

Mastergradsoppgave

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Metal concentrations in
blue mussel (*Mytilus edulis*) in
the Kaldvellfjord, Lillesand county,
affected by metal rich runoff.



Høgskolen i Telemark

Fakultet for allmennvitenskapelige fag

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Sammendrag

I denne masteroppgaven har en studert mulige effekter av sur, metallrik avrenning på blåskjell (*Mytilus edulis*) i Kaldvellfjorden (Lillesand kommune), som følge av sulfidoksidasjon i steindeponier i nedbørsfeltet. Blåskjell fra 6 stasjoner og to dyp, 1 m og 5 m, har blitt undersøkt ved 6 ulike tidspunkter i 2014.

Al, Fe og Mn oppnådde høyest konsentrasjoner i perioder ved høy avrenning i både vann og blåskjellprøver ved 1 m dyp. Ved 1 m dyp viste alle undersøkte metaller en positiv korrelasjon med avrenning. For blåskjell viste de fleste av metallene samme positivt korrelaterte mønster. Resultatene ble sammenliknet med norske konsentrasjonsgrenser for metall i vann og bløtdeler av blåskjell. Median metallnivåene i Kaldvellfjorden viste forurensede verdier, mens noen av metallnivåene i Stordalsbekken var i perioder innenfor den mest forurensede kategorien. Vannprøvene fra 1m dyp i Kaldvellfjorden viste signifikant høyere verdi av alle metallene enn prøvene fra 5m dyp. Nivåene i blåskjell viste heller ingen kritiske verdier, men det ble funnet høyere konsentrasjoner i små blåskjell sammenliknet med store blåskjell i tillegg til at 1 m dybde viste høyere konsentrasjoner enn ved 5 m.

Høye konsentrasjoner av uorganisk, løst Al fra sterkt forsurede bekker regnes som den mest alvorlige biologiske påvirkningsfaktoren i fjorden, særlig i nærheten av ferskvannsutløp. Når fjordvann med høy pH blir tilført metallrikt ferskvann med lav pH, vil mange av metallene polymerisere og felle ut (sedimentere). Blant metaller er aluminium kjent for å kunne være ekstremt giftig for mange gjellepustende organismer under forhold med polymerisering/utfelling. Dette kan være årsaken til den dødeligheten som ble observert hos blåskjell nær bekkeutløpene i Kaldvellfjorden. Siden polymerisering/felling av metaller er svært ustabile prosesser, er det vanskelig både å måle og modellere de faktiske kjemiske forhold. Under slike betingelser er bruk av ulike biomonitorer som blåskjell helt avgjørende for å kunne verifisere de faktiske biologiske effektene av ulike forurensninger på miljøet. Under slike forhold vil biologiske overvåkningsprogrammer derfor være av uvurderlig betydning som referansegrunnlag for en slik effektvurdering.

Abstract

In this master thesis there has been studied possible effects from acidic, metalrich runoff on blue mussels (*Mytilus edulis*) in the Kaldvellfjord (Lillesand county), as a result of sulphide oxidation from deposits in the precipitation area with sulphide-containing bedrock. Blue mussels from 6 stations and two depths 1 and 5 m were investigated at different times in 2014.

Al, Fe and Mn exhibited the highest concentrations during high freshwater runoffs in both water and blue mussels at 1 m depth. Also at 1 m depth, all investigated metal concentrations in water had positive correlations with runoff. For blue mussels most of the metals showed the same positive correlation pattern. The results were compared to the Norwegian pollution guidelines for metal concentrations in water and soft tissue from blue mussels. The median metal concentrations from the Kaldvellfjord showed polluted values, though the stream Stordalsbekken had periodically values within the most polluted category. Water samples from 1m depth in the Kaldvellfjord showed significant higher concentrations than the samples from 5m depth. The concentrations in blue mussels did not show any critical values, although higher concentrations were found in small mussels (20-35 mm) compared to big mussels (>36 mm) in addition to the higher concentrations at 1 m compared to 5 m.

High concentration inputs of inorganic dissolved Al from strongly acidified streams are the factor of most biological concern for this fjord, especially near the fresh water outlets. When the fjord with high pH and poor metal concentrations is supplied with metal rich freshwater with low pH, several metals will start to polymerize and precipitate. Among metals, aluminum is known to be very toxic to many gill-breathing organisms during polymerization/precipitation conditions. This may be the reason for the blue mussel mortality seen near acidic, metal rich stream outlets in the Kaldvellfjord. As polymerization/precipitation are very unstable processes, it is difficult to both measure and model the actual chemical conditions. During conditions like this, the use of different bio-monitors like blue mussels is essential for the ability to verify the actual biological effect of various pollutions in the environment. During conditions like this, biological monitoring programs will be of high importance as a background reference in an effect evaluation.

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Preface

This thesis has been an educational stair for me. It could not have been done without a helping hand from numerous people. I would like to mention and especially thank the blue mussel breeder Per Christian Hoelfeldt Lund for all the help in field, Jarle Haavardstun at the Norwegian Institute for Water Research (NIVA) for both field and laboratory help, and the nice lady at the Norwegian Public Roads Administration (Statens vegvesen), Lene Jacobsen. The Norwegian Public Roads Administration covered both field and analytic costs, and this study would not have been done without their economical support. I also want to acknowledge my supervisor at Telemark University College, Espen Lydersen, my helpful friends and my patient family.

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1 Introduction

Marine pollution is an environmental problem worldwide. Human activities may contaminate all parts of the ecosystem, i.e. air as well as terrestrial and aquatic systems. Contaminants include both artificially produced and natural derived substances. In water contaminants might exist in numerous physical and chemical forms, decisive for the distribution within the abiotic and biotic environment. Most aquatic organisms have the potential to both assimilate particles and dissolved substances (Stewart 1999). If these substances are difficult to eliminate or degrade by organism, they will bio-concentrate and/or bio-magnify up the food web. Different groups of organisms will react differently on various amounts of accumulated contaminants (Clark et al. 1997, Levinton 1995). Subsequently, several organisms can be used in biological monitoring of contaminants in ecosystems (Rainbow & Phillips 1993, Viarengo & Canesi 1991). Metals are natural elements, but primarily due to manmade activities as mining, combustion of fossil fuels and emissions from waste disposal, exposure of high levels of metals have caused adverse effects on flora and fauna worldwide (Valko et al. 2005).

Phillips (1977) suggested three steps for heavy metal monitoring in marine environments, i.e. analysis of metals in sediment, water and aquatic organisms. Normally, it is limitations with the two first steps, as the analyses of compounds in sediment and water often are total concentration analyses, and therefore seldom a good measure of the bioavailability of the metals. As metals can be present as free aqueous monomers, within and associated to colloids or particles, it is a lot of physical and chemical factors in the environment which are essential for the concentrations and physical-chemical forms of metals, and the subsequent distribution in the environment and their bio-availability (Phillips 1977). Regarding analyses of metal concentrations in aquatic organisms, it is important to underline that these values represent a time-integrated value of the local habitats of the investigated organisms (Ritz et al. 1982).

Goldberg (1975) suggested to use mussels in monitoring programmes in coastal and open ocean areas, due to their sedentary and ubiquitous inhabitancy in estuarine and coastal areas. Accordingly, *Mytilus spp* are frequently used as bio-monitors in many monitoring programs in marine environments (Conti & Cecchetti 2003, Dagnino et al. 2007, Orescanin et al. 2006). Their high water filtration capacity, fundamental for their growth, implies significant exposure to many inorganic and organic particle-associated contaminants like heavy metals (Saavedra et al. 2004). It is recognized that biochemical methods help to both reveal the toxic effects of pollutants and explain the mechanism of the organism's response to them, like effects on cellular metabolism and different physiological processes that are involved in development and maintenance of their

population are documented for different metals (Bakhmet et al. 2012, Davenport & Redpath 1984, Fokina et al. 2013, Hoher et al. 2012, Manley & Davenport 1979, Pipe et al. 1999).

For some metals the concentrations increase with size of the organism, while other metals document negative or no correlation with size (Boalch et al. 1981, Phillips 1980, Popham & D'Auria 1983, Ritz et al. 1982, Schulz-Baldes 1974, Saavedra et al. 2004, Wright & Mason 1999).

Blue mussels (*Mytilus edulis*) is one *Mytilus* species often used in environmental monitoring marine programmes. This species is found in sheltered and exposed localities in sub littoral and littoral areas in Europe, from the Mediterranean to the Arctic. It tolerates large variations in both salinity and temperature (Moen 2004, Seed 1976). It is a filter-feeder, and retains algae, detritus and other organic or inorganic particles. Spawning occurs in spring and the larvae settle on solid substrate in shallow water after a week or more, depending on external factors as temperature and food availability (Hågvar 2010, McEdward 1995, Moen 2004, Seed 1976). The physical mobility of the mussels are limited, but they are able to temporarily close their valve gap to avoid pollutants and toxicants, in contrast to other sessile species lacking closing devices (Manley & Davenport 1979).

Among bivalves, blue mussels is one of the most commonly consumed species (Goldberg et al. 1978) and accordingly farmed in both Denmark and Norway. Farming requires clean water to avoid accumulation of metals. As an example, lead (Pb) has been shown to have a bio-concentration factor of 10 000 in blue mussel (Hågvar 2010).

Metals often connect to specific chemical structures in organisms, as proteins or other organic and inorganic particles in cells. Such metal-complexes often result in longer biological half life for many metals (Bjerregaard 2013).

In my study, eleven metals (Al, As, Cd, Co, Cu, Fe, Mn, Ni, Zn, Hg and Pb) have been investigated in blue mussel tissue from the Kaldvellfjord, southern Norway, a fjord impacted by acidic, metal rich discharge from sulphide rich stone deposits. The aim of work was to investigate time and space variations in metal concentrations in water and blue mussels and potential correlation between the concentrations of metals and runoff. This study also looks into the concentrations in mussels of different size and depth classes.

2 Methods

2.1 Sampling and analysis of water

Water sampling and water chemical analyses in the Kaldvellfjord has been administrated by the Norwegian Public Roads Administration, and implemented by other institutions than Telemark University College. However, I have got permission to use the data for interpretation and comparison with the investigation of metal concentrations in blue mussel. In addition runoff data from Birkenes (NIVA) have been used in this study to simulate variations in freshwater input to the Kaldvellfjord during the investigated period.

2.2 Sampling and analyses of blue mussels

Blue mussels were collected from a mussel farm in the Kaldvellfjord. All mussels were kept on the same knitted rope bought from A.S. Fiskevegn as they were grown on the last year to avoid stress and to make a habitat for new larvae. Each rope with mussels was cut in 1m lengths and placed at various stations in a gradient along the shore where freshwater supplies arrive and out to the middle of the fjord (Figure 1). GPS position (Table 1) and depth was measured at each station and mussel ropes were placed at two different depths, at 1 and 5 meter, at each station. Each rope had two 10 L cans as floats and a big stone in the bottom end as anchor. During the monitoring period, some of the stations were lost (boat propels cutting etc) and were replaced by new ropes from the mussel farm that got minimum two weeks of exposure before sampled, as Mao et al. (2011) showed that mussels in Al contaminated water reached its highest concentration in soft tissues and digestive glands after 8-13 days. Still some samples got lost. Mussels were sampled by boat, at six different times from March to October 2014. Each time 50 mussels from all depths at stations were collected. Totally, 3600 mussels were included in this study. To avoid data error due to stomach content variations, all mussels from each station were cleaned and kept in separate plastic boxes with local station water and oxygenated for at least 15 hours before each group were stored in plastic bags in a deep freezer (-18°C). After thawing, length of each mussel was measured with a digital caliper (Cocraft caliper with two decimals), and individual total weight and soft tissue weight were measured by a scale (Metler PE360 with three decimals). Samples from all depths and stations were treated separately and divided into two size groups: 20 -35 mm (*small*) and 36< mm (*big*). Soft tissues were cut free with a scalpel and 15-50 individuals from each size group were pooled together and stored on glass jars in a deep freezer (-18°C). Later the samples were taken out of the freezer and homogenized by a blender (Menuett,

200W), before freeze dried in a vacuum drier (CHRIST, Alpha 1-4) for at least 12 hours. Accordingly, our metal concentration in mussels are dry weight data, which is recommended instead of wet weight (Popham & D'Auria 1983). All freeze-dried samples were analysed for heavy metals by ICP-MS (ELEMENT 2 from Thermo Electronics) at the Norwegian University of Science and Technology (NTNU).

2.3 Calculations

To calculate from wet weight to dry weight in mussels, calculations of the percentage of dry matter from mussels in other studies in this area is used (Schøyen et al. 2012, Schøyen et al. 2013). The percentages were up to 17%. A factor of 5 is therefore used in calculations in this study.

Davenport & Chen (1987) described how to calculate mussel condition indices. This equation was not recommended when freezing before measuring, still it can display difference in shell condition in this study. $Cl = \frac{\text{tissue wet weigh}}{\text{shell weight}} \times 100$

Table 1. Location names and geographical positions of the blue mussel stations in Kaldvellfjorden.

Station name/location	N	E
1 Stordalsbekken outlet	58° 16.103	008° 24.745
2 Smallboat harbour	58° 16.249	008° 25.001
3 Traudalsbekken outlet	58° 16.516	008° 25.266
4 Kaldvellholmene	58° 16.536	008° 25.536
5 Blue mussel farm	58° 16.401	008° 25.837
6 Control	58° 15.909	008° 25.796

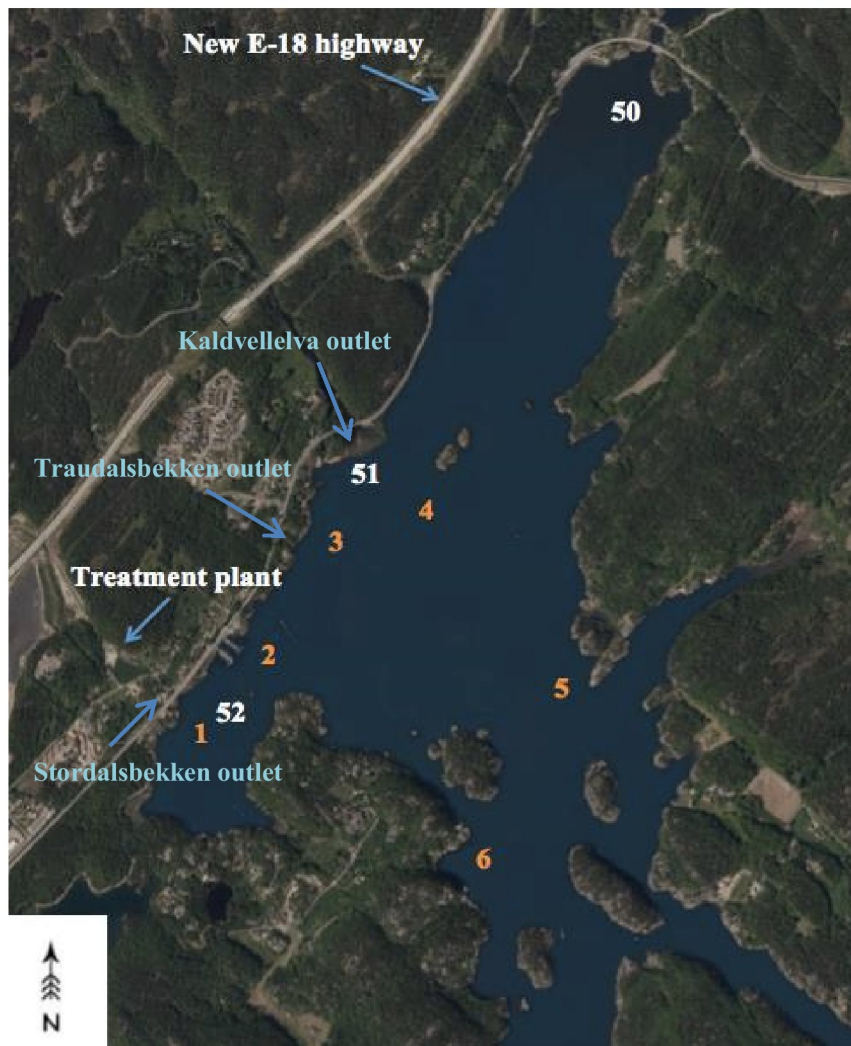


Figure 1. Map of Kaldvellfjorden with the six blue mussel stations investigated (orange numbers) and the three water-sampling stations (white numbers). The new E-18 highway is shown in the uppermost left corner of the picture and the treatment plant on the left side.

2.4 Statistics

Statistical analyses were performed primarily by Minitab version 17. As both normal distributed and not normal distributed variables are compared, there has been used both parametric test (One Way Anova/paired t-test) for normal distributed data and nonparametric tests (Kruskall-Wallis/Mann-Whitney test) for not normal distributed data in all statistical analyzes related to metal concentrations in water and blue mussels between and within stations, depths, and size of mussels. Correlations were calculated using Pearson correlation. A significance level of 0,05 was used as significant criteria in all statistical tests.

3 Results

3.1 Area description and background

The Kaldvellfjord is located in Lillesand county, southern Norway. The maximum depth of the fjord is ≈ 30 m and salinity measured at 14 stations in 2012 was between 24-33‰ (Johnsen et al. 2013, Kroglund et al. 2007). The main fjord basin has a surface area of 2,8 km² and a volume of 36 400 000 km³ (Johnsen et al. 2013). The main river entering the fjord is River Kaldvelleva/Grimeelv, with a catchment of 75 km². This river annually discharge about 73 000 000 m³ of fresh water into the fjord (www.nve.no). The fjord is a partially closed fresh water affected area with shallow drains in the south (Figure 1). Thus natural oxygen depleted deep-water with subsequent formation of hydrogen sulfide (H₂S) naturally occur in the deepest parts of the fjord. This might give an acute toxic effect in parts of the fjord area by a suddenly increased circulation (Johnsen et al. 2013).

The bedrock surrounding the fjord area basically consists of various gneisses, often with relatively high sulphur content. Acidification, as an indirect result of bedrock blasting in these areas, has been reported since the early 1990'thies (Hindar & Lydersen 1994).

In 2009, a new European highway (E18) was established near the fjord (Figure 1). During the construction period, a lot of sulphur containing rocks was blasted and deposited in nearby fjord areas (Hindar 2011). Accordingly, acid metal rich runoff water has entered into the Kaldvellfjord, primarily from small streams and rivers (Figure 1) draining these sulphur rich land deposits (Johnsen et al. 2013). The metal rich runoff has also caused fish mortality in a river nearby (Hindar et al. 2006). Hindar (2011) have described the geochemical acidification processes in the deposits, and the subsequent increase in acidification and metal discharge to the fjord from rivers and streams in the area, and both Hindar & Nordstrom (2014) and Kaste et al. (1995) have proposed measures to reduce these problems.

In 2011, pH about 4,6 and an average labile aluminium concentration of 18 000 $\mu\text{g L}^{-1}$ were documented in Stordalsbekken. This extreme water chemistry might have had negative effects on aquatic life (Johnsen et al. 2013). The benthic fauna in the Kaldvellfjord reflects the natural variations in oxygen in the fjord, earlier supplies of saw dust and the current metal contamination according to Johnsen et al. (2013) with a lack of species diversity, and presence of species that tolerates a polluted and periodically oxygen free habitat.

For the last couple of years, people living around the fjord observed that life in the fjord has

decreased. According to the breeder, the blue mussel farm area in the fjord has experienced a significant lack of larvae and economical loss. Rådgivende Biologer AS examined the area in the years 2012-2013 and reported that this probably was due to a period of oxygen free conditions and formation of hydrogen sulphide (Johnsen et al. 2013), likely with acute toxic effect on some aquatic organisms. They also investigated metal concentrations in knotted wrack (*Ascophyllum nodosum*) from 8 stations in the fjord, and reported low concentrations of cadmium (Cd) and arsenic (As), while chrome (Cr), copper (Cu), nickel (Ni) and aluminum (Al) were high at some of the stations. In areas near Stordalsbekken (Figure 1), the Al concentration in knotted wrack was very high (13 g kg⁻¹ dw) compared with Kaldvellholmene (0,97 g kg⁻¹ dw) (Johnsen et al. 2013).

At the beginning of this study (spring 2014), few organisms was observed along bays and bows in the fjord, especially along the shore line areas, where significant amounts of precipitated Al also were observed (Figure 2).



Figure 2. Precipitation of aluminum (light grey) along the shoreline of the Kaldvellfjord.

The Norwegian Public Roads Administration installed a treatment plant in the valley Stordalen in autumn 2013 after orders from the county governor (Figure 1 and 3, and Table 2). This plant collects all drainage water/leakage from the disposal sites and some storm water from the precipitation catchment area. Downstream from the plant an area with small brooks supplies the stream Stordalsbekken with untreated water. A pump transfers another brook placed downstream, which is known to hold a lot of pollution, back to the treatment plant before it enters Stordalsbekken. Asplan Viak, responsible for the plant, calculated the major transport of Al to the Kaldvellfjord in 2014, from the 3 main streams entering into the fjord (Table 2). The streams Traudalsbekken and Kaldvellelva, do not run through a treatment plant, and it was observed during this study precipitation of Al at the common beach where Traudalsbekken enters the fjord. The treatment plant in Stordalen, is based on addition of sodium hydroxide (NaOH) to the water before passing vertically through a lime filter. The pH increase leads to precipitation (primarily

aluminum) and co-precipitation of metals, which will be retained by the filter. The Norwegian Public Roads Administration internal goal for the pH in the plant is $\text{pH} > 6,0$, during all water regimes. In September 2014 Asplan Viak reported that a pump in the treatment plant was broken and the plant did not function fully until late November.



Figure 3. The two basins holding water after NaOH input in the treatment plant.

Table 2. Calculated transport of aluminum to the Kaldvellfjord in 2014. Table made by Fredrik Ording at Asplan viak.

Source	Watershed area daa	Average flow (NVE) $\text{L s}^{-1} \text{ km}^{-2}$	Average flow L s^{-1}	Al Average $\mu\text{g L}^{-1}$	Al transport to the fjord kg yr^{-1}
Stordalsbekken	850	28	24	1 255	942
Traudalsbekken	350	28	10	4 409	1 363
Kaldvellingva	75000	31	2325	147	10 778

3.2 The investigated metals

Aluminum (Al) is primarily toxic as inorganic cations at low pH, $\text{pH} < 5,0$ (Lydersen et al. 2002), which means that the Al toxicity rarely exists in marine environments where pH normally is between 7,5 – 8,4. Due to this high pH, the solubility of Al is also low and median Al value in sea-water is reported to be about $2 \mu\text{g L}^{-1}$ (Reimann & Caritat 1998). However due to time dependent precipitation kinetics, Al toxicity is reported in marine fjords impacted by acid, Al-rich river water (Teien et al. 2006), similar to the situation in the investigated Kaldvellfjord.

Depending on pH, arsenic (As) usually appears in two chemical forms in marine waters (Bjerregaard 2013, Mandal & Suzuki 2002), i.e. about 80% as arsenate (AsO_4^{3-}), the most stable, thermodynamic form, and about 20% as arsenide (AsO_3^{3-}) (Bjerregaard 2013). For some mollusks in marine waters In fish tissue the organic forms of As dominate with more than 99%, still it has been found in blue mussels that the level of toxic, inorganic As rise when the organic concentration reach a given value (Franzen et al. 2008, Sloth & Julshamn 2008). In waters As is removed relatively fast from the water body and loosely binds into the sediment (Bjerregaard 2013, Reimann & Caritat 1998).

Cadmiums (Cd) behaviour in the sea is known to be dependent on salinity and pH. In particle form, Cd is usually not poisonous for organisms, although dissolved Cd in the water layer can be poisonous to some species (Bjerregaard 2013). For human consume a limit level of $1,0 \text{ mg kg}^{-1}$ ww is set by Commission Regulation EC 1881/2006.

Cobalt (Co) is essential for animals as it is a part of vitamin B12 (Bjerregaard 2013), and usually exists in association with Cu, Fe, Pb, Ni and silver ores (Nagpal 2004). In a study by Amiard (1976), marine shellfish had up to tens of thousands times higher concentrations of Co than its concentrations in the water. Some other marine benthic invertebrates had concentrations of Co ranging to 40,000 times higher than the concentration in the water.

Copper (Cu) is essential for all organisms, but toxic at high doses (Davenport & Redpath 1984, Reimann & Caritat 1998). It is known that Cu is an important component of the oxygen-transporter pigment, hemocyanin, in the mussel's hemolymph (Bakhmet et al. 2012). On boats or other equipment in sea-water, high doses of Cu is used in antifouling (Duinker et al. 2012). In sea waters Cu occurs as free ions, in organic or inorganic complexes, in widespread colloids or adsorbed in organic materials (Davenport & Redpath 1984). 66% of all Cu released in marine water are stored in sediments in costal environments (Bjerregaard 2013).

Iron (Fe) is essential to all organisms and therefore not considered to be toxic, although aquatic organisms is negatively effected when Fe concentrations rise to 100-200 $\mu\text{g L}^{-1}$ (Bjerregaard 2013). It is also known that Fe has a negative correlation with pH (Veselý & Majer 1996).

Manganese (Mn) can be dissolved from sediments and may occur in high concentrations in benthic organisms after a period of oxygen depletion, but still defined to be non-toxic as it is essential to all organisms (Bjerregaard 2013). In sea water it can also occur in particle form (Phillips 1978).

Nickel (Ni) is mobilised in soil by acid rain and can be very toxic to some fish species. Most Ni compounds are soluble at $\text{pH} < 6,5$ and insoluble at $\text{pH} 6,7$ (Bjerregaard 2013).

Zinc (Zn) is a co-factor to a diversity of enzymes and essential to all organisms, but in aquatic environments concentrations above 0,5 - 1 mg L^{-1} are known to be poisonous (Bjerregaard 2013). Zn is known to occur in high concentrations in acid surface waters, affected by low pH, which cause increased solubility, and long range transported air pollutant inputs (Borg 1987, Henriksen & Wright 1978, Johansson et al. 1995).

Mercury (Hg) might be present in high concentrations in sulphide ores (Lydersen et al. 2002), and blue mussel is suitable as bio-indicator of Hg pollution (Davies & Pirie 1978). The limit value for Hg in human consume is 0,5 mg kg^{-1} ww set by Commission Regulation EC 1881/2006.

Lead (Pb) is known to be most soluble and bioavailable under low pH, low organic carbon concentrations, low turbidity, and low concentrations of salts from Ca, Fe, Mn, Zn and Cd (Eisler 1988). Studies done on rats show that low concentrations of calcium in the organism will increase uptake of lead (Bjerregaard 2013). Pb has a limit value of 1,5 mg kg^{-1} ww for human consume set by Commission Regulation EC 1881/2006.

Metal concentration in water, sediment and organisms can be classified as grade of pollution or quality of the environment when compared with natural background levels. Norwegian Water Framework Directive (WFD) use a classification system originally made by Molvær et al. (1997) and re-evaluated for sea and coastal waters by Bakke et al. (2007) and Arp et al. (2014) These guidelines (Table 3) recommends one annual sampling, unless background levels vary significantly between seasons, salinity and particle compounds. Background concentrations (insignificant levels) rely on data from apparently uncontaminated fjords. Limit values for water is used for support to biological factors when monitoring polluted fjords.

Table 3. Quality classes for sea/coastal water according to the Norwegian guidelines (Bakke et al. 2007 and Arp et al., 2014) and for blue mussels (Molvær et al. 1997). I= unpolluted

(background values); II= Good (no toxic effects); III= Moderate (Chronic effects by long-time exposure) values); IV= Bad (Acute toxic effects by short-time exposure); V = Very bad (Extensive toxicity).¹According to Bakke et al, 2007; ²Re-evaluated by Arp et al. 2014.³ According to Guideline 02:2013, where the fraction of Al (LAL) is a measure on cationic, inorganic dissolved Al-forms.

Target	Metal	Concentration	I Background	II Good	III Moderate	IV Bad	V Very bad
Water	As ¹	µg L ⁻¹	< 2	2 - 4,8	4,8 - 8,5	8,5 - 85	> 85
	Pb ^{1,2}	µg L ⁻¹	< 0,05	0,05 - 1,3	1,3-14	14 - 57	> 57
	Cd ^{1,2}	µg L ⁻¹	< 0,03	0,03 - 0,21	0,24 - 1,5	1,5 - 15	> 15
	Cu ¹	µg L ⁻¹	< 0,3	0,3 - 0,64	0,64 - 0,8	0,8 - 7,7	> 7,7
	Cr ¹	µg L ⁻¹	< 0,2	0,2 - 3,4	3,4 - 36	36 - 360	> 360
	Hg ^{1,2}	µg L ⁻¹	< 0,001	0,001 - 0,048	0,048 - 0,071	0,071 - 0,14	> 0,14
	Ni ^{1,2}	µg L ⁻¹	< 0,5	0,5 - 8,6	8,6 - 34	34 - 67	> 67
	Zn ¹	µg L ⁻¹	< 1,5	1,5 - 2,9	2,9 - 6	6 - 60	> 60
	Al ³ (LAL)	µg L ⁻¹	< 5	5 - 30	30 - 65	65 - 95	> 95
Blue mussel	As	mg kg ⁻¹	< 10	10 - 30	30 - 100	100 -200	> 200
	Pb	mg kg ⁻¹	< 3	3 - 15	15 -40	40 - 100	> 100
	Cd	mg kg ⁻¹	< 2	2 - 5	5 - 20	20 - 40	> 40
	Cu	mg kg ⁻¹	< 10	10 - 30	30 - 100	100 - 200	> 200
	Hg	mg kg ⁻¹	< 0,2	0,2 - 0,5	0,5 - 1,5	1,5 - 4	> 4
	Ni	mg kg ⁻¹	< 5	5 - 20	20 - 50	50 - 100	> 100
	Zn	mg kg ⁻¹	< 200	200 - 400	400 - 1000	1000 - 2500	> 2500

3.3 Metal concentrations in the Kaldvellfjord

The stream Stordalsbekken (Table 4), mouting into the Kaldvellfjord about 100 m inside water station 52, mussel station 1 (Figure 1), exhibited high metal concentrations as this stream drains through rock depots with high metal-sulphide content. Oxidation processes within such depots,

with subsequent rain events, result in a very acid and metal rich discharge. Especially the concentrations of Al, Mn and Fe were very high, The most toxic forms of Al, the inorganic, cationic aluminum fraction (LAL) was periodically extremely high with concentrations $> 1000 \mu\text{g L}^{-1}$ in 5 of 11 measurements during 2014. Such concentrations ($\text{LAL} > 1000 \mu\text{g L}^{-1}$) is more than ten times higher than the highest limit set for the most polluted category (very bad) in the Guideline 02:2013 (Table 3). The median concentrations of Cu, Ni and Zn in Stordalsbekken, all fell within the most polluted category V, very bad or heavily polluted, while the median Cd concentration was within category III, moderately polluted. Pb and Hg in water from Stordalsbekken were not measured.

The metal concentrations in the water from the Kaldvellfjord rely on 12 samples taken during period February 3 – December 30, 2014, at 3 stations, St.50, St. 51 and St.52 (Figure 1), and two depths, 1 and 5m. The highest metal concentrations at the 3 stations were generally found at 1 m depth (Table 5), and Fe and Al (both total Al, Al-T, and dissolved Al, Al-F) had the highest concentrations, followed by Mn and Zn. Both Mann-Whitney (not normal distributed) and two-sample T-tests (normal distributed) showed significant higher concentrations of metals at 1 m depth compared with at 5 m. The concentrations of metals in water at 1 m depth were primarily linked to high freshwater runoff to the fjord (Figure 4 and 5). The differences in metal concentrations in water at 1 and 5 m depths ($\delta[\text{Metal}] = [\text{Metal}]_{1\text{m}} - [\text{Metal}]_{5\text{m}}$), were at highest during high runoff periods (Figure 4), most pronounced for total Al, dissolved Al and total Fe, the two metals with the highest concentrations in water samples.

Accordingly, the water samples from 1m exhibited a significant, positive correlation between the concentrations of all investigated metals and runoff, while only dissolved Al (Al-F) showed a similar significant correlation with runoff at 5 m depth (Table 6). The highest metal concentrations in water from the Kaldvellfjord were revealed on March 28, September 1 and November 11.

Both non parametric tests (Kruskal-Wallis) or One-Way Anova (normal distributed data), did not show any significant differences in metal concentrations between the three water stations within the Kaldvellfjord (Figure 1), neither at 1 m depth, nor at 5 m depth, except for total Al at 5 m, where a significant higher concentration were present at station 50 (median: $34,9 \mu\text{g L}^{-1}$; mean: $37,4 \pm 24,9 \mu\text{g L}^{-1}$) compared with station 51 (median: $18,6 \mu\text{g L}^{-1}$; mean: $20,5 \pm 6,5 \mu\text{g L}^{-1}$) and station 52 (median: $14,1 \mu\text{g L}^{-1}$; mean: $14,8 \pm 4,1 \mu\text{g L}^{-1}$).

The guideline for pollution status regarding heavy metals in Norwegian fjords (Molvær et al. (1997), does only incorporate a few metals, but for the 3 metals both measured in the

Kaldvellfjord and incorporated in this quality assessment guideline report (Table 3), i.e. Cu, Ni and Zn, the median Cu concentrations in the Kaldvellfjord ($1,31 \mu\text{g L}^{-1}$) was within category IV: highly polluted. Correspondingly the median Zn concentration of $3,4 \mu\text{g L}^{-1}$ fell within category III: remarkably polluted, while the median concentration of Ni of $0,7 \mu\text{g L}^{-1}$ fell within category II: moderately polluted (Figure 7). Regarding the metals not assessed by Molvær et al. (1997) as Al, Fe and Mn, we have to assume that the concentration levels in the Kaldvellfjord are significantly influenced by the acid, metal rich freshwater inputs as documented in Stordalsbekken.

Compared with a Northern European Lake Survey in 1995, the metal concentrations at 1 and 5 m depth in the Kaldvellfjord did not show any abnormal values. Furthermore, according to the classification of environmental quality in freshwater (Andersen, 1997), background levels for Fe and Mn were assessed to be $< 50 \mu\text{g L}^{-1}$ and $< 20 \mu\text{g L}^{-1}$, respectively. Accordingly, only between 5-10% of the water measurements in the fjord exceeded these values, while the concentrations of the same metals in one of the streams mounting into the fjord, Stordals-bekken, had Mn concentrations far above these values, and Fe well above these values in 2014.

Norwegian guidelines for Al pollution levels are available for freshwater (Guide 02:2013), but not for marine or coastal water. When acid, Al-rich water enters into high pH coastal water, acute toxic conditions occur during polymerization and precipitation processes following such pH increases. (Rosseland et al. 1992; Poleo et al, 1994; Lydersen et al. 2002). Thus, one should avoid using nominal limits for Al in such unstable mixing zones when acid, Al-rich water is mixed with alkaline coastal water, as in the Kaldvellfjord. However, the very high concentrations of cationic small, inorganic forms of Al present in the stream Stordalsbekken, and the relatively high concentration of dissolved Al (Al-F), especially at 1 m depth in the Kaldvellfjord (Figure 4) indicates that significant Al-polymerization/precipitation processes must continuously occur in the fjord water. According to the guideline limits for labile or inorganic monomeric Al (Guide 02:2013), the median Al-F concentrations in the Kaldvellfjord fell in the category 3 (moderate ecological conditions), but about 20 percent of the Al observation made in 2014, corresponded to category V, indicating very bad ecological conditions (Figure 7).

Table 4. Concentrations of metals in the stream Stordalsbekken, mounting into the Kaldfjellfjord inside water station 52, mussel station 1 (Figure 1). Al-T: total Al; Al-F: filtered/dissolved Al; Al-P: Al particles. According to the analytical method by Dougan and Wilson, 1974; Seip et al. 1984 RAL: Total dissolved Al; LAL: labile Al, primarily inorganic cationic dissolved Al.

	Date	Al-T µg L ⁻¹	Al-F µg L ⁻¹	Al-P µg L ⁻¹	RAL µg L ⁻¹	LAL µg L ⁻¹	As µg L ⁻¹	Cd µg L ⁻¹	Co µg L ⁻¹	Cu µg L ⁻¹	Mn µg L ⁻¹	Ni µg L ⁻¹	Zn µg L ⁻¹	Fe µg L ⁻¹
Stordalsbekken	24.01.2014	3680	2790	890	1440	1420	0,30	1,3	57,4	13,8	1300	163	238	146
Stordalsbekken	12.02.2014	7210	6710	500	4810	4773	0,10	1,3	44,5	36,3	744	128	218	145
Stordalsbekken	14.03.2014	1400	497	903	121	121	0,10	0,9	42,9	8,3	830	115	140	128
Stordalsbekken	15.04.2014	1540	494	1046	378	346	0,10	1,1	53,1	8,9	1070	137	206	153
Stordalsbekken	14.05.2014	3130	2390	740	2050		0,09	1,3	47,4	12,2	987	125	232	63
Stordalsbekken	19.06.2014	369	297	72	86	70	0,10	0,4	18,4	3,7	505	50	42	327
Stordalsbekken	18.08.2014	12200	1590	10610	274	240	0,30	3,2	117,0	24,7	2410	298	443	790
Stordalsbekken	29.09.2014	644	379	265	90	61	0,12	0,8	39,9	7,4	1240	121	97	438
Stordalsbekken	30.10.2014	3690	1860	1830	1140	1107	0,10	1,2	46,2	16,5	945	133	199	242
Stordalsbekken	18.11.2014	3690	3050	640	1860	1789	0,10	0,8	30,0	17,1	581	84	146	240
Stordalsbekken	11.12.2014	2650	2600	50	1560	1489	0,08	0,7	21,2	11,8	483	66	111	235
Median	2014	3130	1860	740	1140	727	0,10	1,11	45	12	945	125	199	235

Table 5. Mean (\pm std), median, maximum and minimum values of metal concentration in water at 1 m and 5 m depth from the 3 stations in the Kaldvellfjord during 2014.

Depth		AL-T µg L ⁻¹	AL-F µg L ⁻¹	Co µg L ⁻¹	Cu µg L ⁻¹	Mn µg L ⁻¹	Ni µg L ⁻¹	Zn µg L ⁻¹	Fe µg L ⁻¹
1 m	mean	90	61	0,19	1,3	8,4	0,80	4,7	21,5
	std	58	39	0,16	0,5	6,0	0,33	3,9	24,6
	median	78	52	0,13	1,3	6,4	0,70	3,4	11,6
	max	260	148	0,60	2,1	26,1	1,79	19,6	106,0
	min	18	10	0,05	0,5	0,5	0,50	2,0	4,0
5 m	mean	24	18	0,06	1,0	3,0	0,63	2,6	4,9
	std	18	10	0,02	0,6	2,2	0,22	1,5	1,5
	median	19	15	0,05	0,9	2,4	0,56	2,0	4,0
	max	89	52	0,11	4,0	13,2	1,37	10,4	9,1
	min	9	8	0,05	0,5	0,4	0,50	2,0	4,0

Table 6. Correlation coefficients (r) between metals and runoff in 1m depths (left side) and 5m depths (right side) from the 3 stations in the Kaldvellfjord during 2014. All correlations with $p < 0,05$ are assessed to be significant and present in bold style.

	Al-T µg L ⁻¹	Al-F µg L ⁻¹	Co µg L ⁻¹	Cu µg L ⁻¹	Mn µg L ⁻¹	Ni µg L ⁻¹	Zn µg L ⁻¹	Fe µg L ⁻¹	Runoff L s ⁻¹		Al-T µg L ⁻¹	Al-F µg L ⁻¹	Co µg L ⁻¹	Cu µg L ⁻¹	Mn µg L ⁻¹	Ni µg L ⁻¹	Zn µg L ⁻¹	Fe µg L ⁻¹	Runoff L s ⁻¹	
AL-F	0,754	1,0								AL-F	0,006	1,0								
	0,000										0,974									
Co	0,785	0,728	1,0							Co	0,038	0,300	1,0							
	0,000	0,000									0,827	0,076								
Cu	0,335	0,199	0,302	1,0						Cu	0,144	0,046	-0,010	1,0						
	0,046	0,246	0,074								0,403	0,790	0,954							
Mn	0,832	0,692	0,919	0,284	1,0					Mn	0,183	0,293	0,206	0,069	1,0					
	0,000	0,000	0,000	0,094							0,284	0,083	0,228	0,689						
Ni	0,788	0,665	0,789	0,445	0,782	1,0				Ni	0,337	0,125	0,246	0,064	-0,064	1,0				
	0,000	0,000	0,000	0,007	0,000						0,044	0,466	0,148	0,713	0,709					
Zn	0,647	0,580	0,661	0,315	0,667	0,753	1,0			Zn	0,116	-0,023	-0,075	0,324	-0,003	-0,002	1,0			
	0,000	0,000	0,000	0,062	0,000	0,000					0,501	0,894	0,663	0,054	0,987	0,989				
Fe	0,890	0,649	0,768	0,362	0,905	0,778	0,664	1,0		Fe	0,203	0,130	0,413	0,345	0,334	0,002	0,227	1,0		
	0,000	0,000	0,000	0,030	0,000	0,000	0,000				0,234	0,451	0,012	0,039	0,047	0,993	0,184			
Runoff	0,786	0,554	0,668	0,382	0,672	0,617	0,453	0,761	1,0	Runoff	-0,017	0,730	-0,089	0,077	0,004	-0,124	-0,022	-0,060	1,0	
	0,000	0,000	0,000	0,022	0,000	0,000	0,006	0,000			0,921	0,000	0,606	0,655	0,981	0,470	0,897	0,726		

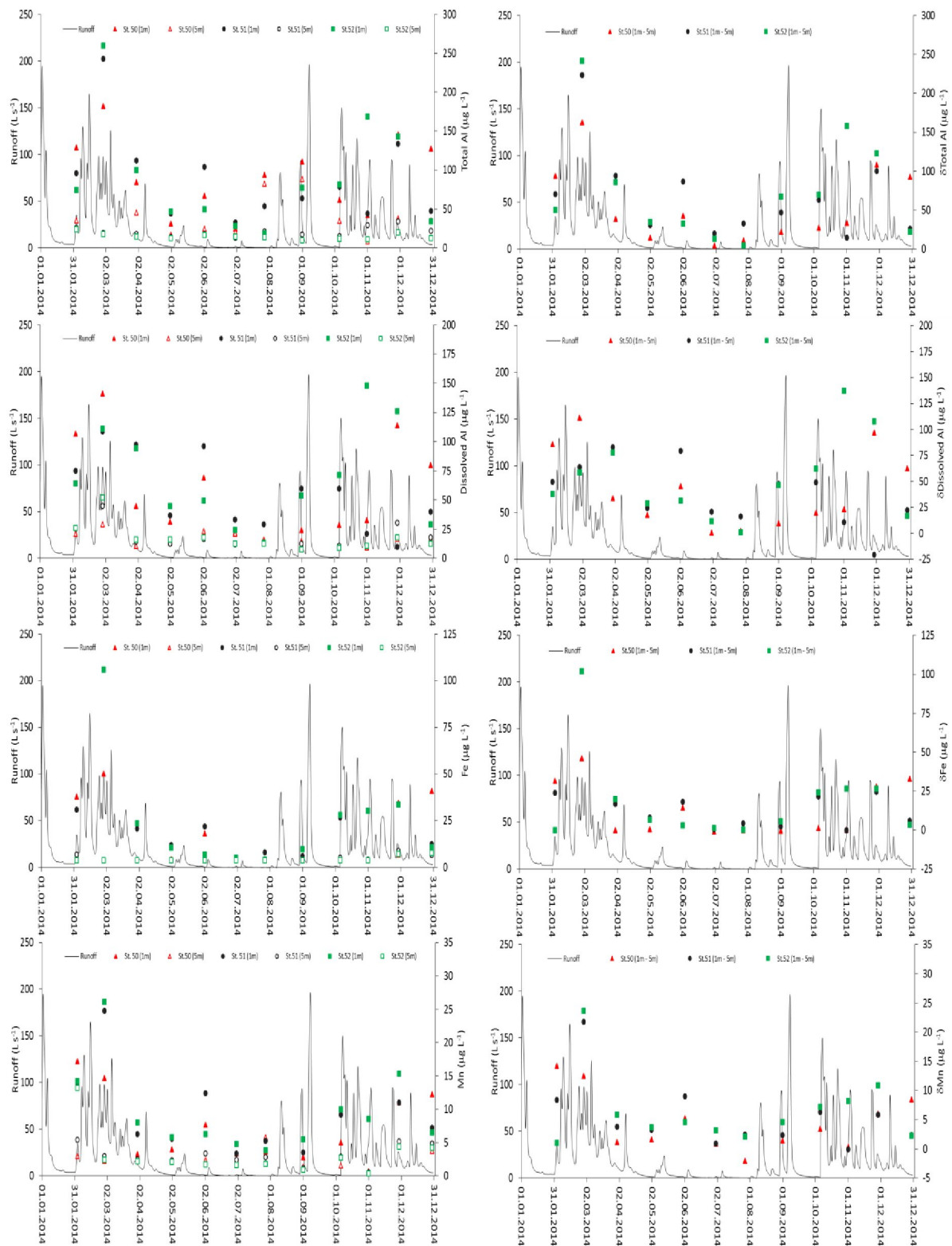


Figure 4 Seasonal variations in runoff and water concentration of total Al, dissolved Al, Fe and Mn from the 3 water stations in the Kaldvellfjord, at 1 and 5 m depth (left side), and the metal concentration differences between depths ($\delta[\text{Metal}] = [\text{Metal}]_{1\text{m}} - [\text{Metal}]_{5\text{m}}$) (right side).

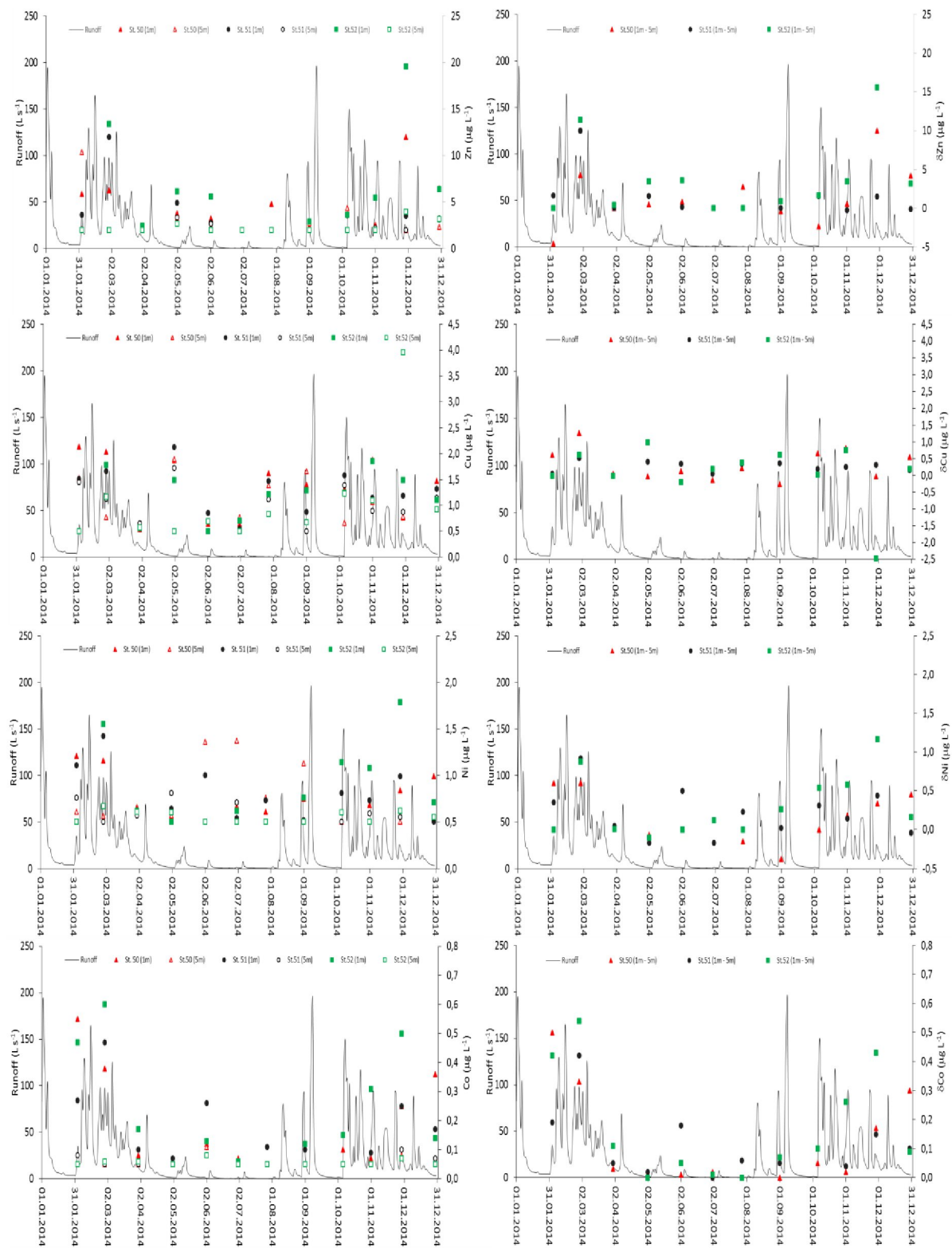


Figure 5 Seasonal variations in runoff and water concentration of Zn, Cu, Ni and Co from the 3 water stations in the Kaldvellfjord, at 1 and 5 m depth (left side), and the metal concentration differences between depths ($\delta[\text{Metal}] = [\text{Metal}]_{1\text{m}} - [\text{Metal}]_{5\text{m}}$) (wright side).

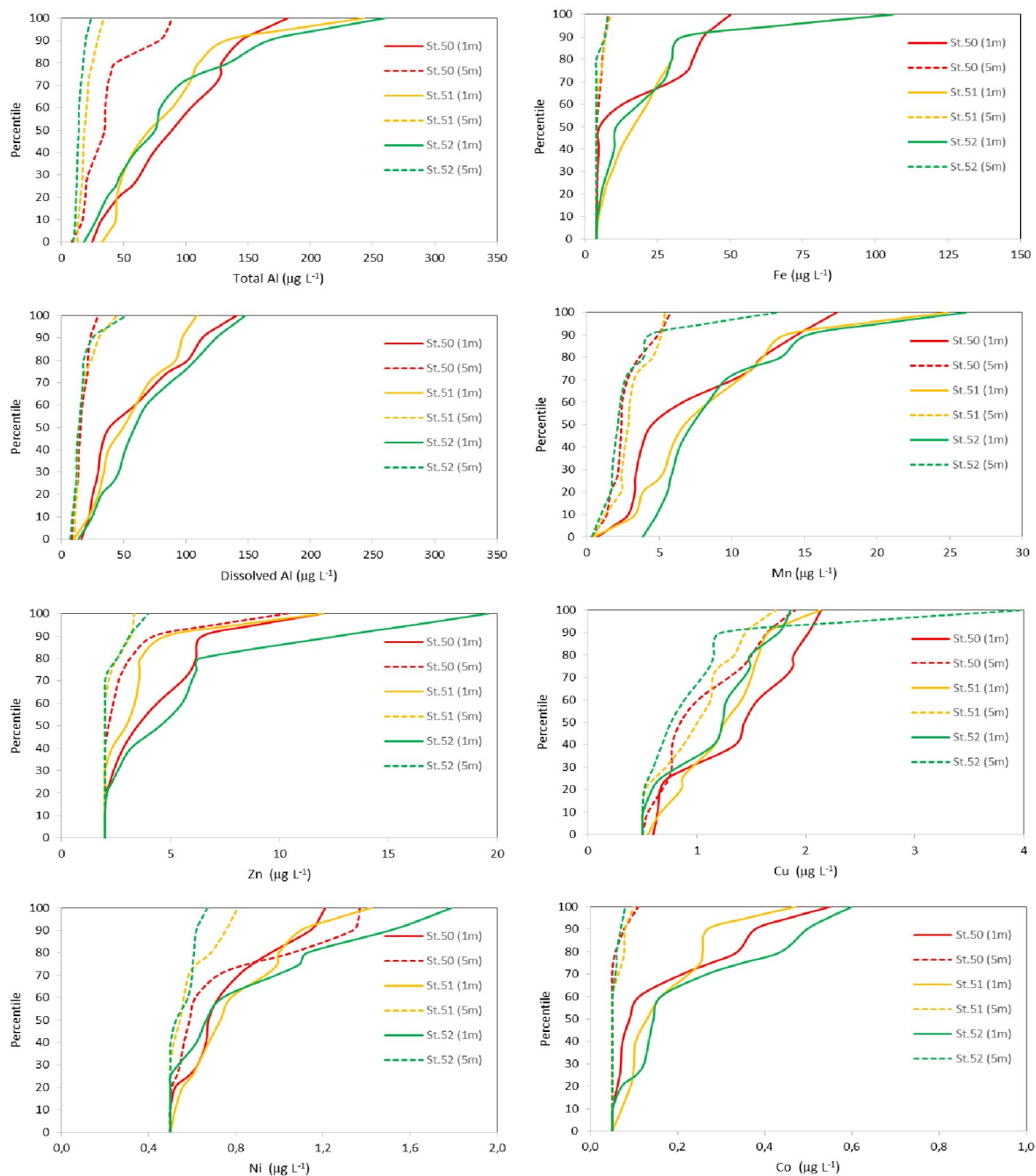


Figure 6 Percentiles of metal concentrations at the 3 water stations in the Kaldvellfjord at 1 and 5 m depths, during the investigated period, February-December 2014.

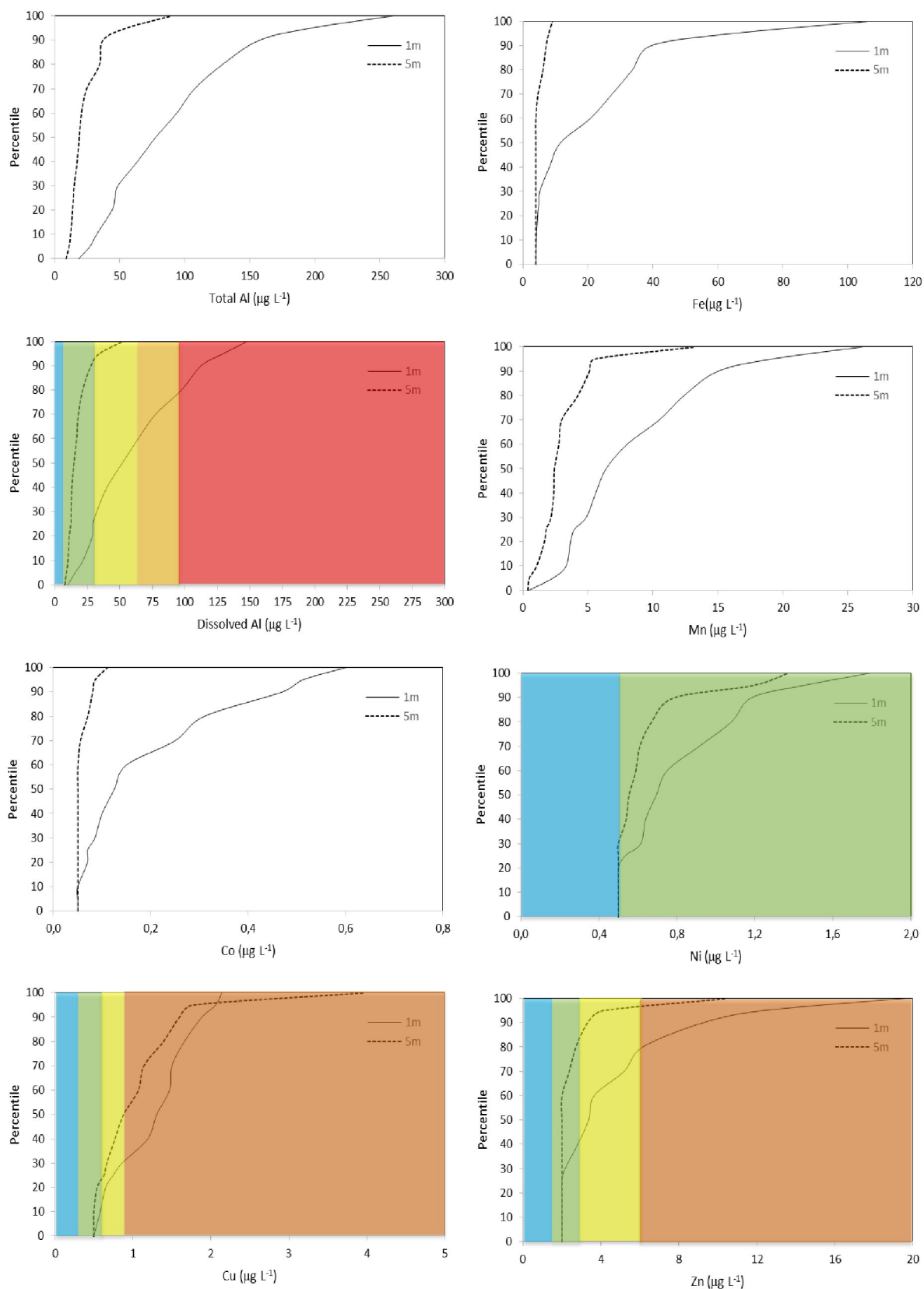


Figure 7. Percentiles for metal concentrations in water at 1 and 5 m depths in the Kaldvellfjord during the investigated period, February-December 2014. Metals evaluated in relation to the guidelines by Bakke et al, 2007 and Arp et al. 2014, presented in Table 3, while aluminium limits are based of freshwater limits in Guide 02:2013.

3.4 Metal concentrations in blue mussels from the Kaldvellfjord

Water and mussel sampling have unfortunately not been implemented at the same stations or dates, as different institutions have participated in different projects in the Kaldvellfjord during the latest years. Furthermore, while water samples in 2014 have been conducted 12 times during the period February 3 – December 30, at 3 stations and two depths (1m and 5m), blue mussels have been sampled 6 times in 2014, during the period April 17 - October 15, at 6 stations and two depths (1m and 5m). At all stations, both small (20-35mm) and big (> 36 mm) mussels were sampled and treated separately. Basic data on the concentrations of metals in blue mussels are presented in Table 7.

The highest concentrations of metals in blue mussels were primarily observed in September and October at 1 m depth, in periods with high freshwater runoff. (Figure 8 and 9). While all investigated metal concentrations (Al-T, Al-F, Co, Cu, Mn, Ni, Zn and Fe) in water from 1 m depth, were significantly and positively correlated with runoff (Table 5), the concentrations of Al, Co, Fe, Mn Ni, Zn and Hg in blue mussels at 1 m depth, also exhibited significant, positive correlations with runoff (Table 8). The concentrations of Cd, Cu, Pb and As in blue mussels from 1m depth were not significantly correlated with runoff. Correspondingly, the concentrations of Al, Co, Fe, Mn and Ni in blue mussels from 5 m depth were significantly and positively correlated with runoff (Table 9), while the correlations between runoff and Zn and Hg in blue mussels were not found to be significant.

Even though some differences in the concentrations in blue mussels between stations were observed (Figure 10 and 11), both small and big mussels, showed no statistical, significant differences in the metal concentrations between the stations. Al, Fe and Zn exhibited the highest concentrations in blue mussel, while the Hg concentrations in blue mussels were far the lowest (Table 7).

The concentration distributions of metals in small and big mussels located at 1 and 5 m depth is presented in Figure 12. Except for As and Mn, all other investigated metals exhibited significantly higher metal concentrations in blue mussel located at 1 m depth (n = 52), compared with at 5 m depth (n = 49). For big mussels, significant higher concentrations were found in mussels located at 1 m (n = 34) compared with at 5 m (n = 34) regarding Al, Cd, Co, Fe, Ni, Zn, Hg and Pb, while the higher concentrations of As, Cu, Mn, found in big mussels at 1 m compared with at 5 m, were not significant. For small mussels, no metal concentrations were found to be

significantly different between the two depths, i.e. at 1 m (n=18) and at 5 m (n = 15). However, a generally higher concentrations of Al, Cd, Co, Cu, Fe, Ni, Zn, Hg and Pb were found in mussels located at 1 m compared with at 5, while As and Mn exhibited lower concentrations in small mussels located at 1 m compared with at 5m.

The concentrations of As, Cu, Zn and Hg were significantly higher in small mussels compared with big mussels, located at both depths (1m and 5m), In addition, significantly higher concentrations of Co, Mn and Ni were measured in small mussels at 1m depth (n =18) compared with big mussels (N = 34) at the same depth, while Al was significantly higher in small mussels at 5 m depth (n = 15) compared with big mussels from same depth (n = 34). For the other metals, Fe, Cd and Pb, the concentrations were higher in small mussels compared with big mussels at both depths, but not statistical significant.

The generally higher metal concentrations found in small mussels compared with big mussels was further supported by the general, negative correlation found between mussel length and metal concentration in tissue from mussels at both depths (Table 8 and 9). The condition index (CI) was significantly higher in April compared with July, the month with lowest average CI, but no significant differences in CI were revealed between stations or depths

Similar to the Kaldvellfjord water, the concentrations of heavy metals in blue mussels in the fjord were generally low in relation to the quality guidelines given by Molvær et al. (1997, Table 3). Based on median concentrations, no metal concentrations in blue mussel samples exceeded the upper limit concentrations for the highest quality class (unpolluted, background values), according to this guideline (Figure 10). Only As, Ni and Pb showed a certain concentration exceedance in relation to the limits for highest quality levels. For As, the percentage concentrations exceedance, in relation to the upper limit assessed for the highest quality class, varied from 5 - 40%, highest regarding samples of small blue mussel individuals located at 5 m. The highest sample As concentrations measured (15-16 ppm) was found in both size classes located at 5 m. For Ni and Pb, only one sample of small blue mussel individuals located at 1 m (Ni: 5,5 ppm; Pb: 3,5 ppm), exceeded the upper limit concentrations for the highest quality class for these elements, i.e. Ni > 5 ppm and Pb > 3 ppm (Figure 10).

Despite the relatively low levels of metals in blue mussels from the Kaldvellfjord, many dead mussels were found, especially at St.1 and St.2, during autumn 2014.

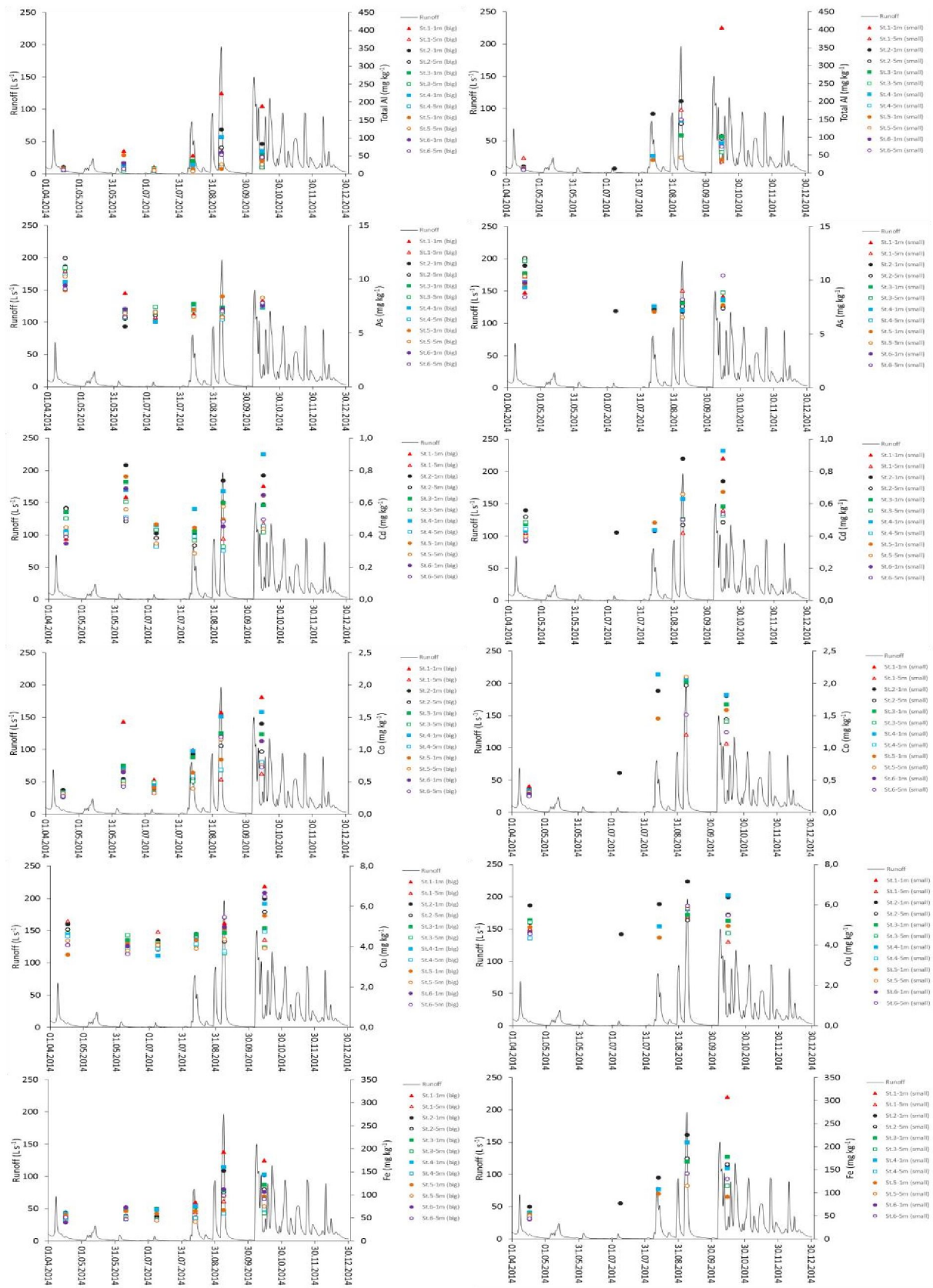


Figure 8. Seasonal variations in runoff, and concentrations of Al, As, Cd, Co, Cu and Fe in big (left side) and small (wright side) blue mussels, at 1 m and 5 m depth, at the different stations.

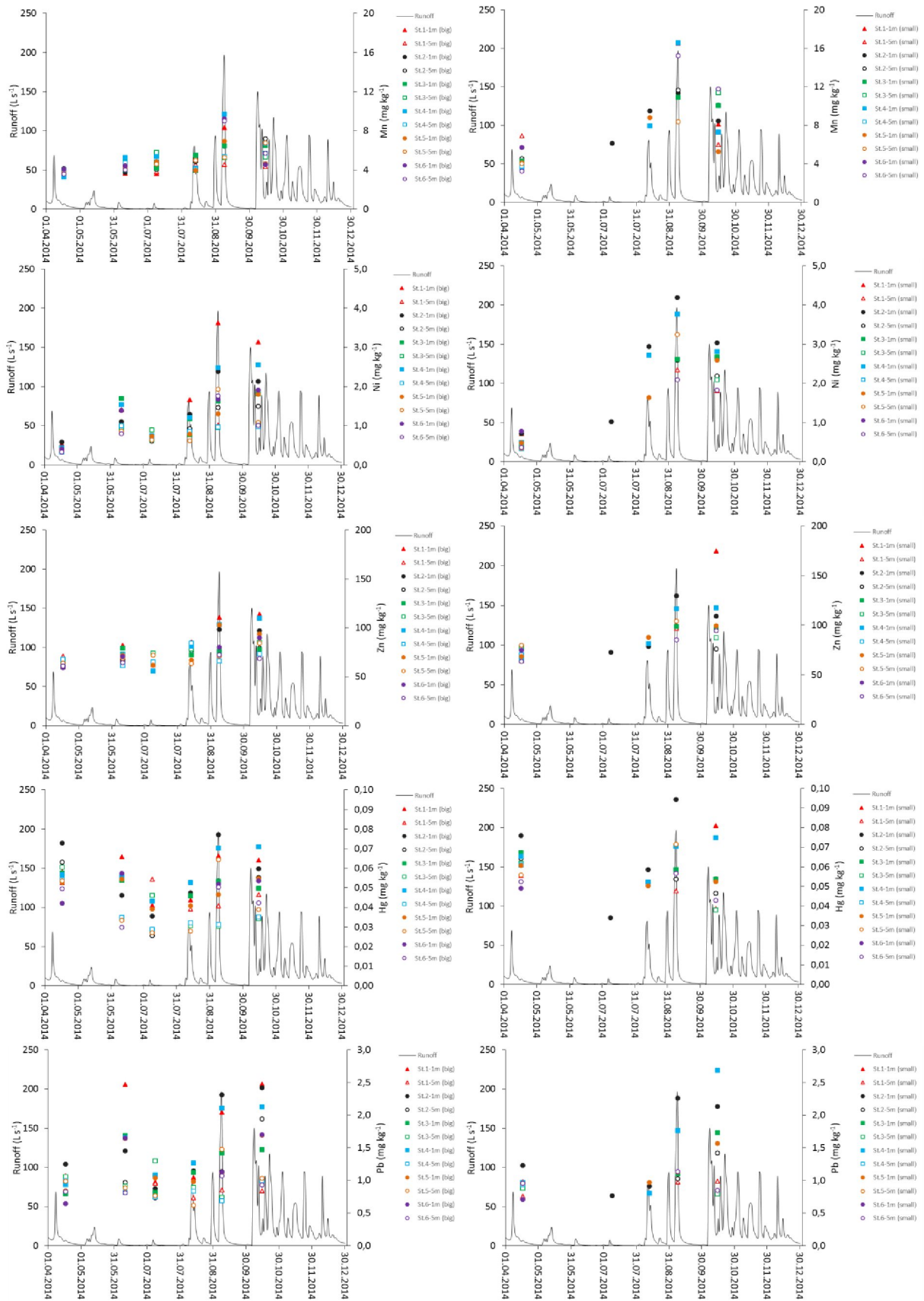


Figure 9. Seasonal variations in runoff, and concentrations of Mn, Ni, Zn, Hg and Pb in big (left side) and small (wright side) blue mussels, at 1 m and 5 m depth, at the different stations.

Table 7. Mean (\pm std), median, maximum and minimum values of in big and small blue mussels located at 1 m and 5 m depth at the 6 stations within the Kaldvellfjord during 2014.

Deep (m)	Size		Al	As	Cd	Co	Cu	Fe	Mn	Ni	Zn	Hg	Pb
			mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹
1	Small (n = 18)	mean	85	8,4	0,6	1,3	5,6	124	7,6	2,2	93	0,06	1,4
		std	100	1,3	0,2	0,9	1,1	73	3,4	1,5	28	0,01	0,8
		median	43	8,0	0,5	1,6	5,2	103	7,6	2,6	85	0,06	1,0
		max	406	11,4	0,9	3,0	8,6	308	16,6	5,5	175	0,09	3,4
		min	12	7,1	0,4	0,3	4,0	42	3,5	0,5	64	0,03	0,7
5	Small (n = 15)	mean	61	9,6	0,5	1,0	5,1	97	8,1	1,4	86	0,06	1,0
		std	55	2,2	0,1	0,7	0,6	50	4,4	1,0	14	0,01	0,2
		median	43	9,0	0,5	1,1	5,1	92	6,9	1,8	85	0,06	1,0
		max	177	15,0	0,8	2,1	5,9	174	16,5	3,2	112	0,10	1,5
		min	9	6,6	0,4	0,3	4,2	44	3,2	0,3	63	0,04	0,8
1	Big (n = 34)	mean	46	7,6	0,6	0,9	4,7	87	5,2	1,4	78	0,05	1,4
		std	49	1,3	0,2	0,4	0,8	40	1,6	0,8	17	0,01	0,5
		median	29	7,2	0,6	0,8	4,6	72	4,8	1,3	74	0,05	1,3
		max	224	11,2	0,9	1,8	7,0	193	9,7	3,6	114	0,08	2,5
		min	9	5,6	0,3	0,3	3,5	40	3,3	0,4	56	0,03	0,6
5	Big (n = 34)	mean	22	7,9	0,4	0,6	4,4	62	4,9	0,9	71	0,04	1,0
		std	17	2,0	0,1	0,2	0,7	19	1,2	0,4	8	0,01	0,3
		median	15	7,3	0,4	0,5	4,3	54	4,6	0,9	69	0,04	0,9
		max	73	15,5	0,7	1,2	6,5	112	9,0	1,9	96	0,08	1,9
		min	7	6,3	0,3	0,3	3,6	42	3,6	0,3	60	0,03	0,6

Table 8. Correlation coefficients (*r*) between metals, length, shell weight, tissue weight and runoff in blue mussel located at 1m depth. All correlations with $p < 0,05$ are assessed to be significant and present in bold stile.

	Al	As	Cd	Co	Cu	Fe	Mn	Ni	Zn	Hg	Pb	Length	Shell weight	Tissue weight	Runoff
	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mm	g (ww)	g (ww)	L s ⁻¹
Al	1,0														
As	-0,177	1,0													
	0,210														
Cd	0,519	-0,225	1,0												
	0,000	0,109													
Co	0,810	-0,301	0,562	1,0											
	0,000	0,030	0,000												
Cu	0,763	0,116	0,571	0,720	1,0										
	0,000	0,415	0,000	0,000											
Fe	0,923	-0,242	0,602	0,902	0,800	1,0									
	0,000	0,080	0,000	0,000	0,000										
Mn	0,581	-0,272	0,270	0,738	0,449	0,746	1,0								
	0,000	0,051	0,053	0,000	0,001	0,000									
Ni	0,899	-0,336	0,643	0,948	0,764	0,952	0,735	1,0							
	0,000	0,015	0,000	0,000	0,000	0,000	0,000								
Zn	0,856	-0,145	0,674	0,865	0,837	0,912	0,619	0,919	1,0						
	0,000	0,306	0,000	0,000	0,000	0,000	0,000	0,000							
Hg	0,570	-0,328	0,595	0,457	0,642	0,584	0,335	0,527	0,590	1,0					
	0,000	0,018	0,000	0,001	0,000	0,000	0,015	0,000	0,000						
Pb	0,732	-0,208	0,864	0,684	0,680	0,760	0,313	0,755	0,809	0,628	1,0				
	0,000	0,139	0,000	0,000	0,000	0,000	0,024	0,000	0,000	0,000					
Length	-0,237	-0,241	0,039	-0,267	-0,357	-0,271	-0,400	-0,285	-0,239	-0,265	0,119	1,0			
	0,091	0,085	0,786	0,055	0,009	0,052	0,003	0,040	0,088	0,058	0,400				
Shell weight	-0,120	-0,108	0,089	-0,066	-0,225	-0,165	-0,296	-0,161	-0,106	-0,097	0,233	0,867	1,0		
	0,396	0,445	0,533	0,640	0,109	0,242	0,033	0,254	0,454	0,495	0,097	0,000			
Tissue weight	-0,203	-0,113	0,047	-0,199	-0,305	-0,257	-0,403	-0,267	-0,208	-0,192	0,155	0,945	0,968	1,0	
	0,149	0,424	0,739	0,156	0,028	0,066	0,003	0,056	0,139	0,174	0,273	0,000	0,000		
Runoff	0,374	-0,256	0,131	0,429	0,208	0,483	0,672	0,438	0,408	0,311	0,181	0,030	0,007	-0,048	1,0
	0,006	0,067	0,353	0,002	0,138	0,000	0,000	0,001	0,003	0,025	0,198	0,833	0,958	0,737	

Table 9 Correlation coefficients (*r*) between metals, length, shell weight, tissue weight and runoff in blue mussels located at 5m depth. All correlations with $p < 0,05$ are assessed to be significant and present in bold stile.

	Al mg kg ⁻¹	As mg kg ⁻¹	Cd mg kg ⁻¹	Co mg kg ⁻¹	Cu mg kg ⁻¹	Fe mg kg ⁻¹	Mn mg kg ⁻¹	Ni mg kg ⁻¹	Zn mg kg ⁻¹	Hg mg kg ⁻¹	Pb mg kg ⁻¹	Length mm	Shell weight g (ww)	Tissue weight g (ww)	Runoff L s ⁻¹	
Al	1,0															
As	-0,016 0,915	1,0														
Cd	0,175 0,229	0,443 0,001	1,0													
Co	0,734 0,000	-0,215 0,138	0,360 0,011	1,0												
Cu	0,530 0,000	0,451 0,001	0,532 0,000	0,467 0,001	1,0											
Fe	0,918 0,000	-0,090 0,537	0,295 0,040	0,888 0,000	0,577 0,000	1,0										
Mn	0,911 0,000	-0,094 0,521	0,127 0,386	0,786 0,000	0,475 0,001	0,883 0,000	1,0									
Ni	0,731 0,000	-0,272 0,059	0,378 0,007	0,974 0,000	0,416 0,003	0,883 0,000	0,783 0,000	1,0								
Zn	0,515 0,000	0,332 0,020	0,543 0,000	0,640 0,000	0,508 0,000	0,585 0,000	0,525 0,000	0,634 0,000	1,0							
Hg	0,181 0,212	0,759 0,000	0,661 0,000	0,154 0,290	0,644 0,000	0,192 0,187	0,049 0,737	0,102 0,485	0,434 0,002	1,0						
Pb	0,289 0,044	0,221 0,127	0,614 0,000	0,354 0,013	0,511 0,000	0,426 0,002	0,178 0,222	0,351 0,014	0,389 0,006	0,600 0,000	1,0					
Length	-0,443 0,001	-0,487 0,000	-0,382 0,007	-0,322 0,024	-0,470 0,001	-0,391 0,005	-0,444 0,001	-0,301 0,036	-0,483 0,000	-0,549 0,000	-0,122 0,403	1,0				
Shell weight	-0,349 0,014	-0,494 0,000	-0,401 0,004	-0,233 0,108	-0,371 0,009	-0,306 0,032	-0,343 0,016	-0,238 0,099	-0,388 0,006	-0,548 0,000	-0,111 0,448	0,960 0,000	1,0			
Tissue weight	-0,416 0,003	-0,470 0,001	-0,429 0,002	-0,333 0,019	-0,418 0,003	-0,398 0,005	-0,419 0,003	-0,335 0,019	-0,461 0,001	-0,576 0,000	-0,179 0,217	0,965 0,000	0,981 0,000	1,0		
Runoff	0,584 0,000	-0,226 0,118	0,005 0,972	0,429 0,002	0,190 0,190	0,558 0,000	0,490 0,000	0,607 0,000	0,294 0,040	0,153 0,294	0,126 0,390	-0,027 0,852	0,031 0,835	-0,059 0,689	1,0	

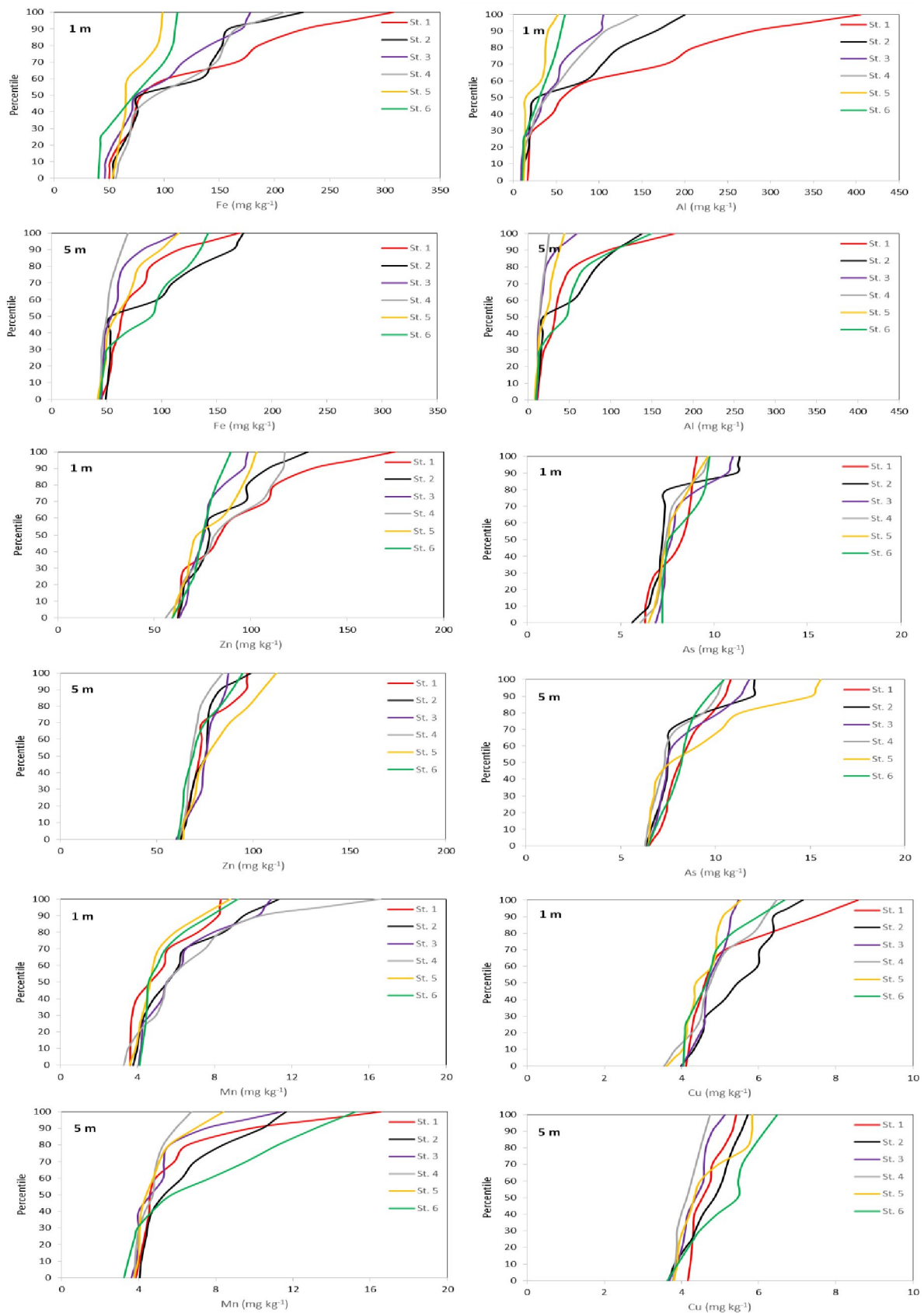


Figure 10. Percentiles of metal concentrations (Al, Fe, Zn, As, Mn and Cu) and size distribution in blue mussels located at both 1m and 5 m depth at the 6 stations in the Kaldvellfjord during 2014.

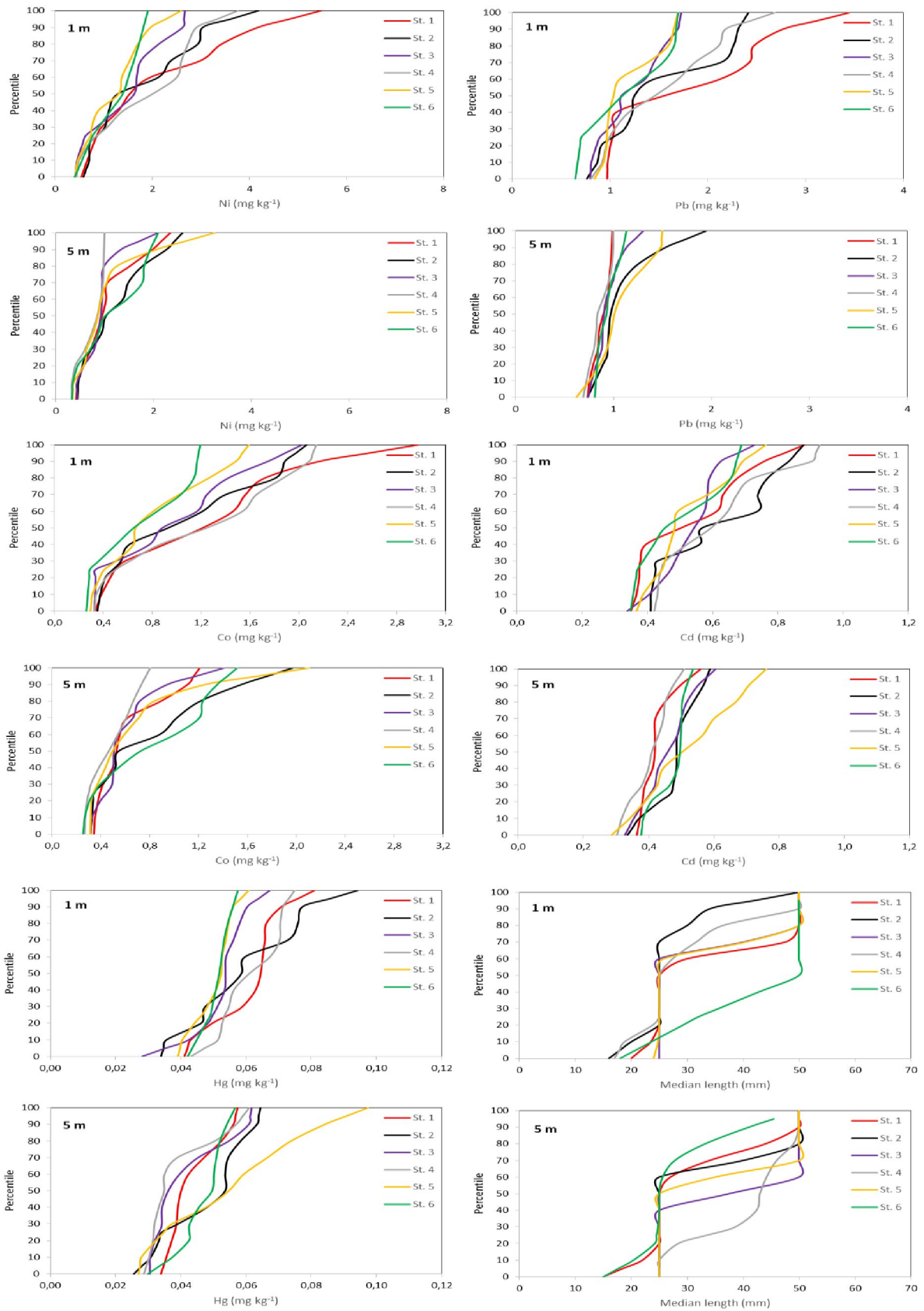


Figure 11. Percentiles of metal concentrations (Ni, Pb, Co, Cd and Hg) and size distribution in blue mussels located at both 1m and 5 m depth at the 6 stations in the Kaldvellfjord during 2014.

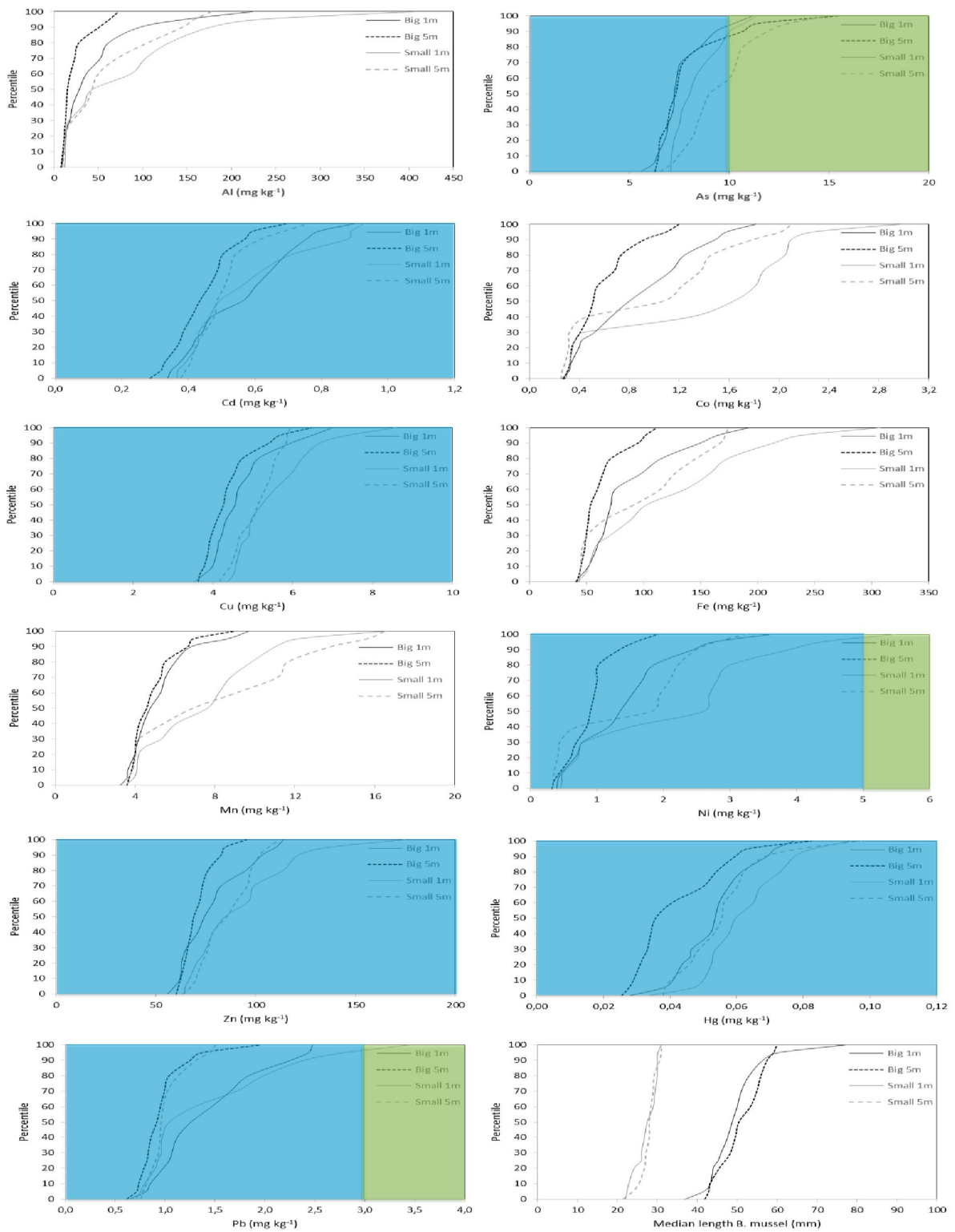


Figure 12. Percentiles for metal concentrations in blue mussels according to the guidelines in Molvær et al. (1997). Small mussels: 20-35 mm. Big mussels: > 36 mm. Small 1m (n=15); Small 5m (n=18); Big 1m (n=34); Big 5m (n =34).

4 Discussion

Based on the water chemical analyses in 2014, from one of the streams entering into the Kaldvellfjord (stream Stordalsbekken), it is well documented that the fjord receives very acidic, metal rich freshwater. This is primarily due to physical and chemical degradation (oxidation) of the sulphide and metal rich stone depots located in the catchment. As the bedrock surrounding the fjord, basically consists of various gneisses, often with relatively high sulphur content, oxidation of metal-sulphides from these rocks will also naturally occur and contribute to higher metal inputs to the Kaldvellfjord compared with other fjords surrounded by less sulphur rich bedrocks. Extreme acidification and metal leakage, primarily aluminium, has earlier been reported after bedrock blasting in the same areas (Hindar & Lydersen 1994).

The concentrations of metals in the Kaldvellfjord water were significantly lower compared with the stream Stordalsbekken. This is expected as the stream-water will be significantly diluted by the recipient, i.e. the metal poor, marine Kaldvellfjord water. Besides dilution, the high pH in marine Kaldvellfjord water implies significant metal precipitation with subsequent adsorption and co-precipitation of metals, when the two water types are mixed. Quantification of these precipitation processes is almost impossible, as significant variations in precipitation kinetics occur as the metal concentrations, pH, temperature and amounts of acidic freshwater runoff continuously change. Despite this fact, we might classify metals in seawater into various groups according to the dominant inorganic ligand that complex them (Byrne et al. 1988):

- a) Hydrolysed (OH^-): Al (III), Fe (III)
- b) Carbonate (CO_3^{2-}): Cu (II)
- c) Chloride (Cl^-): Hg (II)
- d) Free: Mn(II), Fe (II), Co (II)
- e) Transition/mixed: Pb (II)

Although the metals in the transition/mixed category could be placed in the other categories, they are separated because of their unique behaviour (Byre et al. 1988, Byrne, 2002). Metals that form strong complexes with chloride or mainly present in the free form will not be strongly influenced by pH, but metals that form strong complexes with hydroxide and carbonate may undergo significant changes in speciation with pH. However, as mentioned before, the very complexed precipitation/co-precipitation/adsorption – processes in such mixing zones are almost impossible to quantify, as these processes depends on numerous factors where pH, ionic strength, turbidity, ligand composition, temperature, time, are among the most important. All these factors are essential for the quantity and quality of the precipitate formed, and the subsequent transfers of amorphous precipitate to various degrees of more crystalline precipitates. All this factors is again important for the concentration and physico-chemical forms of the remaining dissolved metal

fractions. Accordingly, in such very physical and chemical complex systems, it is important to simplify. One way to simplify is to measure total concentrations of metals in water and in potential target organisms as blue mussel, and assume that the main pathway for assimilation of metals in blue mussel tissue is by uptake of particles (“food”) from the surrounding water. This is to a certain degree revealed in this study, by the positive correlation observed between freshwater runoff and metal concentrations, both in water and blue mussels from the Kaldvellfjord, especially in the uppermost water layer (1 m depth). This documentation indicates that the metals present in fjord are primarily associated to colloids and particles, with the highest concentrations in the uppermost water layers.

Despite anthropogenic discharge of metals into the Kaldvellfjord, the metal concentrations in both fjord water and blue mussels are still generally low compared with the Norwegian pollution guidelines set for these matrixes.

The factor of most concern in the Kaldvellfjord, is likely the high concentration inputs of inorganic dissolved Al from streams strongly impacted by draining water from the metal-sulphide rich bedrock depots. When such water meets water of high pH, significant polymerization with subsequent precipitation occur. Today it is well accepted that acute Al toxicity primarily is related to cationic and thereby predominantly inorganic Al forms, both positively charged monomers and polymers (Lydersen 1992, Poléo 1995). Numerous cationic polymers are known being highly acute toxic to gill-breathing organisms (Biesinger and Stokes 1986; Goodrich et al. 1991; Scott Hall and Mirenda 1991). The physiological and pathological descriptions of fish exposed to many different cationic electrolytes are almost identical to the description of Al toxicity to fish (e.g. compare Biesinger and Stokes 1986 with Rosseland et al. 1992 and/or Poléo et al. 1994). Cationic polyelectrolyte studies conclude that the lowest molecular weight polymers (e.g. Goodrich et al. 1991) and the cationic polymers with highest positive charge densities (Scott Hall and Mirenda 1991) are the most toxic. This agrees well with the chemical and biological relationships in the Al toxicity study reported by Lydersen et al. (1994). There is good evidence that the toxicity of cationic polymers can be eliminated by addition of an anionic polymer (Biesinger and Stokes 1986). Accordingly, as also proposed by them, natural humic acids and clays might be used for detoxifying cationic Al polymers. pH-changes may also reduce or eliminate the cationic properties of aluminium. An example is liming where the cationic properties of monomeric and polymeric Al is significantly reduced or almost eliminated. Accordingly the Al-toxicity is eliminated. Similar water chemical processes occur when acidic, Al-rich water is mixed with coastal water (Teien et al. 2006; Golding et al. 2015). In an unpublished work cited in Golding et al. (2015), *Angel et al. unpublished manuscript*,

seawater was spiked with high concentrations of Al ($> 10\,000\ \mu\text{g L}^{-1}$). Precipitation occurred almost immediately, but a pulse of dissolved Al as high as $1700\ \mu\text{g L}^{-1}$ was sustained for several days, before gradually decreasing to equilibrium concentrations ($500\ \mu\text{g L}^{-1}$). The same precipitation pattern has earlier been revealed by Lydersen et al. (1991) in synthetic freshwater studies, describing Al precipitation as a two-step process, a fast initial precipitation process and a far slower consolidation process. In addition to time, Lydersen et al. (1990) showed that both quality and quantity of the two processes were significantly affected by pH and temperature. At identical pHs, significant amount of high molecular weight Al was precipitated from the solution after 1 month of storage at 25°C , and the solubility product (\log^*K_s) was low (9,0). Substantial amounts of high molecular weight Al was also formed in identical solutions stored at 2°C for 1 month, but the majority of Al was still present as colloids in solution and \log^*K_s was significantly higher (10,2), reflecting a solubility product of an amorphous $\text{Al}(\text{OH})_3(\text{s})$ phase.

It is therefore apparent that at high concentrations of total aluminium, organisms will be exposed to a mixture of both dissolved and particulate forms of Al, both in freshwater and in marine environments. As the Kaldvellfjord receive high amounts of acid, Al-rich water, primarily from point sources (i.e. highly bedrock deposit affected streams), the highest toxic effects in the fjord will obviously occur near the stream outlets, and decline by increasing distance from these sources. The significant Al-precipitation is well illustrated in Figure 2, showing Al-precipitates on stones in the nearest beach areas. However, due to continuously changes in pH, Al concentration, temperature and runoff in the streams, combined with continuous changes in wind and watercurrent regime in fjord, the area in the fjord where potential toxic effects may occur on organisms as blue mussels, will naturally exhibit large spatial and temporal variations. For the last couple of years, people living around the fjord have reported negative effects on the biology in the fjord, and blue mussel is now absent in the nearest areas around the outlet of the most affected streams. In addition a blue mussel farmer, located about 1,2 km from Stordalsbekken outlet, close to mussel station 5, has experienced a significant lack of blue mussel larvae during the latest years. A new developed water quality guideline for seawater (Golding et al. 2015) have anticipated a concentration of $24\ \mu\text{g Al L}^{-1}$ to have a 10% chronic inhibition effect (IC10 or EC10), This study relies on studies of organisms representing 6 taxonomic groups. The blue mussel (*Mytilus edulis planiculatus*) was the second most sensitive species tested, with EC10 = $250\ \mu\text{g Al L}^{-1}$ (72-h embryo development). The highest measured concentration of Al in the Kaldvellfjord was $260\ \mu\text{g Al L}^{-1}$ and $148\ \mu\text{g Al L}^{-1}$ for total and dissolved Al, respectively. These concentrations were measured at St. 52, located ≈ 100 m outside the stream Stordalsbekken

outlet, in November 2014 (Total Al) and February 2014 (dissolved Al). However, as all water samples are analysed several days after sampled, on going precipitation processes might significantly have impacted the analytical results. As over investigations have revealed significant mortality primarily on small blue mussels at St. 1 and St. 2, the nearest stations to Stordalsbekken, especially during autumn 2014, is an indication of more adverse Al-concentrations in situ, than those revealed in water the samples analysed several days after collected. It is therefore likely that the mortality of blue mussel outside Stordalsbekken is a result of the toxic conditions formed when acidic, Al-rich freshwater is mixed with high pH coastal water. Accordingly, we cannot omit that the mortality reported by the blue mussel farmer is a result of similar water chemistry, even though this farm is located > 1 km from the stream Stordalsbekken. However, the liming plant in Stordalsbekken will significantly reduce the ecological effects of the acidic, Al rich runoff to the fjord in the years to come, but the effects of this liming plant is likely significantly reduced during high runoff periods following draught periods, especially during low temperature conditions which primarily occurs between October to May in these areas.

5 Conclusions

Effect studies of metals on biota, are very difficult to perform under unstable water chemical conditions, as in the Kaldvellfjord where acidic, metal rich freshwater is mixed with metal poor, high pH coastal water. As this unstable water chemistry may last for days/weeks, with subsequent changes in the chemistry of metals, the time between sampling and analyses is crucial. Only “in situ” analysis can solve this problem, but this is more or less analytically unattainable. Accordingly, many scientists “solve” this problem by ion interaction modelling calculations based on thermodynamic data. However, as a lot of polymerization/precipitations reactions continuously occur in such systems, with subsequent changes in the quality and quantity of both dissolved and particulate metal species. Under such stochastic conditions, also metal speciation models should be used by care. Thus, the best way to assess biological effects of exposure to very unstable water chemistry, is likely *in situ* biological monitoring over time, to compare biologic post-condition data with pre-condition data. This strongly underline the importance of long term monitoring programs to secure pre-condition data or background reference data.

References

Amiard, J. C. 1976: Experimental study of acute toxicity of cobalt, antimony, strontium and silver salts in some crustaceans and their larvae and in some teleosteans. -Revue Internationale d'Océanographie Médicale 79-95.

Andersen, J. R. 1997: Klassifisering av miljøkvalitet i ferskvann = Classification of environmental quality in freshwater. -Classification of environmental quality in freshwater 97:04.

Arp, H.P., Ruus, A., Macken A., Lillicrap A. 2014: Quality assurance of environmental quality standards. Miljødirektoratet. Report no. M-241, 170 pp + Appendix.

Bakhmet, I. N., Kantserova, N. P., Lysenko, L. A. and Nemova, N. N. 2012: Effect of copper and cadmium ions on heart function and calpain activity in blue mussel *Mytilus edulis*. -Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering 47(11): 1528-1535.

Bakke, T., Oen, A., Kibsgaard, A., Breedveld, G., Eek, E., Helland, A., Källqvist, T., Ruus, A., Hylland, K. 2007: Guidelines on classification of environmental quality in fjords and coastal waters - A revision of the classification of water and sediments with respect to metals and organic contaminants. State Pollution Report TA 2229/2007, 12 pp.

Biesinger, K.E. and Stokes, G.N. 1986: Effects of synthetic polyelectrolytes on selected aquatic organisms. J. Water Pollut. Contam. Fed. 58: 207-213.

Bjerregaard, P. 2013: Økotoksikologi. -Gyldendal, 247:219-223

Boalch, R., Chan, S. and Taylor, D. 1981: Seasonal variation in the trace metal content of *Mytilus edulis*. -Marine Pollution Bulletin 12(8): 276-280.

Borg, H. 1987: Trace-metals and water chemistry of forest lakes in northern Sweden. -Water Research 21(1): 65-72.

Byrne, R.H. 2002: Inorganic speciation of dissolved elements in seawater: The influence of pH on concentrations ratios. Geochemical Transactions 3: 11-16.

Byrne, R.H., Kump, L.R., Cantrell, K.J. 1988: The influence of temperature and pH on trace metals speciation in seawater. Marine Chemistry 25: 163-181.

Clark, R. B., Frid, C. and Attrill, M. 1997: Marine pollution. -Clarendon Press, Oxford,

Conti, M. E. and Cecchetti, G. 2003: A biomonitoring study: trace metals in algae and molluscs from Tyrrhenian coastal areas. -Environmental Research 93(1): 99-112.

Dagnino, A., Allen, J. I., Moore, M. N., Broeg, K., Canesi, L. and Viarengo, A. 2007: Development of an expert system for the integration of biomarker responses in mussels into an animal health index. -Biomarkers 12(2): 155-172.

Davenport, J. and Chen, X. G. 1987: A comparison of methods for the assessment of condition in the mussel (*Mytilus edulis* L.). -Journal of Molluscan Studies 53: 293-297.

Davenport, J. and Redpath, K. J. 1984: Copper and the Mussel *Mytilus edulis*. -Springer Berlin Heidelberg, 176-189:15.

Davies, I. M. and Pirie, J. M. 1978: The mussel *Mytilus edulis* as a bio-assay organism for mercury in seawater. -Marine Pollution Bulletin 9(5): 128-132.

Dougan, W.K., and Wilson, A.L. 1974: The absorptiometric determination of aluminium in water. A comparison of some chromogenic reagents and the development of an improved method. Analyst, 99, 413-430.

Duinker, A., Lunestad, B. T., Svanevik, C. S. and Julshamn, K. 2012: Tilsynsprogrammet for skjell 2011. Fremmedstoffer (tungmetaller og organiske miljøgifter i skjell og tungmetaller i snegler) og mikroorganismer. -Yearly report to the Norwegian Food Safety Authority

EC. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union 20.12.2006 L364/5-L364/24.

Eisler, R. 1988: Lead hazards to fish, wildlife, and invertebrates: a synoptic review. -U.S. Fish and Wildlife Service, Patuxent Wildlife Research Center, Laurel, MD, vii, 134 p.

Fokina, N. N., Ruokolainen, T. R., Nemova, N. N. and Bakhmet, I. N. 2013: Changes of Blue Mussels *Mytilus edulis* L. Lipid Composition Under Cadmium and Copper Toxic Effect. -Biological Trace Element Research 154(2): 217-225.

Franzen, S., Lunestad, B. T., Måge, A., Nilsen, B. and Julshamn, K. 2008: Tilsynsprogrammet for skjell 2007 - fremmedstoffer (tungmetaller og organiske miljøgifter i skjell og tungmetaller i krabbe) og mikroorganismer. -Yearly report to the Norwegian Food Safety Authority

- Goldberg, E. D. 1975: The mussel watch - A first step in global marine monitoring. -Marine Pollution Bulletin 6.
- Goldberg, E. D., Bowen, V. T., Farrington, J. W., Harvey, G., Martin, J. H., Parker, P. L., Risebrough, R. W., Robertson, W., Schneider, E. and Gamble, E. 1978: Mussel watch. - Environmental Conservation 5(2): 101-125.
- Golding, L.A., Angel, B.A., Batley, G.E., Apte, S.C., Krassoi, R., Doyle, C.J. 2015: Derivation of a water quality guideline for aluminium in marine waters. Environmental Toxicology and Chemistry 34: 141-151.
- Goodrich, M.S., Dulak, L.H., Friedman, M.A., and Lech, J.J. 1991: Acute and long-term toxicity of water soluble cationic polymers to rainbow trout (*Oncorhynchus mykiss*) and the modification of toxicity by humic acid. Environ. Toxicol. Chem. 10: 509-515.
- Guide 02:2013. Classification of environmental station in water. Ecological and chemical classification system for coastalwater, groundwater, lakes and rivers, 263 pp.
- Henriksen, A. and Wright, R. F. 1978: Concentrations of heavy metals in small Norwegian lakes. -Water Research 12(2): 101-112.
- Hindar, A. 2011: Highway E18 Grimstad-Kristiansand; effects and quantification of acid runoff from deposits of sulphide-bearing rock. -Norsk institutt for vannforskning,
- Hindar, A., Iversen, E. and Hindar, A. P. M. 2006: Utsprengning i sulfidholdig berggrunn på Storemyr i Lillesand - effekter på vannmiljø og forslag til tiltak. -Norsk institutt for vannforskning,
- Hindar, A. and Lydersen, E. 1994: Extreme acidification of a lake in southern Norway caused by weathering of sulphide-containing bedrock. -An International Journal of Environmental Pollution 77(1): 17-25.
- Hindar, A. and Nordstrom, D. K. 2014: Effects and quantification of acid runoff from sulphide-bearing rock deposited during construction of Highway E18, Norway. -Applied Geochemistry 0):
- Hoher, N., Kohler, A., Strand, J. and Broeg, K. 2012: Effects of various pollutant mixtures on immune responses of the blue mussel (*Mytilus edulis*) collected at a salinity gradient in Danish coastal waters. -Marine Environmental Research 75: 35-44.
- Hågvar, E. B. 2010: Det zoologiske mangfoldet. -Universitetsforlaget AS, 384:129-131

Johansson, K., Andersson, A. and Andersson, T. 1995: Regional accumulation pattern of heavy metals in lake sediments and forest soils in Sweden. -*Science of the Total Environment* 160: 373-380.

Johnsen, G. H., Eilertsen, M. and Haugsøen, H. 2013: Resipientundersøkelse av Kaldvellfjorden, Lillesand kommune. Virkning av avrenning fra deponier med sulfidholdig stein. -Rådgivende Biologer AS,

Kaste, Ø., Hindar, A., Frigstad, O. F. and Kaste, Ø. P. M. 1995: Undersøkelser av avrenning fra sulfidholdige bergarter rundt Travparken/Sørlandshallen i Kristiansand kommune. -Norsk institutt for vannforskning,

Kroglund, T., Molvær, J., Moy, F. and Hindar, A. P. M. 2007: Kjemisk og biologisk tilstand i fjorder før bygging av ny E18 Grimstad-Kristiansand. -Norsk institutt for vannforskning,

Levinton, J. S. 1995: *Marine Biology: Function, Biodiversity, Ecology*. -Oxford University Press.

Lydersen, E., Salbu, B., Poléo, A.B.S., and Muniz, I.P. 1990: The influences of temperature on aqueous aluminium chemistry. *Water Air Soil Pollut.* 51: 203-215.

Lydersen, E., Salbu, B., Poléo, A.B.S. 1991: Formation and dissolution kinetics of $\text{Al}(\text{OH})_3$ (s) in synthetic freshwater solutions. *Water Resour. Res.* 27: 351-357.

Lydersen, E. 1992. Aluminium in dilute acidic freshwaters. Chemical, analytical and biological relevance. PhD thesis, University of Oslo, 132 pp + Appendix.

Lydersen, E., Poléo, A.B.S., Nandrup Pettersen, M., Riise, G., Salbu, B., Kroglund, F., and Rosseland, B.O. 1994: The importance of "in situ" measurements to relate toxicity and chemistry in dynamic aluminium freshwater systems. *J. Ecol. Chem.* 3: 357-265.

Lydersen, E., Lofgren, S. and Arnesen, R. 2002: Metals in Scandinavian surface waters: Effects of acidification, liming, and potential reacidification. 73-295

Mandal, B. K. and Suzuki, K. T. 2002: Arsenic round the world: a review. -*Talanta* 58(1): 201-235.

Manley, A. R. and Davenport, J. 1979: Behavioural responses of some marine bivalves to heightened seawater copper concentrations. -*Bull. Environ. Contam. Toxicol.* 22(6):

Mao, A., Mahaut, M. L., Pineau, S., Barillier, D. and Caplat, C. 2011: Assessment of sacrificial anode impact by aluminum accumulation in mussel *Mytilus edulis*: A large-scale laboratory test. -*Marine Pollution Bulletin* 62(12): 2707-2713.

McEdward, L. 1995: Ecology of marine invertebrate larvae. -CRC Press, Boca Raton,

Moen, F. E. 2004: Marine fish & invertebrates of Northern Europe. -KOM:355

Molvær, J., Knutzen, J., Magnusson, J., Rygg, B., Skei, J. and Sørensen, J. 1997: Klassifisering av miljøkvalitet i fjorder og kystfarvann : veiledning = Classification of environmental quality in fjords and coastal waters : a guide. -Statens forurensningstilsyn, Oslo:36

Nagpal, N. 2004: Technical Report — Water Quality Guidelines for Cobalt. -Ministry of Water Land and Air Protection, Victoria, BC, Canada.

Orescanin, V., Lovrencic, I., Mikelic, L., Barisic, D., Matasin, Z., Lulic, S. and Pezelj, D. 2006: Biomonitoring of heavy metals and arsenic on the east coast of the Middle Adriatic Sea using *Mytilus galloprovincialis*. -*Nuclear Instruments & Methods in Physics Research Section B-Beam Interactions with Materials and Atoms* 245(2): 495-500.

Phillips, D. J. H. 1978: Common mussel *Mytilus-edulis* as an indicator of trace- metals in scandinavian waters. 2. Lead, iron and manganese. -*Marine Biology* 46(2): 147-156.

Phillips, D. J. H. 1980: Quantitative aquatic biological indicators : their use to monitor trace metal and organochlorine pollution. -Applied Science Publishers, London,

Phillips, D. J. H. 1977: The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments—a review. -*Environmental Pollution* (1970) 13(4): 281-317.

Pipe, R. K., Coles, J. A., Carissan, F. M. M. and Ramanathan, K. 1999: Copper induced immunomodulation in the marine mussel, *Mytilus edulis*. -*Aquatic Toxicology* 46(1): 43-54.

Poléo, A.B.S. 1995: Aluminium polymerisation - a mechanism of acute toxicity of aqueous aluminium to fish. *Aquat. Toxicol.* 31: 347-356.

Polèo, A.B.S., Lydersen, E., Rosseland, B.O., Kroglund, F., Salbu, B., Vogt, R., and Kvellestad, A. 1994: Increased mortality of fish due to changing Al-chemistry of mixing zones between limed streams and acid tributaries, *Water, Air, Soil Pollut.* 75: 339-352.

Popham, J. and D'Auria, J. 1983: Combined effect of body size, season, and location on trace element levels in mussels (*Mytilus edulis*). -Archives of Environmental Contamination and Toxicology 12(1): 1-14.

Rainbow, P. S. and Phillips, D. J. H. 1993: Cosmopolitan biomonitors of trace-metals. -Marine Pollution Bulletin 26(11): 593-601.

Reimann, C. and Caritat, P. 1998: Chemical elements in the environment. -Springer 398.

Ritz, D. A., Swain, R. and Elliott, N. G. 1982: Use of the mussel *Mytilus edulis planulatus* (Lamarck) in monitoring heavy-metal levels in sea-water. -Australian Journal of Marine and Freshwater Research 33(3): 491-506.

Rosseland, B.O., Blakar, I., Bulger, A, Kroglund, F., Kvellestad, A., Lydersen, E., Oughton, D.H., Salbu, B., Staurnes, M. and Vogt, R. 1992: The mixing zone between limed and acid river waters: complex aluminium chemistry and extreme toxicity for salmonids. Environmental Pollution: 78, 3-8.

Schulz-Baldes, M. 1974: Lead uptake from sea water and food, and lead loss in the common mussel *Mytilus edulis*. -International Journal on Life in Oceans and Coastal Waters 25(3): 177-193.

Schøyen M, Håvardstun J, Øxnevad S, Borgersen G, Høgåsen T, Oug E. Overvåking av miljøgifter i Kristiansandsfjorden i 2012 : undersøkelse av blåskjell, torsk, taskekrabbe, sedimenter og bløtbunnsfauna. Oslo: Norsk institutt for vannforskning; 2012.

Schøyen M, Håvardstun J, Høgåsen T, Hjermann D, Øxnevad S. Overvåking av miljøgifter i Kristiansandsfjorden i 2013. Undersøkelse av blåskjell. Miljøvern avdelingen hos Fylkesmannen i Vest-Agder; 2013.

Scott Hall, W. Mirenda R.J. 1991: Acute toxicity of wastewater treatment polymers to *Daphnia pulex* and the fathead minnow (*Pimephales promelas*) and the effects of humic acid on polymer toxicity. J Water Pollut Contam Fed 63: 895-899.

Seed, R. 1976: Ecology. In Marine mussels; their ecology and physiology. -Ed. Bayne BL. Cambridge University Press, Cambridge. 13- 65 pp.

Seip, H.M., Muller, L., and Naas, A. 1984: Aluminium speciation: Comparison of two spectrophotometric analytical methods and observed concentrations in some acidic aquatic systems in southern Norway. Water Air Soil Pollution 23, 81-95.

Sloth, J. J. and Julshamn, K. 2008: Survey of total and inorganic arsenic content in blue mussels (*Mytilus edulis* L.) from Norwegian fiords: Revelation of unusual high levels of inorganic arsenic. -*Journal of Agricultural and Food Chemistry* 56(4): 1269-1273.

Stewart, A. R. 1999: Accumulation of Cd by a freshwater mussel (*Pyganodon grandis*) is reduced in the presence of Cu, Zn, Pb, and Ni. -*Canadian Journal of Fisheries and Aquatic Sciences* 56(3): 467-478.

Saavedra, Y., Gonzalez, A., Fernandez, P. and Blanco, J. 2004: The effect of size on trace metal levels in raft cultivated mussels (*Mytilus galloprovincialis*). -*Science of the Total Environment* 318(1-3): 115-124.

Teien, H. C., Standring, W. J. F. and Salbu, B. 2006: Mobilization of river transported colloidal aluminium upon mixing with seawater and subsequent deposition in fish gills. -*Science of the Total Environment* 364(1-3): 149-164.

Valko, M., Morris, H. and Cronin, M. 2005: Metals, toxicity, and oxidative stress. -*Curr Med Chem* 12(116-1208).

Veselý, J. and Majer, V. 1996: The effect of pH and atmospheric deposition on concentrations of trace elements in acidified freshwaters: A statistical approach. -*An International Journal of Environmental Pollution* 88(3): 227-246.

Viarengo, A. and Canesi, L. 1991: Mussels as biological indicators of pollution. -*Aquaculture* 94(2-3): 225-243.

Wright, P. and Mason, C. F. 1999: Spatial and seasonal variation in heavy metals in the sediments and biota of two adjacent estuaries, the Orwell and the Stour, in eastern England. -*Science of the Total Environment* 226(2): 139-156.