.008 ± 0.003 µg/g ww after 264 hours and like in the K34-AS fish the regression analysis indicates high uptake at the beginning of the exposure phase. It does not seem like the trend line has levelled out (fig 22), and it looks like the uptake of uranium in the liver for the Rv.4-AS fish would continue to increase in these conditions over time. The small standard deviation strengthens this. For the Rv.4-AS control there was one measurement above the detection limit, at 96 hours being 0.002 ± 0.001 µg/g ww and the rest of the livers analysed had uranium less than the detection limit, same as in the reference fish. As with the fish from the K34-AS water we can assume that there is a difference between the exposed fish and the control and reference fish with regards to uptake of uranium in the liver.

In the liver collected from the E18-G water there was only one measurement of uranium above the detection limit for the liver and that was after 264 hours at 0.002 ± 0.001µg/g ww and the rest of the measurements was below the detection limit. This was also the case for the E18-G control, where only two measurements were above the detection limit and was 0.002 ± 0.001 µg/g ww and 0.003 ± 0.001µg/g ww respectively. This indicates that the uptake of uranium in the liver for the E18 water is similar to the control and the reference fish. The reason why there is so little uranium in the liver of the E18-G fish compared to the high amount of uranium in the gills indicate precipitate on the gills with limited uptake intracellular and transfer in blood.

For fish, it is found that the kidney is the primary target organ of the uranium taken up by the fish by food-borne long term exposure, together with accumulation of uranium in mineralized tissue like bone and scales (CCME 2011). Consequently, it would be a possibility of finding higher concentrations of uranium if the kidneys were analysed for U, even if the experiment was not long-term.

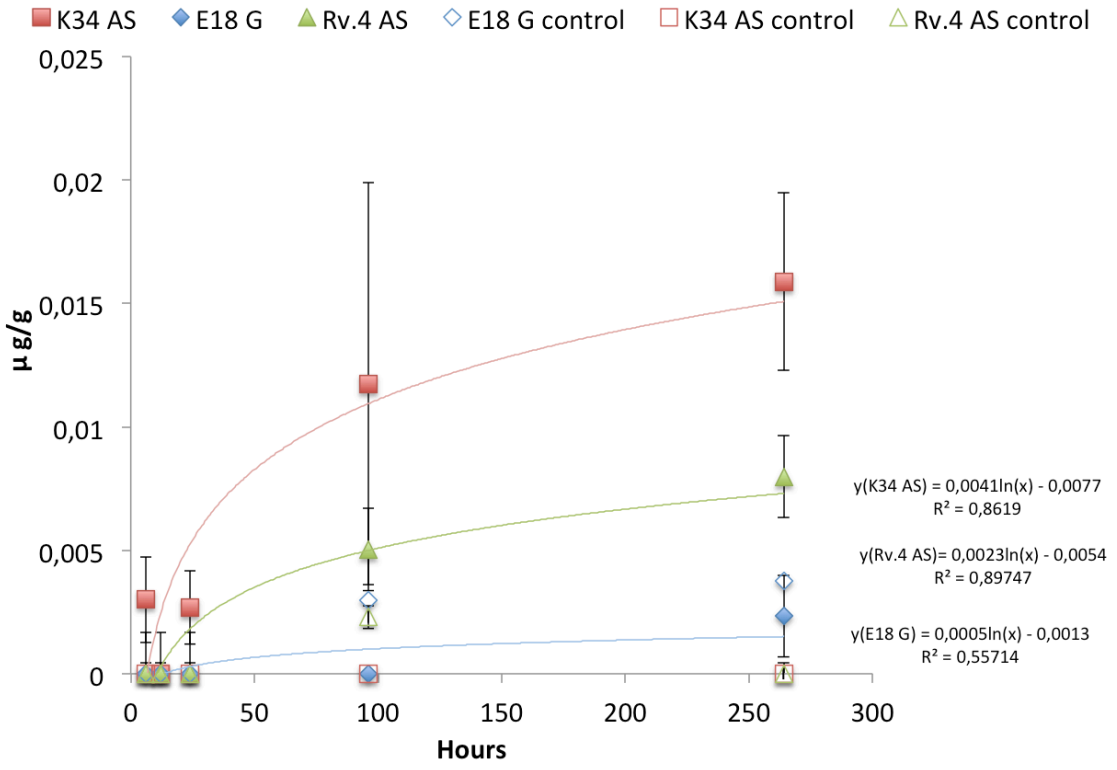

Figure 22 - The average concentrations of uranium in liver (µg/g ww) in control and fish exposed to leachate from the different rocks. The lines were fitted to the data from K34-AS, E18 G and Rv.4 AS using logarithmic regression analysis. The standard deviations given are the standard deviation of the average concentration of uranium in the gills.

Cadmium in gills

The concentration of cadmium in the gills is presented in figure 23. In the gills collected from the fish in the K34-AS exposure water the average concentration of cadmium in the gills after 264 hours was 1.3±0.25 µg/g dw. The regression analysis indicates logarithmic changes with high uptake at the beginning of the exposure phase and then levelling out where the uptake and elimination rates are more similar. The concentration of cadmium in the gills from the K34-AS control water stayed quite constant throughout the exposure experiment with an average of 0.62±0.2 µg/g dw, similar to levels in the reference fish (0.69±0.1 µg/g dw). These results indicate a higher uptake of cadmium in the gills for the exposed fish compared to both the control fish and the reference fish.

The concentration of cadmium in the gills from the Rv.4-AS water showed the same trend as in the K34 water, with increasing concentration over time and with highest uptake at the beginning. After 264 hours the concentration of cadmium in the gills for

the Rv.4-AS exposure fish was 1.45±0.22 µg/g dw compared to 0.55±0.06 µg/g dw in the reference fish. Even though there was higher concentration of cadmium in the K34-AS leachate than in the Rv.4-AS leachate, 0.29±0.003 µg/L and 0.51±0.01 µg/L respectively, the regression model indicate quite similar uptake of cadmium. This is probably because the K34-AS water has a higher concentration of Ca^{2+} as shown in table 12. It is well established that Ca^{2+} competes with cadmium for uptake on the gills (Wood et al. 2012), and in KLIF (2012) classification for cadmium are corrected for hardness of water because of this. The concentrations of dissolved cadmium in both the K34-AS and the Rv.4-AS were so high that they are classified as moderately contaminated and would give chronic long term effects with long term exposure (KLIF 2012).

For the E18-G exposure water the concentration of cadmium in the gills stayed relatively consistent throughout the exposure phase (0.59±0.04 µg/g dw) and similar to the E18-G control fish. We can therefore assume that there was no increased uptake of cadmium in the gills exposed to the E18-G water compared to the control and reference fish.

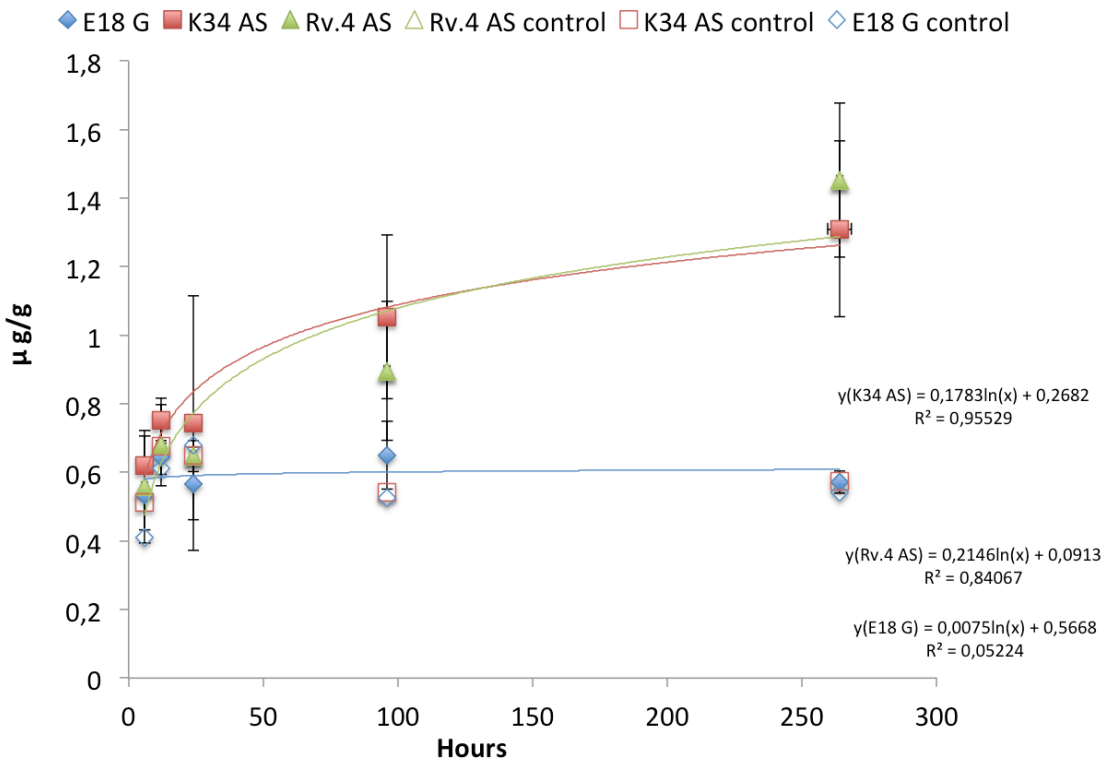

Figure 23 - The average concentrations of cadmium in gills (µg/g dw) in control and fish exposed to leachate from the different rocks. The lines were fitted to the data from K34 AS, E18 G and Rv.4 AS using logarithmic regression analysis. The standard deviations given are the standard deviation of the average concentration of cadmium in the gills.

Cadmium in liver

The uptake of cadmium in the liver of the fish exposed to the leachate from the three different rocks (figure 24) increased with time but there was no clear difference between the rock types or between the rock types and their corresponding controls.

For the K34-AS exposure water the concentration of cadmium was 0.068 ± 0.02 µg/g ww after 264 hours. For the K34-AS control water the concentrations of cadmium at the same time was 0.063 ± 0.007 µg/g ww. This indicates that there is no difference between the exposed fish compared to the control fish, even though the K34-AS exposure water had a higher concentration of low molecular mass cadmium cations than the control water.

The same trends are present for the Rv.4-AS, where the concentrations of cadmium in the livers of the exposed fish are approximately the same as the concentration of cadmium in the livers of the control fish. For the Rv.4-AS exposure water the concentration of cadmium in the livers was 0.056 ± 0.01 µg/g ww compared to 0.055± 0.01 µg/g ww in fish from control water, both after 264 hours.

For the E18-G exposure water the concentration of cadmium in the liver after 6 hours was 0.043 ± 0.004 µg/g ww while 0.065 ± 0.01 µg/g ww in fish of control water. This result is also expected, since the concentrations of LMM cadmium are similar in the two waters. The concentration of LLM cadmium in the E18-G and E18-G control water was 0.03±0.004 and µ/L 0.02 µ/L, respectively. Because the concentration of low molecular mass species of cadmium in the E18-G exposure water are only marginally higher than in the control water, there should not be a difference in the uptake. These concentrations are also below the background concentration of cadmium given in SFT (1997). In the non-diluted E18-G water the concentration of cadmium would have been 100 times higher, and this concentration is so high that it is classified as moderately contaminated and would give chronic effects with long time exposure to this concentration according to KLIF (2012).

The reason why there is not really any apparent difference between the controls/reference fish and the exposed fish regarding cadmium concentrations in the liver might be because cadmium does not accumulate in the liver to the same degree as in the kidney. This was also found in Yesilbudak and Erdrem (2014) where the concentration of cadmium in the kidneys (1.08±0.09 µg/g dw) was higher than in the livers (0.051±0.04 µg/g dw) following an exposure experiment with 0.5 ppm Cd over 30 days. Further focus should then be to determine the Cd in kidney samples to identify if there were any differences.

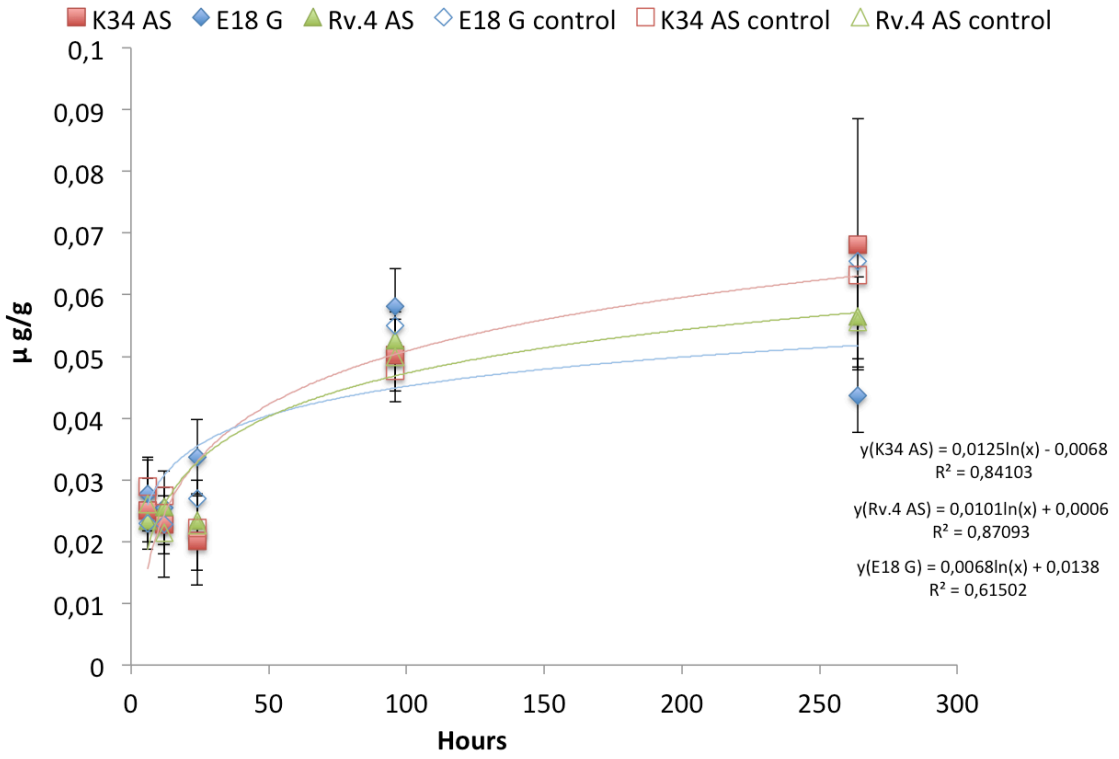

Figure 24 - The average concentrations of cadmium in liver (µg/g ww) in control and fish exposed to leachate from the different rocks. The lines were fitted to the data from K34 AS, E18 G and Rv.4 AS using logarithmic regression analysis. The standard deviations given are the standard deviation of the average concentration of cadmium in the livers.

Aluminium in Gills

From the concentrations of aluminium in the gills it is apparent that the only exposure water giving a significantly uptake in the gills was the E18-G as seen in figure 25. The concentration of aluminium in the gills in the fish from E18-G increased immediately to very high levels and with the highest concentration of 1042 ± 363 µg/g dw at 24 hours. There are high standard deviations for the measurements from 12, 24 and 96 hours, which indicates a big spread in concentration of aluminium on the gills these times. In the E18-G control fish the concentration of aluminium in the gills was below the detection limit for all the measurements but one, which had a concentration of 95 ± 42 µg/g dw at 96 hours. This measurement is abnormally high compared to the other E18-G control fish and the reference fish which had a maximum concentration of 7.8 µg/g dw. This can be caused by contamination of this particular gill under the dissection of the fish.

For the K34-AS exposed fish the concentration of aluminium in the gills was fairly consistent throughout the exposure phase, with concentrations between 19 ± 8.8 µg/g dw and 23 ± 1.6 µg/g dw. The K34-AS control fish all showed concentrations of aluminium in the gills below the detection limit of the ICP-MS.

In the Rv.4-AS exposure fish there was only two measurements with concentrations above the detection limit for aluminium in the gills with 5.5 ± 3.1 µg/g dw after 6 hours and 12 ± 5.6 µg/g dw after 264 hours.

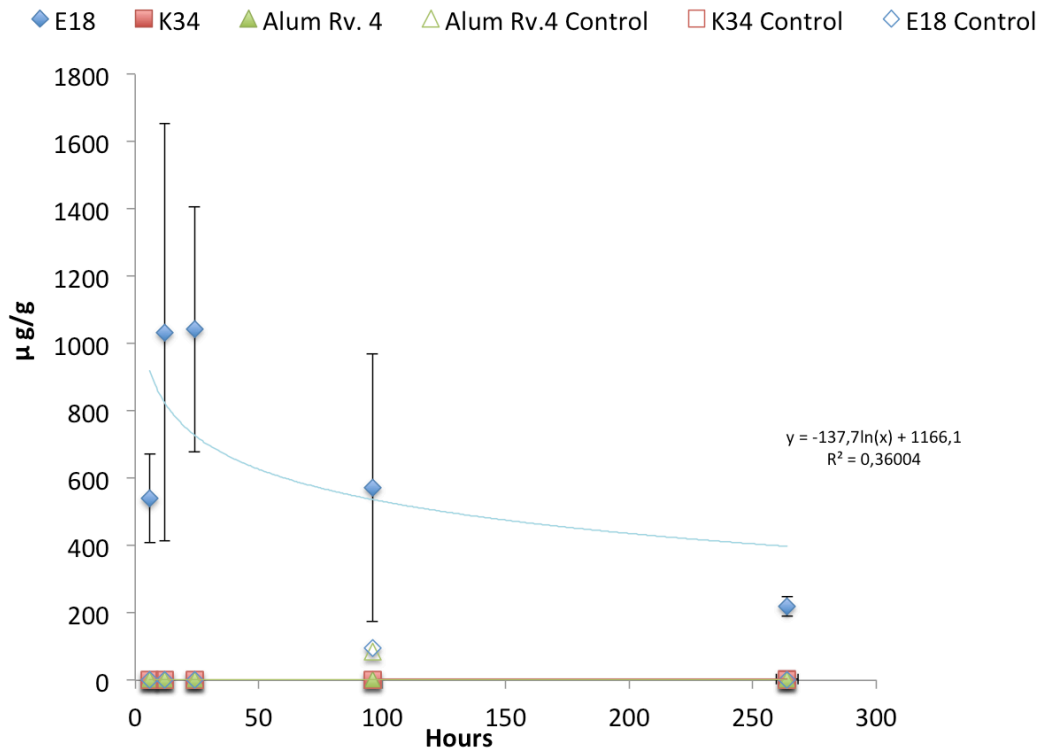

Figure 25 - The average concentrations of aluminium in gills (µg/g dw) in control and fish exposed to leachate from the different rocks. The lines were fitted to the data from E18 G using logarithmic regression analysis. The standard deviations given are the standard deviation of the average concentration of aluminium in the gills.

Aluminium in liver

For the liver analysis the only fish with concentrations of aluminium in the liver above the detection limit for the ICP-MS was the fish from the K34 exposure water with an average concentration of aluminium of 1.81 ± 1.04 µg/g at 12 hours and 2.32 ± 0.86 µg/g at 264 hours.

4.5.3. Effects

Mortality

Three of the fish in the E18 water died between 48 and 96 hours and this is the most significant effect occurring in the exposure experiment. The fact that this water was diluted 1:100 indicates how toxic this kind of water can be to aquatic organisms. The extremely high Al concentration in the gills can explain the mortality, as it is reported that levels of Al higher than 400 µg/g can be lethal to salmonoids (Kroglund et al. 2008).

Blood glucose levels

The other effect endpoint included in this thesis was the level of glucose in the blood, giving an indication of stress response in the fish. The measured glucose in the fish from the 6 different waters is presented in table 18. No glucose was measured at the sampling of the fish at 6 hours.

Based on the control fish, the level of blood glucose decreased from about 5 to 2.4 mM through the exposure period in all control waters. This change in glucose could just be due to increased time of starving or slight increase due to handling stress related to transfer of fish to exposure units. Compared to control fish, the fish from the E18-G exposure water stands out clearly with high glucose levels. The average glucose level in the E18-G fish increased from 5.6 to 20.4 mM after 96 hours of exposure, and this is far above the normal glucose levels in non-fed salmonoids(Kroglund et al. 2008).

Even though the glucose levels for the fish exposed to the shale exposure waters was within the normal range we can not conclude that there are no effects for the fish. The concentration of metals in the waters and the uptake of metals in the fish might be so high that long term/chronic effects can occur and other endpoints affected. This is also

consistent with the concentration of at least cadmium in the K34 water as waters with this concentration of cadmium is classified as bad and might give both acute and long term effects (KLIF 2012)

Table 18 – Glucose levels and mortality of the fish in the exposure experiment. Values marked with red indicate either deaths or glucose above the normal glucose concentration in fish not fed (<5). Values marked with green indicate normal glucose values and no deaths.

		12h	24h	96h	264h
		n=3	n=5	n=5	n=2
E18 G	Glucose mmol/l	5.6±4.5	15.6±3,7	20.4±3,6	16.1±5,1
	Deaths	0	3	0	0
		n=3	n=5	n=5	n=5
E18 G control	Glucose mmol/l	4.3±1,1	6.8±4,2	3.3±0,8	7.0±5
	Deaths	0	0	0	0
		n=3	n=5	n=5	n=5
Rv.4 AS	Glucose mmol/l	4.8±3.4	1.7±0.2	1.5±0.2	2.2±0.3
	Deaths	0	0	0	0
		n=3	n=5	n=5	n=5
Rv.4 AS control	Glucose mmol/l	4.8±1.2	2.2±0.6	2.1±0.4	1.8±0.2
	Deaths	0	0	0	0
		n=3	n=5	n=5	n=5
K34 AS	Glucose mmol/l	5.7±4	2.5±0.3	1.8±0.4	2.1±0.4
	Deaths	0	0	0	0
		n=2	n=5	n=3	n=5
K34 AS control	Glucose mmol/l	5.0±0.3	2.3±0.5	1.8±0.1	2.4±0.2
	Deaths	0	0	0	0

5. Conclusions

The present study demonstrate leaching of radionuclides and metals from shales and sulphur bearing gneiss to water before uptake in fish at levels causing toxic effects.

Results demonstrate the mineral composition of the rock samples plays a major role in the leaching and composition of radionuclides and metals in the leachate. The leaching from the fresh crushed shale samples was dominated by uranium, molybdenum, and cadmium present as LMM species, while aluminium, copper, nickel and manganese dominated in the leachate from the sulphur bearing gneiss.

The pH and major ion concentrations in the leachates were different for the rock samples. The shale samples was characterized by leachate of high concentrations of base cations, combined with high pH (7.5-8.4), while the leachate from the sulphur bearing gneiss was characterized by leachate of low pH (2.8) and low concentrations of most of the base ions, especially Ca^{2+}. The highest concentration of SO_4^{2-} was found in the E18 leachate, indicating sulphuric acid production, probably from the jarosite identified in the rock.

The leachate of uranium increased continually during the seven weeks it was studied, and the leaching was significant for all rocks studied. However, the highest concentration of uranium was from the shales. For fish exposed to the leachates there were a significant uptake of uranium both in the gills and liver. However, for the fish exposed to the 18-G leachate the uptake of uranium in the gills was higher than for the fish exposed to the shale leachates, although the concentration of U in the water was a factor of 350 times lower. This illustrates the importance of understanding underlying mechanisms in transfer. The concentration of uranium in these waters were above the guidelines set by Environment Canada (CCME 2011) for both short time and long time exposure. Stress responses was however not acute in this study.

The leaching of cadmium increased continually during the seven weeks it was studied, and only in the galgeberg shale leachate concentration of cadmium was below the background concentration (SFT 1997). The highest concentration of cadmium was found in the E18-G and the K34-AS. For fish exposed to the leachates there was a significant uptake of cadmium in the gills for the fish exposed to the shale waters. However, no significant uptake was found in the liver of the exposed fish. There was no acute stress response in this study. However, the concentration of dissolved cadmium in the K34-AS water was so high that this water is classified as moderate by KLIF (2012), giving chronic effects with long term exposure.

The E18 gneiss caused very high aluminium concentrations in the leachate, even after 1:100 dilution. The uptake of aluminium on the gills was very high and at levels causing significant increase in blood glucose levels and mortality within 48 hours. The amount of aluminium on the gills observed is also at levels associated with mortality from previous studies. This illustrate the potential toxicity in run-off from such types of rocks, even after significant dilution, are acute toxic. Long-term effects could however not be excluded.

The guidelines set for uranium and cadmium does not take into consideration the water quality parameters like pH and competing ions in the water, which might influence the bioavailability of the radionuclides and metals. This means that there might not be effects in fish exposed to concentrations above the guideline concentrations.

Further work on this subject might include long-term exposure experiments, as it seems likely that the uptake of both cadmium and uranium has not reached equilibrium after 264 hours. In addition it would be interesting to analyse the remaining organs, such as kidneys to look at Cd uptake, and if there was found any difference between exposed fish and control. Further work has already been started on this subject, where a dose-response fish experiment has been performed on leachate from the Rv.4 alum shale, to investigate mixed toxicity of elements leached from the rock. This includes elements that have a protective effect like base cations, and elements with toxic effects. The challenge in this study is to identify which elements have the highest influence on uptake and effects.

Both leaching experiments represent a worst-case scenario, as the water was circulated to keep in contact with the rock samples. This might not be the case in the nature where rainfall passes over the rocks only once. However, in deposits with water surrounding these types of rocks, where there is no circulation of water, these concentrations of radionuclides and metals could occur. This is dependent on the rock to water ratio, and mineral composition of the rocks. In these cases measures needs to be taken to avoid release of these concentrations of radionuclides and metals in to the recipient.

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7. Appendix

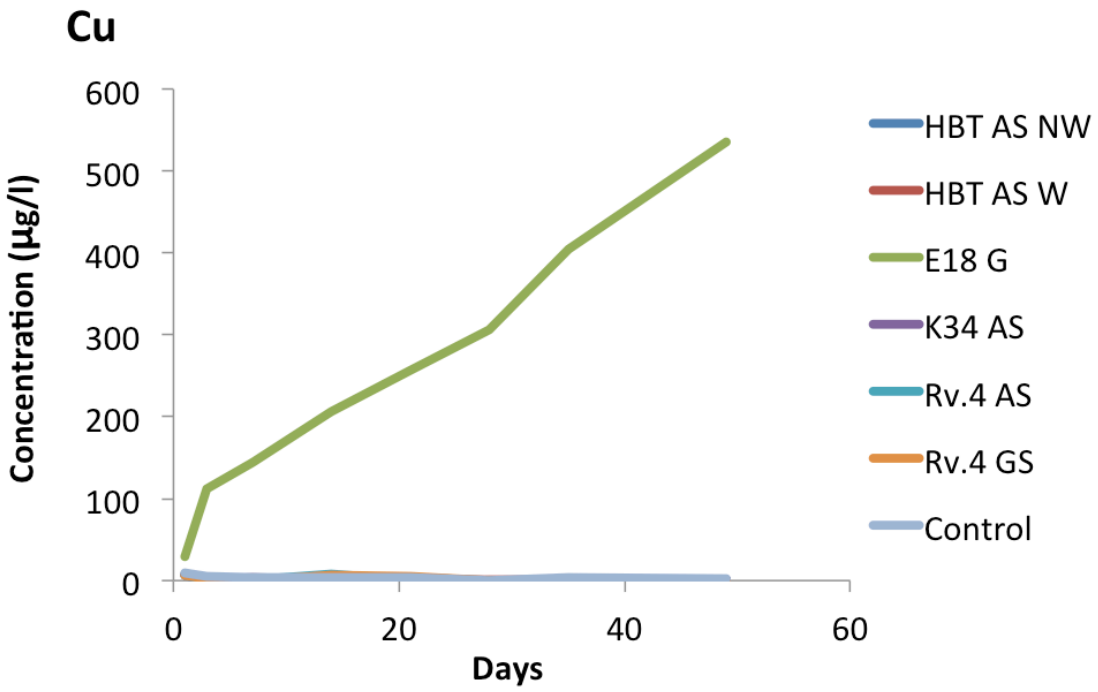


Figure 26 - Pilot leaching experiment. Leaching of copper over time from all six rock samples

As with the aluminium E18-G stood out and leached a substantially bigger amount of copper than the rest of the rock samples, reaching 535 µg/L after seven weeks as seen in fig 26. This concentration is higher than the background concentration of copper in fresh water and waters with this concentration is classified as very strongly contaminated and could induce acute toxic effects in organisms. The next highest copper concentration was leached out from the HBT-AS-W at 0.7 µg/L and the HBT-AS-NW had 0.6 µg/L. Background concentration of copper in Norway is <0.6 according to SFT (1997), so both HBT-AS-W and HBT-AS-NW are just above and on the background concentration. Both K34-AS and Rv.4-GS had 0.04 µg/L copper after seven weeks and the Rv.4-AS leached the least copper with 0.3 µg/L and these three concentrations are within the background concentration range.

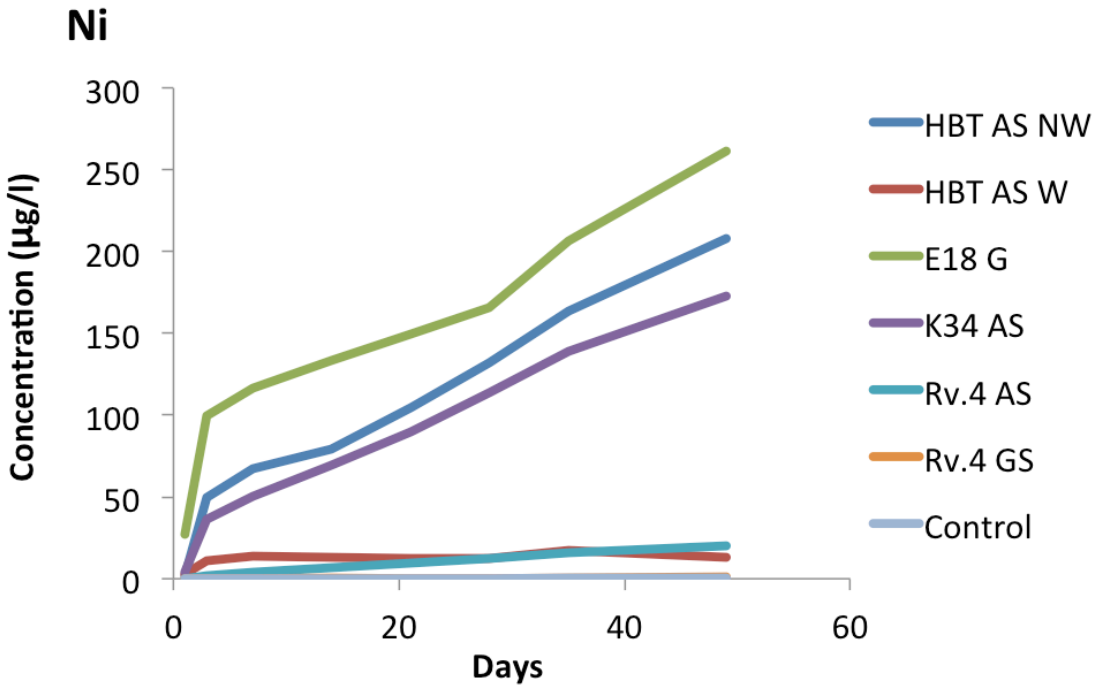


Figure 27 - Pilot leaching experiment. Leaching of nickel over time from all six rock samples

Three of the rock samples leached out the most nickel were E18-G, the HBT AS NW, and the K34-AS as seen in figure 27. E18-G leached the most nickel with the concentration of 261 µg/L after seven weeks. The HBT-AS-W leached 207 µg/L and the K34-AS leached 172 µg/L. The nickel concentration in the Rv.4-AS water was 19 µg/L, in the HBT-AS-NW the concentration was 19 µg/L and in Rv.4-GS the concentration reached 0.8 µg/L. All of these concentrations, except from the Rv.4-GS, gives water with concentrations so high that they are classified as very strongly polluted according to SFT (1997).

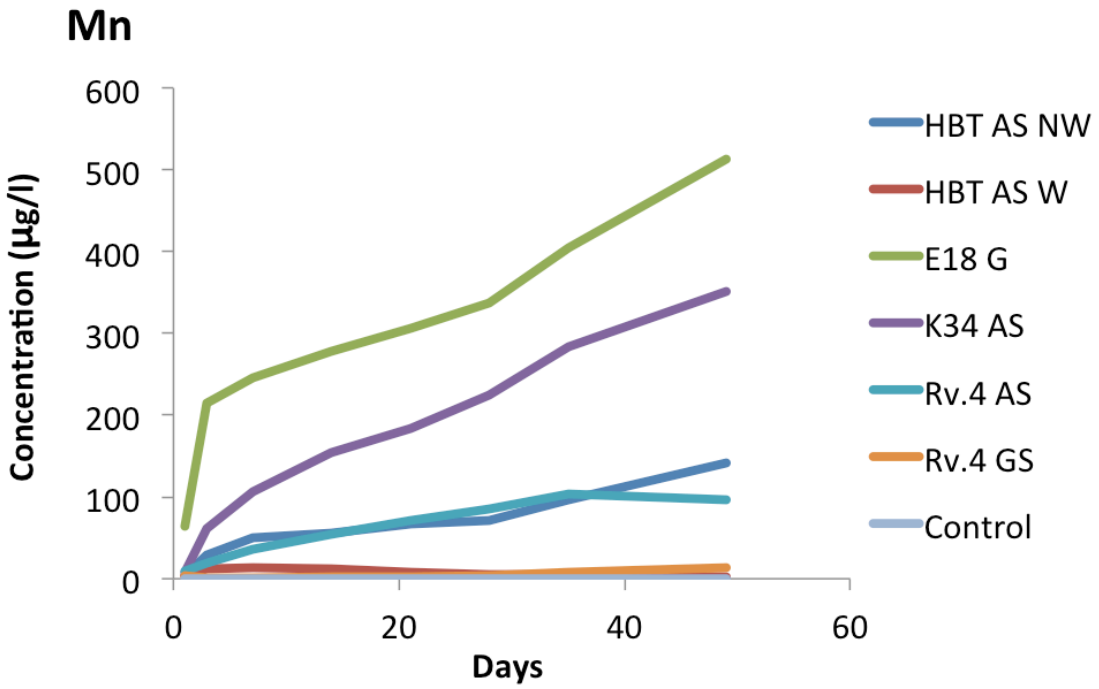


Figure 28 - Pilot leaching experiment. Leaching of manganese over time from all six rock samples

Out of the six rock samples the E18-G leached the most reaching 513 µg/L after seven weeks followed by the K34-AS with 351 µg/L as seen in figure 28. Both the Rv.4-AS and the HBT-AS-NW had quite same leaching pattern throughout the seven weeks, reaching 97 µg/L and 141 µg/L of manganese in the water. The Rv.4-GS leached 13 µg/L and the HBT-AS-W leached 2 µg/L after seven weeks. The control had between <0.12 µg/L and 0.19 µg/L manganese throughout the experiment.

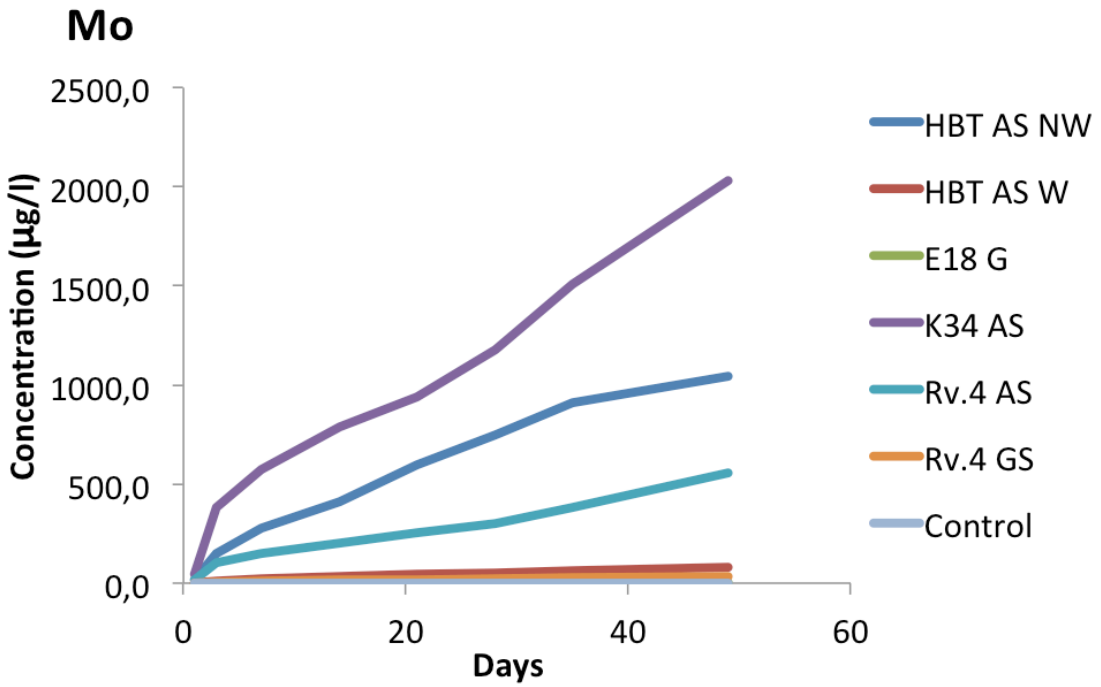


Figure 29 - Pilot leaching experiment. Leaching of molybdenum over time from all six rock samples

Three of the rock samples leached significantly more than the rest, with K34-AS reaching 2032 µg/L, HBT AS W at 1041 µg/L, and Rv.4 AS at 554 µg/L as seen in figure 29. The HBT AS NW leached 77 µg/L, the Rv.4 GS leached 35 µg/L, and the E18-G 4 µg/L. The concentration of molybdenum in the control water was between <0.03 µg/L and 0.04 µg/L throughout the experiment. The concentrations of molybdenum leached from all the rock samples is very high compared to the concentrations of Mo found in surface water in the Oslo area by Reinmann et al. (2009), where the maximum concentration of molybdenum was 15.4 µg/L. The K34-AS and the HBT-AS-W leached such high concentrations that these concentrations are above the limit for release of molybdenum in process wastewater into the environment (*Forskrift om begrensning av forurensning (forurensningsforskriften), Del 7, Kap 28.* 2010).

Table 19 - Average concentration of radionuclides and metals per outtake in the gills of both exposed and control fish. Concentrations measured in µg/g dw

V	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	0,230	0,117	0,129	0,023	0,195	0,055	0,151	0,037	0,343	0,124	0,210	0,057
	12	0,229	0,142	0,287	0,054	0,280	0,118	0,222	0,043	0,185	0,037	0,197	0,108
	24	0,218	0,126	0,216	0,111	0,236	0,125	0,175	0,026	0,450	0,417	0,197	0,089
	96	0,328	0,153	0,222	0,033	0,360	0,185	0,171	0,017	0,244	0,076	0,249	0,057
	264	0,359	0,116	0,237	0,053	0,276	0,079	0,318	0,090	0,242	0,053	0,233	0,066

Mn	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	4,278	0,159	3,826	0,155	4,207	0,670	4,366	0,837	4,750	0,877	4,784	1,581
	12	5,998	0,877	4,664	0,340	5,939	0,970	4,967	0,225	5,025	0,537	5,306	0,703
	24	4,797	0,448	5,158	1,705	4,699	2,160	4,115	0,513	4,601	1,163	4,293	0,538
	96	5,429	0,973	4,492	1,119	5,822	1,117	4,604	0,156	5,091	1,189	4,883	0,464
	264	5,260	0,293	4,120	0,818	5,769	1,624	4,046	0,614	4,816	0,817	4,078	0,494

Fe	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	439,558	105,494	258,219	84,647	295,118	72,833	321,922	10,040	224,330	59,017	277,073	45,661
	12	595,089	328,532	209,535	42,404	169,821	17,326	202,288	49,050	235,211	16,275	212,358	20,275
	24	555,832	108,884	192,405	53,582	174,009	88,295	170,506	48,889	157,589	41,916	230,610	59,005
	96	402,883	219,353	168,402	54,655	235,658	76,141	208,859	28,967	174,671	21,526	181,446	36,796
	264	359,512	61,885	210,627	66,230	218,316	38,136	181,966	44,909	198,736	22,512	181,362	29,576

Ni	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	-	0,000	-	0,000	#DIV/0!	0,000	-	0,000	0,630	0,364	-	0,000
	12	1,613	1,515	-	0,000	#DIV/0!	0,000	2,246	1,297	-	0,000	-	0,000
	24	-	0,000	-	0,000	#DIV/0!	0,000	-	0,000	1,250	0,559	-	0,000
	96	-	0,000	-	0,000	1,239	0,592	-	0,000	1,888	0,717	-	0,000
	264	-	0,000	-	0,000	1,266	0,655	-	0,000	1,685	0,433	-	0,000

Cu	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	4,645	0,484	1,543	0,271	1,648	0,143	1,974	0,037	1,868	0,127	1,805	0,194
	12	8,243	2,603	2,008	0,275	1,832	0,135	1,902	0,147	1,871	0,089	1,736	0,087
	24	9,322	3,325	2,094	0,671	1,655	0,743	1,642	0,115	1,732	0,154	1,920	0,513
	96	6,944	1,229	1,586	0,324	1,791	0,107	1,700	0,076	1,793	0,236	1,734	0,121
	264	6,862	0,863	1,811	0,168	3,752	4,326	1,887	0,140	1,813	0,143	1,910	0,284

Zn	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	345,089	47,860	335,536	16,191	376,598	34,850	388,414	41,875	358,282	70,021	347,323	45,762
	12	372,646	17,039	371,920	33,664	433,045	38,501	399,814	29,521	348,145	67,869	309,847	73,864
	24	405,436	66,252	386,212	53,136	329,023	107,656	366,161	22,036	335,886	113,513	426,172	101,303
	96	405,635	103,038	329,786	88,391	390,392	15,367	462,393	47,077	368,684	132,747	373,812	114,992
	264	366,857	11,624	406,260	26,600	402,161	74,326	372,769	81,667	394,480	72,548	405,095	102,830

As	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	1,411	0,116	1,251	0,252	1,585	0,123	1,495	0,137	1,520	0,025	1,423	0,077
	12	1,712	0,353	1,574	0,065	1,620	0,299	1,599	0,140	1,508	0,134	1,646	0,269
	24	1,705	0,151	1,783	0,405	1,467	0,709	1,563	0,156	1,471	0,198	1,508	0,100
	96	4,048	0,694	1,278	0,140	1,478	0,060	1,369	0,104	1,480	0,104	1,430	0,263
	264	1,512	0,219	1,434	0,105	1,320	0,172	1,514	0,233	1,391	0,085	1,357	0,254

Sr	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	36,683	1,934	40,322	8,968	38,384	2,811	41,974	8,923	42,866	11,433	40,321	6,984
	12	46,314	7,775	47,689	4,937	46,600	4,050	39,696	2,416	42,262	3,358	42,308	2,885
	24	45,730	3,427	44,900	5,893	41,338	18,774	39,988	3,442	40,472	5,808	40,058	4,729
	96	52,142	7,369	44,718	14,459	41,814	4,333	44,076	5,130	38,944	4,518	48,220	7,233
	264	45,901	0,821	39,490	3,743	44,815	3,714	41,533	2,312	42,550	5,421	43,551	5,224

Mo	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	-	0,000	-	0,000	1,734	0,875	-	-	0,793	0,250	-	0,000
	12	0,295	0,171	-	0,000	1,991	0,472	-	-	0,655	0,171	-	0,000
	24	-	0,000	0,320	0,143	1,535	0,760	-	-	0,577	0,103	-	0,000
	96	-	0,000	-	0,000	2,397	0,376	-	-	0,906	0,106	-	0,000
	264	-	0,000	-	0,000	1,909	0,064	-	-	0,915	0,086	-	0,000

Th	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	0,054	0,021	-	0,000	-	0,000	-	0,000	-	0,000	-	0,000
	12	0,121	0,075	-	0,000	-	0,000	-	0,000	-	0,000	-	0,000
	24	0,123	0,043	-	0,000	-	0,000	-	0,000	-	0,000	-	0,000
	96	0,070	0,052	-	0,000	-	0,000	-	0,000	-	0,000	-	0,000
	264	0,017	0,006	-	0,000	-	0,000	-	0,000	-	0,000	-	0,000

Table 20 – Average concentration of radionuclides and metals per outtake in the livers of both exposed and control fish. Concentrations measured in µg/g ww

V	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	0,055	0,021	0,092	0,096	0,046	0,020	0,032	0,014	0,036	0,010	0,053	0,030
	12	0,060	0,057	0,070	0,017	0,057	0,022	0,053	0,017	0,044	0,012	0,042	0,036
	24	0,050	0,029	0,050	0,025	0,048	0,021	0,048	0,023	0,061	0,019	0,051	0,033
	96	0,107	0,026	0,118	0,076	0,083	0,027	0,065	0,007	0,084	0,019	0,079	0,014
	264	0,067	0,016	0,131	0,072	0,083	0,036	0,096	0,041	0,072	0,027	0,127	0,057

Mn	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	1,454	0,081	1,598	0,353	1,416	0,130	1,322	0,181	1,287	0,027	1,212	0,192
	12	1,746	0,169	1,466	0,135	1,431	0,107	1,524	0,149	1,410	0,196	1,105	0,058
	24	1,682	0,341	1,383	0,170	1,226	0,104	1,238	0,122	1,182	0,135	1,351	0,610
	96	2,988	0,300	2,333	0,159	2,576	0,119	2,249	0,125	2,612	0,302	2,373	0,245
	264	0,594	0,007	2,335	0,161	0,458	0,055	1,478	1,077	0,715	0,085	2,652	0,432

Fe	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	44,997	7,870	58,521	10,490	51,406	1,688	42,954	8,012	39,428	3,451	38,435	4,300
	12	47,790	8,563	44,448	13,371	43,037	8,232	52,570	11,428	51,407	11,081	44,864	8,692
	24	57,723	8,445	45,228	7,587	46,377	8,917	47,047	16,537	49,581	14,623	51,717	23,473
	96	89,659	12,471	110,284	40,084	117,045	30,892	124,887	12,542	103,947	23,252	107,409	18,955
	264	27,003	2,206	130,500	25,103	12,502	2,971	84,295	84,746	26,106	6,295	129,756	22,121

Ni	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	-	0,000	-	0,000	-	0,000	-	0,000	-	0,000	-	0,000
	12	-	0,000	-	0,000	0,082	0,047	-	0,000	-	0,000	-	0,000
	24	-	0,000	-	0,000	0,030	0,014	-	0,000	-	0,000	-	0,000
	96	0,094	0,028	0,052	0,013	0,088	0,017	0,064	0,023	0,067	0,019	0,067	0,019
	264	-	0,000	0,054	0,029	-	0,000	0,060	0,035	0,042	0,007	0,042	0,008

Cu	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	19,338	7,384	10,684	3,587	20,367	6,517	9,273	0,900	14,233	5,089	14,953	2,203
	12	20,490	8,086	18,437	13,257	11,887	4,007	22,127	11,220	18,098	0,487	11,086	8,553
	24	21,387	7,297	13,199	7,821	34,840	12,644	17,455	4,435	21,668	6,134	18,312	8,949
	96	104,628	37,303	114,538	21,967	103,136	70,733	144,723	49,954	103,149	50,157	108,586	45,532
	264	1,250	0,442	141,648	69,808	0,453	0,313	34,336	53,115	1,375	0,519	151,812	33,993

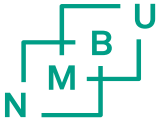
Zn	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	33,083	1,165	33,189	5,702	31,929	1,800	28,357	0,886	31,300	3,141	31,300	3,141
	12	35,321	2,848	29,295	1,771	32,299	2,451	33,907	1,093	29,849	1,800	29,849	1,800
	24	39,456	4,155	30,133	2,489	24,053	2,575	30,361	1,653	28,093	2,133	28,932	12,978
	96	56,602	4,952	54,600	2,052	51,359	2,605	53,431	3,183	54,131	3,002	54,131	3,002
	264	29,156	3,307	52,579	5,239	32,668	1,410	38,345	10,017	30,832	3,022	30,832	3,479

As	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	1,111	0,069	1,114	0,025	1,181	0,189	1,106	0,005	1,030	0,130	1,061	0,213
	12	1,793	1,122	1,109	0,118	1,239	0,220	1,453	0,022	1,148	0,112	1,102	0,124
	24	1,715	0,353	1,324	0,404	0,789	0,126	0,917	0,087	0,899	0,150	1,005	0,461
	96	6,761	1,103	1,550	0,129	1,600	0,165	1,405	0,174	1,648	0,246	1,415	0,195
	264	0,167	0,004	1,372	0,024	0,101	0,025	0,627	0,610	0,170	0,019	1,334	0,168

Sr	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	0,086	0,033	0,078	0,007	0,082	0,006	0,114	0,058	0,074	0,014	0,084	0,013
	12	0,087	0,055	0,070	0,008	0,070	0,013	0,092	0,027	0,087	0,026	0,065	0,002
	24	0,085	0,038	0,070	0,013	0,109	0,070	0,070	0,012	0,091	0,037	0,067	0,032
	96	0,098	0,020	0,110	0,010	0,270	0,187	0,155	0,056	0,176	0,048	0,133	0,028
	264	0,094	0,005	0,094	0,005	1,665	0,820	0,137	0,040	0,148	0,016	0,113	0,016

Mo	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	0,203	0,028	0,201	0,059	0,238	0,012	0,180	0,017	0,211	0,012	0,200	0,031
	12	0,291	0,038	0,259	0,017	0,373	0,059	0,300	0,040	0,297	0,026	0,257	0,005
	24	0,328	0,027	0,263	0,031	0,411	0,032	0,267	0,027	0,338	0,055	0,257	0,118
	96	0,673	0,061	0,676	0,056	1,099	0,098	0,640	0,065	0,890	0,085	0,690	0,078
	264	0,476	0,108	0,704	0,088	1,185	0,103	0,630	0,099	0,721	0,079	0,780	0,086

Th	Hours	E18G	STDV	E18G Control	STDV	K34AS	STDV	K34AS Control	STDV	Rv.4 AS	STDV	Rv.4 AS Control	STDV
	6	-	0,000	-	0,000	-	0,000	-	0,000	-	0,000	-	0,000
	12	-	0,000	-	0,000	-	0,000	-	0,000	-	0,000	-	0,000
	24	-	0,000	-	0,000	-	0,000	-	0,000	-	0,000	-	0,000
	96	-	0,000	-	0,000	-	0,000	-	0,000	-	0,000	-	0,000
	264	-	0,000	-	0,000	-	0,000	-	0,000	0,001	0,000	-	0,000



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