Trace elements in different organs of Atlantic Salmon (Salmo Salar L.) from The River Storelva catchment area.

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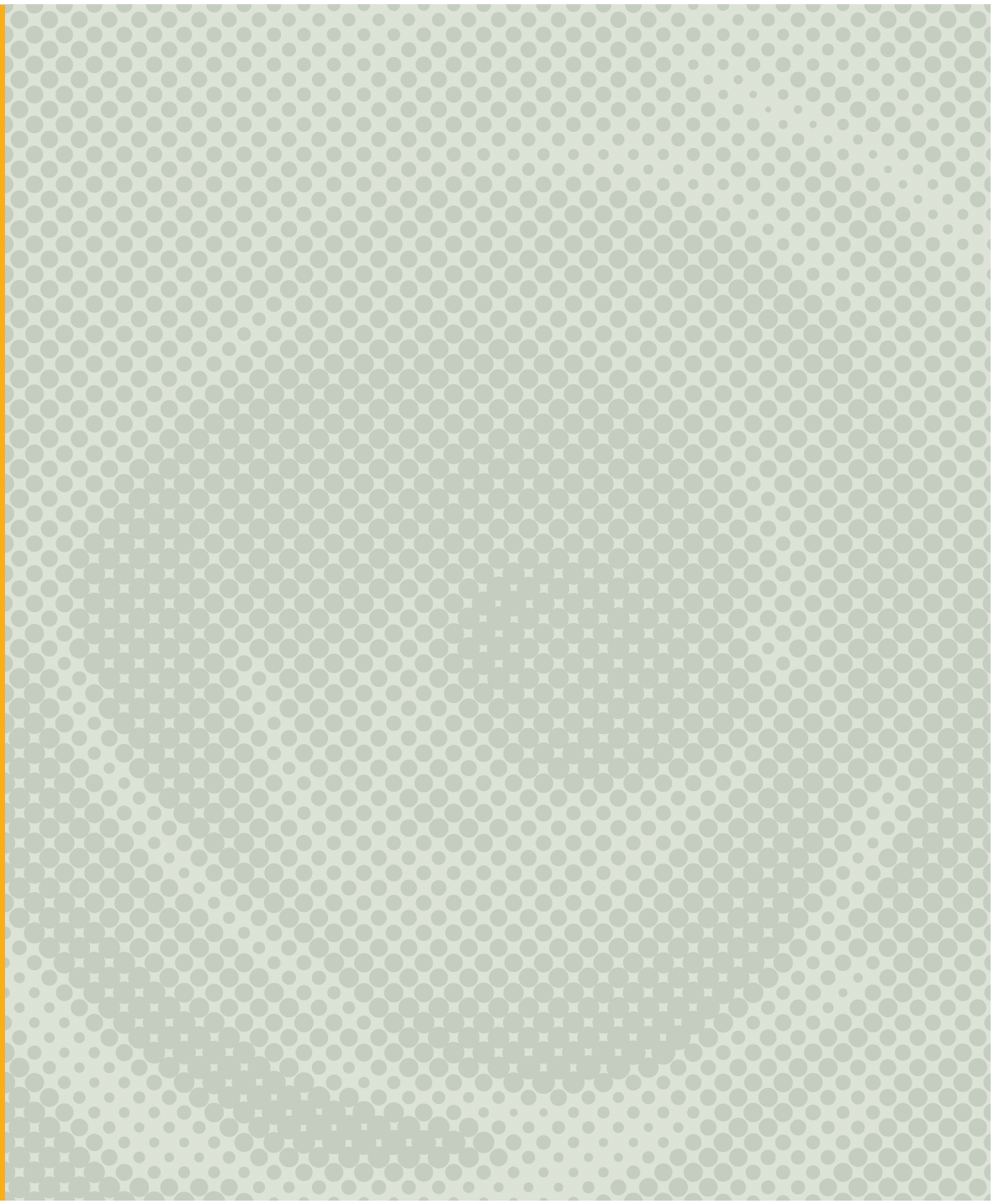

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Preface

This Master thesis represents the end of two years studying General Ecology at the Department of Ecology and Natural Resource Management (INA) at the Norwegian University of Life Sciences (UMB), Norway. The work has been performed within the framework of the Storelva project between UMB, The Norwegian Institute for Water Research (NIVA) and The Norwegian Institute of Nature, with a main focus on Al in brackishwater and its influence on the migration of Atlantic salmon *(Salmo salar L.)* smolts.

I am very thankful for the opportunity to participate in this exciting study and for the great experience that I have gained.

I particularly would like to thank my supervisors Professor Bjørn Olav Rosseland, Professor Brit Salbu (Head of the Isotope Laboratory) and Post Doctor Hans-Christian Teien for their support and comments through the whole period of working in the field, laboratory and writing the thesis. Without all their concern and help, I would not have been able to make it.

I also would like to thank Frode Kroglund from NIVA for his patience and interesting discussions on the boat during the field trip to Sandnesfjord. I would like to thank the research assistant from the Department of Plant and Environmental Scinces (IPM) Merethe Kleiven who helped me during my field work and taught me new techiques in the laboratory, and the graduated Master student Moja Skalsbakken for being that friendly and helpful. Moreover, I would like to send my thanks to the engineer Tove Lovtaas and the pHD students Gjermund Strømman and Lene Sørlie Heier as they guided me through my lab work.

Last but not least, I thank my family for their encouragement during my studies and what is more, for investing their money savings in my education but not in a new car.

List of contents

Summary

In the catchment of River Storelva, situated in the South-East Norway , there have earlier been a mining industry, reflecting a geology rich in metals which might impose a metal stress to the Atlantic salmon. Due to the acidification, an increased trace metal load could be expected, although, the river has been limed for several years. Therefore, the levels of trace metal concentrations (Al, Cd, Cu, Zn, Pb, As, Ni, Cr, Mn, Co, Ti and Cs) in gills, livers and kidneys of Atlantic salmon smolts from River Storelva were investigated in the present study.

The main goal of the present study was to determine the concentrations of metals in the investigated fish organs.

A second goal was to determine the trace metal concentrations in the water where the fish lived prior to dissection to see if this concentration reflected the concentration in the organs.

A third goal was to study whether these metal concentrations in the smolts could be used to estimate in which part of the river the smolts have been raised, as we hypothesised that differences in age and size could occur due to differences in exposure to metals, imposing a stress which potentially could affect the growth, and thus smolt age.

The fish was caught in a smolt trap at the river mouth and placed in cages in different localities along the river and the fjord system one week prior to dissection. The fish was placed in three different types of water : freshwater (fw), brackishwater (bw, salinity < 10‰) and seawater (sw, salinity > 10‰), and dissected at site in the field. The sampling followed an international protocol. The smolts of Atlantic salmon were divided into three different groups of fish : 3 years old fish (16.1cm ± 0.44), 2 years old large fish (14.8cm ± 1.1), and 2 years old small fish (12.1cm ± 0.6).

Water fractionation techniques were applied in the field and the water samples that were fractioned were collected for further analysis in the laboratory at UMB.

The collected organs after dissection were analysed at the Isotope Laboratory at IPM with ICP-MS and the water fractions by using ICP-MS and ICP-OES.

There was a variation between the concentrations of heavy metals within the three types of water. The results obtained with ICP-OES showed higher Al concentration in the brackishwater.

The results obtained with ICP-MC analysis showed that the metal concentrations in fw did not vary significantly.

There was also a variation between the concentrations of heavy metals in the analysed organs within the three groups of fish. The gills accumulated higher levels of metals than the livers and the kidneys. There were significant differences between the concentration of metals accumulated in the gills from the different types of water, and the variation in gill metals was dependent mostly upon the water quality at site, but not upon the age of the fish. This illustrated the link between "water quality, the bioavailability of metals and uptake in fish". However, there were no significant differences found between the concentration of heavy metals accumulated in the livers and kidneys, and the concentration in fresh, brackish or seawater.

There was a positive correlation between the fish age and size, but the size of 3 years old fish overlapped with the size of 2 years old large fish. Based on these data it is, however, difficult to judge if this difference could be attributed to metals exposure, imposing a stress which potentially could affect the growth, and thus smolt age.

One of the most interesting results referred to the concentration of Zn and Pb in the livers, and Zn and Mn in the kidneys. The concentration of Zn was highest in 3 years old fish in bw and sw, and also, the concentration of Pb. This also referred to Mn. As these heavy metals must have bioaccumulated while being in the freshwater, this gave a support to the hypothesis that a metal exposed Atlantic salmon would grow slower and reach the smolt size one year later than smolt in better water quality.

1. Introduction

Salmonidae are indigenous to the temperate or cool northern hemispheric regions of Eurasia and North America, with several species now introduced to the southern hemisphere. Their representatives rank among the most prized of sport fish, and are equally valued in the commercial fisheries and aquaculture arenas. Many are diadromous, leaving the oceans to re-enter their natal rivers or streams in autumn or spring to lay eggs. Some species, including members of each genera, spend their entire life cycles in freshwater, but may still undertake a spawning migration. These latter species are classified as potamodromous, while the former are known as anadromous *(Finn, 2007).*

Atlantic salmon is an anadromous species from the Salmonidae family which is endemic to the Norwegian fauna.

Acidification is an environmental problem that has been a concern of many scientists for many years. In his brief history of research *Gorham (1998)* states that our scientific knowledge of ecosystem acidification goes back at least to the mid-18th century, when Home (1757) applied the concept of acids, bases and neutral salts to soils. Acid rain is a serious environmental problem in large areas of Europe and North America *(Ulgenes and Torrissen, 1989).* It is commonly acknowledged that anthropogenic acidification has affected Atlantic salmon populations on both sides of the Atlantic Ocean *(Haines and Akielaszek, 1984).* Acidification is one of several environmental factors affecting Atlantic salmon populations in Norway *(Kroglund and Finstad, 2003),* and the effects of acidification on fish resources in Norway were probably first noticed for Atlantic salmon, in rivers in southern Norway, as rod catches showed a pronounced decline before the end of the nineteenth century *(Hesthagen and Larsen, 2003).* The low water pH and the high aluminum concentrations occurring in many river systems have aggravated problems for salmonid fish. As a result several populations of salmon and trout are extinct in southern Norway *(Ulgenes and Torrissen, 1989).* Furthermore, acid water has led to the extinction or severe population reductions in 30 rivers in Southern Norway *(Sandøy and Langåker, 2001)* and is regarded as the most likely cause for, or as a contributor to, population reductions in several other rivers *(Rosseland et al., 2007).* The fish population in many rivers in the Agder counties in southern Norway suffered huge loss in fish populations as a consequence of acidification *(Sandøy and Langåker, 2001).* The fish population in Lake Vegår in Vegårsvassdraget (ending in River Storelva) was also affected but it was assumed that there still was some left of the original salmon population in the lower part of River Storelva *(Kaste, 1994).*

River Storelva is one of the rivers in Norway that has had for several years problems with acidification, as well as, with the migration rates of fish which are believed to be a consequence of Al mobilization.

According to *Rosseland et al. (1992)* the mixing zone that occurs where an acidic tributary enters a limed river can be highly toxic to fish. Toxic mixing zones are characterised by a rapid increase in pH and thereby hydrolysis and polymerisation of Al from the acid tributary. The toxicity is most likely to be caused by Al precipitation onto the fish gills, leading to problems related to osmoregulation *(Moiseenko et al., 2006).* The effects of toxic mixing zones on the fish in its natural environment will be dependent on the ability of the fish to avoid these areas *(Åtland and Barlaup, 1995).* Estuarine and coastal areas may represent increased risk to the survival of migrating post-smolts. During their seaward migration, salmonid smolts go through a transitory high-risk life stage and increased salinity subjecting the smolts to physiological (osmotic) stress *(Koed et al., 2006).*

As a part of the collaboration project between UMB, NIVA and NINA, I investigated the levels of accumulation of metals and heavy metals in gills, livers and kidneys of wild Atlantic salmon smolts from the acidified River Storelva catchment area. On entering the estuarine areas, Atlantic salmon can be exposed to a new toxic environment such as organic and colloidal Al (nontoxic in freshwater) that can be mobilised in an estuarine mixing zone *(Bjerknes et al., 2003).*
As mining activity has been performed within the catchment of River Storelva, the geology area is assumed to be enriched in metals. Due to acidification, increased mobilization from soils to run-off water can be expected. Although, the river has been limed for several years the metals load could be significant. Therefore, the objectives of the preset work were to :

- determine the concentrations of metals in the investigated fish organs.
- determine the trace metal concentrations in the water where the fish lived prior to dissection to see if this concentration reflected the concentration in the organs, and
- study whether the metal concentrations in the smolts could be used to estimate in which part of the river the smolts have been raised.

The main hypotheses of the present study were that : 1) metal exposed Atlantic salmon will have to use longer time for reaching smolt size, 2) a 3 years old smolt or a 2 years old small smolt will have a higher metal accumulation in the liver and the kidney, 3) 2 years old smolt will have less metal accumulation and better fitness, and 4) gill accumulation relates to the “present water quality”, and the three groups of smolt would have the same concentration in the same environment.

The field trip was conducted in May, 2009, when the field work in the river catchment area and in the fjord was performed, while the analysis of the collected samples were done in the period - September 2009 - March 2010. By having done analyses of accumulation of trace metals in the fresh, brackish and seawater, I tried to establish a link "water quality – uptake in fish". In addition, I tried to study whether these metals in the organs of fish being exposed to such water qualities were linked to their freshwater history, or the water quality in which they were exposed during the experiment.

2. Heavy metals and major pollutants

2.1 Heavy metals

Although metals are usually considered as pollutants, it is important to recognize that they are natural substances *(Walker and Hopkin, 2006).* Metals differ from other toxic substances in that they are neither created, nor destroyed by humans. Owing to their toxicity persistence and tendency to accumulate in water and sediment, heavy metals and metalloids, when occurring in higher concentrations, can in sufficient concentrations be a hazard for all living organisms *(Castro-Gonzales and Mendez Armenta, 2008).*

Contamination of aquatic ecosystems (e.g., lakes, rivers, streams, etc.) with heavy metals has been receiving increased worldwide attention due to their harmful effects on human health and other organisms in the environment. The main sources of heavy metals in aquatic ecosystems are of the anthropogenic type. Metals after entering the water may precipitate or adsorb on the surface of solids, remain soluble or suspended in it or may be taken up by fauna and flora. One of the most important properties of a toxic pollutant is its ability to accumulate in the tissues of organisms. Over a long period, the pollutants present in the environment at very low levels may accumulate within the body of aquatic species by various mechanisms to the extent that they exert toxic effects. Therefore, it is of great importance to know the bioaccumulation potential of a pollutant *(Palaniappan and Karthikeyan, 2009).*

Heavy metals are one of the most common forms of anthropogenic pollution in the marine environment. Due to variation in local metal inputs and in the biochemical processes operating in

different areas, environmental metal concentrations can vary both spatially and temporally *(Sauer and Watabe, 1989).*

Trace metals are widely distributed and have multiple natural (e.g., degassing of the earth's crust) and anthropogenic inputs (e.g., fossil fuel burning, mining) into the environment (*Dehn et al., 2006).* Trace metals are generally considered as inorganic toxicants. Many trace elements are essential for life (e.g. Cu and Zn), but are all toxic in excess *(Sloman, 2007).* Trace elements in water can be present in a range of different physico-chemical forms, i.e metals species (different size and charge properties, oxidation state, valences) ranging from simple ions, hydrolysis products, colloids or particles *(Salbu and Oughton, 1995).* In addition, elements can be found as cationic, anionic or associated to complex organic molecules, e.g. humic substances *(Heier et al., 2009).*

Toxic effects of metals on organisms depend on the following factors : concentration of metals in water, the speciation of metals variables of environment conditions, ionic structure, pH-value, type of ground, water flowing, etc., and such factors as properties of metals, species of organism, its age, physiological state and type of feeding *(Spry and Wiener, 1991).*

In streams and rivers, the concentrations of toxic metals usually change during the year. At these latitudes, the important factors are snowmelt in the spring and periods with heavy rainfall throughout the year. For shorter periods, i.e. hours or days, metals may reach levels that cause physiological stress and even kill organisms *(Zachariassen et al., 2000).*
In the Norwegian small arms training areas, the deposition of metals in 2006 was estimated to be approximately 103, 73, 12 and 7 t of lead (Pb), copper (Cu), zink (Zn) and antimony (Sb), respectively *(Heier, 2009).* Pb, Cu and Sb are considered to be of major concern, due to the amounts released and the potential negative effect on living organisms. Of the total Norwegian lead emissions in 2005 (total 240 t), ammunition was the major source (66%) followed by fishing equipment (24%),while for instance industrial deposits only constituted 3%. In addition, approximately 200 t of Pb has been deposited as a result of atmospheric long-range transport from other countries in Europe. However, the contribution from long-range transport has been reduced since the late 1970s, and is today only 10% of the 1977 level. The total Norwegian emissions of Cu (2005) was 1067 t, where fish farming net impregnation represented the greatest

source (54%) followed by antifouling preparations (26%). Both Pb and Cu are on the Norwegian Pollution Control Authority priority list of chemicals to be reduced before 2010. Sb and Zn, however, are not considered as key pollutants. Information on Sb is also relatively scarce *(Heier et al., 2009).*

Fish can be exposed to trace metals via two exposure routes, waterborne and dietary. In freshwater, metals can be taken up by a fish directly from its external media, largely across the gills but also across boundaries such as the olfactory system. Uptake can also occur as a result of eating a contaminated diet where metal ions are taken up across the intestine. Toxicity of trace metals to salmonid fish is strongly dependant upon water chemistry, route of exposure and life stage, often making it difficult to compare toxicity values between literature sources *(Sloman, 2007).*

In teleost fish, the gills, liver, kidney and muscles are the tissues most frequently utilized in bioaccumulation studies *(Sauer and Watabe, 1989).* Moreover, in fish the most vulnerable organ to acute exposures is thought to be the gills. As a first defense strategy the fish may try to avoid metal accumulation by secreting gill mucus that binds and immobilizes metals outside the organism *(Rosseland and Staurnes, 1994).* Liver and kidney are vulnerable organs during prolonged metal exposures, both from waterborne and dietary sources. Toxic metals will inevitably cross the boundary layers and enter the organism. Prolonged metal exposure may result in fish that have acquired tolerance to metal toxicity both on physiological and evolutionary time-scales. On a short-term scale, acquired tolerance has been shown for Cd, Cu and Zn. A short pre-exposure to sublethal concentrations of these metals can confer protection against subsequent exposures in freshwater fish *(Zachariassen et al., 2000).*

Salmonids are regarded as "indicator species" due to their sensitivity to water quality and they are well established "model" organisms in aquatic research. In addition, there are commercial interests in salmonid species. Juvenile salmon inhabiting estuarine areas have been shown to have high levels of contamination in regions close to human habitation *(McCain, 1998).* Fish, in comparison with invertebrates, are more sensitive to many toxicants and are a convenient test subject for indication of ecosystem health *(Moiseenko et al., 2008).* Fish gills accumulate bioavailable trace elements, and measurement of metal accumulation on gills can reflect the

speciation of metals in water, and is therefore a useful tool for assessing bioavailability of elements in water *(Heier et al., 2009).*

Atlantic salmon migrates from freshwater to seawater as smolts. Smolts are preadapted to full strength seawater and will leave freshwater within the short time-period called the ‘‘smolt-window’’, a time-period when the fish are both physiologically and ecologically prepared for the high-salinity environment *(Kroglund and Finstad, 2003).* Smoltification in Atlantic salmon involves a series of morphological, physiological, and behavioural transformations required for saltwater adaptation and migration *(Johnston et al., 2004).* This life stage is in the wild only present in spring, a time period when water quality can change rapidly due to snow melt and/or to changes related to acid rainfall and to seasalt episodes *(Rosseland et al., 2007).* Body size is a key factor in determining whether a fish will become a smolt. The process is under complex environmental and endogenous hormonal control *(Johnston et al., 2004),* and smoltification in salmonids results in major changes in endocrine status *(Vieira et al., 2005).* The proximal environmental cue for smoltification is the lengthening of day length in the spring following a period of short days. In the wild, smoltification usually occurs after 1 to 5 years in freshwater *(Johnston et al., 2004).* Salmonids are known to avoid unfavourably cold or warm water, and the preferred temperature for young salmonids ranges from 11 to 14°C *(Åtland and Barlaup, 1995).* Many species during the early life stages are found to be particularly vulnerable to low pH *(Kitamura and Ikuta, 2000).* The youngest stages (eggs, fry and fingerlings) seem to be the most sensitive towards several environmental pollution factors. This is also true for low pH and for high aluminum concentrations *(Ulgenes and Torrissen, 1989).* Death is associated with loss of plasma sodium and chloride, but also severe hampered gas exchange in the gills as a result of extreme mucus production are reported to be a consequence of low environmental pH and elevated aluminum levels *(Ulgenes and Torrissen, 1989).* Adult fish may avoid even weakly acidified environments in selection of spawning site where the offspring develop during their early life stages, this appears to be especially in salmonids, whose spawns spend relatively long durations at the spawning redds *(Kitamura and Ikuta, 2000).*

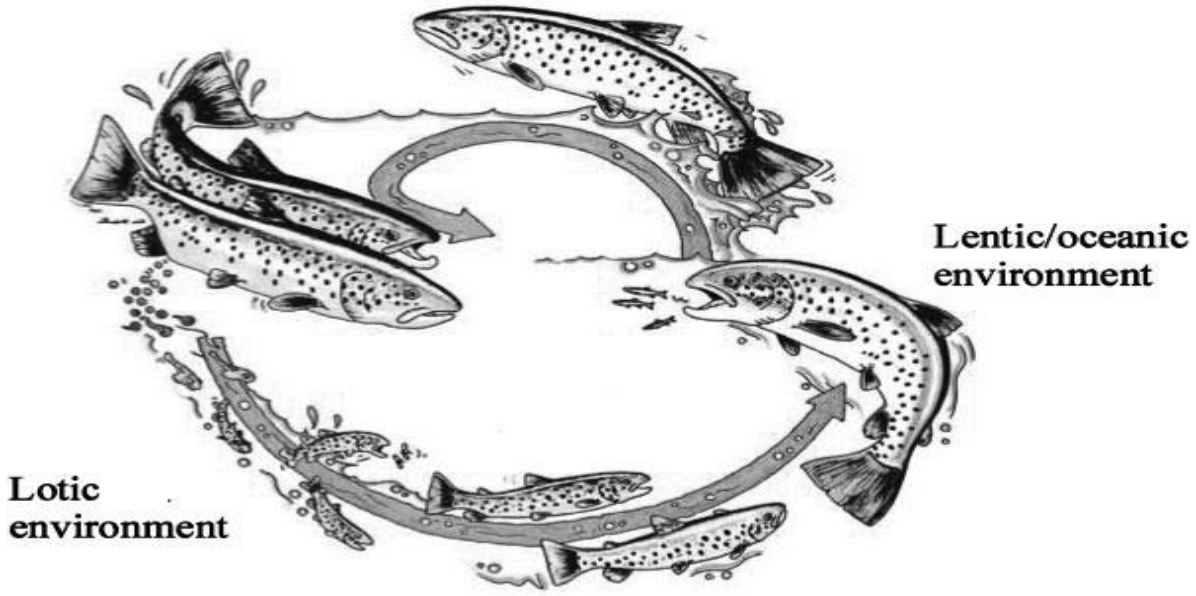

Figure 1. A general view of the life cycle of a salmonid fish. Individuals of sea- or lake-run populations migrate out of their natal rivers to mature in oceanic/lentic environments, and finally return to lotic environments to spawn. Stream resident populations spend their entire life in lotic environments *(Husko et al., 2007).*

2.2 Metals of study

2.2.1 Aluminum (Al)

It is commonly known that the fish populations in Norway have been affected by acid rain in the late 90-100 years *(Sandøy and Langåker, 2001).*

Acidification is the major environmental problem of Norwegian freshwaters, and a recent study concluded that the fish populations were affected within an area of 86 000 km ^{2} *(Åtland and Barlaup, 1995).* High mortality of fish located in nets in fish farms has occurred due to Al in fjord systems *(Bjerknes et al, 2003).*

According to *Walker and Hopkin (2006)* Al is an extremely important pollutant in acidified lakes, where it becomes soluble and is toxic to fauna. The deposition of acid rain has resulted in the acidification of weekly buffered surface waters in many areas, including Scandinavia, eastern Canada, and the northeastern USA. Al is toxic towards fish due to deposition on gills causing stress and even mortality. Al mobilization can occur in fjords where rivers enter coastal water, influencing the migration of smolts. During episodic acidification, Al leaches from the soil and

elevates Al levels of the surrounding watershed. In addition, the solubility of Al increases as a direct result of decreased pH leading to elevated levels of inorganic Al (Ali), the form of Al that is most toxic to fish *(Monette et al., 2008).* According to *Driscoll and Kimberley (1996) and Salbu and Oughton (1995),* Al in aquatic systems can be present in different physico-chemical forms, varying from ions and molecules to high molecular mass species such as colloids and particles. In acid freshwater systems high in organic material Al can be associated with humic substances. Colloidal humic substances and low molecular mass fulvic acids act as transporting agents for trace metals such as Al, and the concentration of colloidal Al can be high in rivers during runoff events due to high erosion and resuspension of sediments. When acid humic rich river water enters estuaries the increase in pH and ionic strenght may affect the Al speciation and the toxicity towards fish. In acidified water dissolved Al is predominantly present as cationic Al-species that is toxic towards fish *(Teien et al., 2006).* Low-molecular mass (LMM) inorganic forms of Al, often referred to as inorganic monomeric Al, are believed to be the most important Al-species causing Al-toxicity in fish *(Heibo et al., 1997).* In order to obtain information on toxic Al species in natural waters, fractionation techniques are needed *(Salbu and Oughton, 1995; Teien et al., 2004).*

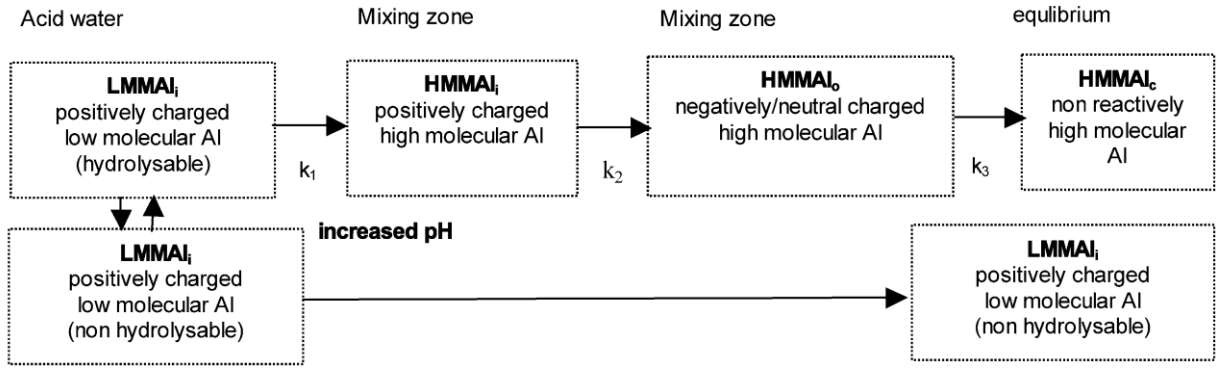

Figure 2. Model describing the transformation process of LMM Ali in the acidic water following an increase in pH *(Teien et al., 2004).*

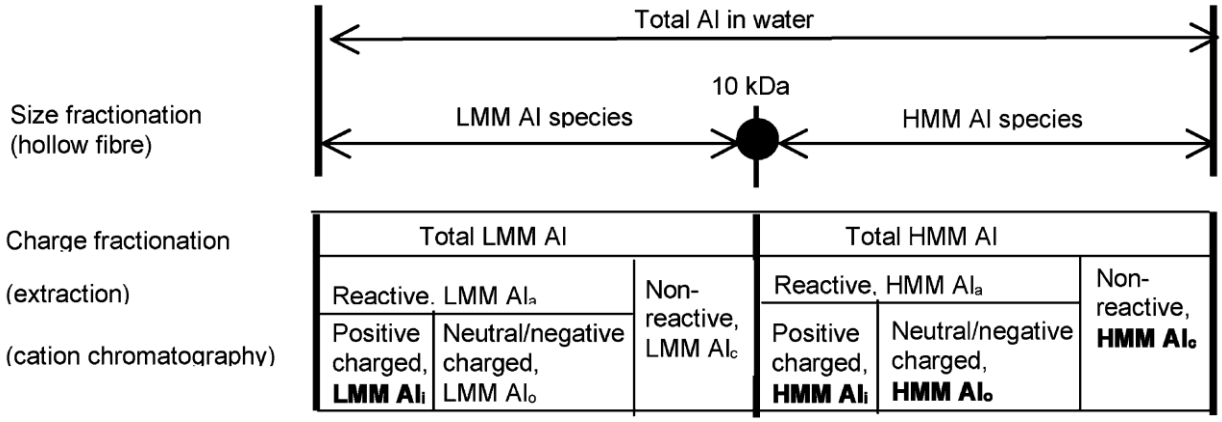

Figure 3. Al fractions obtained using in situ hollow fibre ultra filtration interfaced with cation chromatography and liquids extraction. Hollow fibre with nominal cut-off 10 kDa separates LMM and HMM Al species, liquids extraction with HQ and MIBK separate reactive Al (Ala) and non-reactive Al (Alc), and cation chromatography using Amberlite IR-120 resin, separates positively charged Al species (Ali) and neutralynegatively charged Al species (Alo) *(Teien et al., 2004)*.

Anadromous fishes such as Atlantic salmon may be particularly vulnerable to ion regulatory disturbance resulting from acid Al exposure, as they face severe ion regulatory challenges when migrating from fw to sw *(Monette et al., 2008).* The period from fertilization and embryonic development through to hatching and the alevin stage is very sensitive to acidic soft water for most salmonids, although the smoltification stage is the most sensitive in Atlantic salmon *(Rosseland and Skogheim, 1986).*

The effects of Al for fish, however, seem to differ between species and life stages. Sea trout *(Salmo trutta L.)* seems to have higher resistance than for example Atlantic salmon. Salmon is one of the fish species that's sensitive towards Al-effects in the water systems. When it comes to life stages, parr (salmon) seems to be relatively sensitive for the water quality, but, the smolts seem to be especially sensitive during the migration to the sea *(Kroglund et al., 2007).* Comparative studies of various salmonid fish species have clearly demonstrated that Atlantic salmon, apart from rainbow trout *(Oncorhynchus mykiss),* is the most sensitive salmonid *(Heibo et al., 1997).* Chronic (year-round) acidification and its associated Al toxicity is a cause of Atlantic salmon population decline in Norway. The changing physiology of the Atlantic salmon smolt makes it one of the most sensitive of the salmon life-stages to acid Al *(Rosseland and Skogheim, 1984).* Exposure to acid Al during smolting can disrupt ion regulatory ability in fw,

reduce sw tolerance, decrease growth and impair downstream migration and marine survival *(Rosseland and Staurnes, 1994).*

2.2.2 Cadmium (Cd)

Cd is a widely distributed heavy metal, toxic to terrestrial and aquatic organisms *(Brodeur et al., 1998).* Cd is a metal from group II B that has an atomic weight of 112.41; the ionic form of cadmium (Cd^{2+}) is usually combined with ionic forms of oxygen (cadmium oxide, CdO_2), chlorine (cadmium chloride, $CdCl_2$), or sulfur (cadmium sulfate, $CdSO_4$). There are estimates that 30.000 tons of Cd are released into the environment each year, with an estimated 4000-13.000 tons coming from human activities. Natural, as well as, anthropogenic sources of Cd, which include industrial emissions and the application of fertilizer and sewage sludge to farm land, increased cadmium environmental levels. It has been established that, although Cd occurs in the aquatic organisms and marine environment only in trace concentrations, the salinity can affect the speciation of this metal, and bioaccumulation is affected both by temperature and salinity *(Castro-Gonzales and Mendez-Armenta, 2008).* Contamination of fresh water systems by Cd from industrial and mining activities has been recognized as a global environmental problem *(Hontela et al., 1996).* The occurrence and fate of Cd in the environment has been extensively reviewed. Pollution of aquatic habitats by this metal is therefore a subject of great concern as it possesses no known biological functions *(Teles et al., 2004).*

Cd is toxic to fish, and temperature, pH and water hardness are factors that influence its toxicity. In fish, uptake of Cd occurs across the gastrointestinal tract and gills where it involves Ca^{2+} channels *(Brodeur et al., 1998).* Teleosts are highly sensitive to Cd and markers of toxicity include premature hatching, decreased growth rates and the induction of developmental abnormalities such as cyclopia and spinal lordosis. Recent advances in Cd research have demonstrated that this metal also possesses an endocrine disrupting capacity in teleosts. Coupled with these observations are *in vitro* experiments which provide evidence that endocrine disruption by Cd may be mediated at the molecular level through direct effects on gene transcription *(Teles et al., 2004).* Sublethal doses of Cd cause histological changes in kidney, gills, liver and the gastrointestinal tract of fish, and anemia, osmo-ionic disturbances, hypocalcemia and vertebral deformities are some of the physiological problems of fish exposed to Cd. Abnormal plasma glucose and muscle glycogen levels have also been documented in fish

exposed to Cd in the laboratory or in the field. These fish may grow less and their reproductive competence, including the synthesis of sex steroids, may be impaired *(Hontela et al., 1996).* An exposure of fish to Cd can lead to: a disturbance of ion and water balance; anemia; impaired synthesis of sex steroids; and vertebral deformities *(Brodeur et al., 1998).*

Natural food as well as commercial fish feed may contain significant levels of Cd. Thus the intestine represents an important uptake route for Cd in fish. The gut, kidneys, liver and gills are the organs where toxic actions of Cd first become manifest when it is administrated via food. Dietary Cd can enter the branchial epithelium from the blood compartment and it has been suggested that it is excreted via the gills. Whatever the original source, progressive accumulation of Cd in the gills can induce structural damage to the gills and, as a consequence, plasma ion homeostasis may be disturbed *(Dang et al., 2001).*

2.2.3 Copper (Cu)

Cu is an essential element in the normal metabolism of both plants and animals and a significant portion of the Cu found in both fresh and marine systems may be taken up by the biota. Cu is a micronutrient added to salmon feeds at 1-4g Cu/kg dry feed *(Brooks and Mahnken, 2005).* Though, Cu is one of the more toxic heavy metals and appears in the aquatic environment from both natural sources and anthropogenic origins, such as mine washings and direct application as an algicide and molluscide *(Beaumont et al., 1995).* While Cu is an essential trace element in fish metabolism, its presence in the aquatic environment at relatively low concentrations is known to be harmful. Toxicity effects have been noted in studies of various behavioural patterns, including avoidance, temperature selection, locomotion, feeding refusal, chemosensory ability, and cough frequency *(Owen, 1982).* Impairment of osmoregulation, metabolism, swimming performance, growth, reproduction, disease resistance and development has also been attributed to undesirable levels of Cu *(Spear and Pierce, 1979).* The uptake and toxicity of Cu depend upon its speciation in the environment, the species of fish involved and their stage of development. The influence of Cu on the smolting of coho salmon *(Oncorhynchus kisutch),* and on the immune responses of various species, has been studied (*Peterson et al., 1991).* Various studies have demonstrated that Cu tissue accumulation in fish can be associated with adverse physiological responses *(Lipton et al., 1996).* Metallothionein (MT) induction has been related both to Cu accumulation and growth reductions. This had led to the use of MT as a biomarker of Cu exposure and/or adverse effects.

Alternatively, tissue residues have been used as a means of determining metals exposure associated with adverse response measurements in field-collected fish *(Lipton et al., 1996).* For example, the same authors found elevated tissue concentrations of metals in field-collected trout to be associated with tissue abnormalities and cellular damage. Using Cu tissue residues to predict reduced growth responses could offer advantages over exposure concentrations, because the metabolic demands associated with detoxifying or acclimating to Cu may be more directly related to internal accumulation of Cu. Yet, relationships between tissue Cu and adverse responses have not been adequately quantified. However, a recent toxicokinetic study of Cu in rainbow trout suggested that long-term waterborne Cu exposure resulting in continued Cu accumulation in slowly exchangeable pools should not result in toxicological consequences *(Carbonell and Tarazona, 1994).*

2.2.4 Zinc (Zn)

Zn is an essential metal important for insulin structure and function, and as a co-factor of carbonic anhydrase. It is added to salmon feeds in trace amounts equal to 30–100 mg/kg of feed *(Anderson, 1998).* On the other hand, Zn is one of the most important environmental toxicants, yet also performs essential roles in a wide range of biological processes. Zn pollution rarely occurs in isolation. Aquatic species faced with elevated Zn often must also contend with elevated concentrations of metals such as Cd and Cu *(Glover and Hogstrand, 2003).* Of the metals responding to environmental acidification, Al and Zn are among those that consistently show the most obvious increases in concentration *(Nelson and Campbell, 1991).* This geochemical response is noted both in chronically acidified lakes and streams, and in streams subject to episodic pH depressions. Elevated concentrations of Zn, have been detected in acidified watersheds in Ontario, Scandinavia and in the Adirondacks. Al and Zn concentrations also increase in experimentally acidified lakes (*Roy and Campbell, 1995).*

At neutral pH, the toxicity of mixtures of metals is generally additive, with each metal contributing proportionally, depending on its concentration, to the toxicity of the mixture. As the pH is lowered, the toxicity of metals tested individually tends to decrease but little is known about the effects of acidification on the toxicity of metal mixtures. In a notable exception to this generalization, *Roy and Campbell (1995)* reported that a mixture of Zn, Al and 5 other metals

severely affected the reproduction of flagfish *(Jordanella floridae)* at pH 5.8. Trials with different combinations of metals suggested that Al and Zn were primarily responsible for the observed effects. Similarly, the same authors recently demonstrated that metal concentrations typical of acidified lakes on the Canadian Shield may be lethal to acid-sensitive species, such as the fathead minnow *(Pimepholes promelas).*

2.2.5 Lead (Pb)

Pb is a naturally occurring element; it is a member of Group 14 (IVA) of the periodic table, has an atomic weight of 207.2 and exists in three states: Pb (0), the metal; Pb (II); and Pb (IV). Pb is a blush-gray heavy metal and it is usually found combined with two or more other elements to form lead compounds. Pb is one of the most ubiquitous and useful metals known to humans and it is detectable in practically all phases of the inert environment and in all biological systems. Environmental levels of Pb have increased more than 1000-fold over the past three centuries as a result of human activity; the greatest increase occurred between the years 1950 and 2000 *(Castro-Gonzalez and Mendez-Armenta, 2008).*

2.2.6 Arsenic (As)

The semimetal As is one of the most hazardous substances released in the aquatic environment as a result of both geogenic and anthropogenic processes. In nature As can exist in the inorganic and organic form and the inorganic form has been found to be more toxic *(Datta et al., 2009).* As, a naturally occurring element, is a worldwide contaminant that is found in rock, soil, water, air and food. It has a complex chemical structure and can be found in elemental, trivalent (+3 arsenite), and pentavalent (+5 arsenate) inorganic forms and trivalent and pentavalent organic forms. Organic As is formed when arsenic ions are combined with carbon and hydrogen. Inorganic As is present in groundwater, which is used for drinking in several countries all over the world; whereas organic arsenic compounds are primarily found in fish and shellfish. As is transported in blood by binding to red blood cells and it is distributed throughout the body; once absorbed, arsenites are oxidized to arsenates and methylated. The As (+3) form undergoes enzymic methylation primarily in the liver to form monomethylarsinic acid (MMA) and

dimethylarsinic acid (DMA); this process may then be repeated to result in dimethylated arsenic metabolites *(Castro-Gonzalez and Mendez-Armenta, 2008).*

2.2.7 Nickel (Ni)

Ni is a ubiquitous trace metal and occurs in soil, water, air, and in the biosphere. It is emitted into the environment from both natural and man-made sources. Once released to the environment, Ni readily forms complexes with many ligands, making it more mobile than most heavy metals. The primary sources of Ni emissions into the ambient air are the combustion of coal and oil for heat or power generation, nickel mining, steel manufacture, and miscellaneous sources, such as cement manufacturing. While Ni is an essential element at low concentrations for many organisms (it plays important roles in the biology in microorganisms and plants), it is toxic at higher concentrations *(Clark and Keasling, 2002).* Ni, though essential in trace quantities, is highly harmful to the survival and productivity of aquatic fauna, and in higher concentrations affects populations of commercially important food fishes, both marine and freshwater. It has a high affinity to low molecular weight amino acids and DNA molecules. The most sensitive life stage for fish regarding Ni exposure is the embryonic stadium, because it affects hatching progress and causes a delay in hatching and increased mortality in embryos *(Sreedevi et al., 1992).* Numerous studies have confirmed the carcinogenic potency of Ni compounds in experimental animals *(Palaniappan and Karthikeyan, 2009).* As for most metals, it is also documented that the toxicity of Ni decreases as the hardness increases *(Lydersen et al., 2002).*

2.2.8 Chromium (Cr)

Cr is a relatively common element in nature and at the trivalent form Cr (III) it is as an essential element in mammals by maintaining efficient glucose, lipid, and protein metabolism. The hexavalent form, Cr (VI), is toxic to organisms due to the oxidative power of these compounds *(Lydersen et al., 2002).* Cr compounds are frequently encountered as environmental pollutants and have been known to produce toxic, mutagenic, and carcinogenic effects in biological systems, although Cr is an essential nutrient *(Palaniappan and Karthikeyan, 2009).* Plating and electroplating factories, leather tanneries, textile manufacturing facilities, cooling tower blow down, rinse waters, steel producing factories, etc. are most often the anthropogenic sources.

Relatively little is known about the relation between concentration of total Cr in an aquatic environment and biological effects. However, the toxicity of Cr to aquatic biota is significantly influenced by abiotic variable such as hardness, temperature, pH, salinity, and biotic factors such as species, population variations, and life stage *(Lydersen et al., 2002).*

Studies with perfused gills showed that the transfer of Cr was directly coupled with the transfer of oxygen from the external solution to the internal perfusion medium, and that this transfer was significantly more rapid at pH 6.5 than at higher pH *(Van der Putte and Pärt, 1982*). The accumulation of Cr seems to be highest in gill, liver, kidney, and digestive tracts, after the transfer of rainbow trout to Cr-free media. The most sensitive stadium for fish regarding hexavalent Cr exposure seems to be the yolk-sac fry and swim-up fry stages *(Grande and Andersen, 1983).*

2.2.9 Manganese (Mn)

Mn has three oxidation states of importance to aquatic chemistry, Mn (II), Mn (III), and Mn (IV) *(Lydersen et al., 2002).* High concentrations of Mn in surface waters are normally present in high TOC and/or low pH lakes *(Borg, 1987).*

2.2.10 Cobalt (Co)

Beside other metals such as Ni, Cd or Cr for which the genotoxic and carcinogenic properties have been extensively studied, Co and Sb have received relatively less attention, probably because their commercial and industrial applications are more limited *(Boeck et al., 2003).*

Co is considered a biological essential element, which occurs in trace concentrations in plants, animals and microorganisms *(Kunze et al., 1978)* since it is required in vitamin B12 formation *(Diamond et al., 1992).* Co is widely distributed but usually occurs in trace amounts (0.001-0.002% of earth's crust) *(Diamond et al., 1992).* Radionuclides of the element are known to appear in waste water resulting from activities of nuclear technology *(Kunze et al., 1978).* As a consequence of the emission of nuclear power plants, the uptake and accumulation of such nuclides by aquatic flora and fauna are of great interest. Animal tissues contain traces of Co and chromium. Exogenous cobalt chloride has been reported to concentrate in the pancreatic tissue of several fish *(Hertz et al., 1989).*

2.2.11 Titanium (Ti)

As the ninth most abundant element in the Earth's crust, Ti is a ubiquitous constituent of rocks, soils, and sediments. Titanium-bearing phases are chemically refractory during weathering; consequently, only very low concentrations of dissolved Ti are released into natural waters *(Skrabal, 1995).*

Literature about Ti and its accumulation in fish is very limited. Most of it that one can find is about titanium nanoparticles, titanium oxide, etc.

The acid waste water from titanium dioxide (TiO_2) producing industries form, when neutralized in the receiving water body, a fluffy, reddish brown precipitate. This precipitate is known to cause respiratory disturbances in fish by mechanical blockage of the gill epithelium. Such a mechanical action of the precipitate formed on the gills, was suggested as the main reason for the marked effects on the ion balance, the hematology and the carbohydrate metabolism in exposed fish and for the impaired capacity to manage in a rotatory flow test. In addition, some of the noted sublethal physiological responses in the exposed fish seem to be attributable to a generally decreased physiological condition due to the exposure *(Klingsted et al., 1984).*

2.2.12 Cesium (Cs)

Cs was originally discovered in a Bavarian mineral spring by Bunsen and Kirchhoff in 1860. However, until the advent of nuclear power, Cs attracted little interest as the stable isotope, ^{33}Cs, is the rarest of the alkali metals, has little economic value and to date no essential biological role of Cs has been elucidated although trace quantities of stable caesium do occur in most living organisms *(Avery, 1996).* ^{137}Cesium has been introduced into the environment by global fallout resulting from atmospheric nuclear weapon testing during the 1950s and 1960s and from nuclear accidents such as Chernobyl. Concerns associated with this fallout stem from four properties of the isotope: (a) high fission yield, (b) moderately long halflife (30 years), (c) high solubility and potential mobility in the marine environment, (d) high biological availability due to metabolic similarity with potassium. Hence, ^{137}Cs remains one of the most problematic of the radionuclides in terms of radioactive waste management *(Sawidis et al., 2003).* Contamination of marine waters by radionuclides is a major concern in coastal areas which receive radioactive inputs from industries, accidents and fallout from nuclear weapon testing. Surveys estimating concentrations of such radionuclides in water or sediments are often complemented by biomonitoring programs,

and marine mussels have been used as biological monitors for radionuclides and heavy metals *(Bustamante et al., 2006).* The radionucleide ^{137}Cs has been the major contributor to contamination of the marine environment after the reactor accident at Chernobyl, and it is also the main long-lived component of radioactive fallout which has arisen from nuclear weapon tests *(Topcuoğu, 2001).*

In the natural environment, fish take up radionuclide from water and food. After leaving the contaminated area, fish eventually lose much of their radioactivity. A study on the elimination of ^{137}Cs from fish is needed to gather data for the assessment of the expected levels of contamination to predict the time required for fish to be adequately free from contamination through biological elimination and physical decay. Accurate prediction of ^{137}Cs retention is important in gauging potential health effects of environmental releases and for estimating food consumption by free ranging fish *(Malek et al., 2004).*

3. Area description

The study was conducted in River Storelva and Sandnesfjord (58° 42' 0 N 9° 13' 0 E). The river is located South-East of Norway and has its source from Lake Vegår, and descends into the fjord between the towns Risør and Tvedestrand, a few miles east of Arendal. Lake Vegår is located in the municipality of Vegårshei (58º 48' 27″ N 8 º 51' 29″ E) in Aust-Agder county and it has a total catchment area of about 141km² with a maximum depth of 102m.

The Southern Norway lake-district comprises the 4 counties Telemark, Aust-Agder, Vest Agder and Rogaland. This lake-district receives high levels of acid deposition and many of the lakes have lost their trout populations due to acidification. The 23 lakes in the district used for the regional MAGIC simulations have been sampled annually since 1986 as part of the Norwegian Monitoring Programme for Long-Range Transported Polluted Air and Precipitation. Most of the lakes are acid sensitive due to thin and patchy soils derived from granites and gneiss *(Patrick, 2003)*

In addition, according to *Kaste (1994)* many rivers in Southern Norway were affected by acid rain in the 1980's, and fish populations in many rivers were strongly reduced.

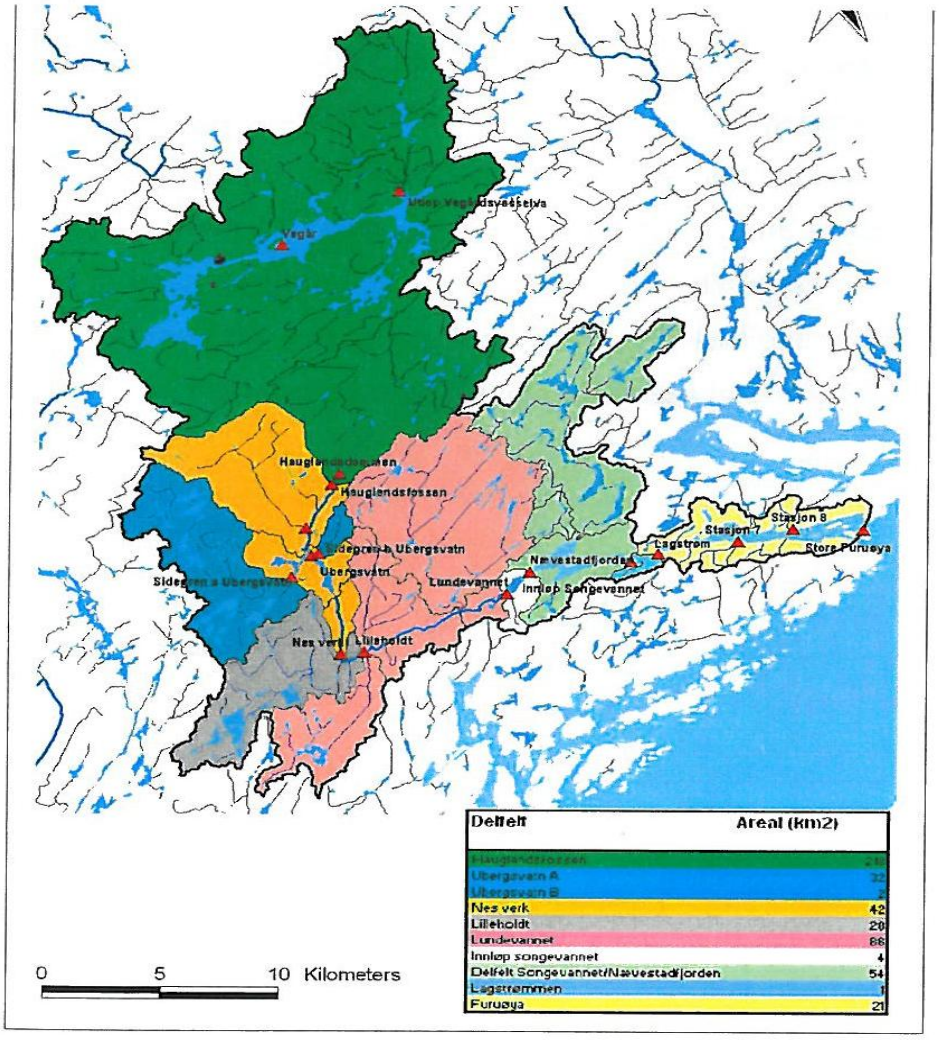

Figure 4. Map over Vegårvassdraget (*Kroglund et al., 2007*).

4. Materials and Methods

4.1 Sampling by stations

The sampling of Atlantic salmon smolts was performed during two periods – from May 4-th to May 10-th, 2009, and from May 24-th to May 25-th, 2009. All the dissected fish was caught in a smolt trap at the river mouth and placed one week prior to dissection in different localities along the water system. Drums of washing machines were used as cages to keep the fish inside. Moreover, in the river system there were three stations where fish was placed, and in the fjord there was a set up of 15 stations with different salinity (bw < 10 ‰ and sw > 10 ‰), and depths

of 0,5m ; 2m and 4m. During the whole study the fish were not fed. The total amount of dissected fish was 285.

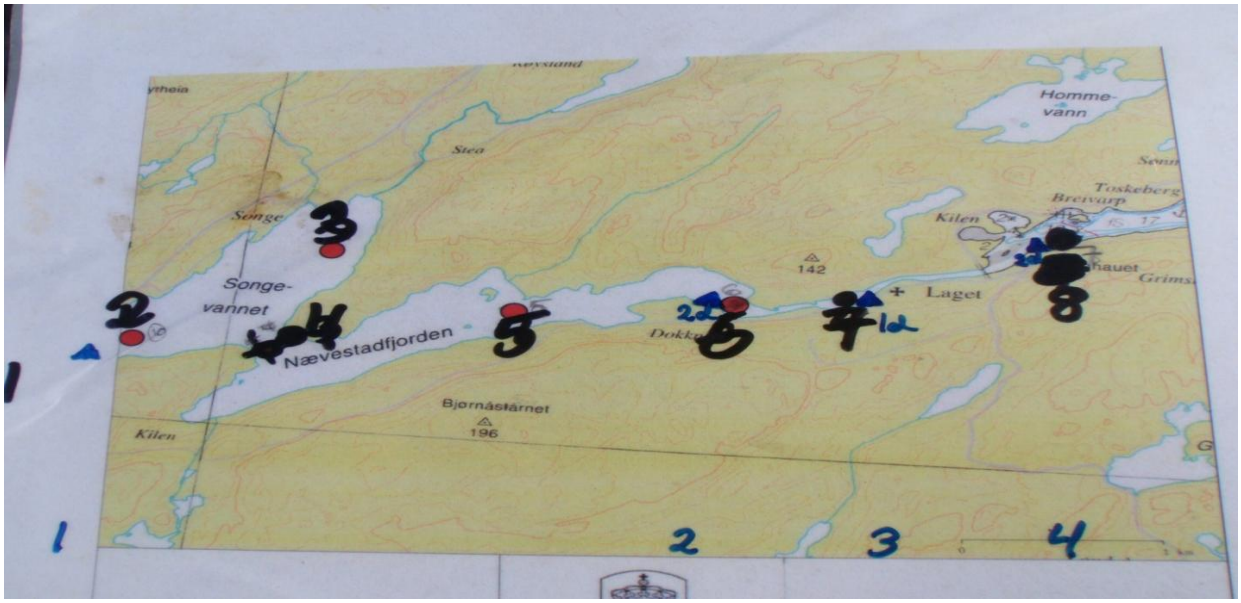

Figure 5. Map showing the stations in the fjord system (inner part of the fjord, bw) *(Photo Vesela Yancheva).*

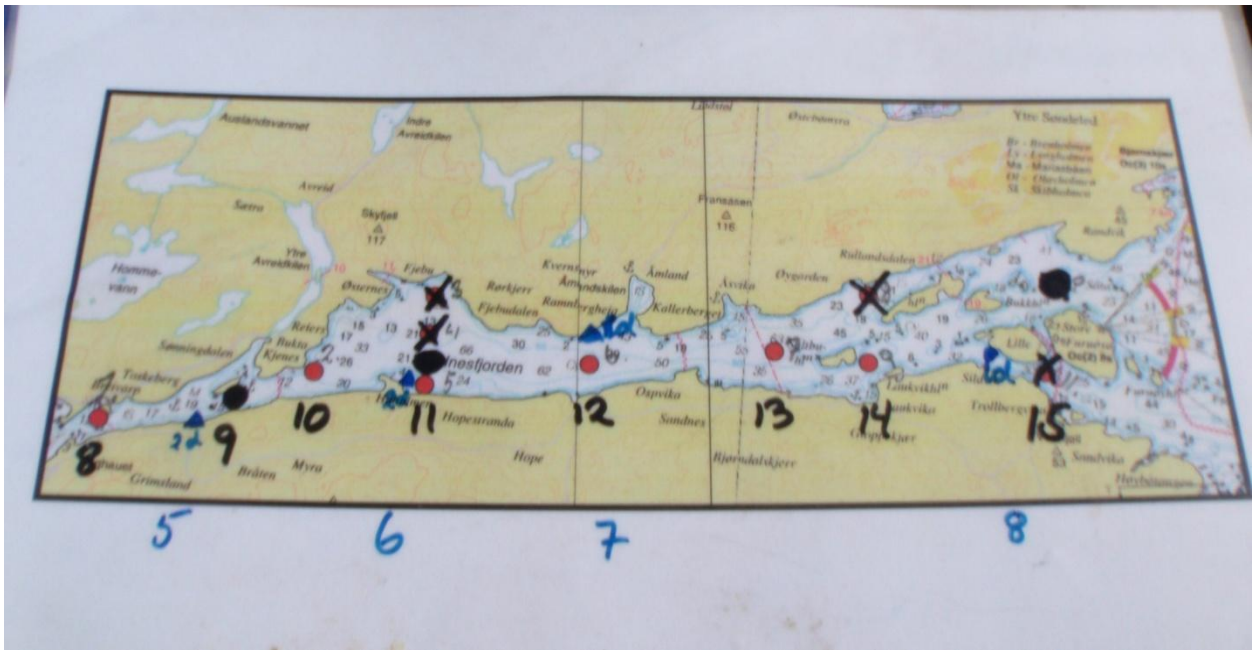

Figure 6. Map showing the stations in the fjord system (outer part of the fjord, sw) – continued *(Photo Vesela Yancheva).*

4.2 Fish sampling

All fish samples were collected according to the EMERGE sampling protocol of live fish procedure *(Rosseland et al., 2003)* for determination of metal accumulation. The dissection of fish was performed in an open laboratory. Prior to dissection, the fish were sacrificed with a single sharp hit to the head. Blood was sampled from the caudal vein using a syringe and directly analysed for plasma ion parameters by an I-STAT portable analyser with a cassette. Then the fish were dissected as firstly, the gills were taken for accumulated trace metals analysis and secondly, the liver and kidney. Lastly, dorsal muscle tissues that were closest to the fish tail were also taken for Persistent Organic Pollutatnts (POPs), mercury (Hg) and isotopes analyses. Clean stainless steel scalpel blades, scissors and tweezers were used. Before dissecting, preweighted marked vials for storing the gills, aluminium foil pieces to wrap the organs and tissues with, envelopes on which all the required data was marked such as : fish number (every single fish had its own individual number), weight, length, area, date, etc., and plastic bags for each sample were prepared. The organs and the tissues were wrapped in the foil with the shiny side towards them, put in the plastic bags and stored on ice. The gill arch was put in the preweighted marked vials and stored on ice before bringing all samples back to the freezer where they were kept until the preparation for analyses began. Scales from the mucus were used for age determination, and were placed in the marked envelopes. The weight of the fish was measured to the nearest gram using a scale and the length was measured to the nearest millimeter from the fork to the tail using a ruler, and recorder for each individual. Photos of all dissected fish were taken.

Figure 7. A killed smolt prior to dissection from Sandnesfjord *(photo Vesela Yancheva).*

Figure 8. Collecting fish samples on the boat in Sandnesfjorden *(photo Hans-Christian Teien).*

4.3 Age determination

The fish age was read by scale analysis. The scales of teleost fish consist of an upper, highly calcified, osseous layer, and a lower, collagenous, fibrillary plate. The sequential formation of scale surface features has led to the wide spread use of scales for purposes for age determination *(Sauer and Watabe, 1989).* The scales were taken during the fish dissection procedure in the field work and stored in the envelopes that were prepeared in advance. The scales were dried by the time the age determination analysis began. By taking small dried flat pieces of the fish skin, a microscope for age determination in the laboratory at INA at UMB was used.

Figure 9. Scale of Atlantic salmon *(Photo Craig A. Tinus and Russell W Brown, US National Oceanic and Atmospheric Administration)*

4. 4 Water Chemistry

4. 4.1 Collecting water samples

During the field work, water samples were fractioned *in situ*. The water samples were taken from the different stations in fresh, brackish and seawater where the fish was placed. Using fractionation techniques, the changes in metal species in the water with respect to size (molecular mass) and reactivity (charge properties) were followed.

To obtain information on the size distribution of metal species, 0.45μm membrane filters (Millipore, d: 45 mm) and 10 kDa ultrafiltration membrane (Amicon H1P10-20 hollow fibre operating at 10–15 psi) were used. Thus, metal species in waters were separated into three different size classes: particles ($\geq$ 0.45μm), high molecular mass (HMM) fraction, such as colloids ($\leq$ 0.45μm and $\geq$ 10 kDa), low molecular mass (LMM) fraction ($\leq$ 10 kDa) *(Teien et al., 2004).*

pH, temperature, salinity and conductivity (μS/cm) were recorded, simultaneously, using a pH-meter.

4.4.2 Water chemistry analysis

To obtain information about major cations (Ca, K, Mg, Na, Si), ICP-OES was used, and to obtain information on major anions (Cl, NO_3, SO_4, Br, F) – ion chromatography. Total Organic Carbon

(TOC) was measured with TOC-analyzer. ICP-MS was used to investigate the levels of trace metals in bw and sw. However, the salinity was too high and the obtained results could not be used. This analysis was performed twice but eventually, the results were still not satisfactory. Therefore, ICP-OES (Perkin-Elmer) was used to determine the levels of accumulation of trace metals in the bw and the sw. ICP-MS (Perkin-Elmer) was used to determine the levels of accumulation of trace metals in the fw and the dissected fish organs.

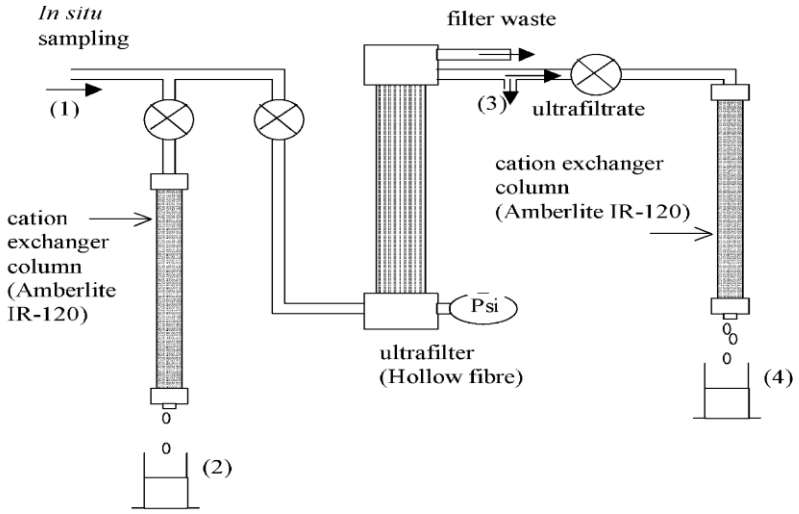

Figure 10. Copy of the picture presenting In situ ultrafiltration system interfaced with cation chromatography, sample positions for Al speciation (1–4) are indicated. 1. Unfiltered (total water sample), 2. Unfiltered and present in eluate of the Amberlite column (Alo), 3. Ultrafiltered (nominal mass -10 kDa), and 4. Ultrafiltered and present in eluate of the Amberlite column *(H.Christian Teien et al., 2006).*
Chelex 100 was used but not Amberlite IR-120 in the present project.

4.5 Determination of accumulated trace metals in organs

The collected organs were analysed for accumulated trace metals with ICP-MS. The gills, kidneys and livers of 3 years old fish (n=8), 2 years old large fish (n= 37) and 2 years old small fish (n=35) from fw, bw and sw were analysed.

4.5.1 Determination of accumulated trace metals in gills.

Care was taken in order to avoid contamination during sample processing and analyses. The gills that were cut out of the fish were stored in preweighted vials in a freezer. In order to use them for determination of accumulated trace metals they were freeze-dried in the original vials thus any

contamination would be avoid. The weight afterwards was calculated once more in order to get the actual gill weight.

Weight of gills = Weight of vials with dried gills – Weight of empty vials.

The freeze-dried gills were digested (1ml 2% HNO_3 and 2 drops of H_2O_2), heated in a sand bath at 100 C˚ for 6h with the vials opened. Then the residuals were digested once more (1ml 2% HNO_3 and 2 drops of H_2O_2), heated at a sand platform at 60˚ C for 2h with the caps on the vials), and diluted with 9ml MQ-water. The samples were analysed by usuing ICP-MS and reported as µg/g dry weight (dw) gill.

4.5.2 Determination of accumulated trace metals in livers and kidneys

The livers and kidneys were cut out of the fish after the gills were removed in a similar way. However, they were wrapped and stored in aluminium foil pieces and plastic bags until the lab work started, but not in preweighted vials.

When the preparation procedure began, the liver and kidney samples were moved into Teflon tubes very carefully. In addition, firstly the weight of the empty tubes was measured and secondly, the weight of the tubes with the samples. Then the samples were freeze-dryed and the weight was measured once more in order to get the actual liver and kidney weight. The livers and kidneys were digested in a similiar manner as the gills and reported also as µg/g dry weight.

Dry weight of liver/kidney = Weight of Teflon tube after freeze-drying – Weight of empty Teflon tube.

Wet weight of liver/kidney = Weight of Teflon tube with sample – Weight of empty Teflon tube.

UltraClave for digesting was used. Briefly, in the liver samples 0.25ml internal standart, 2.5ml 5% Ultra Pure HNO_3 were added and diluted to 50ml with MQ-water. In the kidney samples 0.05ml internal standart, 0.5ml 5% Ultra Pure HNO_3 were added and diluted with MQ-water to 10ml before digesting for 2h. All samples were analysed using ICP-MS.

4.6 Accuracy

During the analysis of accumulated trace metals in the fish organs and water samples, blank samples (n=5 or 10) were analysed every single time ICP-MS was used. Internal standard was also added in each gill, liver and kidney sample – 1mg/L Yttrium in the gills, 0.05ml 4mg/L Te,

4mg/L In, 4mg/L Tl, 4mg/L Rh and 2% HNO_3 in the kidneys and 0.25ml 4mg/L Te, 4mg/L In, 4mg/L Tl, 4mg/L Rh and 2% HNO_3 in the livers. The internal standard prevented any losses and controlled the digestion and dilution procedure. In addition, for the liver and kidney samples reference material was used - DOLT 4 (dogfish's liver) and DORM 3 (dogfish's protein). Dogfish Liver Certified Reference Material and Fish Protein Certified Reference Material for trace elements are primarily intended for use in calibration of procedures and the development of methods for the analysis of marine fauna and materials with similar matrix *(Sturgeon, 2008).* Determination of the detection limit was accomplished by multiplying the standard deviation of the blank samples by 3.

4.7 Statistical analysis

MINITAB realese 15 was the programme used to conduct the statistical analysis on the heavy metals concentration data for the fish from the River Storelva catchement area.

1) The differences in the concentration of the heavy metals in the investigated organs due to the water type exposure independant of the fish age, and 2) the differences in the concentration of the heavy metals in the investigated organs due to the fish age independant of the water type were tested by using ANOVA and General Linear Model.

Prior to using ANOVA, the data was tested for normality. If the data was not normally distributed, Johanson Transformation was used to transform the data before running the test. P-value < 0.05 and Confidence Interval of 95 % were used to distinguish the significant differences between the tested groups.

Regressions between the concentration of every single element and the organs (gills-kidneys ; gills-livers ; kidneys-livers) were also done. In the end, the distribution of the elements within the organs was followed by using Principal Component analysis

5. Results

5.1 Correlation between fish age and fish size.

The estimated age of the catched smolts after age determination analysis by reading scales was performed was 2 years. However, there were few exceptions of 3 years old fish. The 2 years old fish was divided into 2 groups – small and large based on their length. The average length was

16±0.4cm for 3 years old, 14.9±1.1cm for 2 years old large and 12±0.6cm for 2 years old small fish. There was a positive correlation between the fish age and length for 2 years old fish. However, the fish size of 2 years old large and 3 years old fish overlapped, see Table 11 and 12.

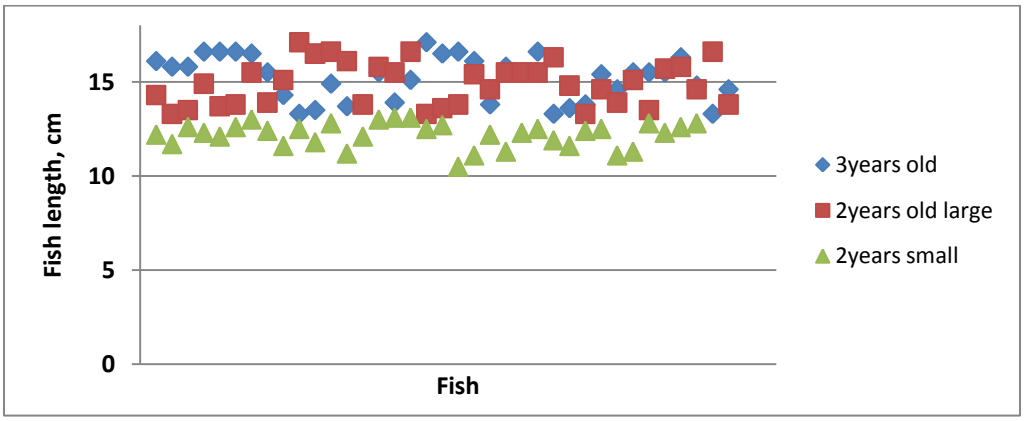

Figure 11. Fish length and age.

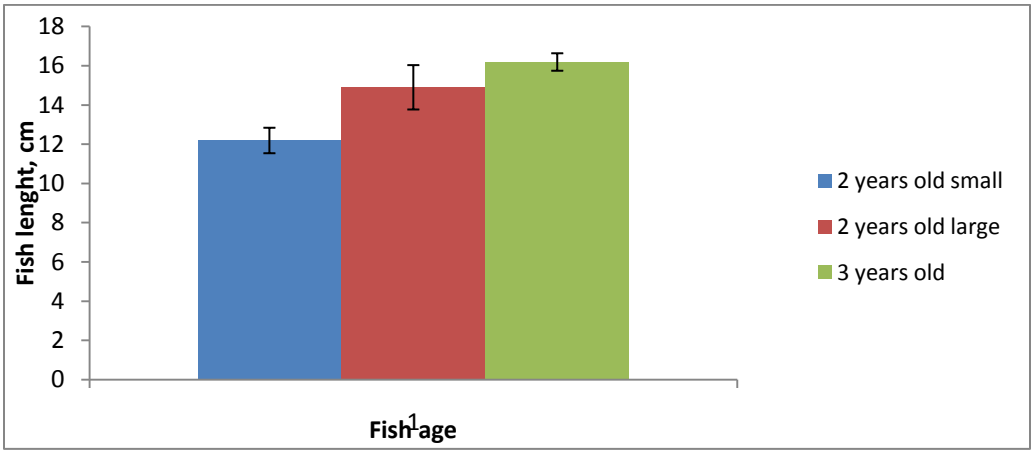

Figure 12. Mean fish length and age. Length is shown as mean with SD indicated.

5.2 General water chemistry analysis

The water used in this experiment had temperature 13.9±1.5 °C, conductivity 24.7±3.5 µS/cm and pH 6.6±0.3, see Table 1. The concentration of major anions in fw was measured with ion chromatography and TOC with TOC analyser, see Table 1.

Table 1. General FW chemistry

Date	Geographic position	Temperature (°C)	Conductivity(µS/cm)	pH
10.05.2009	Storelva outlet	12.2	25	6.53
23.05.2009	Above the lime doser	15	21	6.29
23.05.2009	Fosstveit	14.5	28	6.85
average±st.dev.		13.9±1.5	24.7±3.5	6.6±0.3

Table 1. General FW chemistry - continued

Date	Geographic position	TOC (µg/L)	Cl (µg/L)	NO_3 (µg/L)	SO_4 (µg/L)	Br (µg/L)	F (µg/L)
10.05.2009	Storelva outlet	no data	no data	no data	no data	no data	no data
23.05.2009	Above the lime doser	3902	2516	106	2158	<80	<40
23.05.2009	Fosstveit	no data	no data	no data	no data	no data	no data

5.3 Water chemistry analysis – ICP-OES

Due to very high salinity, the results obtained from the analysis with ICP-MS for bw and sw were not used. Therefore, only the results from ICP-OES were presented for these water samples and they referred only to the Al and Cu concentration, see Table 3. All the fw samples were analysed with ICP-MS, see Table 4.

The total concentration of Al in bw was 135±17µg/l and in sw 47±29µg/l. The most toxic form of Al, considered to be 0.45µm Ali was higher than the toxic level of 20µg/l in bw - 46±22 µg/l and in sw - 28±15 µg/l.

Table 2. –Water chemistry, FW (ICP-OES).

Date	Water quality (Rygg)	Ca(µg/L)	K(µg/L)	Mg(µg/L)	Na(µg/L)	Si(µg/L)
6.05.2009	Total					
average		851	125	189	2446	903
st.dev.		685	110	147	1182	18
7.05.2009	Total	457	133	347	2771	496
average		1497	248	345	2277	923
st.dev.		280	72	100	1062	66
7.05.2009	LMM	924	179	246	2050	884
8.05.2009	Total					
average		1321	219	324	2284	1590
st.dev.		27	20	30	39	535
8.05.2009	LMM	790	175	205	1322	864

Table 3. – Water chemistry, BW and SW (concentration of Al and Cu) (ICP-OES).

Water quality	Tot Al	0,45 µm Al	0.45 µm Ali	LMM Al	LMM ione Al	Ali	Ali (after 0.45µm)	LMM Ali	Tot Cu
bw									1.02
average	135	107	55	69	28	76	46	41	
st.dev.	17	12	32	16	20	20	22	21	
sw									
average	47	36	8	35	7	39	28	28	1.42
st.dev.	29	22	4	16	2	21	15	15	0.52

Table 4. - Retention in chelex 100

Water quality	Retention in chelax 100 (Total Al -Total 0.45µm ione Al)	Retention in chelax 100 (LMM Al-LMM ione Al)
bw		
average	80	41
st.dev.	21	21
sw		
average	39	25
st.dev.	25	16

* 0.45 µm Al chelex ≥ LMM Al chelex

5.4 Water chemistry analysis – ICP-MS

The trace metals concentration in fw was analysed with ICP-MS and the total, and LMM concentrations were determined, see Table 5.

Table 5. – Concentration of heavy metals in FW (µg/L) (n=22)

	Al		**Ti**		**Cr**		**Mn**		**Co**	
	µg/L Total	**µg/L** LMM	**µg/L** Total	**µg/L** LMM	**µg/L** Total	**µg/L** LMM	**µg/L** Total	**µg/L** LMM	**µg/L** Total	**µg/L** LMM
average	141	100	1	1	0.5	0.5	0.3	0.2	0.1	0.1
st.dev.	34	98	0.3	0.9	0.09	0.07	0.05	0.07	0.02	0.07

Table 5. – Concentration of heavy metals in FW (µg/L) (n=22) – continued

	Ni		**Cu**		**Zn**		**As**		**Cd**	
	µg/L Total	**µg/L** LMM	**µg/L** Total	**µg/L** LMM	**µg/L** Total	**µg/L** LMM	**µg/L** Total	**µg/L** LMM	**µg/L** Total	**µg/L** LMM
average	1	1	0.8	1	0.2	0.2	0.1	0.07	0.1	0.1
st.dev.	0.1	0.04	0.2	0.7	0.1		0.03	0.02	0.05	0.08

Table 5. – concentration of heavy metals in FW (µg/L) (n=22) - continued

	Cs		**Pb**	
	µg/L Total	**µg/L** LMM	**µg/L** Total	**µg/L** LMM
average	0.03	0.03	0.24	0.1
st.dev.	0.008	0.01	0.14	0.1

5.5 Concentration of trace metals in gills

Fish gills are multifunctional organs involved in ion transport, gas exchange, acid–base regulation and waste excretion *(Dang et al., 2001).* The gills are regarded as the important site for direct uptake from the water, whereas the body surface is generally assumed to play a minor role in heavy metal uptake of fish *(Popova et al., 1997).*

The gill is considered to be an important site for direct toxic effects to metals in high concentrations, for sub-lethal effects at lower metal concentrations, and, along with uptake from

food, an important point of entry into the organism for both essential trace elements (Cu, Zn, Se, Mn, Fe) and non-essential elements (Al, As, Cd, Cr, Ni, Pb) *(Rosseland et al., 2007).*

The results from the trace metal analysis in gill are presented in Tables 6, 7 and 8.

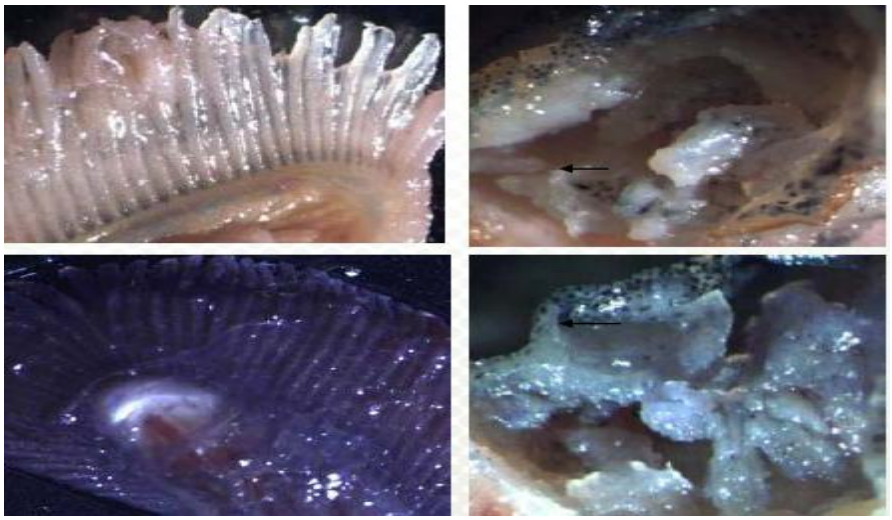

Figure 13. Heavy metal accumulation in gills – effect of Al *(photo – Teien and Salbu)*

Table 6. – Mean concentration of trace metals in gills in 2 years old large fish in FW, BW and SW, µg/g dw (average±standart deviation) (n=37).

		Al	Ti	Cr	Mn	Co	Ni
FW	**2years old large**	**50±32***	26±2	2±0.5	60±6	0.6±0.3	1.8±0.4
BW	**2years old large**	233±111	27±4	2±0.9	96±131	0.7±0.3	3.5±2
SW	**2years old large**	**76±28 ***	28±5	3.6±1	62±19	0.79±0.4	5±2.5
		Cu	**Zn**	**As**	**Cd**	**Cs**	**Pb**
FW	**2years old large**	2.6±0.3	688±170	**0.07±0.01***	6±1.5	0.1±0.02	0.6±0.1
BW	**2years old large**	**3±0.8***	1434±2722	**0.08±0.03***	7±1.8	0.2±0.5	1.5±2.8
SW	**2years old large**	**4±1***	830±264	**0.1±0.04***	7.5±2	0.1±0.06	0.7±0.2

***Al – FW - 2 years old large fish - 3 fish had a concentration less than the detection limit.**

SW - 2 years old large fish - 6 fish had a concentration less than the detection limit.

***Cu – BW – 2 years old large fish - 5 fish had a concentration less than the detection limit.**

SW - 2 years old large fish - 6 fish had a concentration less than the detection limit.

***As – FW - 2 years old large fish - FW, BW, SW - all fish had a concentration less than the detection limit.**

Table 7. – Mean concentration of trace metals in gills in 2 years old small fish in FW, BW and SW, µg/g dw (average±standart deviation) (n=35).

		Al	Ti	Cr	Mn	Co	Ni
FW	**2years old small**	69	22	1	54	0.5	2
BW	**2years old small**	**204±89***	24±6	3±0.7	60±25	0.6±0.2	2.5±1
SW	**2years old small**	**95±48***	31±4.9	4±1.5	64±20	0.6±0.3	3±1
		Cu	**Zn**	**As**	**Cd**	**Cs**	**Pb**
FW	**2years old small**	**2***	771	**0.06***	5	0.09	0.3
BW	**2years old small**	3±0.6	727±225	**0.09±0.02***	8±2	0.1±0.03	0.8±0.7
SW	**2years old small**	**4±1.6***	833±165	**0.1±0.05***	8.5±1	0.1±0.03	3±7

***Al – BW - 2 years old small fish - 2 fish had a concentration less than the detection limit.**

SW - 2 years old small fish -2 fish had a concentration less than the detection limit

***Cu – FW – 2 years old small fish - 6 fish had a concentration less than the detection limit.**

SW – 2 years old small fish- 6 fish had a concentration less than the detection limit.

***As – FW – 2 years old small fish - 1 fish had a concentration less than the detection limit.**

BW – 2 years old small fish - 9 fish had a concentration less than the detection limit.

SW – 2 years old small fish - 8 fish had a concentration less than the detection limit.

Table 8. – Mean concentration of trace metals in gills in 3 years old fish in BW and SW, µg/g dw (average±standart deviation) (n=8).

		Al	Ti	Cr	Mn	Co	Ni
BW	**3years old**	217±74	26±4.9	3±0.5	69±33	0.7±0.4	6±2
SW	**3years old**	**63±33***	29±11	4±1	57±4.9	0.4±0.2	4.5±1
		Cu	**Zn**	**As**	**Cd**	**Cs**	**Pb**
BW	**3years old**	**3.9±2***	868±139	**0.1±0.066***	8±1	0.2±0.033	0.6±0.1
SW	**3years old**	**4±1***	729±171	**0.1±0.039***	142±261	0.1±0.08	0.8±0.4

***Al – SW - 3 years old fish - 1 fish had a concentration less than the detection limit.**

***Cu – BW - 3 years old fish - 2 fish had a concentration less than the detection limit**

SW- 3 years old fish - 2 fish had a concentration less than the detecetion limit.

***As – BW - 3 years old fish - 3 fish had a concentration less than the detection limit.**

SW – 3 years old fish - 4 fish had a concentration less than the detection limit.

5.5.1 Concentration of Al

The results showed that there were no significant differences found between the concentration of Al in the gills and the fish age. The differences were more significant between the three types of water – fw, bw and sw. The concentration of Al in 3 years old fish, 2 years old large and small fish in fw, bw and sw seemed similar. The highest concentrations were found in bw, see Table 6, 7, 8 and Figure 14. When MINITAB release 15 was used to run a test and investigate the differences between the water type and the trace metals concentration in the gills, it was observed that the p-value showed a significant difference for Al in bw and fw (p = 0.00) and bw and sw (p-value = 0.0).

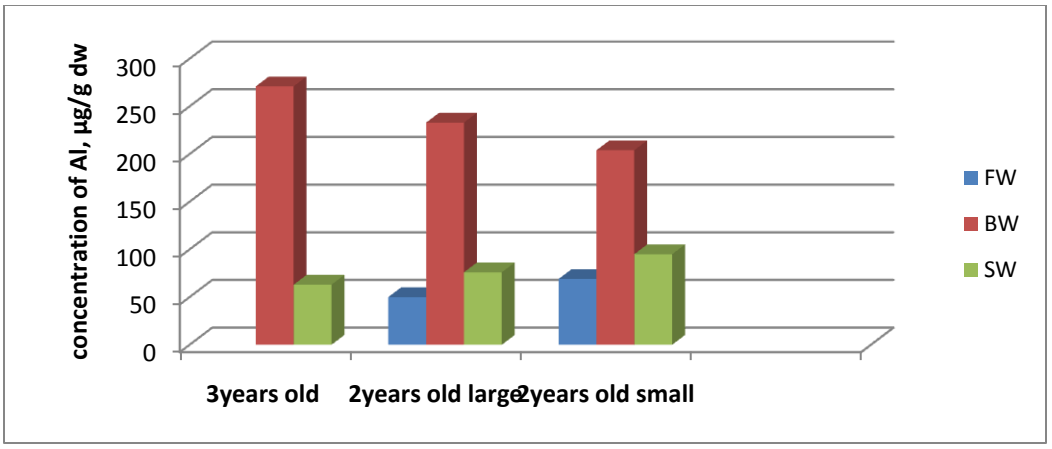

Figure14. Mean concentration of Al, µg/g dw in gills in FW, BW and SW for 3 and 2years old fish.

5.5.2 Concentration of Cd

The results showed that the concentration of Cd in gills was quite similar for the fish in fw and bw but the concentration of Cd in gills of 3 years old fish in sw was few times higher than in 2 years old large and small fish, see Table 6, 7, 8 and Figure 15.

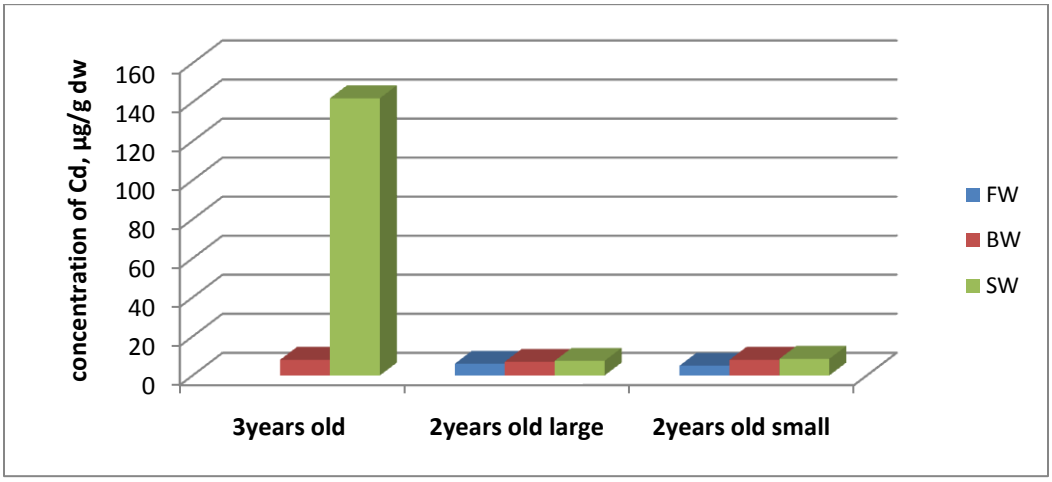

Figure 15. Mean concentration of Cd, µg/g dw in gills in FW, BW and SW for 3 and 2 years old fish.

5.5.3 Concentration of Cu

The results showed that the concentration of Cu in gills varied between the groups of fish in the different types of water; see Table 6, 7, 8 and Figure 16. When MINITAB release 15 was used to test and investigate the differences between the water type and the concentration of the trace metals in the gills, a significant difference between the concentration of Cu into the gills in bw

and sw (p-value = 0.001) and fw and sw (p-value = 0.0001) was found. There was no difference in Cu concentration related to the fish age.

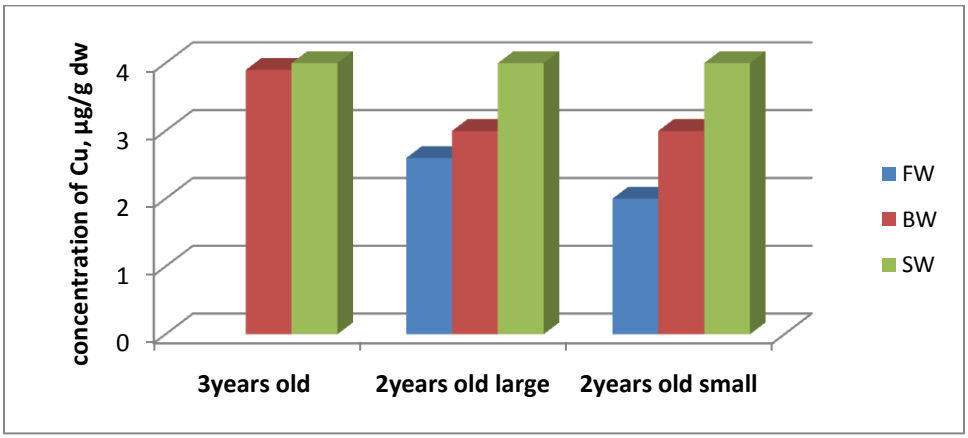

Figure 16. Mean concentration of Cu, µg/g dw in gills in FW, BW and SW for 3 and 2 years old fish.

5.5.4 Concentration of Zn

The results showed that the concentration of Zn in gills was highest in 2 years old large fish in bw and this was an extreme difference, see Table 6, 7, 8 and Figure 17. The rest of the concentration of Zn in 3 years old fish and 2 years old large fish in fw and sw did not show any significant differences.

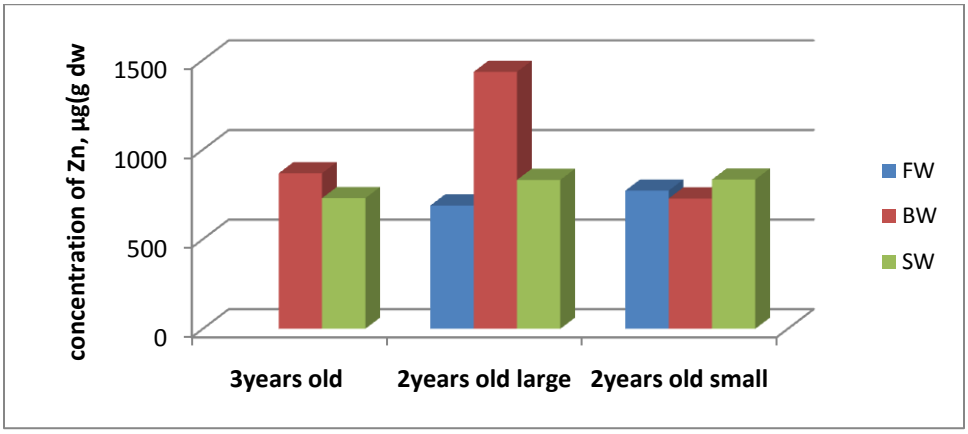

Figure 17. Mean concentration of Zn, µg/g dw in gills in FW, BW and SW for 3 and 2 years old fish.

5.5.5 Concentration of Pb

The results showed small differences in the concentration of Pb in gills between the groups of fish in the different types of water. The highest concentration was detected in 2 years old large fish in bw and in 2 years old small fish in sw, see Table 6, 7, 8 and Figure 18.

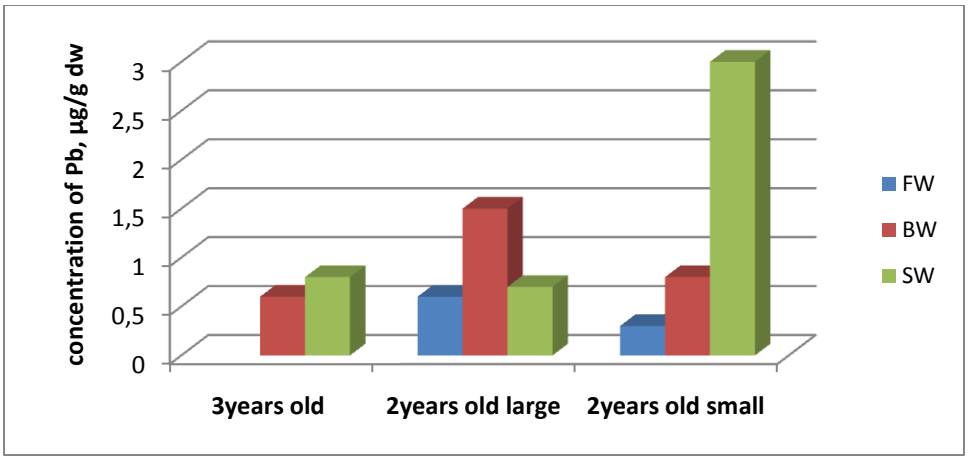

Figure 18. Mean concentration of Pb, µg/g dw in gills in FW, BW and SW for 3 and 2 years old fish.

5.5.6 Concentration of As

The results showed that the concentration of As in gills were low and varied little between the groups of fish in the different types of water and age groups, see Table 6, 7, 8 and Figure 19. The highest concentration of As in gills was found in 2 years large fish in sw and in 2 years old small fish in sw. There was a difference in As concentration in gills in 2 years large and 3 years old fish (p-value = 0.04) in bw.

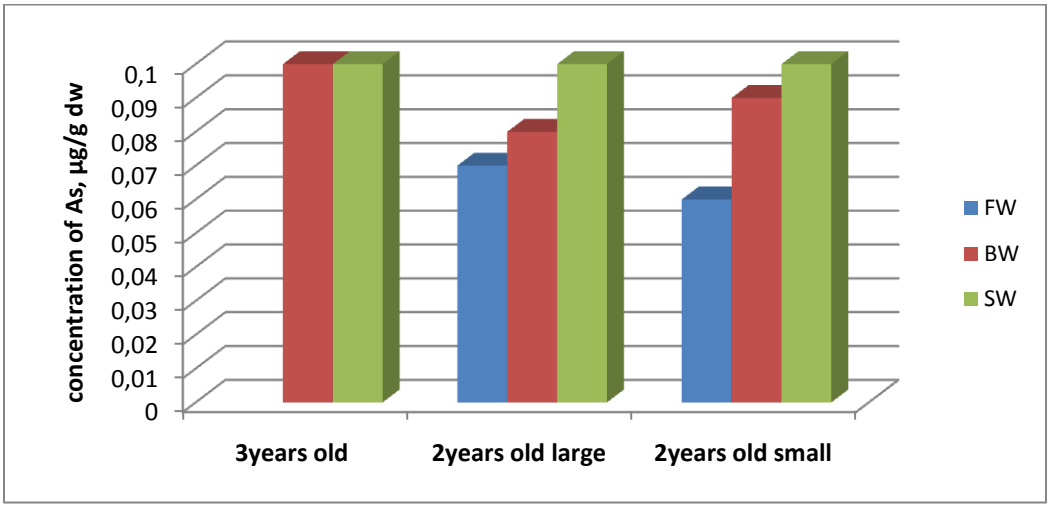

Figure 19. Mean concentration of As, µg/g dw in gills in FW, BW and SW for 3 and 2 years old fish.

5.5.7 Concentration of Ni

The results showed small differences in concentration of Ni in gills between the groups of fish in the different types of water, see Table 6, 7, 8 and Figure 20. The highest concentration of Ni was found to be in 3 years old fish in bw and in sw. In 2 years old small fish the concentration was

lowest. The statistical test, however, pointed out a difference between the concentration of Ni in the gills in fish in fw and sw (p-value = 0.005). There was also a significant difference when the concentration of Ni in the gills and the fish age were tested – in 2 years small and 3 years old fish the (p-value = 0.02).

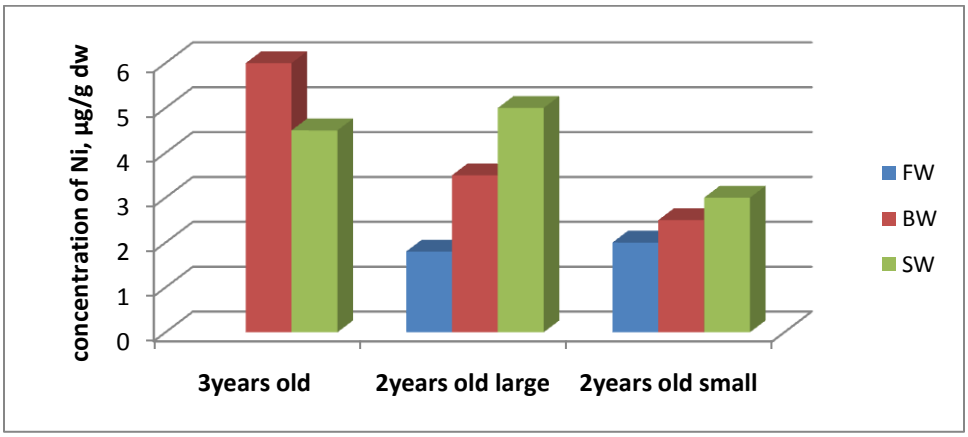

Figure 20. Mean concentration of Ni, µg/g dw in gills in FW, BW and SW for 3 and 2 years old fish.

5.5.8 Concentration of Cr

The results showed that the concentration of Cr in gills did not vary very much between the groups of fish in the different types of water, see Table 6, 7, 8 and Figure 21. The concentration of Cr in sw fish was highest. The concentration in fish in bw was lower and similar within the 2 years old fish groups but it was different in fw between the groups of 2 years old large and small fish. The test done with MINITAB realease 15 showed a siginifcant difference between the concentration of Cr in the gills and the bw and sw (p-value = 0.001) and fw and sw (p-value = 0.0001). There was no significant difference found between the concentration of Cr in the gills and the fish age.

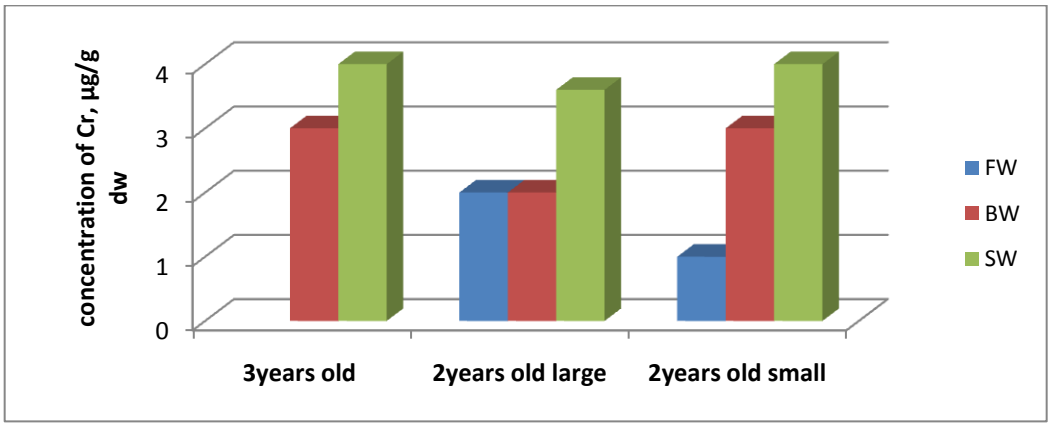

Figure 21. Mean concentration of Cr, µg/g dw in gills in FW, BW and SW for 3 and 2 years old fish.

5.5.9 Concentration of Mn

The results showed that the concentration of Mn in gills did not vary significantly between the groups of fish in the different types of water, see Table 6, 7, 8 and Figure 22. The highest concentration of Mn was detected in 2 years old large fish in bw.

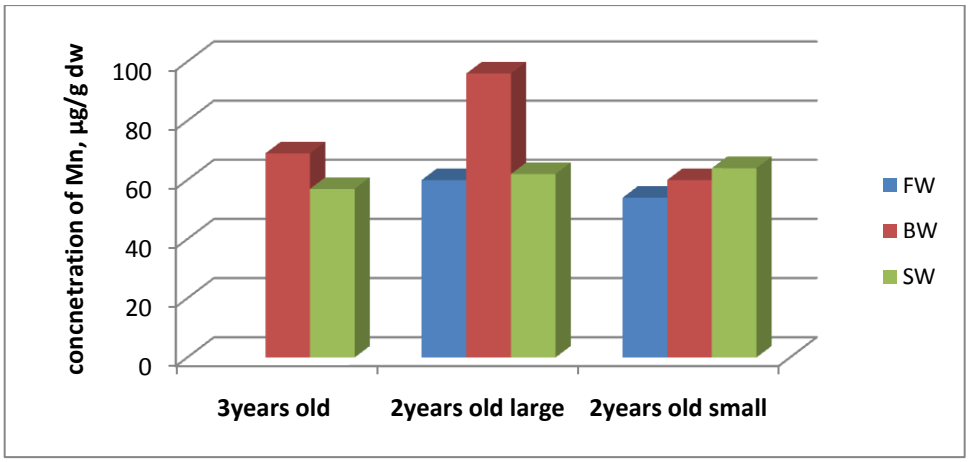

Figure 22. Mean concentration of Mn, µg/g dw in gills in FW, BW and SW for 3 and 2 years old fish.

5.5.10 Concentration of Co

The results showed that the concentration of Co in gills did not vary significantly between the groups of fish in the different types of water, see Table 6, 7, 8 and Figure 23.

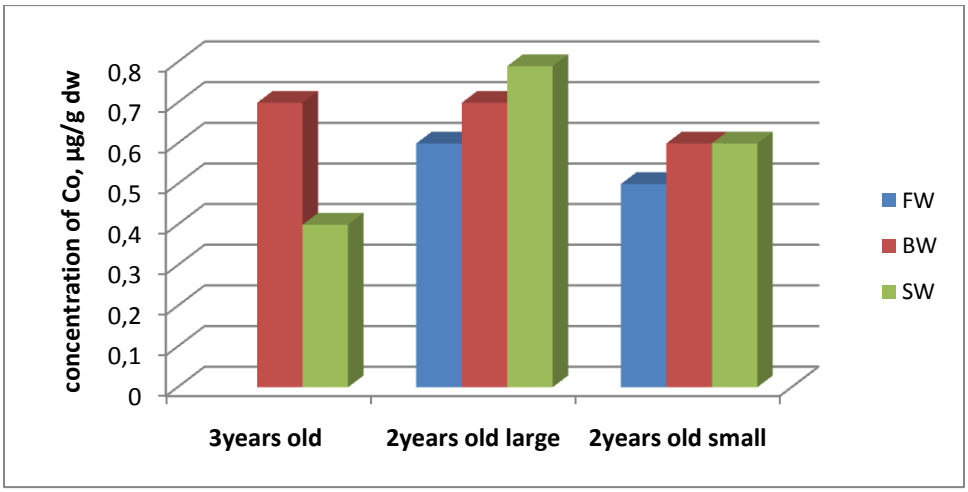

Figure 23. Mean concentration of Co, µg/g dw in gills in FW, BW and SW for 3 and 2 years old fish.

5.5.11 Concentration of Ti

The results showed that the concentration of Ti in gills did not vary significantly beween the groups of fish in the different types of water, Table 6, 7, 8 and Figure 24. The highest

concentration was found in the group of 2 years old small fish in sw. The concentration of Ti in fw and bw fish were similar but lower. There were no significant differences found between the Ti concentration and the fish age, or the water type.

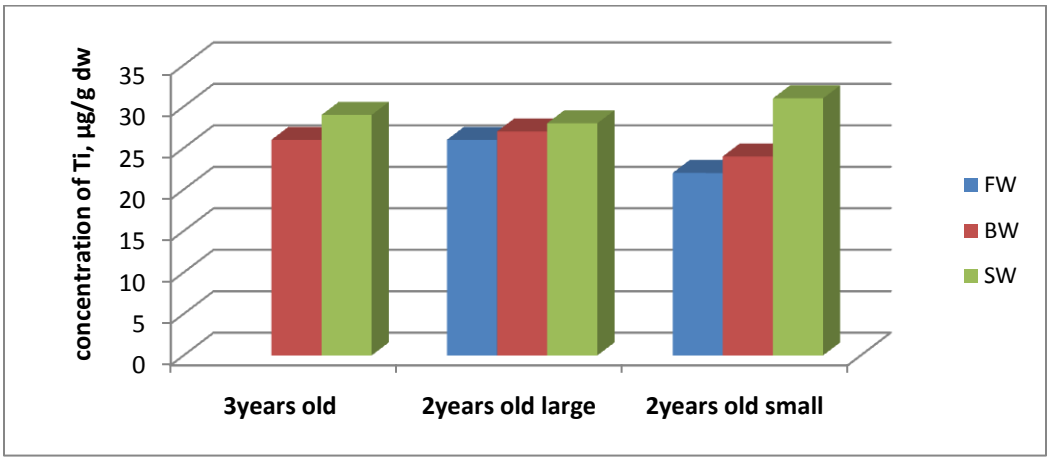

Figure 24. Mean concentration of Ti, µg/g dw in gills in FW, BW and SW for 3 and 2 years old fish.

5.5.12 Concentration of Cs

The results showed that the concentration of Cs in gills did not vary significantly between the groups of fish in the different types of water, Table 6, 7, 8 and Figure 25. The highest concentration was found to be in fish – 3 and 2 years large large fish in bw, but there were no significant differences found between the age groups or water quality. The results for fw and sw fish for all three groups related to the fish age did not have any significant differences.

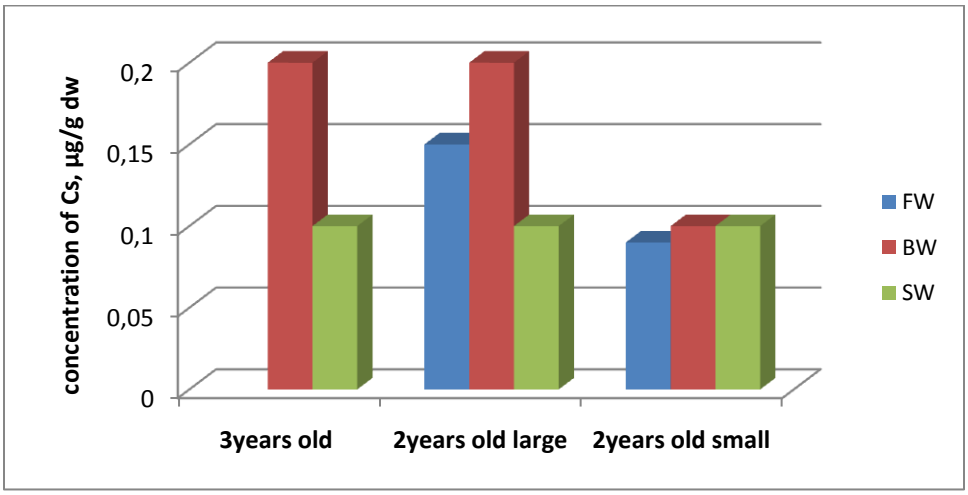

Figure 25. Mean concentration of Cs, µg/g dw in gills in FW, BW and SW for 3 and 2 years old fish

5.6 Concentration of trace metals in livers

The results from the ICP-MS analysis of trace metals in livers are presented in Tables 9, 10 and 11.

Table 9. - Mean concentration of trace metals in livers in 2 years old large fish in FW, BW and SW, µg/g dw (average±standart deviation) (n=37).

	Al	Ti	Cr	Mn	Co	Ni
FW 2years old large	79±44	2±1	0.2±0.1	1±3	0.5±0.2	0.03±0.04
BW 2years old large	66.9±57	3 ±1	0.3±0.1	4 ±5	0.4±0.1	0.2±0.8
SW 2years old large	107±111	2.9±0.5	0.2±0.1	3.9±4	0.3±0.1	0.01±0.05

	Cu	Zn	As	Cd	Cs	Pb
FW 2years old large	57±40	15±41	0.2±0.1	3±1	0.2±0.1	0.1±0.07
BW 2years old large	62±28	69±94	0.2±0.1	5±2	0.2±0.1	0.3±0.1
SW 2years old large	67 ±44	65 ±74	0.2±0.1	3±0.8	0.2±0.09	0.1±0.06

Table 10. - Mean concentration of trace metals in livers in 2 years old small fish in FW, BW and SW, µg/g dw (average±standart deviation) (n=35).

	Al	Ti	Cr	Mn	Co	Ni
FW 2years old small	175±196	2.5±1.8	**0.3±0.3***	0.16±0.1	0.3±0.3	0.2±0.18
BW 2years old small	112±125	2.9±1	1±3	4±5	0.4±0.1	1±3
SW 2years old small	115±224	2.6±1.8	**0.1±0.3***	4±4.9	0.3±0.2	**0.1±0.2***

	Cu	Zn	As	Cd	Cs	Pb
FW 2years old small	71±90	2±1	0.15±0.16	3.18±3.10	0.13±0.11	0.1±0.08
BW 2years old small	92±54	69±78.9	**0.3±0.2***	4.5±2	0.3±0.1	0.29±0.29
SW 2years old small	64±68	76±80	0.2±0.1	0.2±2	0.2±0.1	0.2±0.13

***Cr – FW - 2 years old small fish – 1 fish had a concentration less than the detection limit.**

SW - 2 years old small fish - 2 fish had a concentration less than the detection limit.

***As – SW - 2 years old small fish - 2 fish had a concentration less than the detection limit.**

***Ni - SW - 2 years old small fish – 1 fish had a concentration less than the detection limit.**

***Pb – FW - 2 years old small fish – 1 fish had a concentration less than the detection limit.**

SW - 2 years old small fish – 1 fish had a concentration less than the detection limit.

Table 11. – Mean concentration of trace metals in livers in 3 years old fish in BW and SW, µg/g dw (average±standart deviation) (n=8).

	Al	Ti	Cr	Mn	Co	Ni
BW 3years old	37±16	2.7±0.2	-0.01±0.1	10±1	0.4±0.2	0.02±0.04
SW 3years old	31±17	2±1	0.1±0.08	5±3	0.2±0.1	0.03±0.04

	Cu	Zn	As	Cd	Cs	Pb
BW 3years old	58±39	150±25	0.2±0.05	5.9±1	0.3±0.1	0.8±0.5
SW 3years old	53±41	107±63	0.2±0.19	2±1	0.2±0.1	0.8±0.4

5.6.1 Concentration of Al

Since all the livers and kidneys samples were wrapped in Al foil pieces, it was considered that the results for accumulated Al in these organs might have been contaminated from the foil, and thus the results could have been affected. The highest concentration was found in 2 years small fish in fw, bw and sw. The concentration of Al in 2 years old large and 3 years old fish was lower, see Table 9, 10, 11 and Figure 26.

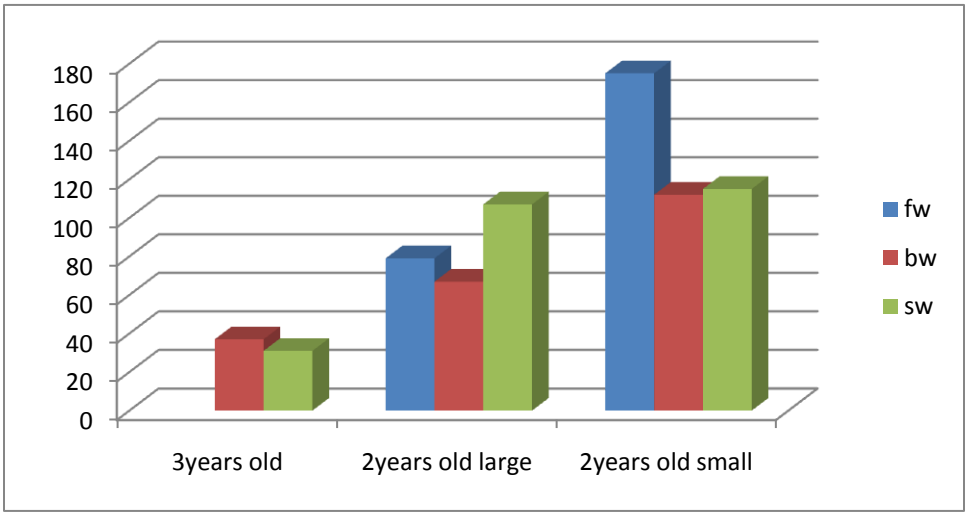

Figure 26. Mean concentration of Al, µg/g dw in livers in FW, BW and SW for 3 and 2 years old fish.

5.6.2 Concentration of Cd

The results showed that the concentration of Cd varied between the groups of fish in the different water types. In the liver it was highest in 3 years old fish in bw, see Table 9, 10, 11 and Figure 27.

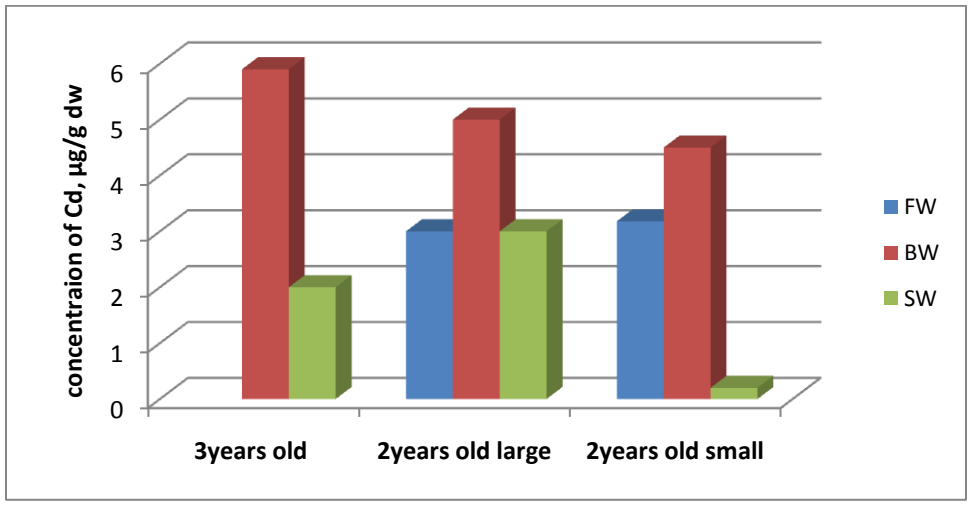

Figure 27. Mean concentration of Cd, µg/g dw in livers in FW, BW and SW for 3 and 2 years old fish.

5.6.3 Concentration of Cu

The results showed that the concentration of Cu in 2 years old small fish was highest in bw, see Table 9, 10, 11 and Figure 28. The results for Cu concentration in livers in fw, bw and sw in 2 years old large and 3 years old fish were lower, and did not show any significant differences between the different age groups and water quality.

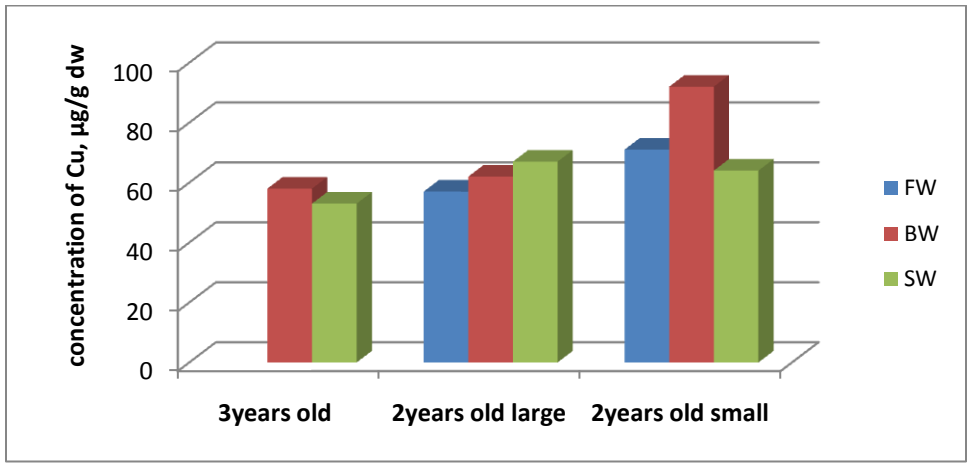

Figure 28. Mean concentration of Cu, µg/g dw in livers in FW, BW and SW for 3 and 2 years old fish.

5.6.4 Concentration of Zn

The results showed that the concentration of Zn in bw and sw in 3 years old fish was highest. The results for bw and sw in 2 years old large and 3 years old fish were lower but similar within the two groups. The concentration of Zn in 2 years old large and small fish in fw was different, and lowest in 2 years old small fish, see Table 9, 10, 11 and Figure 29.

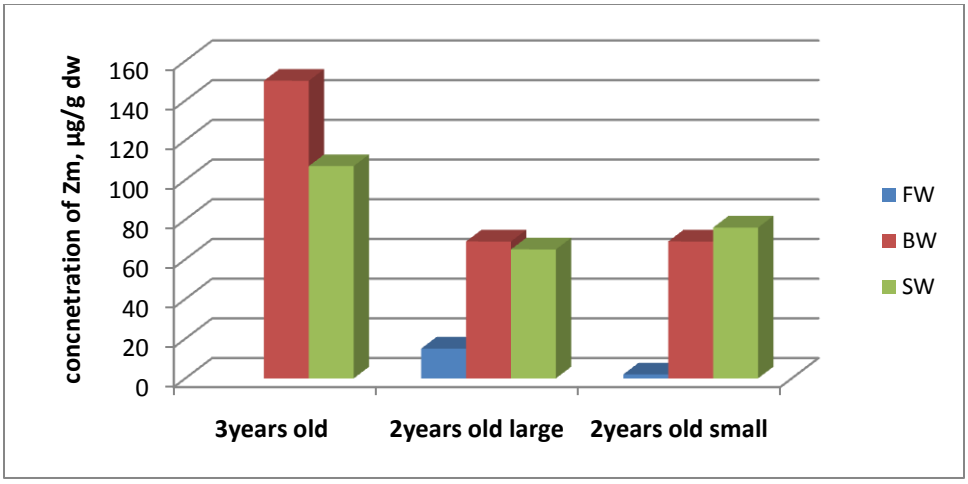

Figure 29. Mean concentration of Zn, µg/g dw in livers in FW, BW and SW for 3 and 2 years old fish.

5.6.5 Concentration of Pb

The results showed that the concentration of Pb in 3 years old fish was highest and much higher that the other two groups, see Table 9, 10, 11 and Figure 30. The results for bw and sw in 2 years old large and 2 years old small fish were similar within the two age groups. The concnetration of Pb in 2 years old large and small fish in fw was lowest and seemed to be similar between the two groups. The statistical analysis showed a difference between the concentration of Pb in the livers of fish that was in bw and fw (p-value = 0.0001) and in fw and sw (p-value = 0.01) There was also a difference between the concentration of Pb in the livers of 2 years small and 3 years old fish (p-value = 0.002), and 2 years large and 3 years old fish (p-value = 0.0002).

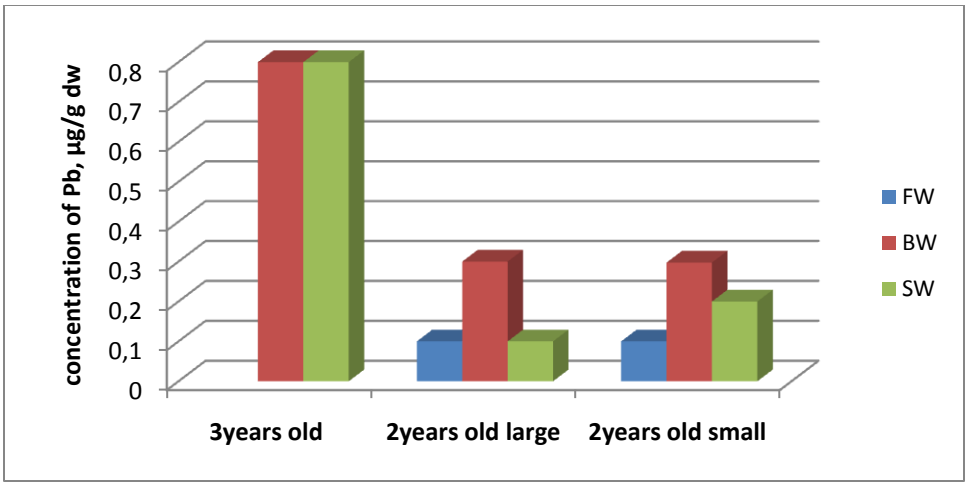

Figure 30. Mean concentration of Pb, µg/g dw in livers in FW, BW and SW for 3 and 2 years old fish.

5.6.6 Concentration of As

The results showed that the concentration of As was highest in 2 years old small fish in bw, see Table 9, 10, 11 and Figure 31. In general, the results for As were not that significant.

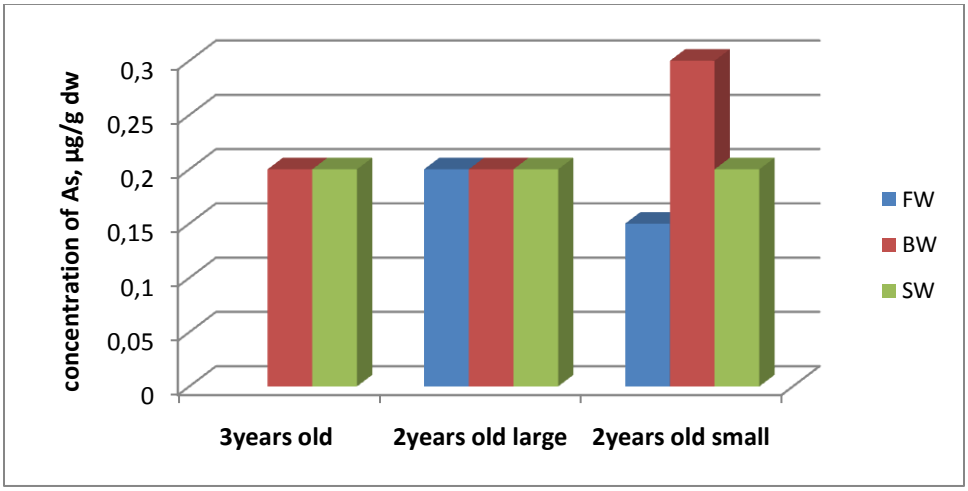

Figure 31. Mean concentration of As, µg/g dw in livers in FW, BW and SW for 3 and 2 years old fish.

5.6.7 Concentration of Ni

The results showed that the concentration of Ni in 3 years old fish was lowest, see Table 9, 10, 11 and Figure 32. The results for fw and bw in 2 years old large and 2 years old small fish were very similar within the two groups, but the concentration of Ni in 2 years small and large fish in sw were different.

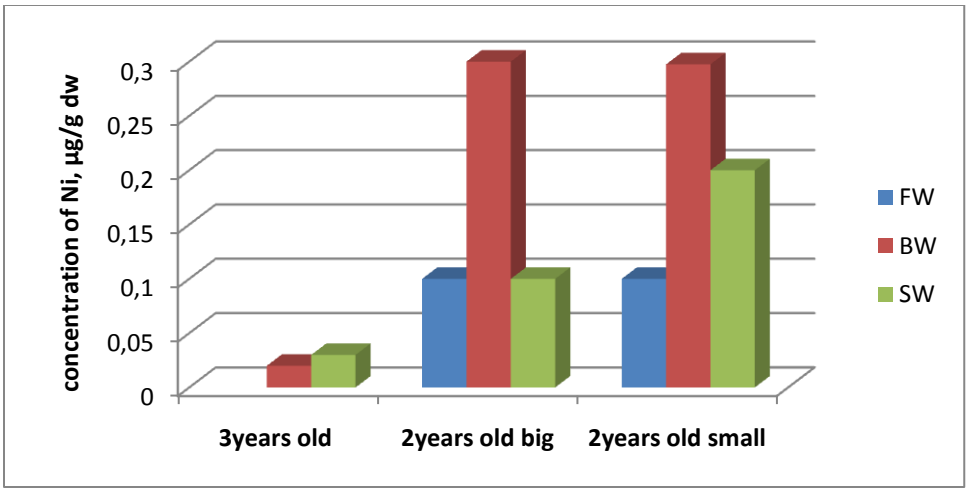

Figure 32. Mean concentration of Ni, µg/g dw in livers in FW, BW and SW for 3 and 2 years old fish.

5.6.8 Concentration of Mn

The results showed that the concentration of Mn in 3 years old fish in bw was highest and that was a significant difference, see Table 9, 10, 11 and Figure 33. The results were similar in bw and sw in 2 years old large and 2 years old small fish, and they were higher than in fw.

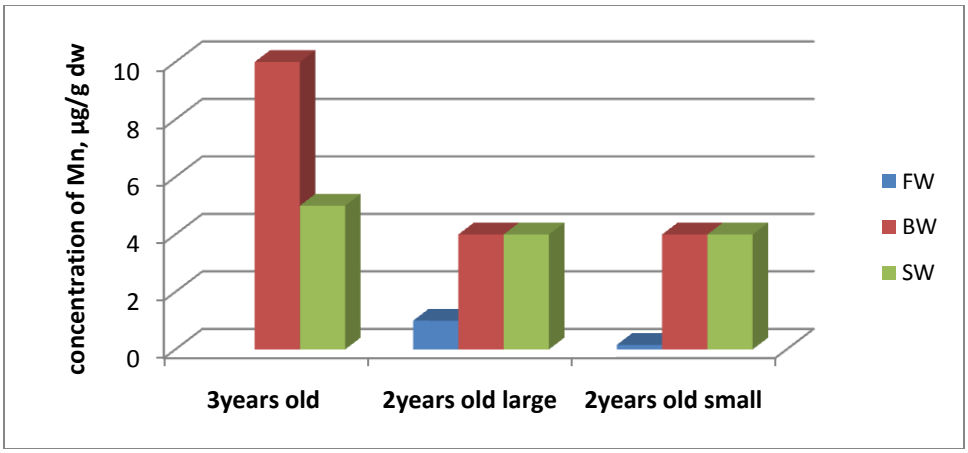

Figure 33. Mean concentration of Mn, µg/g dw in livers in FW, BW and SW for 3 and 2 years old fish.

5.6.9 Concentration of Cr

The results that showed a large variation - the concentration of Cr in 3 years old fish was lowest (< detection limit), see Table 9, 10, 11 and Figure 34. The results in 2 years old large and 2 years old small fish in fw were higher and similar within the two groups. The concentration of Cr in 2 years old small fish in bw was highest.

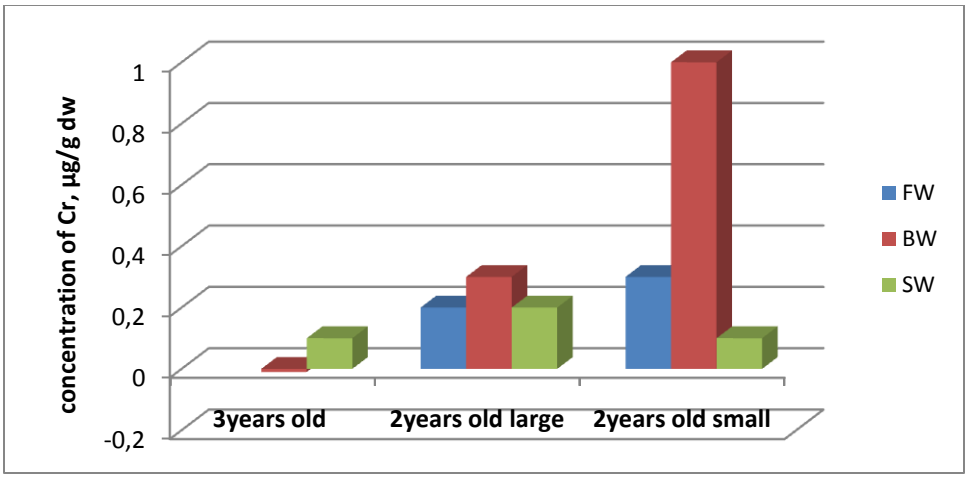

Figure 34. Mean concentration of Cr, µg/g dw in livers in FW, BW and SW for 3 and 2 years old fish.

5.6.10 Concentration of Co

The results showed that the concentration of Co between all three investigated groups of fish in all three types of water was quite similar, see Table 9, 10, 11 and Figure 35. There was a significant difference between the concentration of Co in the livers in fish in bw and sw (p-value = 0.04). There was no difference found in the concentration of Co related to the different fish age.

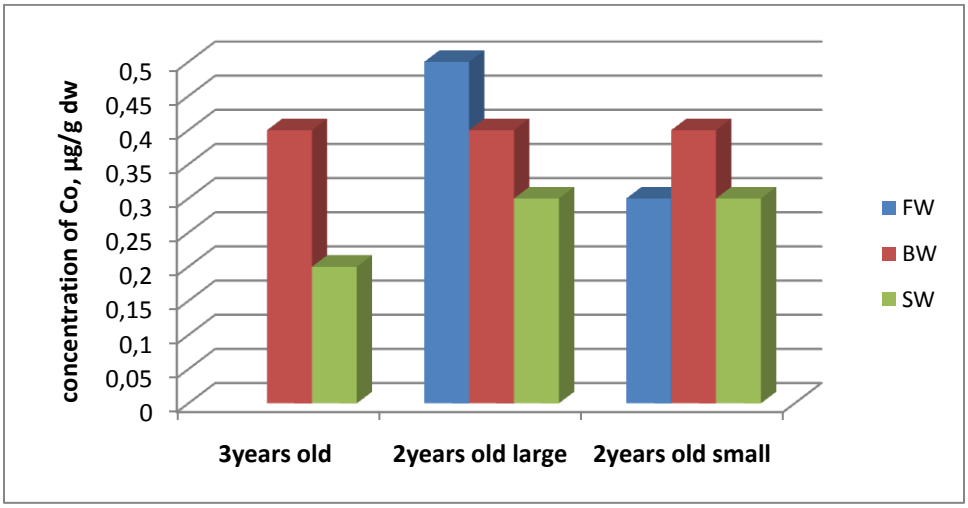

Figure 35. Mean concentration of Co, µg/g dw in livers in FW, BW and SW for 3 and 2 years old fish.

5.6.11 Concentration of Ti

The results showed that the concentration of Ti in all three investigated groups of fish were similar and there were no significant differences, see Table 9, 10, 11 and Figure 36.

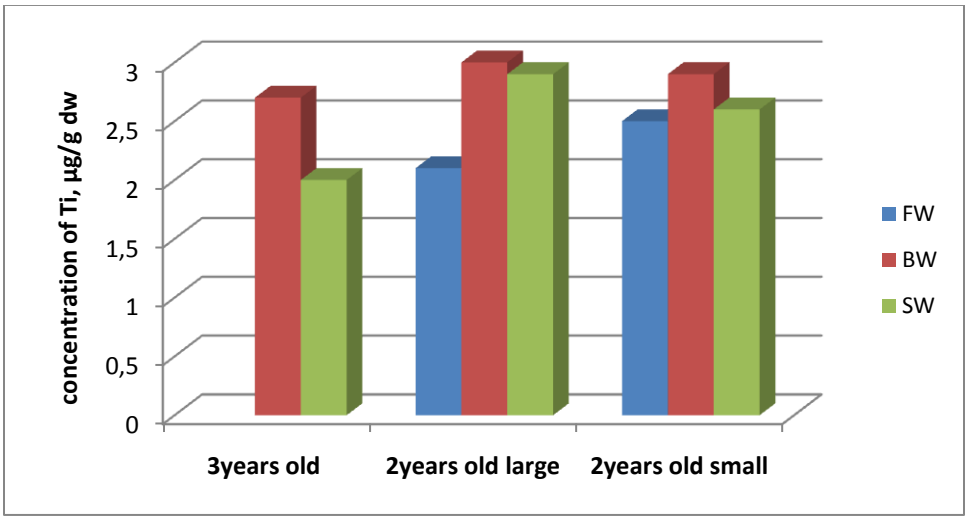

Figure 36. Mean concentration of Ti, µg/g dw in livers in FW, BW and SW for 3 and 2 years old fish.

5.6.12 Concentration of Cs

The results showed that the concentration of Cs in all three groups of fish in all the three types of water were similar, see Table 9, 10, 11 and Figure 37. There were no significant differences found.

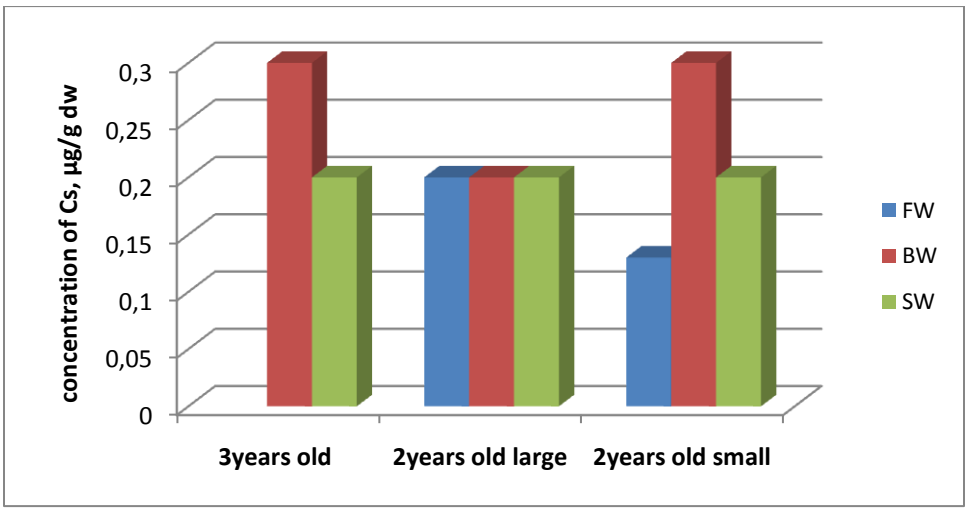

Figure 37. Mean concentration of Cs, µg/g dw in livers in FW, BW and SW for 3 and 2 years old fish.

5.7 Concentration of trace metals in kidneys

The results from the ICP-MS analysis of metals in kidneys are presented in Tables 12, 13 and 14.

Table 12. - Mean concentration of trace metals in kidneys in 2 years old large fish in FW, BW and SW, µg/g dw (average±standart deviation) (n=37).

	Al	Ti	Cr	Mn	Co	Ni
FW 2years old large	569±405	5±1	0.6±1	1±3	**1 ±0.9***	3 ±8
BW 2years old large	284±166	6 ±1	0.7±1	3 ±5	2±0.8	1±2
SW 2years old large	407±195	9±4	0.5±0.3	5±7	2±1	1±0.7

	Cu	Zn	As	Cd	Cs	Pb
FW 2years old large	5±1	29±77	0.3±0.09	23±9	0.30±0.1	0.4±0.1
BW 2years old large	8±2	124±166	0.4±0.1	36±10	0.3±0.1	0.7±0.6
SW 2years old large	9±4	195±247	0.3±0.1	29 ±15	0.3±0.1	0.6±0.4

***Co – FW - 2 years old large fish – 1 fish had a concentration less than the detection limit.**

Table 13. Mean concentration of trace metals in kidneys in 2 years old small fish in FW, BW and SW (average±standart deviation) (n=35).

	Al	Ti	Cr	Mn	Co	Ni
FW 2years old small	1413±1282	22±23	1.25±1.27	0.8±0.7	**5±4***	4±11
BW 2years old small	480±373	5±2	0.3±0.2	4.5±4.8	**2±0.5***	2.04±2.01
SW 2years old small	567±792	11±14	1.1±1.7	4 ±5	**2±1***	1±2

	Cu	Zn	As	Cd	Cs	Pb
FW 2years old small	36±38	17±13	0.7±0.5	55.5±55	0.75±0.71	1.54±1.53
BW 2years old small	9±4	162±212	0.3±0.1	31±13	0.2±0.1	0.6±0.7

***Co – FW - 2 years old small fish – 6 fish had a concentration less than the detection limit.**

BW – 2 years old small fish - 1 fish had a concentration less than the detection limit.

SW – 2 years old small fish - 2 fish had a concentration less than the detection limit.

***As – SW – 2 years old small fish - 2 years old small fish – 1 had a concentration less than the detection limit.**

Table 14. Mean concentration of trace metals in kidneys in 3 years old fish in BW and SW (average±standart deviation) (n=8).

	Al	Ti	Cr	Mn	Co	Ni
BW 3years old	196±147	7±2	0.7±0.5	11±3	2±0.7	1.8±1.7
SW 3years old	187±36	6±1	0.6±0.2	7±1	1±0.3	1.2±0.5

	Cu	Zn	As	Cd	Cs	Pb
BW 3years old	11±4.9	369±163	0.4±0.08	42±20	0.4±0.2	0.5±0.2
SW 3years old	9±1	290±59	0.3±0.1	27±10	0.3±0.1	0.4±0.1

5.7.1 Concentration of Al

There was a significant difference between the concentration of Al in kidneys in bw and fw (p-value = 0.02). There was also a difference between the concentration of Al and the fish with a different age - 2 years old small and 3 years old fish (p-value = 0.01), see Table 12, 13, 14 and Figure 38.

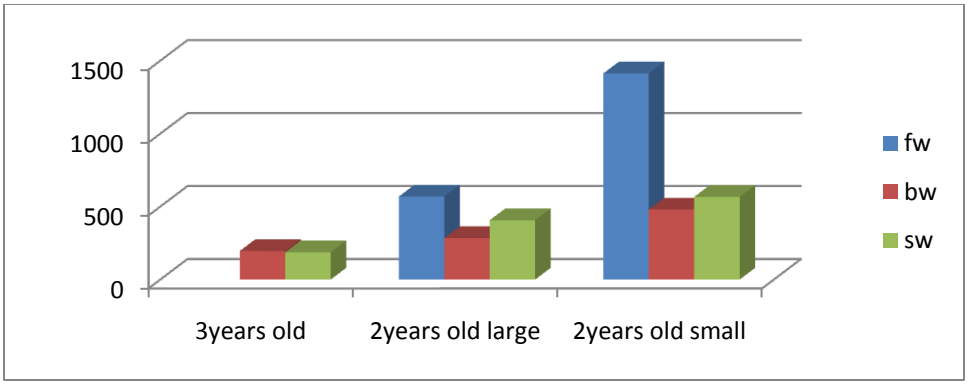

Figure 38. Mean concentration of Al, µg/g dw in kidneys in FW, BW and SW for 3 and 2 years old fish.

5.7.2 Concentration of Cd

The results showed that the concentration of Cd in 3 years old fish in sw was lowest, see Table 12, 13, 14 and figure 39. The concentration of Cd in 2 years old small fish in fw was higher than in the 2 years old large fish in fw.

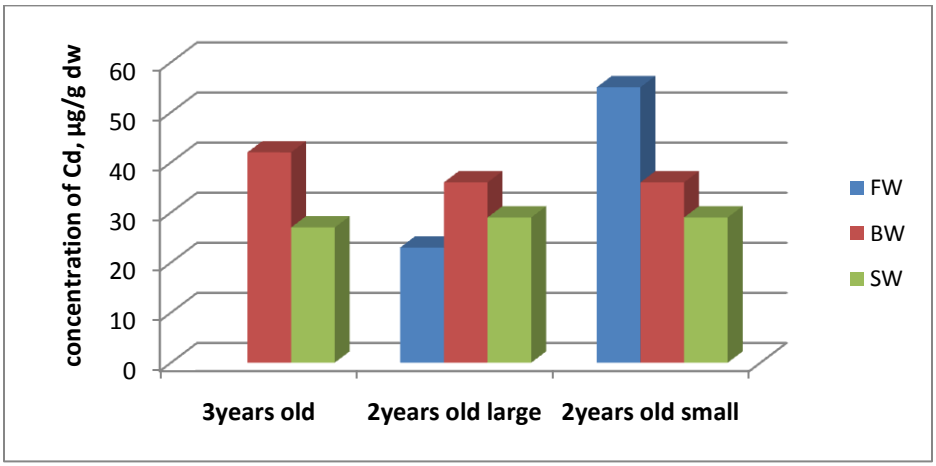

Figure 39. Mean concentration of Cd, µg/g dw in kidneys in FW, BW and SW for 3 and 2 years old fish.

5.7.3 Concentration of Cu

The results showed that the concentration of Cu in in 2 years old small fish in fw was highest, see Table 12, 13, 14 and Figure 40. The statistical test showed a difference between fw and bw (p-value = 0.01). There was no difference related to the fish age.

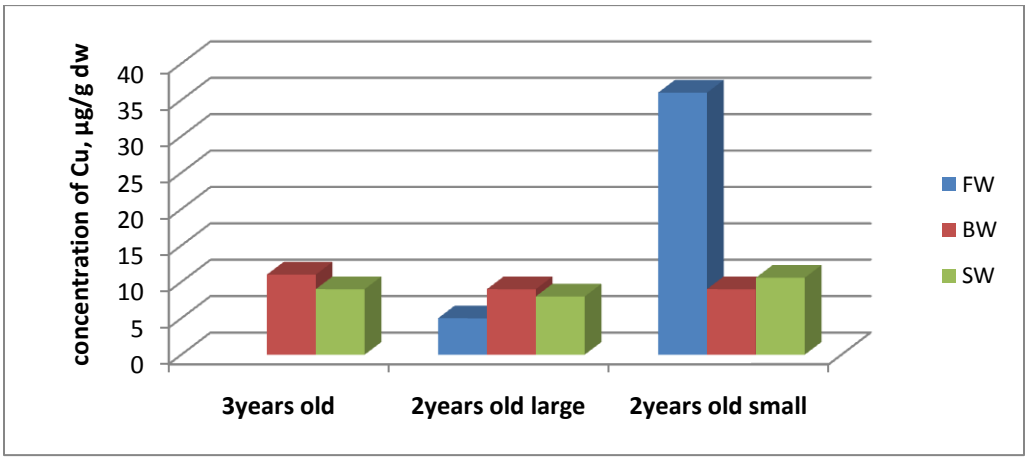

Figure 40. Mean concentration of Cu, µg/g dw in kidneys in FW, BW and SW for 3 and 2 years old fish.

5.7.4 Concentration of Zn

The results showed that the concentration of Zn in bw and sw was highest in 3 years old fish, see Table 12, 13, 14 and Figure 41. The results for 2 years old large and 2 years old small fish in bw and sw were similar within the two groups. The concentration of Zn in 2 years in fw old large and small fish was lowest.

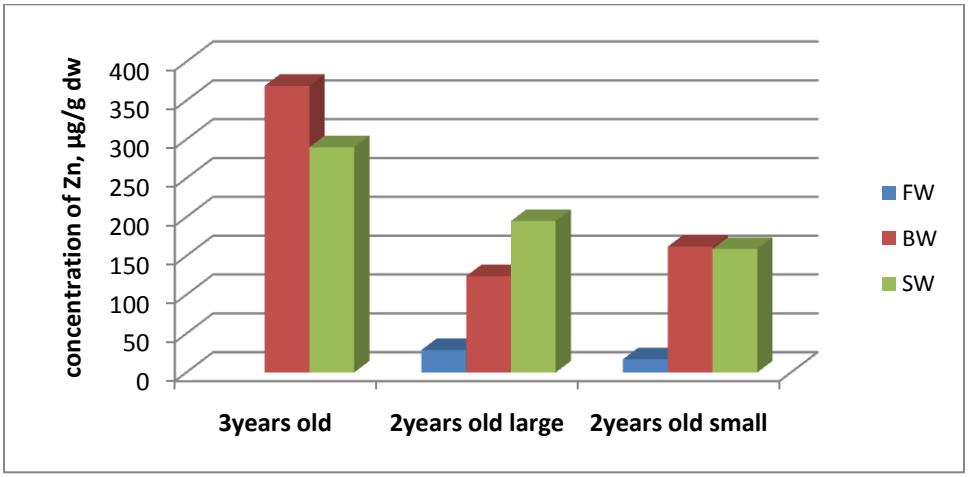

Figure 41. Mean concentration of Zn, µg/g dw in kidneys in FW, BW and SW for 3 and 2 years old fish.

5.7.5 Concentration of Pb

The results showed that the concentration of Pb in 3 years old fish and 2 years old large fish in bw and sw were similar, see Table 12,13, 14 and Figure 42. Highest concentration was found to be in 2 years old small fish in fw.

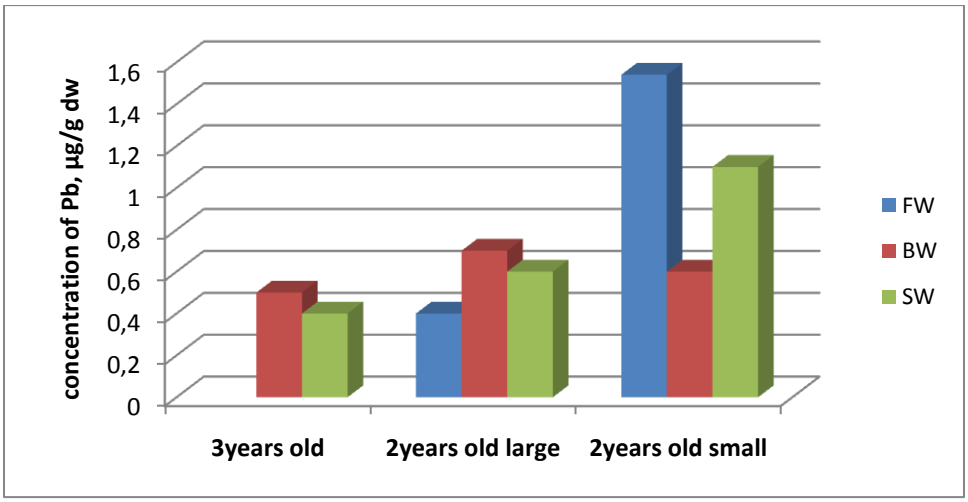

Figure 42. Mean concentration of Pb, µg/g dw in kidneys in FW, BW and SW for 3 and 2 years old fish.

5.7.6 Concentration of As

The results showed that there were some differences between the investigated groups - the concentration of As in 3 years old fish in bw was highest, see Table 12, 13, 14 and Figure 43. The concentration in 2 years old small fish was highest in fw.

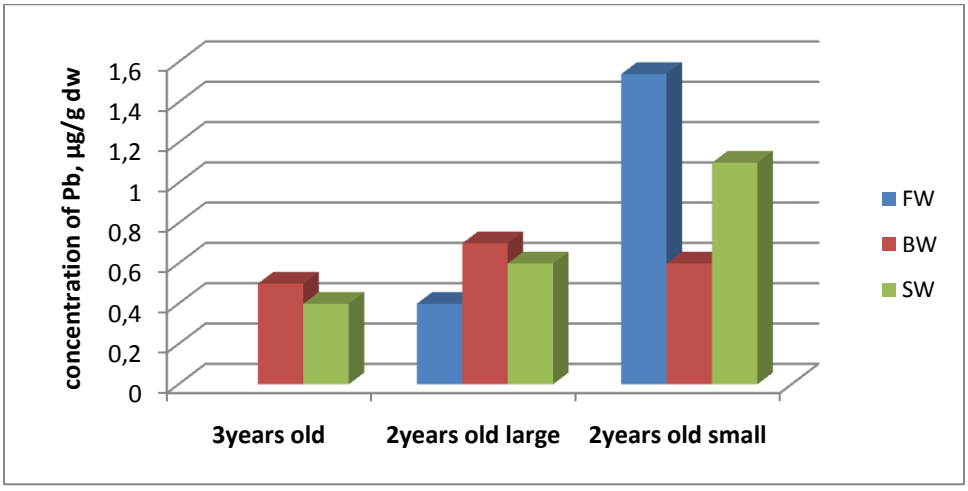

Figure 43. Mean concentration of As, µg/g dw in kidneys in FW, BW and SW for 3 and 2 years old fish.

5.7.7 Concentration of Ni

The results showed that the concentration of Ni in 3 years old fish in sw was highest, see Table 12, 13, 14 and Figure 44. The concentration was highest in 2 years old small fish in bw and also the concentration of Ni in 2 years old small fish in fw.

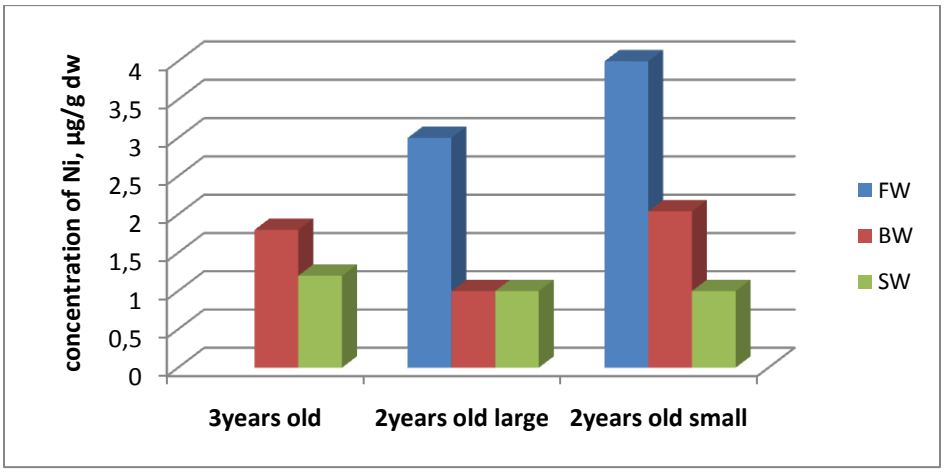

Figure 44. Mean concentration of Ni, µg/g dw in kidneys in FW, BW and SW for 3 and 2 years old fish.

5.7.8 Concentration of Cr

The results showed that the concentration of Cr in 2 years old small fish in fw and sw was highest, see Table 12, 13, 14 and Figure 45. However, the concentration of Cr in bw in that group of fish was lowest. The concentration in 3 years old and 2 years old large fish in bw was the same.

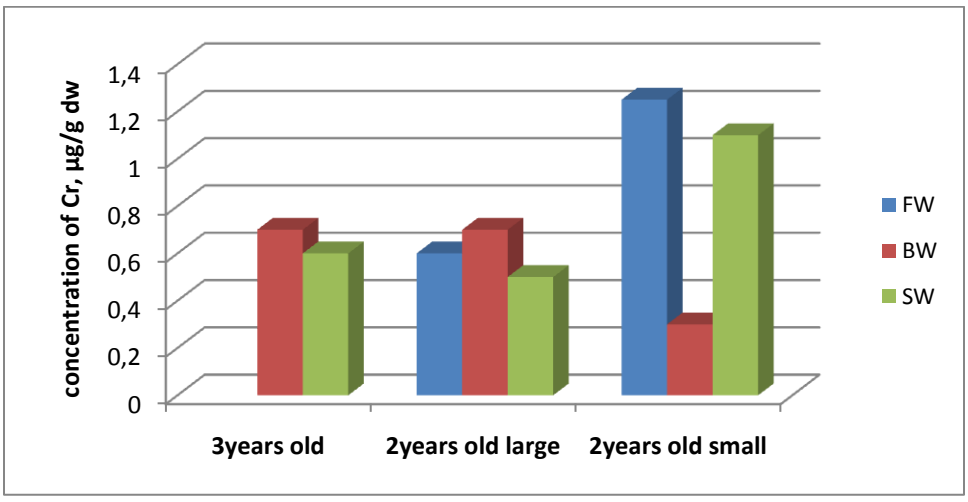

Figure 45. Mean concentration of Ni, µg/g dw in kidneys in FW, BW and SW for 3 and 2 years old fish.

5.7.9 Concentration of Mn

The results showed that the concentration of Mn in 3 years old fish was highest in bw and sw, see Table 12, 13, 14 and Figure 46. The concentration of this metal in 2 years old large fish in fw was higher than in 2 years old small fish. The concentration in 2 years old large and small fish in bw and sw did not show any significant differences.

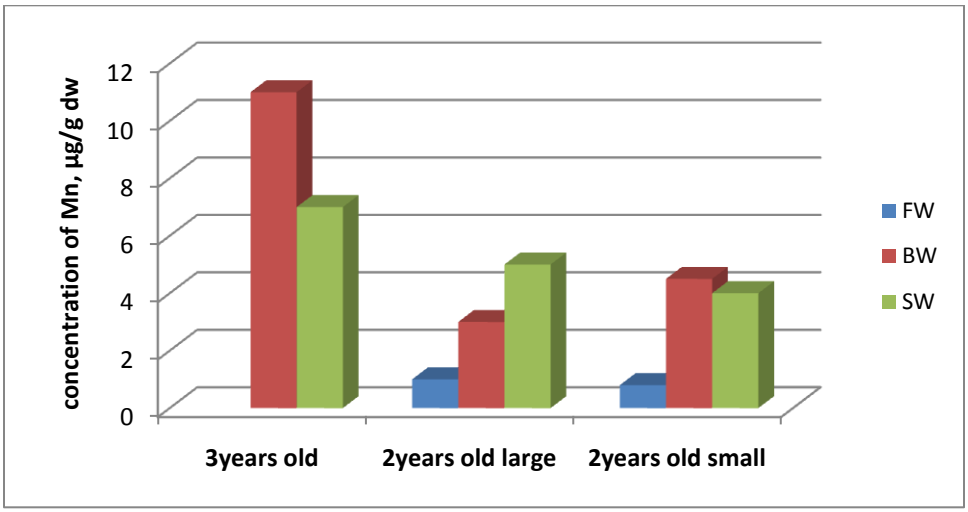

Figure 46. Mean concentration of Mn, µg/g dw in kidneys in FW, BW and SW for 3 and 2 years old fish.

5.7.10 Concentration of Co

The results showed that the concentration in fw was much higher in 2 years old small than in 2 years old large fish, see Table 12, 13, 14 and Figure 47.

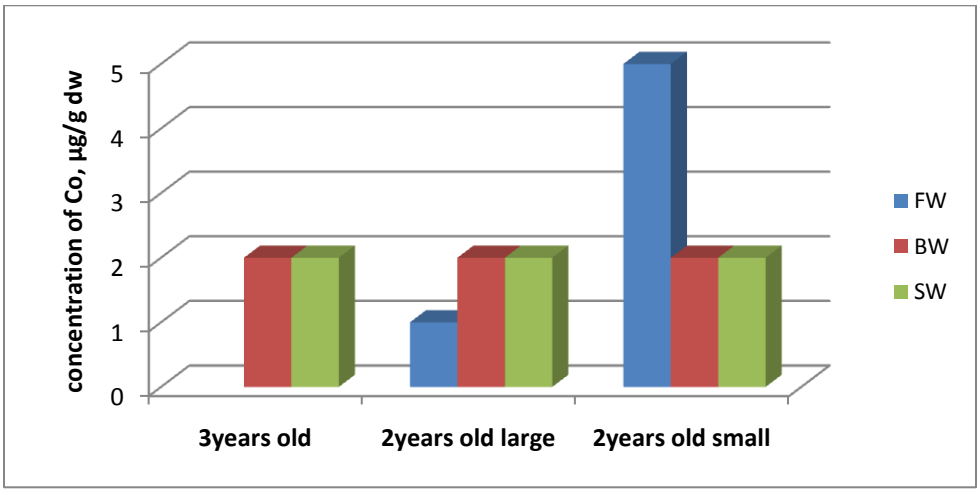

Figure 47. Mean concentration of Co, µg/g dw in kidneys in FW, BW and SW for 3 and 2 years old fish.

5.7.11 Concentration Ti

The results showed that the concentration of Ti in fw was highest in 2 years old small fish, see Table 12, 13, 14 and Figure 48. The concentration of Ti in the 3 years old fish group in sw was lowest.

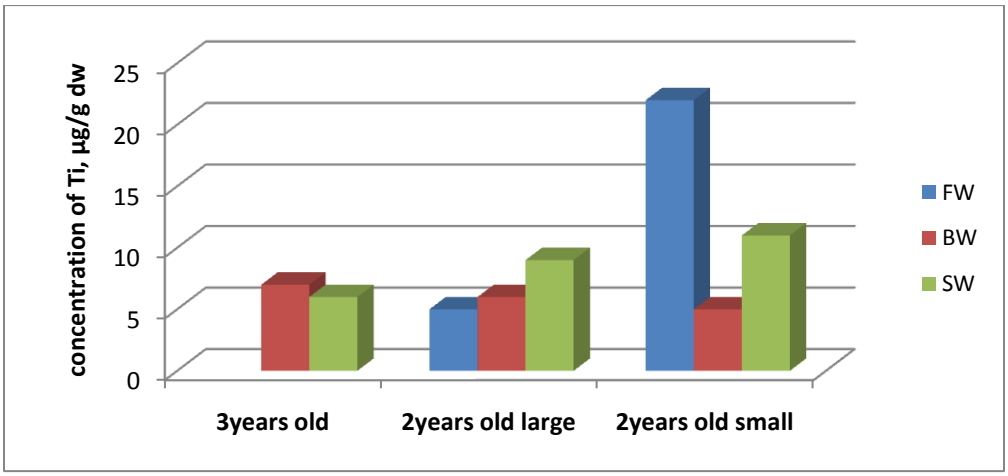

Figure 48. Mean concentration of Co, µg/g dw in kidneys in FW, BW and SW for 3 and 2 years old fish.

5.7.12 Concentration of Cs

The results showed that the concentration of Cs in 3 years old fish in bw was highest, see Table 12, 13, 14 and Figure 49. The concentration in 3 years old and 2 years old large fish in sw was the same. The concentration was detected to be highest in 2 years old small fish in fw and much higher than the one in 2 years old large fish.

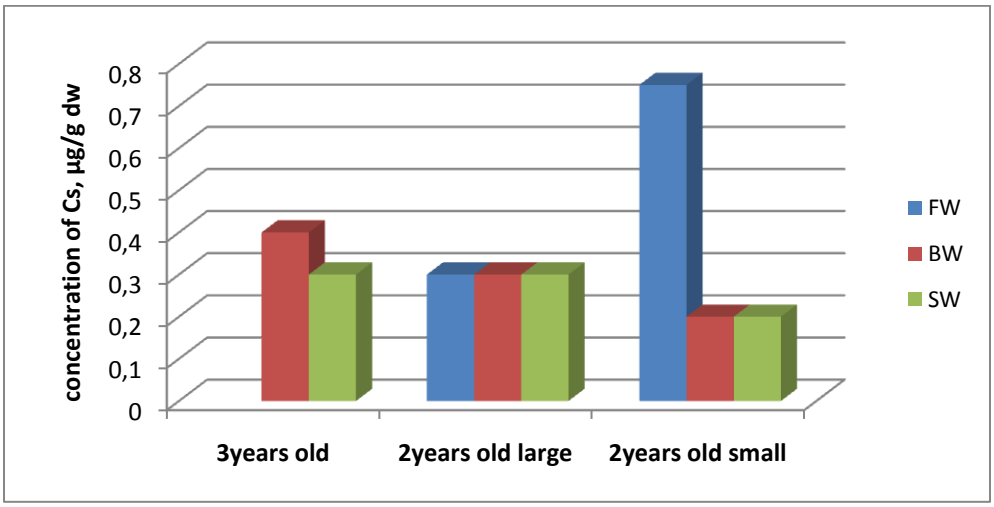

Figure 49. Mean concentration of Cs, µg/g dw in kidneys in FW, BW and SW for 3 and 2 years old fish.

6. Accuracy

During the process of analyzing the liver and kidney samples, certified reference material from dogfish's liver (DOLT 4) and dogfish's protein (DORM 3) was used. The more detailed information about this certified material is presented in Table 12 and 13.

Table 15. - Information value for DOLT 4 *(Sturgeon, 2007)*

DOLT 4	Element	Mass fraction (mg/kg)
	Na	6800
	Mg	1500
	Al	200
	Ca	680
	V	0.6
	Cr	1.4
	Co	0.25
	Sr	5.5
	Mo	1
	Sn	0.17
	K	9800

Table 16. – Certified value for DOLT 4 and DORM 3 *(Sturgeon, 2007)*

DOLT 4	Trace element	Mass fraction (mg/kg)	DORM3	Trace element	Mass fraction (mg/kg)
	As	9.66±0.62		As	6.88±0.30
	Cd	24.3±0.8		Cd	0.29±0.02
	Co	31.2±1.1		Cu	15.5±0.63
	I	1833±75		Cr	1.89±0.17
	Pb	0.16±0.04		I	347±20
	Hg	2.58±0.22		Pb	0.395±0.05
	Ni	0.97±0.11		Hg	0.382±0.06
	Se	8.3±1.3		Ni	1.28±0.24
	Ag	0.93±0.07		Sn	0.66±0.012
	Zn	116±6		Zn	51.3±31
	CH3Hg	1.33±0.12		CH3Hg	0.355±0.056

By using MINITAB release 15 regressions between the concentration of every single element and the organs (gills-kidneys ; gills-livers ; kidneys-livers) were done, see Table 17. Significant differences between the concentration of the elements and the tested organs were found. The gills had a higher concentration of accumulated metals than the livers and the kidneys, and the kidneys had a higher concentration than the livers. In addition, there was correlation found between the Co concentration in the gills and the livers and between the Al, Mn and Zn concentration in the kidneys and the livers.

Table 17. – Regression model, MINITAB 15 release

Regression equation : Organ 1= const + slope * organ 2

	Al	**Ti**	**Cr**	**Mn**	**Co**	**Ni**
gills/kidneys						
const	136*	26*	3.37*	75.4	0.53*	3.74*
slope	0.00722	0.16	0.11	-0.74	0.07	0.147
gills/livers						
const	137*	29.9*	3.44*	78.3*	0.27*	4*
slope	0.04	-0.8	0.034	-1.31	1.04**	-0.02
kidneys/livers						
const	260*	11.7*	0.79*	0.38	2.95*	2.51*
slope	2.96**	-0.69	-0.041	0.993**	-0.96	-0.243

	Cu	**Zn**	**As**	**Cd**	**Cs**	**Pb**
gills/kidneys	2.76*	1106*	0.09*	29.7	0.02	1.37*
const	0.009*	-0.852	0.02	-0.36	0.48	-0.014
slope	0	0	0	no difference	no difference	0.02
gills/livers						
const	3.47*	1118*	0.09*	23.7*	0.7*	1.34*
slope	0.003	-2.09	0.05	-1.33	-0.26	0.09
				no difference		
kidneys/livers						
const	13.4*	15	0.476*	34.9*	0.384*	0.84*
slope	-0.008	2.04*	-0.019	0.09	0.017	-1.842

p-value < 0.05 - difference * - significant difference
p-value > 0.05 - no difference **- correlation

The distribution of the investigated metals within the three organs was followed by using Principal Component analysis, see Figure 50, 51, 52, 53. The main goal of usuing this analysis was to explain the variance-covariance structure of the variables in this study through linear combinations.

Firstly, the distribution of the meatls within the gills was presented, see Figure 50. It showed that the elements Ni, As, Cr, Cu, Cs, Pb and Ti followed each other. This pattern also referred to Mn, Zn, Al and Co. However, when the concentration of Cd was high, the concentration of Mn, Zn, Al and Co was low.

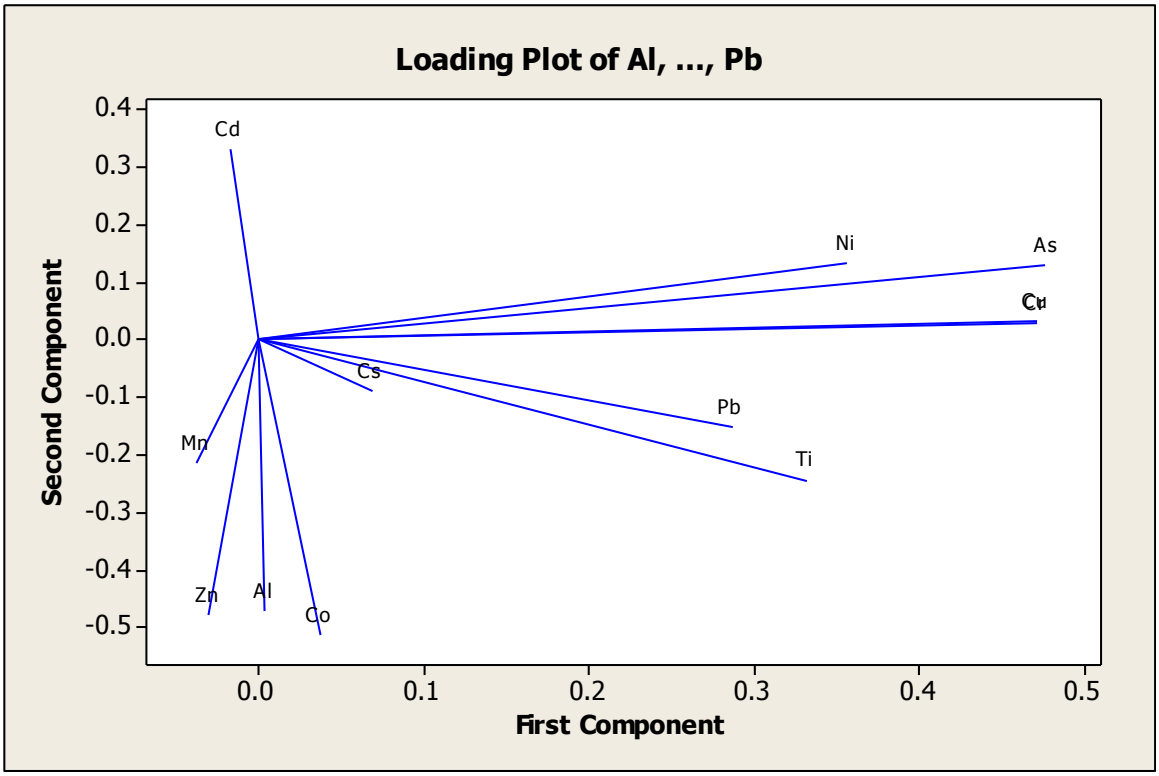

Figure 50. Distribution of the trace metals within the gills (first component = 32%, second component = 14%).

Secondly, the distribution of the metals within the livers was presented, see Figure 51. It showed that the elements As, Cd, Cs, Co, Cu and Ti followed each other. This pattern also referred to Ni

and Cr, and Zn and Mn. Al was excluded because the livers were wrapped in Al foil and it might have contaminated the samples. Thus, its distribution was not presented.

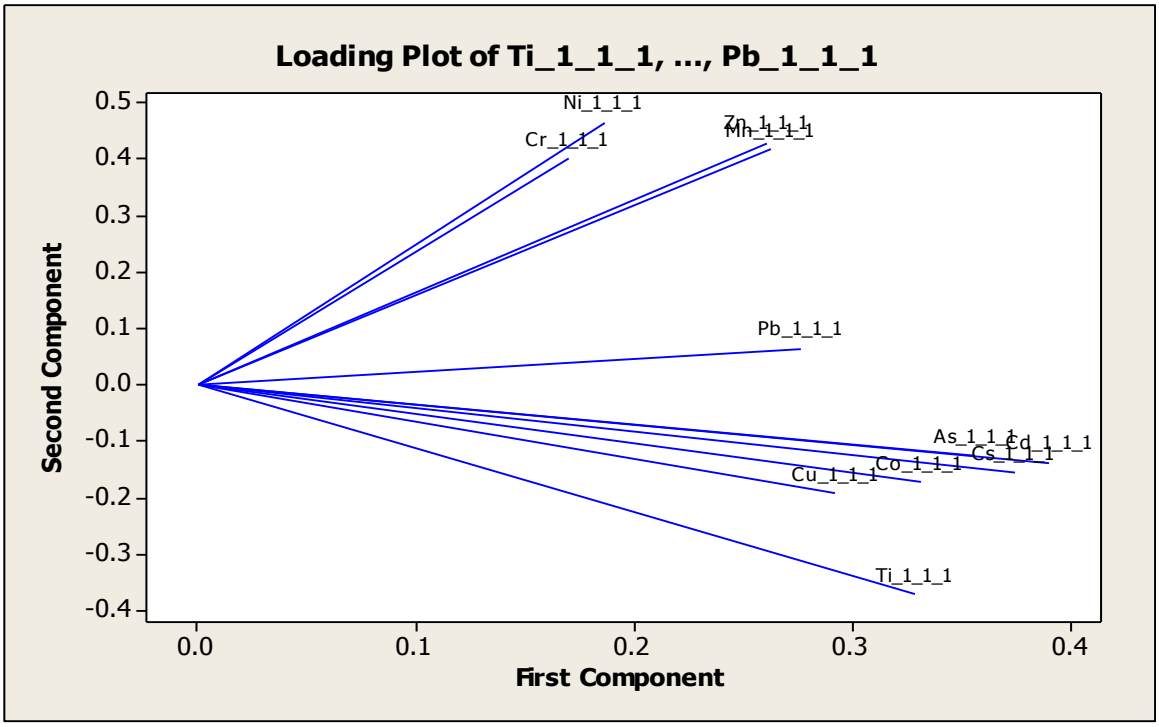

Figure 51. Distribution of the trace metals within the livers (first component = 36%, second component = 18%).

Thirdly, the distribution of the metals within the kidneys was presented, see Figure 52. It showed that the elements Ni, Cr, Cu, As, Pb, co, Cs, Ti and Cd followed each other. This pattern also referred to Zn and Mn. Al was excluded because the kidneys were wrapped in Al foil and it might have contaminated the samples. Thus, its distribution was not presented.

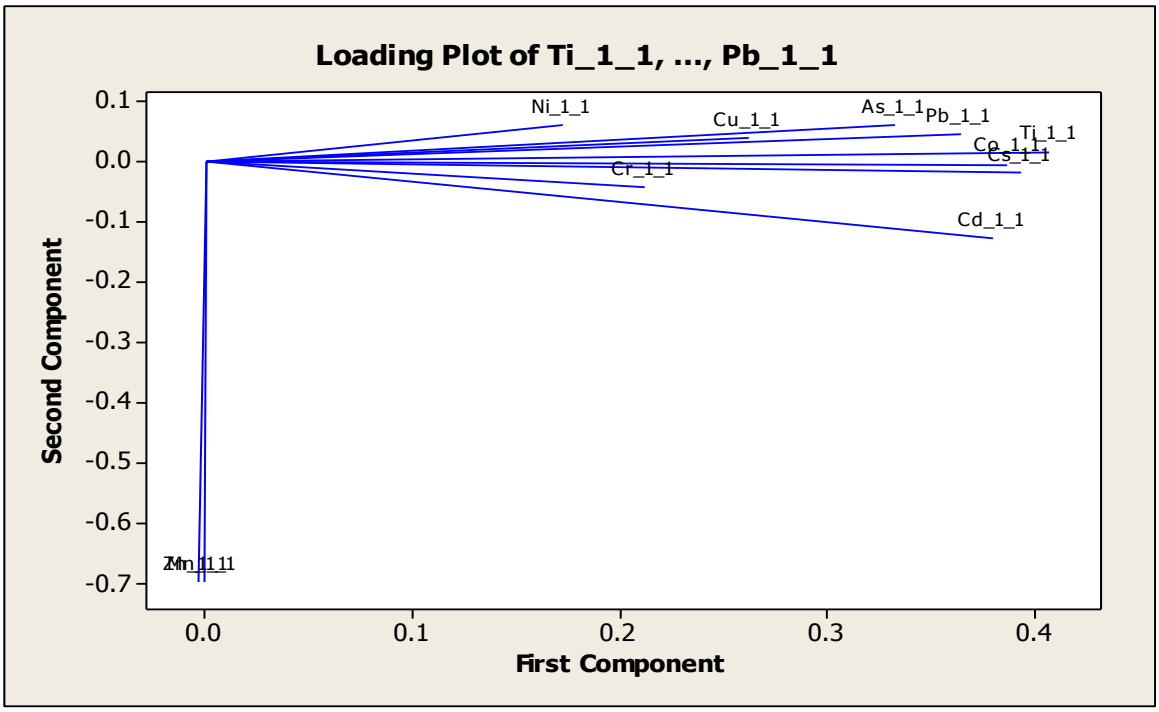

Figure 52. Distribution of the trace metals within the kidneys (first component = 48%, second component = 17%).

Lastly, the distribution of the metals within the three organs was followed, see figure 53.

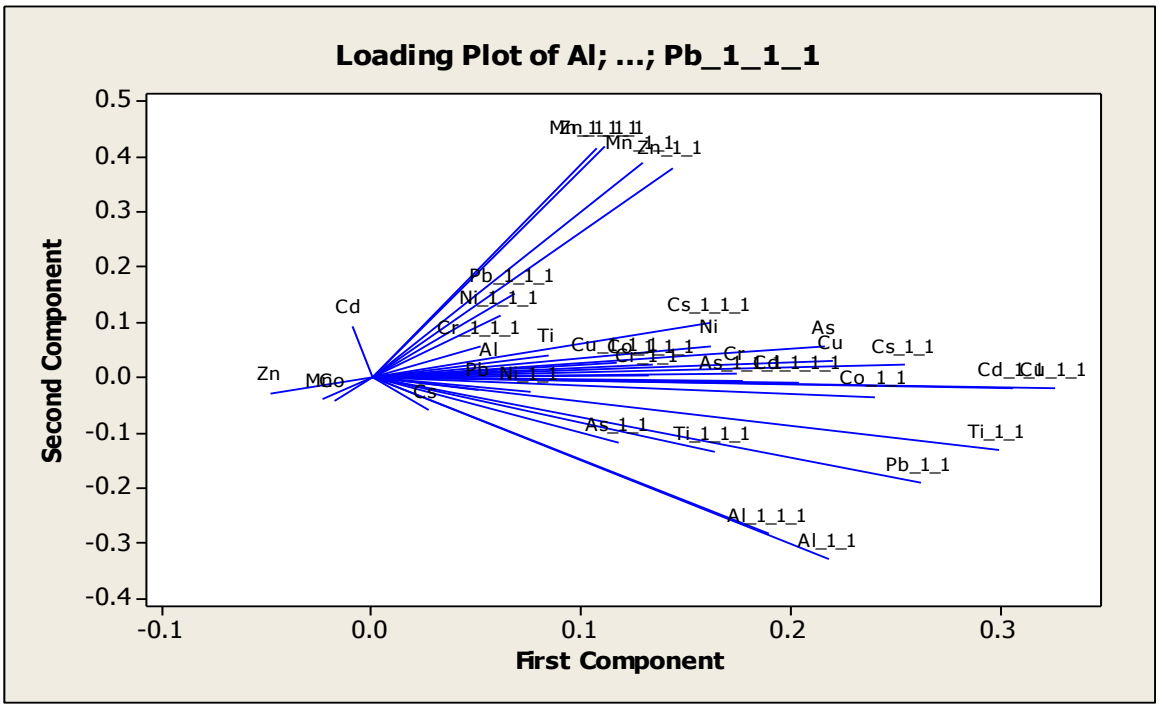

Figure 53. Distribution of the heavy metals within the organs (gills; kidneys 1_1 ; livers 1_1_1).

However, here the Al concentration in the livers and the kidneys was presented and it showed that Al in the livers followed Al in the kidneys. In contrast, this metal was distributed differently in the gills. This proved an example of a possible Al contamination in the livers and the kidneys samples.

7. Discussion

7.1 Water

The investigation of the water quality aimed to indicate if it reflected significantly the accumulation of trace metals in the analysed 3 years, 2 years large and small fish gills, livers and kidneys in fw, bw and sw. Metals bioaccumulate and have negative effects in fish, therefore, they could reduce their growth as energy is put into detoxification.

Smolts are raised in certain areas in a river and these areas may have different concentration of metals and heavy metals. By having done metal analysis in organs, I tried also to see where the individual smolt had been raised in the river.

The concentration of the trace elements in the water influenced the concentration of the metals into the gills, in particular Al. In gills of fish exposed to bw the levels of concentration of the investigated trace elements were higher than in gills of fish in fw and sw. In addition, most of the gills of 3 years old fish from bw had also higher trace metal levels, but these levels were close to the concentration in gills of 2 years large and small fish. This supported one of the hypotheses that a metal exposed Atlantic salmon would have to use a longer time for reaching smolt size. This means they will be older.

The concentration of the trace metals in the water did not reflect significantly the concentration of these metals in the livers and the kidneys. The concentration of the trace elements in the analysed livers and kidneys varied among the fish groups related to the age and the water quality. It was considered that this concentration was not dependant upon the water quality or the age of the fish. It could have been dependant upon a diet, genetic factors, behavior patterns, etc. Zn and Pb showed highest concentration in livers of 3 years old fish in bw and sw, and also Pb and Mn in kidneys. These significant differences might have been dependant upon the fw history of this particular fish.

The measured pH of the fw in this experiment was 6.6±0.3, the total Al 141±34µg/l and the LMM - 100±98µg/l The total concentration of Al in bw was 135±17µg/l and in sw 47±29µg/l. In addition, the concentration of low molecular inorganic Al (LMM Ali) were 41±21µg/l in bw and 28±15µg/g in sw. This form of Al is considered to me the most toxic to aquatic organisms and its levels were above the levels of toxicity. The total Cu concentration in bw was estimated to be 1±0.2µg/l and in sw - 1.42±0.52µg/l.

In comparison with this data, *Skalsbakken (2009)* measured pH of River Storelva in laboratory experiments to be 5.7 (November, 2008), 5.5-5.85 (January, 2008) and 6.06 (April, 2009). She also measured the total Al and LMM Al to be 131µg/l and 40 µg/l (November, 2008); 107-209µg/l (January, 2009) and 53-79µg/l (January, 2009); 145µg/l and 63µg/l (April, 2009), see Table 18.

Table 18. – General water chemistry of freshwater from the Storelva River used in laboratory experiments *(Skalsbakken, 2009).*

			Concentrations (mg/L)										
	pH	Conductivity (µS/cm)	Al	Ca	Mg	Na	K	Si	TOC	F	NO_3	SO_4	Cl
River Storelva, November 2008	5.70[1]	-	0.131	1.5	0.30	1.7	0.32	1.1	4.7	0.023	0.12	2.10	2.51
River Storelva, 26th January 2009	5.85	26	0.177	1.8	0.39	2.0	0.25	1.6	5.2	0.023	0.20	2.40	3.32
River Storelva, Mid-January 2009	5.50[2]	29	0.209	0.2	0.41	2.4	0.22	1.7	5.6	0.022	0.20	2.36	4.20
River Storelva, 1st April 2009	6.06	24	0.145	1.6	0.33	2.0	0.25	1.4	5.4	0.040	0.18	2.37	2.98

[1]The raw water had originally a pH of 6.20 before it was acidified with HCl to pH 5.7. The pH of 5.7 is chosen since this is the normal pH on the water in the spring (Figure 3).
[2]The raw water had originally a pH of 5.76 before it was acidified with HCl to pH 5.5, in order to simulate an episodic event with low pH.
- Value not determined.

Other authors have presented data for metals in water. For example, in the experiment of *Rosseland et al. (2007)* the Imsa strain (South-Western Norway) had pH 5.8; 5–15µg LAl l^{-1}. In the Al toxicity experiment to rainbow trout by *Dietrich and Schlatter (1984)* it was stated that slightly enhanced production of mucus was recorded at pH 5.4 and 200µg A1/1, while moderate and extensive mucification resulted from exposing the fish to pH 5.4 and 400µg A1/1, pH 5.6 and 200µg Al/l, and pH 5.6 and 400µg Al/l, respectively. Fish dying at pH 5.2 and 200µg Al/l did not exhibit gaping opercula, although this was seen at pH 5.4 and 400µg Al/l, pH 5.6 and

200µg A1/1, or 400µg Al/l. 200µg Al/l was lethal to 50% of the exposed fish within 64 h at pH 5.2, no deaths occurred at pH 5.4, and three fish out of ten died within 96 h at pH 5.6 .

The total Cd concentration in fw in this study was 0.1±0.05µg/l and LMM Cd 0.1±0.08µg/l, which is considered as low.
Dissolved Cd levels in fresh water generally range from 10 to 500ng l-¹, but there have been recorded levels >17mg l-¹ in industrialized areas *(Jones et al., 2005).* Unpolluted lake waters have concentrations of Cd generally lower than 0.1mg l^{-1}. Concentrations of Cd of 0.4-0.5mg l^{-1} are frequently observed in areas where mining and smelting activities occur. Concentrations of 5mg l^{-1} and more have been reported *(Brodeur et al., 1998)*

The total Pb concentration in this study fw was 0.24±0.14µg/l and LMM Pb was 0.1±.1µg/l. Pb reaches the aquatic system because of superficial soil erosion and atmospheric deposition. The concentration of Pb in deep oceanwaters is about 0.01-0.02µg/l, but in surface ocean waters is about 0.3µg/l *(Castro-Gonzalez and Mendez-Armenta, 2008).*

The total As concentration in this study in fw was 1±0.1µg/l and LMM As was 0.07±0.02µg/l. To date more than 25 different arsenic species have been identified in the environment. In seawater and marine sediments typical total As concentrations in the range of 1-2µg l^{-1} and 3-15mg kg^{-1}, respectively. Marine organisms are capable of bioaccumulating arsenic and concentrations of total As in the range of 1-100mg kg^{-1} ww have been reported in marine animals and plants *(Edmonds and Francesconi, 2003).* In freshwater, As occurs in both inorganic and organic forms, as well as in dissolved and gaseous states. The chemical forms of As depend on Eh, pH, organic content, suspended solids, microbial activity, etc. In Norway, high As concentrations seem to be more abundant in low TOC lakes with pH values < 6 *(Lydersen et al., 2002).* Aresenic naturally occurs as sulfides and as complex sulfides of Fe, Ni, and Co. Soil arsenic levels are normally elevated near arseniferous deposits and in mineralized zones containing gold, silver, and sulfides of lead and zinc. Secondary iron oxide formed from the weathering of pyrite act as scavengers of As. Scandinavian moss analysis in 1995 showed elevated As concentrations in the vicinity of large point sources such as the smelter in Rönnskär (Sweden) and industries at Årdal (Norway). However, in Norway the As distribution was

dominated by atmospheric long-range transport with elevated concentrations (≈1 mg kg^{-1} dw) at the southern coast. In remote areas not affected by local pollution and with low long-range transport, the As concentrations in moss varied between 0.2 to 0.6mg kg^{-1}dw in both Norway and Sweden *(Lydersen et al., 2002).*

The total Ni concentration in this study in fw was 1±0.1µg/l and LMM Ni was 1±0.04µg/l. In unpolluted stream and river sediments, the Ni concentrations generally are in the range of 1 to 150 mg kg^{-1} dw, but with values up to 1000 mg kg^{-1} dw in the vicinity of nickeliferous deposits *(Lydersen et al., 2002). Lydersen et al. (2002)* also reported lowest known effect at 23µg Ni l^{-1} and assume risk of biological effects in sensitive Swedish waters at 15µg Ni l^{-1}. The same authors reported maximum acceptable toxicant concentration for Atlantic salmon of 50µg Ni l^{-1} or somewhat lower.

The total concentration of Mn in this study in fw was 0.3±0.05µg/l and LMM Mn was 0.3±0.07µg/l. High concentrations of Mn in surface waters are normally present in high TOC and/or low pH lakes and is normally well correlated with iron (Fe). The concentration of Mn in surface water and the subsequent efflux are highly dependent on the concentration of Mn^{2+}. Low concentration of oxygen (low Eh), low pH, and high TOC (reductant) increases the possibility of high Mn^{2+} concentrations. The highest Mn concentration should be expected in shallow acidic, high TOC lakes *(Lydersen et al., 2002).* The same authors assumed Mn^{2+} being the main toxic compound for brown trout mortality during springmelt in two mountain streams in central Sweden.

7.2 Gills

7.2.1 Concentration of Al

In neutral or non-acidified waters, gill Al concentration in Atlantic salmon is always < 1-2µg g^{-1} gill (ww) or < 5-10µg g^{-1} gill (dw). In brown trout, a gill Al concentration of 4.9±2.7µg g^{-1} gill (dw) was found on reference fish from neutral waters *(Rosseland et al., 2007).*

In this project Al accumulation levels into gills was observed for 3 years old and 2 years old fish (large and small). The results showed that in bw the mean levels of accumulation were highest - 233±11µg/g dw for 2 years old large fish ; 204±89µg/g dw for 2 years old small fish and

217±74µg/g dw for 3 years old fish. This data proved firstly, the acidification problem in the river and secondly, the high toxic Al levels that accumulates in gills and therefore, can cause fish death. Several authors have documented Al accumulation in gills. Compared with other experiments, in this the concentration of Al accumulated into Atlantic salmon was higher. For example, Al concentrations in the gills of brook trout *(Salvelinus fontinalis)* from acidic (pH 4.7-5.8) Lake Tantare in Quebec (Canada) averaged 106µg g^{-1} dry weight *(Spry and Weier, 1991).* Exposure to low concentrations of Ali raised the gill-Al concentration by 20-30µg Al g^{-1} gill dry weight compared to control fish having < 10µg Al g^{-1} gill dry weight in the experiment made by *Kroglund and Finstad (2003).* The concentration of Al in fish gills was relatively low (28±20µg Al g^{-1} dry weight (dw) for fish, but high for pre-exposed fish (River Ritlandsona spring; 220 ± 35µl g^{-1} dw) prior to mixing zone exposure in the experiment of *Kroglund et al. (2006).* In addition, in the experiment of *Kroglund et al. (2007)* fish exposed to Lake Imsa water (pH > 6.5 and < 5µg LAl l^{-1}) acted as controls. Control fish had gill-Al concentrations in the range of 5 to 10µg Al g^{-1} gill dry weight (dw), while Al-exposed fish had gill-Al concentrations exceeding 20 µg Al g^{-1} gill dw prior to seawater release. Accumulation of Al on gills was high, and increased from 4 to a maximum of 350µg/g in brown trout in water draining a shooting range *(Heier et al., 2009).* The concentration of gill Al in Lochnagar was 21.6±4.8µg g^{-1} gill (ww) and showed no bioaccumulation *(Rosseland et al., 2007).* This referred to 108±24µg/g dw.

7.2.2 Concentration of Cd

Fish readily absorb aqueous Cd^{2+}, and direct uptake across the gills has been widely considered to be the primary influx of the metal for fish in dilute waters *(Spry and Weier, 1991).* The same authors, however, exposed rainbow trout and lake whitefish *(Coregonus clupeaformis)* to dietary and aqueous cadmium and found that both species accumulated more Cd from food than from water.

In this experiment highest mean Cd concentration into gills was discovered in 3 years old fish in sw - 142±261 µg/g dw. Based on the 2 years old groups the Cd levels were highest aslo in fish gills from sw, but they were much lower than in 3 years old fish in sw. This mean concentration ranged beween 5 and 8µg/g dw. These results were close to the mean gill concentration of Cd in brown trout from Lochnagar found by *Rosseland et al. (2007)* was 2.1 µg/g (ww) (10.5µg/g dw). Gills take up Cd from the water and histological changes in the gills are detected in rainbow trout

exposed to Cd within 1 h. It has been shown by others that Cd interferes with the active uptake of Ca in the gills by blocking Ca^{2+} channels and causes hypocalcemia *(Hontela et al., 1996).* *Popova et al. (1997)* found in an experiment 0.02-0.28 µg/g dw Cd in gills in fresh water fish. In their study *Zachariassen et al. (2000)* investigated the concentration of Cd in two Norwegian rivers and in brown trout during a run-off episode. Gills Cd for RR was 0.55 (0.48/0.64), for RN - 0.59 (0.54/0.60), for NN - 2.34 (1.94/3.59), for NR - 3.45 (3.15/3.74) (µg/g tissue wet weight), and the values were given as medians, first and third quartiles (in parentheses) (RR, Rugla trout in Rugla water; RN, Rugla trout transferred to Naustebekken water; NN, Naustebekken trout in Naustebekken water; NR, Naustebekken trout transferred to Rugla water).

7.2.3 Concentration of Cu

Cu can be acutely toxic to freshwater fish, the toxicity depending on species, age, size, water temperature and ionic composition *(Pilgaard et al., 1994).* The uptake of Cu by the fish is considered to be passive, involving diffusion down gradients made by adsorption or binding of the metal to tissues or cell surfaces. The gill is an obvious site of Cu uptake, because of its great surface area and intimate contact with the external environment *(Meador, 1991).* The mucous covering the gills accumulated Cu to a great extent. This binding of Cu to mucous might hinder the most toxic states of Cu, the Cu ion and the Cu hydroxides in binding to the apical membrane of the gill epithelium. An elevated rate of mucus secretion appears to be a defense mechanism against several toxic substances, including acid aluminium zinc *(Pilgaard et al., 1994).*

In this study highest mean Cu levels of accumulation were detected in 3 years old fish in sw fish – 4±1µg/g dw, in 2 years old large fish 4±1µg/g dw and 2 years old small fish. The mean concentration of Cu within the three age groups and water quality, however, ranged between 2-4µg/g dw. These results were close to the mean gill concentration of Cu in brown trout from Lochnagar found by *Rosseland et al. (2007)* which was 0.4µg (ww) (2µg/g dw), and the gill concentration of Cu 2-4µg/g dw gill in brown trout in the study of *Heier et al. (2009).* *Zachariassen et al. (2000)* investigated the concentration of Cu in two Norwegian rivers and in brown trout during a run-off episode. Gill Cu for RR was 1.91 (1.72/2.04) , for RN - 1.68 (1.50/1.97), for NN -1.51 (1.18/1.68), for NR -2.63 (2.17/3.15) (µg/g tissue wet weight), and the values were given as medians, first and third quartiles (in parentheses) (RR, Rugla trout in Rugla

water; RN, Rugla trout transferred to Naustebekken water; NN, Naustebekken trout in Naustebekken water; NR, Naustebekken trout transferred to Rugla water).

7.2.4 Concentration of Zn

High Zn levels were determined in this fish experiment. The mean concentration of Zn was higher in gills of fish in bw and in particular, in 2 years old big fish - 1434±2722µg/g dw. The rest of the mean Zn levels ranged between 688 and 833µg/g dw. Even though, they were lower than in the 2 years old large fish gills, these numbers were higher than the mean gill concentration of Zn in brown trout from Lochnagar found by *Rosseland et al. (2007)* which was 78.9µg (ww) (394.5µg/g dw), and concentration of 313-767µg/g dry gill found in brown trout in the experiment, made by *Heier et al. (2009). Popova et al. (1997)* found in fresh water fish in gills 75-675µg/g dw Zn. *Adeyeye et al. (1996)* investigated the levels of heavy metal accumulation in three fish species and the levels for Zn in gills were - 1.41 (0.01) ppm dry weight for Common carp *(Cyprinus carpio),* 0.80 (0.00) ppm dry weight for African Sharptooth catfish *(Clarias guriepinus),* and 3.00 (0.00) ppm dry weight for Nile tilapia *(Oreochromis niloticus). Zachariassen et al. (2000)* investigated the concentration of Zn in two Norwegian rivers and in brown trout during a run-off episode. Gill Zn for RR was 152.97 (139.20/181.98), for RN - 146.45 (142.81/150.99), for NN - 473.73 (380.25/503.43), for NR - 450.91 (405.19:503.77) (µg/g tissue wet weight), and the values were given as medians, first and third quartiles (in parentheses) (RR, Rugla trout in Rugla water; RN, Rugla trout transferred to Naustebekken water; NN, Naustebekken trout in Naustebekken water; NR, Naustebekken trout transferred to Rugla water).

7.2.5 Concentration of Pb

Fish readily absorb aqueous Pb^{2+}, and direct uptake across the gills seems to be the primary mode of uptake of lead in fresh water. Cd and Pb do not biomagnify in aquatic food chains. The concentrations of Cd and Pb in fish do not, therefore, increase with increasing trophic level. Furthermore, concentrations of these two metals within a fish population do not typically increase with increasing body size or age on gill *(Rosseland et al., 2007).* The accumulation of Cd and Pb by fish typically increases with increasing exposure concentration in water *(Spry and Weier, 1991).*

In this study it was found out that highest mean Pb concentration was in gills in 2 years old small fish in sw - 3±7µg/g dw. The mean concentration in gills in bw varied between the range 0.6-1.5µg/g dw and in fw – 0.3-0.8µg/g dw. The mean Pb concentration in gills in fw was lowest. The concentarions were lower than the mean gill concentration of Pb in brown trout from Lochnagar found by *Rosseland et al. (2007)* which was 3.9µg (ww) (19.5µg/g dw). *Adeyeye et al. (1996)* investigated the levels of heavy metal accumulation in three fish species and the levels for Pb in gills were significant only for Nile Tilapia - 1.58 (0.04) ppm dry weight.

7.2.6 Concentration of As

High concentrations of As are found in marine biota, where concentrations of total arsenic typically are in the range of 1– 100 mg kg^{-1} (fresh weight) in marine animals and plants. Arsenobetaine is the predominant and non-toxic species in fish, bivalves and crustaceans, while the more toxic inorganic arsenic species, arsenite (As(III)) and arsenate (As(V)), usually constitute a minor amount of the total content *(Larsen et al., 2005).*

The highest mean concentration of As in this experiment was detected in fish gills in bw and sw in the range 0.08-0.1µg/g dw. In general, the concentration of As in the gills were low. These results were lower than the the mean gill concentration of As in brown trout from Lochnagar found by *Rosseland et al. (2007)* - 0.2µg (ww) (1µg/g dw).

7.2.7 Concentration of Ni

In this experiment highest mean Ni concentration was found in 3 years old fish gills in bw - 6±2µg/g dw. For the rest of the fish groups the Ni levels were higher in gills from sw (the concentration ranged between 3-5µg/g dw). The mean gill concentration of Ni in brown trout from Lochnagar found by *Rosseland et al. (2007)* was 0.3µg (ww) (1.5µg/g dw), which was lower that the concentration of Ni in gills in this study. Concentration of Ni in gills in fresh water fish in the experiment made by *Popva et al. (1997)* was 0.4-9.13µg/g dw Ni.

7.2.8 Concentration of Cr

The highest mean concentration of Cr acuumulated in gills was found to be in 3 years old and 2 years old small fish in sw - 4±1µg/g dw and 4±1µg/g dw. The mean Cr levels in gills in 2 years large fish in fw and bw were lower. These results were close to the mean gill concentration of Cr in brown trout from Lochnagar found by *Rosseland et al. (2007)* of 0.6µg (ww) (3µg/g dw). 0.64-2.0 µg/g dw Cr in gills was found by *Popova et al. (1997)* in fresh water fish.

7.2.9 Concentration of Mn

In this study highest mean Mn concentration was found in gills of 2 years old large fish in bw - 96±131µg/g dw. The mean concentration in gills in sw was between 57-64µg/g dw and in fw 54-60µg/g dw. These results showed higher accumulation of Mn into gills than the results of *Rosseland et al. (2007).* The mean gill concentration of Mn in brown trout from Lochnagar found by *Rosseland et al. (2007)* was 4.2µg (ww) (21µg/g dw). *Adeyeye et al. (1996)* investigated the levels of heavy metal accumulation in three fish species and the levels for Mn in gills were - 2.04 (0.03) ppm dry weight for Common carp, 0.29 (0.02) ppm dry weight for African Sharptooth catfish, and 0.92 (0.02) ppm dry weight for Nile Tilapia.

7.2.10 Concentration of Co

There is very limited literature on Co toxicity if fish and in particular, on Co accumulation in gills. The concentration of Co in this experiment did not vary much between the age groups and the water quality. The mean concentration in the gills in all types of water ranged between 0.4-0.79µg/g dw. The highest mean concentration was detected in 3 years old fish – 0.7±0.4µg/g dw in bw and in 2 years old large fish in sw – 0.79±0.4µg/g dw. *Moiseenko et al. (2008)* found Co in fish from the upper Volga < 0.01-0.98µg/g dw (min-max), middle Volga – 0.17-0.98µg/g dw (min-max) and in lower Volga - < 0.01-0.98µg/g dw (min-max).

7.2.11 Concentration of Ti

There is also very limited literature on Ti toxicity if fish and in particular, on Ti accumulation in gills. The results showed highest concentration of Ti in 3 years old and 2 years old small fish in sw - 29±11µg/g dw and 31±4.9µg/g dw. The mean Ti concentration in fw and bw was lower.

7.2.12 Concnetration of Cs

The literature about Cs accumulation in gills is limited. The highest mean Cs concentration in gills from River Storelva was found in 3 years and 2 years old large fish in bw – 0.2±0.03µg/g and 0.2±0.5µg/g dw. The rest of the Cs levels in the gills were lower.

7.3 Livers

7.3.1 Concentration of Al

Most of the literature about Al toxicity in fish deals with gills. The highest mean concentration of Al in livers in this study was in fish in fw - 175±196µg/g dw. The concentration of Al in 2 years old large and small fish had higher Al concentration in sw. The 3 years old fish group had lowest mean concentration of Al, both in bw and sw - 37±16µg/g and 31±17µg/g dw. *Moiseenko et al. (2008)* found Al in fish livers from the upper Volga 1.59-15.7µg/g dw (min-max), middle Volga –1.86-14.1/g dw (min-max) and in lower Volga – 3.05-14.3µg/g dw (min-max).

7.3.2 Concentration of Cd

In this study highest mean concentration of Cd in livers was detetcted in fish in bw – 3 years old - 5.9±1µg/g dw, 2 years old large - 5±2µg/g dw and 2 years old small fish – 4.5±2µg/g dw. *Popva et al. (1997)* detected 0.4-4.3µg/g dw Cd in liver in fresh water fish from a border region between Russia and Norway. The highest levels of Cd are detected in kidney, liver and gills and liver Cd is considered a good indicator of exposure, together with induction of liver metallothioneins *(Hontela et al., 1996).*

7.3.3 Concentration of Cu

Cu will accumulate in highest concentrations in the intestinal tissues, liver and gall bladder (bile) and in lower concentrations in the gill, muscle and kidney *(Clearwater et al., 2002).* In this experiment highest mean Cu concentration in liver was found in 2 years small fish, especially in bw - 92±54µg/g dw. The Cu concentration was also high in livers of 2 years old large and small fish in bw. In general, the mean Cu concentration ranged between 53-92µg/g dw for all three fish groups. 11-180 µg/g dw Cu in liver in fresh water fish was found by *Popva et al. (1997).* In the study of *Handy (1993)* the liver and the gills of rainbow trout showed progressive contamination, while the kidney did not accumulate Cu significantly.

7.3.4 Concentration of Zn

The highest mean Zn concentration was found to be in livers in bw, in paricular, 3 years old fish - 150±25µg/g dw. The mean concentration of Zn in livers in sw was also highest in 3 years old fish - 107±65µg/g dw. The Zn results showed that there might be a Zn source in fw. As comparison, 98-614µg/g dw Zn was found in liver in fresh water fish by *Popva et al. (1997). Adeyeye et al. (1996)* investigated the levels of heavy metal accumulation in three fish species and the levels for Zn in liver were significant for Common carp - 0.30 (0.05) ppm dry weight and for African Sharptooth fish - 0.48 (0.03) ppm dry weight . *Hodson (1988)* found that zinc toxicity was not influenced by size of juvenile Atlantic salmon while *Farmer et al. (1979)* found that smaller fish are most sensitive to this metal. These observations indicate a need for further investigation of the relationships among zinc toxicity, season and size of juvenile Atlantic salmon *(Hodson, 1988). Farmer et al. (1979)* observed that the incipient lethal level (ILL) of zinc for juvenile Atlantic salmon in water of 10 °C and hardness of 14 mg/l ranged from 0.15 to 1.0mg/l zinc as a function of the season and size of the fish.

7.3.5 Concentration of Pb

Pb accumulates chiefly in the bone, scales, gill, kidney, and liver. Fish readily absorb aqueous Pb^{2+}, and direct uptake across the gills seems to be the primary mode of uptake of lead in fresh water *(Spry and Weier, 1991).* In this study highest mean concentration of Pb in livers was discovered in 3 years old fish, both in bw (0.8±0.5µg/g dw) and sw (0.8±0.4µg/g dw). In general,

the levels of Pb were higher in livers in bw. The results for Pb may indicate that there probably is a Pb source in fw. Pb is most intensely accumulated by the kidneys, liver, and muscle (the respective concentrations were up to 1.3, 0.75, and 0.18µg/g dry weight) related to the findings of *Moiseenko et al. (2008).* For the brown trout from Lochnagar, in the 2-4 year old fish, weighing between 41 and 296g, the Pb in the liver ranged from 0.52-0.77µg g^{-1} (ww) (2.6±3.85µg/g dw) *(Rosseland et al., 2007).* In the Atlantic salmon liver from River Storelva, the mean Pb concentration was lower. *Jagoe et al. (1998)* investigated the levels of Pb in fish and pond sediments in an inhabited region of the Ukraine near Chernobyl and he stated that detectable amounts of Pb were found in most fish. Concentrations ranged from below the detection limit (25ng g^{-1}) to 1760ng Pb g^{-1} dry mass. Fish excrete Pb rapidly, and levels in fish muscle tend to be fairly low compared to kidney, liver, gill and bone *(Sorensen, 1991).*

7.3.6 Concentration of As

The mean concentration of As in the analysed livers from River Storelva was quite similar between livers in fw, bw and sw. It was in the range 0.15-0.2µg/g dw in fw, 0.2-0.3µg/g dw in bw and 0.2µg/g dw in sw (mean concentration). For example, for the brown trout from Lochnagar, *Rosseland et al. (2007)* detected in kidney mean concentration of As to be 0.15µg g^{-1} (ww) (0.75µg/g dw).

7.3.7 Concentration of Ni

The highest mean concentration of Ni was found in 2 years old small fish –in fw (0.2±0.18µg/g dw), bw (1±3µg/g dw) and sw (0.1±0.2µg/g dw). *Moiseenko et al. (2008)* stated that the concentration of Ni in the muscles and liver of bream inhabiting River Volga did not exceed 0.60µg/g dry weight; for kidneys, the value was somewhat higher. 0.58-9.0µg/g dw Ni in liver was the detected concentration in the study of *Popva et al. (1997)* in fresh water fish from the border between Russia and Norway. In addition, regarding fish age, *Sreedevi et al. (1992)* reported that rainbow trout were more sensitive when 12 months old than when 3 months old. They reported reduced weight of rainbow trout at Ni concentration of 35µg l^{-1}, while a decrease in survival was reported at concentrations of 134µg Ni l^{-1}.

7.3.8 Concentration of Cr

Studies on histopathological responses of vital organs like gills, kidney and liver to Cr toxicity are almost lacking in fish. Few reports are only available on alteration in histomorphology of gill *(Mishra and Mohanty, 2008).* In this experiment the highest mean concentration of Cr in livers was found in 2 years old small fish. It was 0.3±0.3µg/g dw in fw, 1±3µg/g dw in bw and 0.1±0.3µg/g dw in sw. The mean cocnentration of Cr in livers in 3 years old fish in bw was under the the detection limit. In experiment of *Popova et al. (1997)* the concentration of Cr was 19-0.46µg/g dw in liver. Among sensitive fish species, Cr (VI) concentrations between 16 to 21µg l^{-1} have been reported to reduce the growth of rainbow trout and chinook salmon *(Oncorhynchus tshawytscha)* fingerlings during exposures of 14 to 16 weeks, and altered plasma cortisol metabolism in rainbow trout after 7 days *(Lydersen et al., 2002).*

7.3.9 Concentration of Mn

Mn is usually considered to be of low toxicity *(Moiseenko et al., 2008).* According to *Moiseenko et al. (2008)* Mn reduces the toxicity of such elements as Cu and Al, i.e., Mn possesses antagonistic properties in multicomponent water contamination. In the experiment on Atlantic salmon from River Storelva highest mean concentration of Mn in livers was found in fish in bw and sw. In 3 years old fish in bw the concentration of Mn was 10±1µg/g dw and in sw - 5±3µg/g dw which was higher than in 2 years old large and small fish livers.

In the experiment of *Adam et al. (1997)* the uptake of water, tissue distribution and release of ^{54}Mn in rainbow trout was investigated. At the end of the accumulation phase the tissues with the highest concentration of ^{54}Mn, are the fins (214Bqg-^{l}), the gills (167Bqg-^{l}), the bones (145Bqg^{-1}) and the head (139Bqg-^{l}), whereas the concentration is low in the liver, kidneys and other viscera (about 60Bqg-^{l}).

7.3.10 Concentration of Co

There is scarce literature on Co toxicity in general. The findings in this experiment showed that highest mean concentration of Co was in livers of 2 years large fish in fw – 0.5±0.2µg/g dw. However, the concentration within the three age groups and types of water was similar (range – 0.2-0.5µg/g dw, mean concnetration). As a rule Co accumulate in fish liver, where metabolic prosseses take place *(Moiseenko et al., 2008). Moiseenko et al. (2008)* found Co in fish livers

from the upper Volga < 0.01-0.30µ/g dw (min-max), middle Volga – 0.17-0.83µg/g dw (min-max) and in lower Volga – < 0.01-0.98µg/g dw (min-max).

7.3.11 Concentration of Ti

In this experiment highest mean concentration of Ti was found in livers of fish in bw – 3 years old - 2.7±0.2µg/g dw, 2 years old large - 3±1µg/g dw and 2 years old small – 2.9±1µg/g dw. There is also very limited literature on Ti accumulation on livers of Atlantic salmon smolts but liver cells exposed to TiO_2 NPs showed minor fatty change and lipidosis, and some hepatocytes showed condensed nuclear bodies (apoptotic bodies) *(Shaw et al., 2007). Klingstedt et al. (1984)* studied the physiological disturbances in rainbow trout exposed at two temperatures to effluents from a titanium dioxide industry. Rainbow trout were exposed to the supernatant waste water (260µg/1) from a TiO_2 producing plant in a continuous flow test (Diluent: brackish water 7‰) for 2 wk at two temperatures (7-8 and 13-15°C). After 2 wk the exposed fish showed a significantly increased hematocrit value at 13.1-14.4°C, but not at 7.4-7.9°C. Liver glycogen content was 28-34°70 lower in the exposed fish, most pronounced at low temperature.

7.3.12 Concentration of Cs

^{133}Caesium is the only stable isotope of caesium, although the element can exist in over 20 isotopic forms, including the complete series from ^{123}Cs to ^{144}Cs, with the exception of ^{124}Cs *(Avery, 1996).* Uptake and accumulation of radionuclide are usually expressed as concentration factor (CF) which is the ratio of the radionuclide in tissue to that in environment *(Man and Kwok, 2000).*

CF **=Activity concentration per kg of fish (wet wt) / Activity concentration per litre of water** X **l kg-1**

Radiocaesium accumulation by fish is a complex dynamic process, which is determined by both environmental and physiological factors such as contamination of feedstuffs, feeding intensity, position in the food chain etc. In turn, the physiological characteristics of fish growth and metabolism depend on fish age (size). As a consequence, a specifc phenomenon, called the "size effect" is observed. More often, a "positive size effect" has been revealed - older fish are

more contaminated with ^{137}Cs than younger members of the same species. Such a phenomenon was reported for perch, silver carp, brown trout, bream, roach, pike-perch *(Kryshev and Ryabov, 1999).*

In the present study, the Cs accumulation in the livers did not show any significant differences between the fish groups and the water quality. The highest mean concentration was found in all fish from bw – 0.3±0.1µg/g dw and the mean concentration in livers in fw and bw was 0.2±0.1mg/g dw.

7.4 Kidneys

7.4.1 Concentration of Al

There is scarce literature about accumulation on Al in fish kidneys but the data from River Storelva experiment showed high levels of Al. The highest mean concentration was found in 2 years old small fish in fw - 1413±1282µg/g dw. The mean concentration of Al within the fish groups in bw and sw was in the range – 196-480µg/g dw and 187-577µg/g.

7.4.2 Concentration of Cd

In this study highest mean Cd concentration was found in kidneys of 3 years old fish in bw - 42±20µg/g dw and in 2 years old small fish in fw – 55.5±55µg/g dw. For example, for the brown trout in Lochnagar, in the 2-4 year old fish, weighing between 41 and 296g, the Cd in the kidney ranged from 1.33 – 2.58µg g^{-1} (ww) (6.65-12.9µg/g dw) *(Rosseland et al., 2007). Moiseenko et al. (2008)* found Cd in fish livers from the upper Volga to be 0.31–3.56µg/g dw dw (min-max), middle Volga 0.73–1.93µg/g dw (min-max) and in lower Volga - 1.78–5.66µg/g dw (min-max).

7.4.3 Concentration of Cu

The highest mean concentration of Cu from River Storelva was found in 2 years old small fish in fw - 36±38µg/g dw. In bw highest mean concentration was detected in 3 years old fish - 11±4.9µg/g dw and in sw in 2 years old small fish – 10.57±10.53µg/g dw. In comparison with this, in the experiment of *Moiseenko et al. (2008)* the concentration of Cd, recorded in kidney was 5.66µg/g dw.

7.4.4 Concentration of Zn

The concentration of Zn in kidneys of Atlantic samlon smolt from River Storelva was highest in 3 years old fish, both in bw and sw - 369±163µg/g dw and 290±59µg/g dw. This concentration might be linked to the fw history of the fish. The results for 2 years old fish (large and small) in bw and sw were similar. The mean Zn concentration in fish in fw was lowest. *Moiseenko et al. (2008)* found that in kidneys from River Volga the concentration of Zn was 78-112µg/g dw (min-max in Upper Volga), 59-107µg/g dw (min-max in Middle Volga) and 63-106µg/g dw (min-max in Lower Volga).

7.4.5 Concnetration of Pb

In the present experiment highest mean concentration of Pb was detected in 2 years small fish in fw and sw – 1.54±1.53µg/g dw and 1.1±1.7µg/g dw. *Moiseenko et al. (2008)* found that in kidneys from River Volga the concentration of Pb was 0.03-1.31µg/g dw (min-max in Upper Volga), 0.16-1.31µg/g dw (min-max in Middle Volga) and 0.05-0.24µg/g dw (min-max in Lower Volga).

7.4.6 Concentration of As

The concentration of As was low in the kidneys in all fish in all types of water. The highest mean levels were found in 2 years old small fish in fw and sw – 0.7±0.5µg/g dw and 0.6±0.7µg/g dw.

7.4.7 Concentration of Ni

Ni accumulates intensely in fish, mainly in the gills and kidneys *(Moiseenko and Kudryavtseva, 2002).* The results in this study showed highest mean concentration of Ni in 2 years old small fish in fw - 4±11µg/g dw and in bw – 2.04±2.01µg/g dw. *Moiseenko et al. (2008)* found that in kidneys from River Volga the concentration of Ni was 0.27-1.71µg/g dw (min-max in Upper Volga), 0.28-1.99µg/g dw (min-max in Middle Volga) and 0.44-2.35µg/g dw (min-max in Lower Volga).

7.4.8 Concnetration of Cr

The highest mean concentration in kidneys of Atlantic salmon was in 2 years old small fish in fw – 1.25±1.27µg/g dw and in sw – 1.1±1.7µg/g dw. The highest meanconcentration of Cr in bw was found in 3 years old and 2 years old large fish – 0.7µg/g dw. *Palaniappan and Karthikeyan (2009)* found that the kidney of freshwater fish *(Cirrhinus mrigala)* accumulated the highest level of chromium - 97.326 and 162.637µg/g. Next to kidney, the liver accumulated the highest level - 87.325 ± 3.683µg/g, whereas the muscle accumulated 91.227µg/g. *Moiseenko et al. (2008)* found that in kidneys from River Volga the concentration of Cr was < 0.01-1.18µg/g dw (min-max in Upper Volga), < 0.01-0.73µg/g dw (min-max in Middle Volga) and 0.14-1.26µg/g dw (min-max in Lower Volga).

7.4.9 Concentration of Mn

In this study the highest mean Mn concentration was in 3 years old fish, both in bw and sw - 11±µg/g dw and 7±1µg/g dw. *Moiseenko et al. (2008)* found that in kidneys from River Volga the concentration of Mn was 4.35-8.64µg/g dw (min-max in Upper Volga), 2.38-8.03µg/g dw (min-max in Middle Volga) and 1.18-5.75µg/g dw (min-max in Lower Volga).

7.4.10 Concentration of Co

The kidneys of 2 years small fish in fw had highest mean Co concentration - 5±4µg/g dw. The concentration of Co in fish in bw had mean concentration of 2µg/g dw. *Moiseenko et al. (2008)* found that in kidneys from River Volga the concentration of Co was 0.40-1.87µg/g dw (min-max in Upper Volga), 0.50-2.09µg/g dw (min-max in Middle Volga) and 0.81-1.83µg/g dw (min-max in Lower Volga).

7.4.11 Concentration of Ti

The literature about Ti accumulation in fish and in particular, in kidneys is very limited. Therefore, I could not compare my results with other authors' work. The highest mean concentration of Ti was found in 2 years small fish in fw - 22±23µg/g dw and in sw - 11±14µg/g dw. The highest mean concentration in bw was in 3 years old fish - 7±2µg/g dw.

7.4.12 Concentration of Cs

The literature about Cs accumulation in different Atlantic salmon organs is also very scarce. In this experiment the highest mean Cs levels were detetcted in 2 years small fish in fw – 0.75±0.71µg/g dw.

8. Conclusions

In sum, the results in this study showed that there were not many significant differences between the concentration of the trace metals related to the fish age, or the concentration of the trace metals related to the water quality.

However, the results in this study showed that the gill concentration of trace metals was influenced strongly by the Al concentration in the water. Its concentration was always higher in brackish water and these levels were above the levels where toxic effects may occur, dependant on exposure time. In addition, most of the gills of the 3 years old fish had higher metal concentration. The results in this study about trace metal concentration in livers and kidneys showed a variation within the organs and the water quality. The hypothesis that 2 years old large fish have less accumulation and better fitness than 2 year old small and 3 year old smolt could not be validated properly because some 2 years old large fish had high trace metal levels, and some low. This referred also to the hypothesis that an older smolt – 3 years old or a smaller sized 2 years old, would have a higher metal accumulation in the liver and kidney than 2 years large. It was suggested that the concentration of the metals in these organs might have been influenced by other factors.

One of the most interesting results referred to the concentration of Zn and Pb in the livers, and Zn and Mn in the kidneys. The concentration of Zn was highest in 3 years old fish in bw and sw, and also, the concentration of Pb. This also referred to Mn. As these heavy metals must have bioaccumulated while being in the freshwater, this gave a support to the hypothesis that a metal exposed Atlantic salmon would grow slower and reach the smolt size one year later than smolt in better water quality.

We were not able to collect water samples and presmolt samples from different parts of River Storelva in spring 2010 due to the high snow masses and late spring and we could not point out which part of the river the 3 years old smolts had been raised. We, therefore, suggest further

investigations in this particular direction in order to learn if the 3 years old Atlantic salmon smolts come from a specific polluted area in the river.

9. Appendix

In this chapter the more detailed tables with the results from the fw, bw, sw analysis are presented (ICP-MS and ICP-OES).

Table 19. - Gneneral water chemistry (FW)

Date	Geographic position	Temperature (°C)	Conductivity(µS/cm)	pH
10.05.2009	Storelva outlet	12.2	25	6.53
13.05.2009	Above the lime doser	no data	22	6.23
13.05.2009	Inlet of Ubergsvann	no data	22	6.67
13.05.2009	Songedalselva	no data	no data	6.55
13.05.2009	"Songeøygard"	no data	no data	6.6
13.05.2009	"Angelstad"	no data	no data	6.61
average±st.dev.				**6.5±0.16**
23.05.2009	Above the lime doser	15	21	6.29
23.05.2009	Songedalselva	12.1	23	6.45
23.05.2009	"Songeøygard"	15.3	22	6.69
23.05.2009	Inlet of Ubergsvann	14	23	6.59
23.05.2009	Næs verk	16.2	25	6.7
23.05.2009	Fosstveit	14.5	28	6.85
average±st.dev.		**14.5±1.4**	**23.7±2.5**	**6.6±0.2**

Table 19. - continued

Date	Geographic position	TOC (µg/L)	Cl (µg/L)	NO3 (µg/L)	SO4 (µg/L)	Br (µg/L)	F (µg/L)
10.05.2009	Storelva outlet	no data	no data	no data	no data	no data	no data
13.05.2009	Above the lime doser	no data	no data	no data	no data	no data	no data
13.05.2009	Inlet of Ubergsvann	no data	no data	no data	no data	no data	no data
13.05.2009	Songedalselva	no data	no data	no data	no data	no data	no data
13.05.2009	"Songeøygard"	no data	no data	no data	no data	no data	no data
13.05.2009	"Angelstad"	no data	no data	no data	no data	no data	no data
average±st.dev.							
23.05.2009	Above the lime doser	3902	2516	106	2177	<80	<40
23.05.2009	Songedalselva	7184	3236	39	2011	<80	<40
23.05.2009	"Songeøygard"	5010	2481	92	2152	<80	<40
23.05.2009	Inlet of Ubergsvann	5180	256	105	2129	<80	<40

23.05.2009	Næs verk	4930	2589	146	2158	<80	<40
23.05.2009	Fosstveit	no data	no data	no data	no data	no data	no data
average±st.dev.		**5241±1070**	**2261±1018**	**98±34**	**2125±59**	**<80**	**<40**

Table 20. - Water chemistry (FW)

Date	Water quality (Rygg)	Ca(µg/L)	K(µg/L)	Mg(µg/L)	Na(µg/L)	Si(µg/L)
	Total					
6May2009	fw+cons si(3)type 1	1380	237	300	2397	2307
6May2009	fw+diluted si(3)type 2	1377	221	418	3484	2312
6May2009	fw+cons si(2)type 3	1359	238	386	3319	1831
6May2009	fw	1419	190	308	1841	907
6May2009	fw	1357	218	280	1623	921
6May2009	fw	30.5		15	4586	916
6May2009	fw	891	147	212	1551	875
6May2009	fw				3063	886
6May2009	fw	1433	221	324	2017	918
average		1156	211	284	2653	1319
st.dev.		487	32	124	1027	638
	Total					
7May2009	fw	1299	197	274	1526	876
7May2009	fw	1695	299	416	3029	970
average		1497	248.5	345	2277	923
st.dev.		280	72	100	1062	66
7May2009	fw+conc. Si(3)type 1	1327	273	295.7	2452	2030
7May2009	fw+diluted Si(3)type 2	1324	247	301	2759	2712
7May2009	fw+conc. Si(2)type 3	1340	246	327	2535	1598
7May2009	fw+conc. Si(2)type 3	1639	342	510	4686	1613
7May2009	fw+diluted Si(3)type 2	1539	287	450	4063	2051
7May2009	fw+conc. Si(3)type 1	1599	459	812	6770	2034
7May2009	fw+conc. Si(3)type 1	1590	418	810	6596	2020
	LMM					
7May2009	fw				3740	859
7May2009	fw	924	179	246	2050	884
7May2009	fw		17	5	3986	858
average			98	125	3258	867
st.dev.			114	170	1053	14
7May2009	fw+conc. Si(2)type 3		108		6422	1713
7May2009	fw+conc. Si(2)type 3	966	202	339	3658	1553
7May2009	fw+diluted Si(3)type 2	29	90	13	5014	1782
7May2009	fw+conc. Si(3)type 1	1585	589	1339	11690	1861
7May2009	fw+conc. Si(3)type 1		452	15	17530	1867

	Total					
8May2009	fw	1283	218	286	1548	872
8May2009	fw+conc. Si(2)type 3	1342	191	349	2652	1700
8May2009	fw+diluted Si(3)type 2	1339	237	312	2217	1624
8May2009	fw+conc. Si(3)type 1	1322	232	350	2720	2167
	LMM					
8May2009	fw	790.3	175	205	1322	864
8May2009	fw		25		2674	874
average			100		1998	869
st.dev.			106		956	7

*** Type 1 concentrated – Na2O / Sio2 ratio = 3.4**

***Type 2 diluted (1:200) – Na2o / Sio2 ration = 3.4**

***Type 3 concentrated Na2o / Sio2 ration = 2.1**

Table 21. - Concentration of Al and Cu (µg/L) in BW and SW (ICP-OES)

Date	Geographic position	Depth (m)	Salinity	Water quality	Tot Al	0,45 µm Al	0.45 µm Ali
09.05.2009	SongNæv	4	7.2	bw	137	109	34.68
09.05.2009	Doknes	0.5	0.8	bw	168	125	111.18
09.05.2009	Hopestranda	0.5	26.8	sw	23	19	8.16
09.05.2009	Pålene	2	30.4	sw	71	59	13.26
13.05.2009	Pålene	0.5	4.9	bw	143	115	40.8
13.05.2009	Pålene	2	19.4	sw	37	29	6
13.05.2009	Bunker	0.5	17.8	sw	54	42	5
13.05.2009	Bunker	2	20.8	sw	31	20	4
14.05.2009	Doknes	0.5	0.8	bw	148	117	96
14.05.2009	Doknes	2	1.4	bw	149	115	95
24.05.2009	Song	0.5	2.2	bw	131	94	49
24.05.2009	Første ekstra	0.5	2.2	bw	127	125	75
24.05.2009	SongNæv	4	13.2	sw	88	73	12
24.05.2009	SongNæv	2	7.3	bw	110	92	20
24.05.2009	SongNæv	0.5	2.1	bw	136	100	55
24.05.2009	Holmene	2	8.7	bw	130	108	19
24.05.2009	Holmene	0.5	1.7	bw	142	99	66
24.05.2009	Doknes	2	6.1	bw	113	90	28
24.05.2009	Pålene	0.5	10.9	sw	90	63	14
24.05.2009	Bunker	2	26.9	sw	8	8	4
24.05.2009	Bunker	0.5	19.6	sw	46	34	9
24.05.2009	Hopestranda	0.5	25.5	sw	19	14	3
25.05.2009	Song	2	7.9	bw	114	92	23

Table 21. - continued

Date	Geographic position	Depth (m)	Salinity	Water quality	LMM Al	LMM ione Al	Ali	Ali (after 0.45µm)	LMM Ali
09.05.2009	SongNæv	4	7.2	bw	102	39	103	74	62
09.05.2009	Doknes	0.5	0.8	bw	83.6	64	57	14	19
09.05.2009	Bunker	0.5	18.1	sw	8.1	4			4
09.05.2009	Hopestranda	0.5	26.8	sw	21.4	4	15	11	17
09.05.2009	Pålene	2	30.4	sw	58.1	11	58	45	46
13.05.2009	Pålene	0.5	4.9	bw	81.6	21	103	74	60
13.05.2009	Pålene	2	19.4	sw	31.6	8	31	23	23
13.05.2009	Bunker	0.5	17.8	sw	40.8	8	48	37	32
13.05.2009	Bunker	2	20.8	sw	24.4	6	27	16	18
14.05.2009	Doknes	0.5	0.8	bw	66.3	62	52	20	4
14.05.2009	Doknes	2	1.4	bw	49.98	34	54	19	15
24.05.2009	Song	0.5	2.2	bw	53.04	21	81	44	31
24.05.2009	Første ekstra	0.5	2.2	bw	56.1	15	52	49	40
24.05.2009	SongNæv	4	13.2	sw	12.24	68	76	61	
24.05.2009	SongNæv	2	7.3	bw	10.2	84	89	72	
24.05.2009	SongNæv	0.5	2.1	bw	56.1	13	81	45	42
24.05.2009	Holmene	2	8.7	bw			111	88	
24.05.2009	Holmene	0.5	1.7	bw	59.16	16	76	33	42
24.05.2009	Doknes	2	6.1	bw	69.36	11	84	62	58
24.05.2009	Pålene	0.5	10.9	sw	59.16	7	76	48	52
24.05.2009	Bunker	2	26.9	sw	5.1	11	4	4	
24.05.2009	Bunker	0.5	19.6	sw	33.6	8	37	25	25
24.05.2009	Hopestranda	0.5	25.5	sw	14.28	5	16	11	9
25.05.2009	Song	2	7.9	bw	80.58	10	90	69	70

Table 21. - continued

Date	Geographic position	Depth (m)	Salinity	Water quality	Cu
09.05.2009	SongNæv	4	7.2	bw	1
09.05.2009	Doknes	0.5	0.8	bw	
09.05.2009	Bunker	0.5	18.1	sw	
09.05.2009	Hopestranda	0.5	26.8	sw	2
09.05.2009	Pålene	2	30.4	sw	2
13.05.2009	Pålene	0.5	4.9	bw	1
13.05.2009	Pålene	2	19.4	sw	1
13.05.2009	Bunker	0.5	17.8	sw	1
13.05.2009	Bunker	2	20.8	sw	1
14.05.2009	Doknes	0.5	0.8	bw	1
14.05.2009	Doknes	2	1.4	bw	1
24.05.2009	Song	0.5	2.2	bw	1
24.05.2009	Første ekstra	0.5	2.2	bw	1
24.05.2009	SongNæv	4	13.2	sw	1
24.05.2009	SongNæv	2	7.3	bw	1
24.05.2009	SongNæv	0.5	2.1	bw	1
24.05.2009	Holmene	2	8.7	bw	1
24.05.2009	Holmene	0.5	1.7	bw	1
24.05.2009	Doknes	2	6.1	bw	1
24.05.2009	Pålene	0.5	10.9	sw	1
24.05.2009	Bunker	2	26.9	sw	2
24.05.2009	Bunker	0.5	19.6	sw	1
24.05.2009	Hopestranda	0.5	25.5	sw	2
25.05.2009	Song	2	7.9	bw	1

Table 22. - Retention in chelex 100

Date	Geographic position	Depth (m)	Salinity	Water quality	Retention in chelax 100 (Total Al -Total 0.46µm ione Al)
09.05.2009	SongNæv	4	7.2	bw	103
09.05.2009	Doknes	0.5	0.8	bw	57
09.05.2009	Hopestranda	0.5	26.8	sw	15
09.05.2009	Pålene	2	30.4	sw	58
13.05.2009	Pålene	0.5	4.9	bw	103
13.05.2009	Pålene	2	19.4	sw	31
13.05.2009	Bunker	0.5	17.8	sw	48
13.05.2009	Bunker	2	20.8	sw	27
14.05.2009	Doknes	0.5	0.8	bw	52
14.05.2009	Doknes	2	1.4	bw	54
24.05.2009	Song	0.5	2.2	bw	81
24.05.2009	Første ekstra	0.5	2.2	bw	52
24.05.2009	SongNæv	4	13.2	sw	76
24.05.2009	SongNæv	2	7.3	bw	89
24.05.2009	SongNæv	0.5	2.1	bw	81
24.05.2009	Holmene	2	8.7	bw	111
24.05.2009	Holmene	0.5	1.7	bw	76
24.05.2009	Doknes	2	6.1	bw	84
24.05.2009	Pålene	0.5	10.9	sw	76
24.05.2009	Bunker	2	26.9	sw	4
24.05.2009	Bunker	0.5	19.6	sw	37
24.05.2009	Hopestranda	0.5	25.5	sw	16
25.05.2009	Song	2	7.9	bw	90

Table 22. - continued

Date	Geographic position	Depth (m)	Salinity	Water quality	Retention in chelax 100 (LMM Al-LMM ione Al)
09.05.2009	SongNæv	4	7.2	bw	62
09.05.2009	Doknes	0.5	0.8	bw	19
09.05.2009	Bunker	0.5	18.1	sw	4
09.05.2009	Hopestranda	0.5	26.8	sw	17
09.05.2009	Pålene	2	30.4	sw	46
13.05.2009	Pålene	0.5	4.9	bw	60
13.05.2009	Pålene	2	19.4	sw	23
13.05.2009	Bunker	0.5	17.8	sw	32
13.05.2009	Bunker	2	20.8	sw	18
14.05.2009	Doknes	0.5	0.8	bw	4
14.05.2009	Doknes	2	1.4	bw	15
24.05.2009	Song	0.5	2.2	bw	31
24.05.2009	Første ekstra	0.5	2.2	bw	40
24.05.2009	SongNæv	4	13.2	sw	
24.05.2009	SongNæv	2	7.3	bw	
24.05.2009	SongNæv	0.5	2.1	bw	42
24.05.2009	Holmene	2	8.7	bw	
24.05.2009	Holmene	0.5	1.7	bw	42
24.05.2009	Doknes	2	6.1	bw	58
24.05.2009	Pålene	0.5	10.9	sw	52
24.05.2009	Bunker	2	26.9	sw	
24.05.2009	Bunker	0.5	19.6	sw	25
24.05.2009	Hopestranda	0.5	25.5	sw	9
25.05.2009	Song	2	7.9	bw	70

* 0.45 μm Al chelex ≥ LMM Al chelex

Table 23. - Concentration of heavy metals in FW (µg/L)

Date	Geographic position	Al µg/L Total	µg/L LMM	Ti µg/L Total	µg/L LMM
23May2009	Above the lime doser	102		0.7	
23May2009	Songedalselva	195		1.0	
23May2009	"Songeøygard"	131		1.4	
23May2009	Inlet of Ubergsvann	160		1.6	
23May2009	Næs verk	137		1.4	
23May2009	Steaelva	217		1.8	
6May2009	FW(control)	47		0.6	
6May2009	FW+conc.Si(type3)	158		1.4	
6May2009	FW+conc.Si(type3)	163		1.5	
6May2009	FW+dilutedSi(type2)	159		1.2	
6May2009	FW(control)		170		1.8
7May2009	FW+conc.Si(type3)	161.1		1.4	
7May2009	FW+dilutedSi(type2)	115.6		0.8	
7May2009	FW(control)	159.05		1.2	
7May2009	FW+conc.Si(type3)	161.1		1.4	
7May2009	FW(control)	115.6		0.8	
7May2009	FW(control)		3		0.4
8May2009	FW(control)	157		1.2	
8May2009	FW+dilutedSi(type2)	160		1.5	
8May2009	FW+conc.Si(type3)	162		1.5	
8May2009	FW+conc.Si(type3)	164		1.4	
8May2009	FW(control)		5		0.7

Table 23. - continued

Date	Geographic position	Cr µg/L Total	µg/L LMM	Mn µg/L Total	µg/L LMM
23May2009	Above the lime doser	0.4		0.2	
23May2009	Songedalselva	0.5		0.2	
23May2009	"Songeøygard"	0.6		0.1	
23May2009	Inlet of Ubergsvann	0.7		0.1	
23May2009	Næs verk	0.7		0.1	
23May2009	Steaelva	0.5		0.2	
average		**1**		**0.2**	
st.dev		**0.12**		**0.05**	
6May2009	FW(control)	0.7		0.2	
6May2009	FW+conc.Si(type3)	0.5		0.3	
6May2009	FW+conc.Si(type3)	0.6		0.3	
6May2009	FW+dilutedSi(type2)	0.5		0.3	
6May2009	FW(control)		0.6		0.3
7May2009	FW+conc.Si(type3)	0.6		0.3	
7May2009	FW+dilutedSi(type2)	0.5		0.4	
7May2009	FW(control)	0.5		0.3	
7May2009	FW+conc.Si(type3)	0.6		0.3	
7May2009	FW(control)	0.6		0.3	
7May2009	FW(control)		0.5		0.2
8May2009	FW(control)	0.6		0.3	
8May2009	FW+dilutedSi(type2)	0.7		0.3	
8May2009	FW+conc.Si(type3)	0.5		0.3	
8May2009	FW+conc.Si(type3)	0.4		0.3	
8May2009	FW(control)		0.3		

Table 23. - continued

Date	Geographic position	Co µg/L Total	µg/L LMM	Ni µg/L Total	µg/L LMM
23May2009	Above the lime doser	0.2		1.2	
23May2009	Songedalselva	0.2		1.3	
23May2009	"Songeøygard"	0.1		1.1	
23May2009	Inlet of Ubergsvann	0.1		1.5	
23May2009	Næs verk	0.1		1.5	
23May2009	Steaelva	0.2		1.6	
average		**0.2**		**1**	
st.dev		**0.05**		**0.20**	
6May2009	FW(control)	0.1		0.9	
6May2009	FW+conc.Si(type3)	0.2		1.0	
6May2009	FW+conc.Si(type3)	0.2		1.1	
6May2009	FW+dilutedSi(type2)	0.2		1.3	
6May2009	FW(control)		0.2		1
7May2009	FW+conc.Si(type3)	0.2		1.5	
7May2009	FW+dilutedSi(type2)	0.2		1.0	
7May2009	FW(control)	0.2		1.3	
7May2009	FW+conc.Si(type3)	0.2		1.5	
7May2009	FW(control)	0.2		1.4	
7May2009	FW(control)		0.1		1
8May2009	FW(control)	0.2		1.2	
8May2009	FW+dilutedSi(type2)	0.2		1.2	
8May2009	FW+conc.Si(type3)	0.2		1.1	
8May2009	FW+conc.Si(type3)	0.2		1.3	
8May2009	FW(control)		0.01		0.6

Table 23. - continued

Date	Geographic position	Cu µg/L Total	µg/L LMM	Zn µg/L Total	µg/L LMM
23May2009	Above the lime doser	0.5		0.3	
23May2009	Songedalselva	1.1		1.2	
23May2009	"Songeøygard"	0.7		0.2	
23May2009	Inlet of Ubergsvann	0.9		0.3	
23May2009	Næs verk	0.9		0.1	
23May2009	Steaelva	0.8		0.2	
average		**1**		**0.4**	
st.dev		**0.2**		**0.4**	
6May2009	FW(control)	0.7		0.1	
6May2009	FW+conc.Si(type3)	1.0		0.2	
6May2009	FW+conc.Si(type3)	1.0		0.2	
6May2009	FW+dilutedSi(type2)	0.8		0.2	
6May2009	FW(control)		0.8		0.2
7May2009	FW+conc.Si(type3)	1.0		0.3	
7May2009	FW+dilutedSi(type2)	0.3		0.3	
7May2009	FW(control)	0.8		0.2	
7May2009	FW+conc.Si(type3)	1.0		0.3	
7May2009	FW(control)	0.5		0.3	
7May2009	FW(control)		0.4		0.2
8May2009	FW(control)	0.8		0.15	
8May2009	FW+dilutedSi(type2)	1.2		0.5	
8May2009	FW+conc.Si(type3)	0.9		0.3	
8May2009	FW+conc.Si(type3)	0.8		0.2	
8May2009	FW(control)		1.9		

Table 23. - continued

Date	Geographic position	As µg/L Total	µg/L LMM	Cd µg/L Total	µg/L LMM
23May2009	Above the lime doser	0.1		0.2	
23May2009	Songedalselva	0.3		0.1	
23May2009	"Songeøygard"	0.1		0.2	
23May2009	Inlet of Ubergsvann	0.2		0.04	
23May2009	Næs verk	0.2		0.03	
23May2009	Steaelva	0.2		0.03	
average		**0.2**		**0.1**	
st.dev		**0.05**		**0.07**	
6May2009	FW(control)	0.07		0.02	
6May2009	FW+conc.Si(type3)	0.13		0.13	
6May2009	FW+conc.Si(type3)	0.13		0.18	
6May2009	FW+dilutedSi(type2)	0.14		0.08	
6May2009	FW(control)		0.1		0.2
7May2009	FW+conc.Si(type3)	0.15		0.13	
7May2009	FW+dilutedSi(type2)	0.14		0.21	
7May2009	FW(control)	0.14		0.08	
7May2009	FW+conc.Si(type3)	0.15		0.13	
7May2009	FW(control)	0.13		0.2	
7May2009	FW(control)		0.064		0.04
8May2009	FW(control)	0.2		0.2	
8May2009	FW+dilutedSi(type2)	0.1		0.2	
8May2009	FW+conc.Si(type3)	0.1		0.2	
8May2009	FW+conc.Si(type3)	0.2		0.2	
8May2009	FW(control)		0.06		0.1

Table 23. - continued

Date	Geographic position	Cs µg/L Total	µg/L LMM	Pb µg/L Total	µg/L LMM
23May2009	Above the lime doser	0.03		0.01	
23May2009	Songedalselva	0.08		0.5	
23May2009	"Songeøygard"	0.03		0.3	
23May2009	Inlet of Ubergsvann	0.04		0.4	
23May2009	Næs verk	0.03		0.3	
23May2009	Steaelva	0.02		0.2	
average		**0.04**		**0.3**	
st.dev		**0.02**		**0.18**	
6May2009	FW(control)	0.02			
6May2009	FW+conc.Si(type3)	0.04		0.3	
6May2009	FW+conc.Si(type3)	0.03		0.3	
6May2009	FW+dilutedSi(type2)	0.04		0.3	
6May2009	FW(control)		0.03		0.2
7May2009	FW+conc.Si(type3)	0.03		0.35	
7May2009	FW+dilutedSi(type2)	0.02		0.003	
7May2009	FW(control)	0.04		0.2	
7May2009	FW+conc.Si(type3)	0.03		0.3	
7May2009	FW(control)	0.02		0.003	
7May2009	FW(control)		0.04		
8May2009	FW(control)	0.04		0.2	
8May2009	FW+dilutedSi(type2)	0.04		0.3	
8May2009	FW+conc.Si(type3)	0.04		0.3	
8May2009	FW+conc.Si(type3)	0.04		0.3	
8May2009	FW(control)		0.02		0.02

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