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Concentrations and speciation of Cu, Zn, Cd, and Al in mine-polluted Norwegian rivers: Influence of main water parameters and consequences to fish

by

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Preface and acknowledgements

This work was carried out at the Department of Chemistry, Norwegian University of Science and Technology (NTNU) in Trondheim and was financed mainly by the Norwegian Research Council. Samples were collected at mine polluted sites in the Røros area, Central Norway. Part of the present thesis forms the water chemistry contribution to a study related to the adaption of freshwater fish to heavy metal pollution. The major findings of that project were published as a thesis in November 2000, with the title “Biochemical impacts of Cd, Cu, and Zn on brown trout (Salmo trutta) in two mining-contaminated rivers in Central Norway”. I thank the author of that thesis, Pål Olsvik for good cooperation and a nice companionship during fieldwork and discussions. I would also like to thank Karl Erik Zachariassen for initiating and leading this research project, and the associated members Bjørn Olav Rosseland, Brit Salbu and Rolf A. Andersen for their contributions. I am also grateful to Trond Peder Flaten for very useful corrections and Magnar Lillegård for statistical advice.

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Paper 1: Gundersen P., Olsvik P. A. and Steinnes E. (2001) Variations in heavy metal concentrations and speciation in two mining polluted streams in central Norway. *Environmental Toxicology and Chemistry* **20**, 5.

Paper 2: Olsvik P. A., Gundersen P., Andersen R. A. and Zachariassen K. E. (2000) Metal accumulation and metallothionein in two populations of brown trout, Salmo trutta, exposed to different natural water environments during a run-off episode. *Aquatic Toxicology* **50**, 301-316.

Paper 3: Gundersen P. and Steinnes E. (2001) Influence of Temporal Variations in River Discharge, pH, Alkalinity, and Ca on the Speciation and Concentration of Heavy Metals in Some Mining Polluted Rivers. *Aquatic Geochemistry* **7**, 173-193.

Paper 4: Gundersen, P. Steinnes, E., Influence of pH and TOC concentration on Cu, Zn, Cd, and Al speciation in rivers. *Water Research* (in press).\

Summary

This thesis presents concentration and speciation data for Cu, Zn, Cd, and Al in eight highland rivers and streams in the Røros area, central Norway. About 16 sampling campaigns were performed before, during and after floods during spring and autumn of 1997. Due to rain- and snowmelt-induced flushout from weathered mine tailings, the flood episodes were expected to produce high concentrations of metals in the local rivers. The examined river sites represent highly different degrees of pollution, height above sea level, annual discharge, pH, etc., and the project is aimed towards producing general information about the temporal variations of the metal chemistry and parameters important for the metal chemistry in this and similar regions. Dissolved species of the metals were fractionated by dialysis *in situ*, colloidal species by filtration, and total (more precisely; soluble in 0.1 M HNO_3) concentrations were determined directly after acidification. Ca concentration, pH, river discharge, water temperature, and to a lesser extent precipitation and TOC were also monitored. In addition Cu and Zn/Cd metallothioneins were studied in kidney, liver and gills in trout (salmo trutta) populations in two of the rivers characterized by completely different metal concentration fingerprints.

The results showed that Cu and Al, and possibly Zn and Cd as well, were practically completely in particulate or colloidal form at pH values of 7 and above. At pH levels one or a few pH units lower, the trace metals shifted to occur almost completely dissolved. The pH range at which the change from colloidal/particulate to dissolved species occurred, depended on the metal concerned and the TOC in the water. High TOC concentrations (> 8) seemed to accompany low fractions of dissolved metals, probably because the metals adsorbed on high molecular weight organic compounds or organic coatings on inorganic particles. At TOC concentrations lower than 8 mg/L, a 50 % dissolved fraction was estimated at pH ~7.2 and ~5.8 for Cu and Al respectively, whereas for Cd and Zn, a 50 % dissolved fraction was estimated at pH 7.7. The latter is a pH slightly higher than the highest value observed in the present investigation. Higher TOC concentrations (>8 mg/L) increased metal adsorption and made adsorption start up to one pH unit lower than in low TOC waters (<8 mg/L).\

Total metal concentrations were generally elevated during flood conditions in the pH neutral rivers, whereas pH was significantly lowered. In spite of the low pH, the dissolved fractions of Zn, Cd, and Al decreased during flood periods, probably due to enhanced particle concentrations. Thus flood conditions apparently brought metals into a less acute toxic state. However pH may have influenced metal toxicity in other ways as well; e.g. the free metal ion activity *in* the dissolved fraction could have increased during flood due to the decreased pH. But even if that was the case, metal toxicity would not necessarily be higher since H^+ competes with free metal ions for uptake sites on biological membranes.

Alkalinity and Ca reduce negative effects of metals, and both were low during flood conditions. This is obviously unfortunate for aquatic organisms. Generally however, total metal concentration peaks occurred at the beginning of rising floods, followed by a very low pH, alkalinity, and Ca concentration a few weeks later and the spring discharge maximum a few weeks after that. Thus Ca^{2+} and pH had not yet reached their spring minimum, that is; the most unfavorable condition to protect organisms against metals, at the metal concentration maximum.

The snowcap covering River Orva accumulated and contained huge amounts of Cu, and may have substantially increased the Cu concentration in the river during snowmelt. This also impacts reaches of the large river Glåma which receives water from Orva. It is suggested that the hydroelectric power plant Kuråsfossen in Glåma should regulate river runoff in a different manner in order to smooth out metal concentration peaks.

Gill concentrations of Cu metallothionein (MT) in Rugla and Cd/Zn MT in Naustebekken were appreciably elevated during run-off episodes. The Cu MT and Cd/Zn MT concentrations in gills and kidneys were high enough to account for all or almost all Cu and Cd but only for a minor fraction of the Zn present in these organs. For Zn this indicates that other detoxifying mechanisms may be more important than MT.\

Introduction

The objective of this thesis was to examine the composition of Cu, Zn, Cd, and Al species in mine-polluted rivers and lakes. The dissolved, colloidal, and particulate species were fractionated by the dialysis *in situ* method (Beneš and Steinnes, 1974). Special emphasis has been paid on how the percent dissolved metal fraction changed during changes in river discharge, pH, total organic carbon concentration, alkalinity, and Ca concentration, generally and in individual rivers. Metal and main water chemistry patterns were investigated, e.g. to make results from monitoring studies easier to interpret and expected hazards to aquatic life more precisely predictable. The section "Suggested applications of…" in the Results and discussion chapter exemplifies how results from the present work may be used.

Water organisms may be influenced by metal concentrations varying temporally on a seasonal or diurnal scale, or due to weather related variations in temperature and rainfall. Due to genetic diversity, some individuals from a population may die and some survive during intermediate to highly toxic episodes. Populations may thus adapt rather rapidly to chemical pollution that is not extensive enough to erase the whole population. On a longer time scale, populations may also adapt to lower metal concentrations that do not kill fish directly. Such adaptations however are less likely in the present study area during the, evolutionary speaking, short time since mining pollution became a problem. Two of the rivers have been thoroughly examined in order to interpret to what extent genetic adaptations to metal pollution may have occurred, and if so, whether such adaptations could be metal specific and connected to one of the major interior metal defense mechanisms in fish; the methallotheioneins (MT). MTs are proteins that attach to and detoxify metals inside body tissue. To study metal specific adaptation in trout, we searched for two different aquatic habitats; one high in Cu, low in Cd and Zn, the other with an opposite composition. Other criteria were (1) that the metal concentrations should be high enough to wipe out parts of the trout populations during episodes, (2) that the rivers would have to be available for monitoring most of the year, and (3) that the fish stocks should not be able to rescue in attached clean creeks during critical episodes. Parameters such as pH, Ca concentration, accessibility from road, distance from other considered sites, river discharge, lakes in the stream path and numerous practical features were also considered. No ideal habitats were found in Norway for this experiment, but two streams located a few km from each other near the town of Røros in central Norway\

constituted the closest match to the criteria. In Paper 1 the water chemistry of these two streams is discussed in detail. Their fish populations and the influence of metals on them are discussed in Paper 2, and in two other studies that are not part of the present thesis (Olsvik *et al.*, 2001a-b). Six additional rivers were studied in Paper 3 to reveal how parameters affecting toxic stress from metals covary and correlate during changing river flow. Results from the same eight rivers were also analyzed to reveal how TOC and pH influence the partition of the metals between the dissolved, the colloidal and the particulate fraction (Paper 4).

Mining

Man has extracted Cu from rocks for 6-7000 years and Fe for about 4000 years. During the last centuries, however, mining has been much more extensive throughout the world and has caused major impacts on nature. Water, air, and soil pollution have resulted from extraction, smelting and waste deposition from the mining activity. Coal combustion and the use, processing and deposition of refined products have caused further, yet indirect, damage on the environment. Mineral extraction and processing activities prior to recent decades have left a legacy of acid drainage from waste rock and ore bodies, mercury pollution from amalgam processing of precious metals, other heavy metals pollution from residual minerals in mill tailings, and, in some instances, cyanide toxicities from early gold leaching operations (Moore, 1997). Accumulation of heavy metals and resulting physiological impacts have been reported for a variety of fish species and other aquatic organisms from mining and smelting areas (Bradley and Morris, 1986; Hillis and Parker, 1993; Moore *et al.*, 1991; Somers and Harvey, 1984). Mining operations may also cause an increased potential for erosion that can increase sediment loading to surface waters. Such eroded minerals may be abrasive and can kill aquatic organisms directly or their spawning areas or prey may be influenced (Johnson, 1997b).

A large number of abandoned mining sites exist throughout Norway. From 1941 to 1997 the activity of the nine most important Norwegian mining sites ceased (Dahl, 2001) and today only two metal producing mines are still operated (Carstens, 2000). Activity at the last sulfide ore mine in Norway, Nikkel Olivin A/S in Ballangen ceased in 2001 due to lacking reserves and low Ni richness in the deposits. According to Foslie (1926), mining activity in Norway started in the middle of the 17th century. At that time, only Cu was extracted from the ores. Two hundred years later sulfur also became extruded, and Norway became a major exporting\

nation of mining products during the 18th and 19th century. Cu has been the major product, but also Ni, Mo, Ti, Ag, Zn, and Fe/pyrite have been produced in quantities of national-economic importance (Foslie, 1925). By 1926 the mines in the Røros district had contributed with about 1/3 of the total Cu production in Norway (Foslie, 1926). All mines in the area are now abandoned, but due to new technology and rising prices, Zn deposits are being considered for extrusion.

During the last two decades great effort has been made to reduce acid mine drainage from Norwegian mining areas. From 1985 to 1996 the total amount of Cu and Zn released from the nine largest mining areas was reduced by 75 and 60 % respectively (Dahl, 2001), but there are some new problems arising. Mining shafts, e.g. at the Bjørgåsen site which is investigated in the present work, may some time in the future be filled to the edge with acidic and metal polluted water; endangering downstream trout and salmon populated stretches of Gaula river.

Most of the extrudable ore deposits in Norway are located along the Caledonian Mountain Ridge, stretching from Stavanger in the SW of Norway in a NNE direction along the whole country (Foslie, 1925). Metal sulfide ore deposits are usually very long, moderately broad, and rather shallow. The huge mine “Kongensgruva”, which is located in the drainage area to Orva is a typical example, covering an area of 3000 x ~75 meters and with a depth of 1 to 4 meters (Foslie, 1926).

Mining pollution

Low pH and high heavy metal concentrations characterize mining influenced waters. Precipitation of Fe and Al hydroxides ("yellow-boy") are common in the watersheds, as well as high concentrations of colloids, particles, alkali metals, alkaline-earth metals, and high and conservatively mixed sulfate concentrations (Schmiermund, 1997).

Acid mine drainage is produced by the exposure of pyrite (FeS_2) and other sulfide minerals to air and water. The resulting sulfur oxidation and production of acidity entails elevated concentrations of iron, sulfate and other metals in drainage waters (Sengupta, 1993). Waters where considerable acidity is caused naturally, by acidic rocks, are very rare (Schmiermund, 1997), probably since sulfidic ores generally covers small areas and are not crushed to small\

stones/gravel in the way mining tailings are. Acid mine drainage may originate from underground or open pit workings, as leachate from waste rock dumps, mill tailings, ore stockings, and smelting residuals, or from lake deposits made for reducing oxygen access to the metal containing materials. The most acidic waters known are mine waters found underground in the Richmond Mine at Iron Mountain in California, USA. Total dissolved metal concentrations as high as 200 g/L, pH values as low as -3.6 (using the Pitzer method for pH calibration), and sulfate concentrations as high as 760 g/L were encountered (Nordstrom *et al.*, 2000). There is a strong negative correlation between pH and heavy metal concentrations in waters influenced by mining activity, however some waters draining mining areas may reach pH levels ~8, and still be influenced by metal pollution (Johnson, 1997a).

The reactions of acid and metal sulfides may be illustrated by the oxidation of pyrite. Pyrite reacts with oxygen and water to produce ferrous iron, sulfate, and hydrogen ions (Johnson, 1997a);

$$FeS_2 + {}^{7}/{}_{2}\,O_2 + H_2O = Fe^{2+} + 2SO_4^{2-} + 2H_2O + 2H^+ \qquad \text{(Eq. 1)}$$

Some of the released ferrous iron (Fe^{2+}) will be oxidized by oxygen to ferric iron (Fe^{3+}). Ferric iron may precipitate as iron hydroxide ($Fe(OH)_3$), producing acid, or being a catalyst for further oxidation of pyrite. Either way, due to intermediate rate determining reactions and very effective bacterial catalysis, the production of acid mine drainage is a rapid and self-perpetuating process, which continues as long as air, water, and pyrite are available. Similar and more or less simultaneous reactions will also release high amounts of metals such as Cu, Co, As, Ni, Mo, and Sb to aquatic environments for decades or even centuries after the operation has ceased. The high acidity in mine effluents will often release other adjacent acid consuming minerals such as hydroxides of Al and Mn, increasing the concentration of these metals as well in downstream water systems (Johnson, 1997a; Schmiermund, 1997). Acid mine drainage may also dissolve carbonate minerals such as calcite ($CaCO_3$), increasing the concentrations of Ca^{2+} in the water or producing gypsum ($CaSO_4$) precipitation.\

The production of acid mine drainage is occasionally reduced by covering or surrounding mining residuals with membranes and gravel. Such remediations prohibit rain and air to access, and water to flush through the residuals. Some residuals are instead covered completely with water in ponds to reduce oxygen access. Acid mine drainage may be treated by liming ($Ca(OH)_2$ or CaO) or other chemicals which rise the pH, followed by oxygenation or other oxidants. These procedures cause neutralization of pH, and, subsequently, precipitation of metal contaminants (Kleinmann, 1997).

Other factors influencing metal concentrations in rivers and lakes

Precipitation chemistry

Trace substances, both gases and particles, are removed from the atmosphere by precipitation (wet deposition), cloud or fog impaction (cloud deposition), or by impaction onto surfaces in the absence if precipitation or clouds (dry deposition). For many metals (e.g. Zn and Cd), the atmosphere is a significant transport medium to the biosphere (Ross and Vermette, 1995). Al on the other hand is a major constituent of many commonly occurring minerals, and although this element may be considerably enriched in rainwater close to e.g. smelters (Reimann *et al.*, 1997), riverborne Al will generally almost exclusively originate from the watershed. Al leaching to surface waters may still be strongly influenced by anthropogenic influence through acid pollution. A huge number of fish populations in the southern part of Norway have become extinct due to the combination of acid rain and Al leaching (Dalziel *et al.*, 1995; Staurnes *et al.*, 1995). The present study area however is not much influenced by long range atmospheric transport of pollutants (Steinnes *et al.*, 1992) and it is unlikely that any considerable part of the acidity, Cu, Zn, or Cd observed in these mine-polluted rivers originates from this source. Previous local mining and smelting activity in the area may however have caused some airborne transport of metals to the watersheds.

Mechanisms in the watershed

Topographic, geological, and biological conditions may vary highly within, as well as between, watersheds: a) the landscape may be flat or steep, b) rocks may contain different combinations of minerals. Moraine and soil material may be c) deep or shallow, d) porous or more impermeable and e) consist of numerous combinations of grain sizes. Finally, f) trees and other vegetation may vary, which is important for the water chemistry since plants withdraw a lot of water from the ground and absorb/release compounds from/to the water. The solid states the water contacts along its underground path, and the retention time in each of the zones it passes, will also vary highly; temporally as well as from site to site. These variations will strongly influence the composition of the water that finally reaches lower altitude river systems.

Once introduced into a watershed as rain, water may flow towards the sea along the surface (surface water), below the surface in the unsaturated zone (subsurface water) or in the saturated zone (groundwater). All waters at any point in or on the ground will shortly after precipitation begin to mix with other waters with different composition. Thus, water at any point along its pathway is generally not in complete equilibrium with the various geological phases it is surrounded by (Allard, 1995). Water slowly flowing through easily weathered materials with large surfaces (fine-grained) will generally contain the highest amounts of material from the solid states it passes.

Weathering kinetics and content of metals in the different minerals and rocks may vary considerably, and are important factors determining the flux of metals from the watershed to the river systems. Shale formed from clay minerals is e.g. occasionally enriched in Cd and As, whereas this rock normally has moderate to low concentrations of Cu, Cr, and Ni. Secondary mineral formation and adsorption to other solid states may as well alter the water chemistry considerably after the metal containing solids are dissolved.

The snowpack may have a crucial impact on the water chemistry in arctic and subarctic regions. Pollution compounds may be covered and restrained under snow during winter (e.g. mining pollution) or stored within the snowpack (e.g., atmospheric metal pollution and acid precipitation). Different elements may respond differently to snowpack melting during spring flood (Caritat *et al.*, 1996): Some elements/parameters show a short-lived sharp peak during spring flood, which is indicative of a lower value in summer and winter baseflow than in the meltwater. This pattern is frequently observed for metal contaminants or acidification products. Some parameters may be highest during winter and summer baseflow (e.g. Ca and alkalinity) because of dilution by lower concentrations in meltwaters. Other elements/parameters show a peak in the beginning of the snowmelt, followed by a dilution\

through the flood peak. This pattern is abundant for elements that mobilize from snow before the melting is completed, and is occasionally observed for major elements.

In a river or a lake, water masses from different chemical environments in a watershed will mix. New equilibria will result, and the water composition may be oversaturated with respect to some minerals. Due to this fact and to the complex physico-chemical and biological mechanisms discussed above, the watershed influence on the water chemistry in lakes and streams is poorly predictable. The watershed characteristics in the study area will however be briefly discussed since they may indicate to what extent the conclusions drawn may apply in similar regions as well.

Interactions with bottom sediments

The chemical composition in rivers and lakes will influence and be influenced by bottom sediments. In lakes and slowly flowing stretches of rivers, sedimentation will remove particles and colloids supplied by groundwater or incoming rivers. Such old sedimentation deposits may later on be excavated along the outer curves of rivers, or, during flood conditions, along the whole riverbed. At flood conditions, particles will be transported in rivers to and from the river banks, and into the river from acres and soils in the watershed. Considerable amounts of heavy metals transported to and in rivers by these mechanisms may be found in the particulate fraction (Bradley, 1988). The coarser the particles, the lower their ability to stay in the water masses. Particles containing metals may therefore fall down and be released several times from their origin to more lasting storages in slow-flowing stretches of the rivers or on the ocean floor. Especially during flood conditions, huge amounts of particles may be released from streambeds to the aquatic phase (Bradley, 1988). By two different mechanisms this may lead to elevated particulate fractions of the metals: a) The resuspended particles contain metals. b) dissolved species may adsorb to the particle surface. Other chemical mechanisms however as well as adsorption will influence on the water chemistry during changing water discharges. Dissolved metal species may e.g. be removed from the water by precipitation/coprecipitation on particles and changing concentrations of ligands- and counterions.

Metal concentrations may be several orders of magnitude higher in interstitial waters compared to overlying waters (Hong *et al.*, 1995), and dissolved species may be released from lake as well as river deposits through diffusion from sediment interstitial waters. In some marine systems as much as 80-90 % of the metals deposited on the bottom may be released by upward diffusion (Gobeil *et al.*, 1987; Heggie, 1983). Organic substances are reported to be important in binding metals such as Cu, Pb, and Cd to the sediment particles (Schintu *et al.*, 1991).

In addition to those discussed above, other mechanisms may contribute to the release of particulate or dissolved metal species from earlier sediment deposits; biological activity, changing flow paths, temperatures, or light, seasonal, diurnal or more casual, e.g. rain induced, variations in the general water chemistry (Kimball *et al.*, 1992; Wielinga *et al.*, 1999). In rivers covered by ice during the winter, it is also reported that spring ice-jam episodes may mobilize large amounts of metal containing, fine grained sediments (Moore and Landrigan, 1999). Hart and Hines (1995) suggest that Cu, Zn, and Cd chemistry in aquatic systems is probably more likely to be controlled by physico-chemical than by biological processes.

Factors influencing metal speciation in natural waters

Metal speciation and toxicity in natural waters are influenced by many variables. Some variables may influence the distribution of metal species available for uptake, some may influence biota uptake channels, and some may influence to what exctent organisms may deal with accumulated metals. Some, such as pH, may at the same time exert both beneficial and harmful effects on organisms exposed to metal pollution. Significant variables such as organic compounds, pH, particles, hardness, and alkalinity; their temporal trends and how they influence metal speciation and toxicity, will be briefly discussed in the next sections.

River discharge, particles, and temporal trends

As discussed above, the elevated water velocity in a river during flood will cause resuspension of previously deposited particles and lead to higher concentrations of suspended particles. Maximum sediment concentrations occasionally precede the river discharge peak, as observed by Miller and Piest (1970). Coinciding sediment concentrations and river discharge peaks have however also been observed, as well as maximum sediment loads at flood ebb (Bradley, 1982). Temporal variability in grain size distribution may also differ between rivers. Suspended particles may become coarser, finer grained or unaffected by floods (Walling and Moorehead, 1989).

Main water chemistry constituents such as pH and Ca concentration may also respond differently to discharge variations in different rivers. Flood released particles may decrease the percent fraction of dissolved metals by two mechanisms: a) low metal content particles because they can offer binding sites to dissolved metals b) high metal content particles because they are directly introduced to the water column.

Due to all these possible variations, and because the water chemistry as well as the particulate quantity and quality will influence the metal chemistry in the water, the metal speciation in each river is unique, and so is each and every flood incident within it. Some general features about metal fractionation and variability in natural fresh waters are still evident. By investigating temporal variations in eight rivers with very different chemical fingerprints, an attempt is made in this work to reveal general trends concerning the covariation of parameters influencing metal chemistry, and probably toxicity, during a year.

pH

Groundwater reaching the rivers usually contributes to high alkalinity. During flood episodes, the groundwater fraction is usually low compared to surface runoff (Stumm and Morgan, 1996), leading to a pH drop (Bergström and Lindström, 1987). Such a pH drop may be additionally decreased in mining areas where rainwater flushes through oxidized metal sulfides. Decreased pH may as well lead to the release of adsorbed metals from colloids and particles. In fact, for most metals, adsorption changes from almost none at low pH to almost 100 % a few pH units higher (Allard *et al.*, 1986; Mouvet and Bourg, 1983; Schindler *et al.*, 1976; Stumm and Morgan, 1996).

The size distribution of metals may also be influenced indirectly by pH; the H^+ ion competes with metals for ligands such as OH^-, Cl^-, CO_3^{2-}, HCO_3^-, HS^-, S^{2-}, sulfate, and phosphate, and metal complexation will change adsorption abilities as well as bioavailability of the metals. In most cases, pH is the most important variable influencing metal speciation (Stumm and Morgan, 1996).

Organic compounds

Organic compounds may be found in considerable amounts both in the dissolved, colloidal, and particulate fraction (Patel *et al.*, 1999; Tanizaki *et al.*, 1985; Thorsen, 2000), and the metals may be attached to either of them. The organic content of water may, depending on pH, influence significantly the size distribution of all metals investigated. Generally humic substances tend to enhance metal cation sorption on particles at low pH and reduce metal cation sorption at high pH, though many exceptions are reported (Zuyi *et al.*, 2000). Other chemical parameters will probably also influence metal attachment to organic material; during mixing of fresh and salty waters, the partition of metal species between solution and suspended particles is controlled by two counteracting, non-biological processes: a. Removal from the dissolved fraction by precipitation/coprecipitation mechanisms or flocculation of humic and fulvic acid – metal complexes (Hoyle *et al.*, 1984; Sholkovitz, 1976). b. Gain to the dissolved fraction by desorption mechanisms (Li *et al.*, 1984; Van der Weijden *et al.*, 1977). It is possible that the ionic strength variation which may be observed between rivers and temporally within rivers may cause some similar influences on the bounding of metals to organic compounds in fresh waters.

Hardness and alkalinity

Water hardness is the sum of [Ca] and [Mg], predominantly existing as free Ca^{2+} and Mg^{2+} ions in most freshwaters (Sigg and Xue, 1994). Ca^{2+} and Mg^{2+}, as well as most other major cations in water, generally form less stable organic complexes than trace metals (e.g. Cu(II) and Zn(II)). Occasionally however, due to their much higher concentrations, Ca^{2+} and Mg^{2+} may occupy much more of the organic ligand sites than the trace metals they compete with (Stumm and Morgan, 1996). The major metal ions such as Ca^{2+} may therefore make metal ions more bioavailable through ion exchange/desorption reactions. Despite the fact that water hardness or $[Ca^{2+}]$ may increase the concentration of dissolved or free metal ion species, high Ca concentrations are found beneficial for most organisms in waters affected by metal pollution. A suggested mechanism for this has been that Ca^{2+} competes with metal ions for uptake sites on cell membranes (Campbell and Stokes, 1985; Pagenkopf, 1983).

Considerable amounts of HCO_3^- and CO_3^{2-} is present in neutral and alkaline waters. Complexes with these ligands dominate speciation of many metals under certain conditions (Stumm and Morgan, 1996). Carbonato and bicarbonato metal species are generally less bioavailable than metal hydroxo complexes and free metal ions (Luoma, 1983). This is one of the reasons why high alkalinity as well as high hardness is considered favorable in metal polluted rivers and lakes. Hardness or alkalinity are even accounted for in some water quality guidelines (e.g. United States Environmental Protection Agency, 1998).

The aqueous concentrations of Ca^{2+}, H^+, OH^-, HCO_3^- and CO_2 may also influence the surface and adsorption properties of mineral particles, e.g. the surface charge of calcium carbonate (Stumm and Morgan, 1996; Thompson and Pownall, 1989).

Other parameters influencing metal speciation

Some additional parameters should be mentioned which affect water speciation. Light conditions have proved to be important for iron chemistry (Brick and Moore, 1996; Kimball *et al.*, 1992; Sullivan *et al.*, 1998) whereas water temperature may influence the metal speciation of many metals to some extent (Byrne *et al.*, 1988; Hawke and Hunter, 1992).

Since natural water masses continuously mix with each other, they are generally not in complete equilibrium according to all possible reactions that may take place within the water, or with the atmosphere or bottom sediments. Kinetics may thus be important for many water chemistry processes. Complexation reactions usually proceed towards equilibrium within a second or less (Buykx *et al.*, 1999), whereas adsorption and especially desorption reactions may be rather slow (Stumm and Morgan, 1996).

Biota may affect metal speciation as well as concentrations. In addition to "conventional" uptake and excretion of metals between the aquatic phase and the organsms living within it, it has been suggested that some organisms produce and excrete organic ligands into the bulk water. In this manner they regulate their own metal uptake through controlling the bulk water chemistry they live in (Xue and Sigg, 1993).

Metal toxicity

Metal speciation and biovailability

Usually metal pollution in rivers and lakes is monitored by analyzing samples for total concentrations or filtered <0.45 µm fractions. However only a small fraction of the measured quantities may penetrate biological membranes. Truly dissolved metal species are generally considered more readily bioabsorbed than metals attached to high molecular weight material

(Batley, 1983), and probably dissolved species contribute most of the toxicity experienced by aquatic organisms during acute metal exposure episodes. Several investigations have concluded that the most readily bioavailable and toxic metal species is the free metal ion species (e.g. Cu^{2+}) (e.g. Campbell, 1995; Florence, 1983). Thus the free metal ion activity is considered a good approach to the toxicity of a certain metal. This is called the Free Metal Activity Model (FIAM). The FIAM theory has been widely used in toxicity considerations (e.g., Vercauteren and Blust, 1996; Brown and Markich, 2000; Sunda and Hanson, 1976). Some studies stresses exceptions and difficulties with FIAM, such as complexing of metals with dissolved organic matter (Campbell, 1995; Pagenkopf, 1983; eij *et al.*, 1992; Campbell *et al.*, 2000). Luoma (1983) however suggests that hydroxo complexes as well as free metal ions are relatively bioavailable whereas carbonato and bicarbonato complexes are bioavailable only to a minor degree. Several other investigations discuss the bioavailability of dissolved metal species and in particular free metal ions (Brown and Markich, 2000; Cope *et al.*, 1994; Mason and Jenkins, 1995; Stumm and Morgan, 1996; Sunda and Hanson, 1976; Vercauteren and Blust, 1996). Generally they conclude that these metal species are more readily accumulated in fish than particulate metal species.

Determination of the free metal ion activity in fresh water is difficult, thus in the present work the dissolved fraction as obtained by dialysis in situ was measured. This is obviously a less accurate estimate of the readily bioavailable fraction than the free metal ion, still it is far better than the total metal concentration. Small inorganic species (e.g. hydroxo, carbonato and bicarbonato complexes) and some organic complexes (e.g. chelates with fulvic acids), due to their size, will be included in the dissolved fraction (Steinnes, 1983; Stumm and Morgan, 1981). The work has been focused on bioavailable metal species and how they vary and covary with other factors influencing on metal toxicity. The role of pH and organic matter in controlling the metal speciation is also emphasized.

Metal uptake, accumulation and biological response

Huge differences in metal tolerance and uptake between biological species are frequently observed (e.g., Williams *et al.*, 1991; Masnado *et al.*, 1995). This indicates that uptake/excretion mechanisms specific for each biological species are important for toxicity responses and accumulation in water organisms. Metals may enter the bodies of fish as well as most water organisms through gills, the integument, or the intestine, and accumulate and influence differently on different organs.

Generally gills are probably the most vulnerable organ in fish towards acute exposures (McDonald and Wood, 1993), whereas liver and kidney are more likely to be damaged during prolonged dietary or waterborne exposures (Spry and Wiener, 1991). Uptake through the gastrointestinal tract can be important for some aquatic organisms. The uptake of Cu and Cd via the gastrointestinal tract has been shown to be considerable in trout from metal spiked feed, and identifiable as such through elevated metal levels in the guts compared to the gills (Handy, 1992). Food uptake may be increasingly important during prolonged exposures and during the winter season when lakes are covered by ice. Köck *et al.* (1998) e.g. reported that Pb uptake in arctic char was significant in lakes during the ice-covered season. Especially for Cd, body burdens are reported to increase after exposure to Cd-loaded food (Kumada *et al.*, 1980).

Numerous passive and active uptake channels are available on cell surfaces (Simkiss and Taylor, 1995). Generally uptake through gills is expected to be most significant in fish, especially during acute exposures (McDonald, Reader and Dalziel, 1989; Wood, 1992) and summer season (Köck *et al.*, 1998)(Pb). The fish skin is probably rather impermeable to most harmful metals in water (Dallinger *et al.*, 1987).

The route of uptake may influence the final tissue distribution of metals. In salmonids e.g., dietary cadmium is retained principally in the gut, kidney, and liver, whereas exposure to dissolved cadmium mainly results in gill and kidney accumulation (Segner, 1987). The process of uptake of a metal into a cell is generally considered to be a two-step process: 1. Initial surface binding onto specific transport sites on the cell wall. 2. Transport across the cell membrane.

Organisms have evolved a number of mechanisms to prevent or limit excessive uptake at these binding or transport stages (Mason and Jenkins, 1995). But also after biouptake, different strategies are prevailed to withstand toxic stress. To acclimate to metal pollution, fish may e.g. use metal binding proteins to bind and detoxifying intracellular metals (Stone and Overnell

1985; Cope et al., 1994). Metallothioneins (MTs) are one group of low molecular weight proteins with a high ability to sequester excessive heavy metals such as Cu, Zn, Cd, and Hg. MTs are assumed to be especially efficient in detoxifying sudden bursts of intracellular metals (Cherian and Cahn 1993), and the production of MT may be induced by metal stress. Tort et al., (1996) e.g. found three times higher levels of liver MT in fish exposed to cadmium as compared to controls.

The last strategy of metal detoxification is excretion, which is reviewed elsewhere (Mason and Jenkins, 1995).

Parameters influencing metal toxicity

Given two water bodies with similar metal regimes, the toxicological response of an organism may still be different. The reason may be that positive metal species such as Me^{2+} and $Me(OH)^+$ must compete with H^+, Ca^{2+}, and Mg^{2+} for metal binding sites on fish gills or other biological surfaces where the metal uptake occurs (Campbell and Stokes, 1985). The effect of the H^+ competition on uptake as well as on toxic responses, was reviewed by Campbell and Stokes (1985): The toxicity of Cu, Zn, and Cd decreased at low pH in most studies on Salmo Gairdneri, as well as many other aquatic organisms. The studies cited were however mostly performed in artificial water *in vitro*. Artificial water normally contains natural levels of main water components such as pH, alkalinity, hardness etc., but cannot account for the complex mixtures of particles, colloids, and organic ligands typical of natural waters. The enhanced toxicity expected at decreasing pH from dissolving metal complexes and desorbing colloidal/particulate metals might therefore have been less than in natural waters. Nevertheless Campbell and Stokes (1985) clearly state that although a low pH incident might lead to a increased dissolved fraction of a metal, it does not necessarily lead to a higher metal toxicity.

Water hardness ($[Ca^{2+}]+[Mg^{2+}]$) and alkalinity are occasionally both quoted as mg $CaCO_3$/L, and in nonpolluted rivers and lakes the two parameters are usually similar, (Davies *et al.*, 1993). In heavily polluted streams however, a low alkalinity may be accompanied by high hardness.

Many studies confirm that hardness and/or alkalinity reduce the toxicity of metals in aquatic environments (Köck *et al.*, 1995; Moore and Ramamoorthy, 1984; Spry and Wiener, 1991) (review), but the mechanisms of the two are different. Whereas Ca and to a minor extent Mg competes for adsorption sites and uptake channels in cell membranes, alkalinity increases complexation of readily adsorbed free metal ions (Köck *et al.*, 1995). Davies *et al.* (1993) propose that antagonistic properties of water hardness reduce toxicity of Cd in acute toxicity tests, but may not reduce toxicity significantly during long-term exposure.

Organic compounds and Ca^{2+} may reduce the toxic response of fish exposed to low pH and/or high Al concentrations (Lien *et al.*, 1996).

Aims and hypothesis of the present study

The main purposes of this thesis were: 1) to reveal how the organic content and pH together influence the dissolved/particulate metal concentration ratio in rivers. 2) To estimate the dissolved, colloidal, and particulate fraction of Cu, Zn, Cd, and Al in natural waters. 3) To study metal speciation differences between sites with different pH, TOC, alkalinity, and Ca concentrations. 4) To reveal temporal covariation patterns concerning metal concentrations, metal speciation, and other parameters influencing metal toxicity. 5) To study metallothionein concentrations and organ distribution in fish during a river runoff episode. 6) To reveal to what extent metallothioneins are capable of detoxifying significant amounts Cu, Zn, and Cd accumulated in trout tissues.

Study area

The study aimed at providing water chemistry data to biochemical studies on fish, as well as studying the metal chemistry itself. Therefore many criteria had to be considered while choosing the study area. For the cross exposure work published in Olsvik *et al.* (2000) (Paper 2) and Olsvik *et al.* (2001a-b), two trout populated rivers with a completely different metal chemistry were required; one with high Cd/low Cu concentrations and another with the opposite ratio. This criterion was however difficult to fulfill, because the [Cu]/[Cd] ratio in most Norwegian mine polluted rivers appeared to be rather constant at about 100-200. Ca concentrations should be intermediate or low, and the populations were ideally supposed to live on the edge of extinction. The latter was to achieve a remaining fish stock that was genetically adapted through partial deteriorations during high toxic stress episodes. The two rivers found, Rugla and Naustebekken matched these criteria fairly well, however genetical adaptation was somewhat uncertain since the fish stocks to some extent had access to unpolluted side-creeks during critical episodes. Practical considerations such as availability via road, available cabins, distance to laboratories, and distance between river sites were considered as well. The study area and the river sites (Figure 1) finally chosen were a compromise between all these considerations.

The water chemistry of six other rivers in the area, which differed highly concerning levels of pollution, river discharges, pH, and TOC, were studied as well. The metal chemistry variations and covariations of all eight rivers are described in Papers 3 and 4.

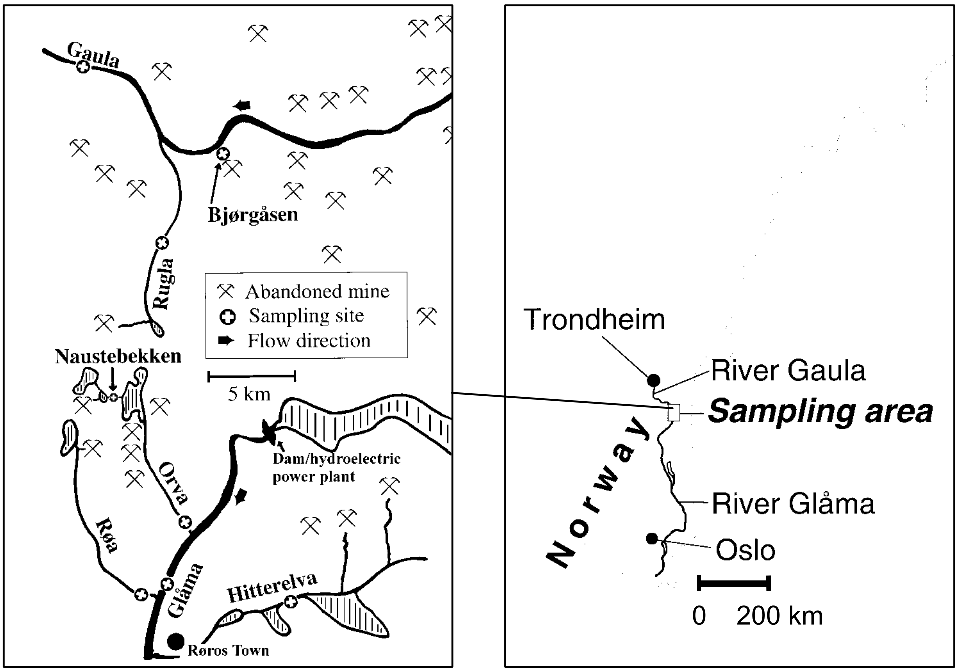

Figure 1: Map of the study area

Table 1 shows major characteristics of the selected sites. Orva, and especially the very small (nameless) creek running from the Bjørgåsen mining area, are very heavily polluted by acid mine drainage. These two streams, as well as river Glåma around the chosen sampling stretch, do not contain any fish population. Glåma and the five other streams have rather neutral pH values and moderate to low Ca concentrations. The stream discharge varies highly from stream to stream as well as temporally within each of them; from less than 1 to more than 100 L s^{-1} in Bjørgåsen, and from 10^4 to more than 10^5 L s^{-1} in the largest river, Glåma. The sizes of the other rivers may be estimated by comparing watershed sizes (Table 1). In two of the rivers, Naustebekken and Hitterelva, lakes are located between the polluting mines and the sampling sites. Sedimentation in these lakes may cause depletion of particulate metals from the rivers. Sampling sites are located in headwater streams, all except Gaula at altitudes ranging from 500 to 900 meters (see Table 1). Tree vegetation reaches an altitude of 800-900 meters in the area, and the bulk of snow accumulated in the watersheds during winter is melted by the end of June. The area has an inland climate with relatively warm summers (~10 to 30 °C) and cold, dry winters with temperatures occasionally reaching below –40 °C. Annual precipitation (1961-1990) is 504 mm (Aune, 1993), which is very low for Norway.

Table 1: Topographic characteristics of the sampling sites and chemical quality of stream water.

Site	**Altitude (m)**		**Distance mine - sampl. site (km)**	**Water-shed size[1)] (km^2)**	**Lake in river path**	**Average pH**	**Average Ca (mg/L)**	**Pollut-ion level**
	Sampl-ing site	**Main mine**						
Røa	640	860	11	90	N	7.14	3.7	Low
Nauste-bekken	820	860	1	8	Y	6.90	2.3	Low
Gaula	360	1040	30	290	N	7.02	3.5	Low
Hitter-vassdraget	700	820	7	140	Y	7.35	6.4	Medium
Rugla	620	880	7	70	N	7.01	4.3	Medium
Glåma	620	800-900	10	1000	N	7.13	5.0	Medium
Orva	620	800-900	5	25	N	5.52	4.4	High
Bjørgåsen	500	580	2	1	N	3.19	9.8	Very high

1)Above the sampling site.

Pictures showing the study sites and illustrating some of the challenges during fieldwork are presented in Figures P1 to P24.

Experimental

In this chapter a short description of the methods used is given. Complete descriptions of the methods are given in each of the papers. In addition a minor study in Orva is described (not in any of the papers). Pictures showing the dialysis and filtration process are presented in Figures P.25 to P.30. Sampling and analysis quality control are accounted for in each of the published papers.

The dissolved species of the metals were fractionated by dialysis *in situ*. Suspended matter was removed by filtration, and the colloid fraction was defined as the difference between the filtered and the dialyzed fraction. Unfractionated samples were collected for total concentration determination, or more precisely; acid extractable concentrations, since all samples were acidified to 0.1 M HNO_3. Ca concentrations, pH, river discharge, water temperatures, and (to a lesser extent) precipitation and TOC were also monitored.

Three samples were collected from red deposits observed in the snowcap covering stream Orva (Figure P.23): A fraction of the snowcap was shovelled away to form a vertical wall from the stream surface to the top of the remaining snow. Wide-neck 50 ml polyethylene bottles were then pushed into this snow-wall at ~0 cm, 10 cm, and 20 cm from underneath. The samples were acidified to 0.1 M HNO_3 and Cu, Zn, and Cd were determined by flame atomic absorption spectrophotometry.

Results and discussion

Metal accumulation and metallothionein in fish

Biological uptake, accumulation, and biochemical response (MT-production) during a run-off episode were studied in the streams Rugla and Naustebekken (Paper 2). Rugla was highly polluted by Cu and intermediate by Zn and Cd. For Naustebekken the metal fingerprint was opposite. The run-off episode caused enhanced concentrations of Cu, Zn, and Cd in the water, and generally corresponding metal accumulation in fish gills, liver, and kidney. It was shown that Cu, Cd, and Zn concentrations in all three organs also reflected well the general metal concentrations in each of the rivers, however the kidney in Rugla trout unexpectedly contained less Cu than the kidney in Naustebekken trout. A negative correlation between age and Cu concentration was found in all organs except in the Rugla trout kidney. For Zn no agedependence was established, whereas for Cd the general trend was opposite of that for Cu; a positive correlation between age and fish organ concentrations was found in liver and kidney however not in gills.

Intracellular MT bound to Cd or Zn was found by ^{109}Cd replacement, whereas Cu bound MT is the difference between total and Cd/Zn MT (Paper 2). As for the totals, the Cd and Zn fractions bound to MT in fish reflected well the corresponding metal concentration in the rivers. The concentrations of Cd/Zn MT in gills, liver, and kidney were three- to fourfold higher in Naustebekken compared to Rugla trout, whereas the corresponding water concentrations were six- to tenfold higher in Naustebekken. For Cu this pattern was less obvious; it seemed to coincide for gills but not for liver. Although Naustebekken trout may have been adapted to high levels of Cd and Zn, and minor levels of Cu, its MT production system still seemed capable of coping with Cu stress; after transfer to Rugla this trout experienced a pronounced higher Cu concentration, and a marked increase in Cu MT was observed.

Gill concentrations of Cu MT in Rugla and Cd/Zn MT in Naustebekken was clearly enhanced during run-off episodes, indicating the significance of MT in detoxifying sudden bursts of metal concentrations. Generally the Cu MT and Cd/Zn MT concentrations in gills and kidneys were high enough to account for all or almost all Cu and Cd but only for a minor fraction of the Zn present in these organs. For Zn this indicates that other detoxifying mechanisms may be more important than MT.

Metal speciation

Dialysis *in situ* and filtration separated the metals in three fractions; dissolved (< 4 nm), colloidal (>4 nm, <0.45 µm) and particulate (>0.45 µm). At the Bjørgåsen and Orva sampling sites, average pH levels were 3.1 and 5.5 respectively (Figure 3, Paper 4). Because of this low pH, more than 90 % of Cu, Zn, and Cd occurred in dissolved form in these streams. Average pH in the six other streams studied was in the range 6.9 to 7.4, and for this reason the dissolved fractions were lower with an average of 54, 79, and 79 % for Cu, Zn, and Cd respectively. In the same streams a major part of Al, 55 %, was colloidally bound, whereas the dissolved and particulate fractions were 21 and 23 % respectively.

In Figure 4 (Paper 4), data from the present study is combined with data from similar studies to reveal relations between TOC, pH, and metal size distribution. The results support equilibrium models implying that the percent fraction of metals bound to particles (for simplicity called adsorbed) rises steeply from almost zero to almost 100 % within a narrow and element-specific pH range. Changes in TOC concentrations however seem capable of shifting the metal absorption curves by up to one pH unit. The scattered points reveal that other parameters as well as pH and TOC, e.g. metal concentrations, organic matter composition, and hardness/alkalinity, probably also influence the metal speciation.

50 % of Cu and Al are in dissolved form at about pH 7.2 and 5.8 respectively in low TOC (< 8 mg/L) waters (Figure 4, Paper 4). Zn and Cd on the other hand adsorb at slightly higher pH levels: For Zn and Cd the corresponding 50 % dissolved fraction seems to occur at higher pH than accounted for by own and literature data; extrapolation suggests pH 7.6-7.8. The rather short distance on the pH scale between Cu adsorption and Zn/Cd adsorption may seem insignificant at first glance. It may however explain the fact that the percent dissolved fraction of Cu is generally found to be some 30 % lower than the case for Zn and Cd in pH neutral surface waters (Figure 4, Paper 4).

TOC alterations may also significantly influence Cu, Cd, and Al speciation (Figure 4, Paper 4). High TOC concentrations (> 8) seem to accompany low fractions of dissolved metals. For Cu and Cd, this is probably because the metals adsorbed on high molecular weight organic compounds or organic coatings on inorganic particles. The influence of organic material on Al may be somewhat less clear because, as described above, that element may precipitate as well. For Cd, 10 to 60 % is adsorbed at high TOC (> 8 mg/L) in the pH range 6-7. However in corresponding low TOC samples (< 8 mg/L), as much as 75 to 100 % is adsorbed. For Al, a dissolved fraction of 50 % seems to occur at about pH 6 at low TOC. This is one pH unit or more higher than in the high TOC waters. For Zn only a small number of high TOC data exist, and the influence of TOC on Zn size distribution is less obvious.

In short, a narrow pH range almost completely governs the dissolved/particulate distribution for many metals. The location of this range along the pH axis is specific for each element and may be considerably altered by the organic matter content. In particular during sudden metal concentration bursts this could be significant, since dissolved species probably exert most of the acute toxic stress in waters.

Dissolved fraction modelling

As discussed below, models predicting the water chemistry from easily attainable data would be very beneficial in future monitoring studies. Multivariate regression analysis (Fugleberg and Kristianslund, 1995) on data from Paper 4 revealed a general equation for the dissolved fraction of Cu, based on pH and TOC concentrations (mg/L)(Eq. 2).

$$\text{Dissolved fraction of Cu (\%)} = (61.894 - 6.482\ \text{pH} - 0.644\ \text{TOC})\ \ln(\text{pH}^2) \qquad \text{(Eq. 2)}$$

The model explained 69 % of the variance in Cu dissolved fraction in the pH range ~ 4 to 7.2 (ANOVA analysis). The equation should however be used with precaution since in many cases dissolved fractions predicted by the model deviated strongly from the experimentally determined ones. Other parameters such as metal concentrations and alkalinity probably influenced the dissolved fraction as well as pH and TOC, and could possibly have been included as model variables. Examples of dissolved fractions calculated by the model are presented in Table 2, and are in reasonable agreement with measured dissolved fractions in Figure 4, Paper 4. Successful multivariable regression models were not achieved for the other elements.

Table 2: Dissolved fractions of Cu from Equation 2 at different combinations of pH and TOC

	Model Cu dissolved fractions			
TOC (mg/L)	pH 4	pH 5	pH 6	pH 7
12	78	70	55	34
6	89	82	69	49
2	96	91	78	59
0.5	99	94	81	63

Temporal variations in metal chemistry and parameters influencing metal toxicity

All water chemistry data collected for this thesis are given in Appendix 1 (Table A1.1 to A1.18). The most important parameters are plotted as a function of time as well (Appendix 2, Figures A2.1 to A2.8). Temporal variations are discussed in Paper 3, Appendix 2 contain additional figures not printed in that paper. This chapter shortly summarizes conclusions from Paper 3, with some additional information from Appendix 2 and views temporal trends in the light of Paper 4.

In most rivers, low winter discharges were succeeded by rain induced flood peaks at May 7-8. However in Glåma, a hydroelectric power plant regulation smoothed out this peak. In most streams the May 7-8 incident led to elevated, or even year 1997 maximum, total metal concentrations (Figure A2.1 to A2.8 in Appendix 2). As the spring flood discharge proceeded to its maximum, the metal concentrations generally declined; exceptions were Naustebekken and Hitterelva (Figure A2.2 and A2.3 respectively in Appendix 2). In these two streams the metal peaks were delayed by lakes in their path. In Bjørgåsen (Figure A2.8 in Appendix 2), the total metal concentrations were clearly lower during flood incidents; opposite to other sites. Probably clean rain- and meltwater diluted water from a contaminated and relatively steadily seeping groundwater source in Bjørgåsen during the floods. During the rather extraordinarily dry summer in 1997 the metal concentrations declined or were rather steady in most of the streams and rose again during autumn rainfalls.

Early stages of flood episodes have been reported to accompany elevated metal concentrations in several studies (Cortesao and Vale, 1996; Grimshaw *et al.*, 1976; Sullivan *et al.*, 1986). For organisms in moderately mine-polluted streams, such incidents could therefore be critical. Generally total Al showed higher temporal variations than the other elements.

Another parameter that may influence metal toxic stress in natural waters is pH. As discussed in Paper 4, pH is probably the most important parameter determining the dissolved to particulate metal distribution. Within a rather narrow range, pH may reduce the dissolved fraction from ~100 % (low pH) to ~0 % (high pH). Low pH however may have other effects on metal speciation and toxicity; the H^+ ion may e.g. act directly toxic on aquatic life. At decreasing pH, metals will also be increasingly present in the bioavailable free ionic state (Stumm and Morgan, 1996). But the H^+ ion may also protect aquatic life from metal uptake, by competition for surface uptake sites (see Introduction). The total concentrations of Cu, Zn, Cd, and Al seem to covary rather closely, Al deviating somewhat from the others (Figure 3a in Paper 3). But each of the metals also correlate negatively with pH (Table II, Paper 3). Whereas the metal concentrations have an early peak during the spring flood, the pH minimum seems to occur a few weeks later, still a few weeks before the river discharge peak (Figure 3a and 4 in Paper 3). However this does not necessarily apply for all sites. In Naustebekken e.g., the pH trough induced by the spring flood coincides with the maximum for total Al, and even occurs one month prior to the maxima of the other elements (Figure A2.2 in Appendix 2).

High river discharges usually accompany increased concentrations of particles and colloids. This material may contain metals or introduce adsorption surfaces to dissolved metal species. Probably due to one or both of these, the dissolved fractions of Zn, Cd, and Al showed a significant negative correlation with river discharge (Figure 3c and Table II in Paper 3). The desorbing effect from a lower pH observed during flood (Table II, Paper 3) could be expected to counteract this trend, but did not seem strong enough. Total and dissolved Cu generally follows the same trend as corresponding Zn, Cd, and Al concentrations (Figure 3a and 3b in Paper 3). Still no correlation was found between river discharge and the dissolved fraction of Cu. It is suggested in Paper 3 that particle concentrations may be less important, on the expense of pH dependence (discussed below) for Cu. The deviating behavior of Cu however may be further understood from results in Paper 4: In that paper it is concluded that the dissolved fraction of the metals may change rapidly from ~100 % at a low pH to ~0 % a few pH units higher. The change appears steepest at about 50 % adsorption (see Figure 4, Paper 4), that is, for Cu, at about pH 7.2. Most streams and lakes discussed in the present work show pH values around this level, and this may be the reason why the Cu speciation seems so strongly correlated to pH.

As discussed in the Introduction, alkalinity and Ca may protect aquatic organisms against metal pollution. Unfortunately however the temporal variation pattern for alkalinity and Ca concentration seems to be opposite to that of the metals; alkalinity and Ca concentration are high at low river discharges during the winter and dry summer and low during spring and autumn flood peaks (Figure A2.1 – A2.8 in Appendix 2). Because of this pattern, there were significant negative correlations between alkalinity and Ca concentration on one side and Cu and Al on the other (see Paper 3). Generally, though, the minimum levels of alkalinity and Ca concentration occurred one month or more delayed compared to the spring maximum of the total metal concentrations. This contributed to intermediate Ca and alkalinity levels during the critical metal concentration maximum in spring.

As discussed below, organic substances may decrease the metal toxicity. Three sampling campaigns included TOC analysis, unfortunately too few to reveal any temporal trends. Some investigations report elevated concentrations of organic substances during flood (Borg, 1986; Borg *et al.*, 1995) whereas others report the opposite (Bishop *et al.*, 1995; Sanden *et al.*, 1997). In another investigation (Bishop and Pettersson, 1996) it seemed that during floods, TOC in forested subcatchments was high, whereas the opposite seemed evident in a mire subcatchment. TOC is therefore not connected to river discharge in any consistent manner. Some Norwegian investigations in the present study area (Kjellberg and Løvik, 1997) or in adjacent regions (Grande and Romstad, 1993; Kjellberg, 1991) still indicate that TOC most likely will be higher during flood or increasing flood conditions.

Normalized values (z-scores) describe how high a certain concentration is in a stream compared to the average in that stream. In short, a z-score of 0 (zero) means that a certain sample contains exactly the average (e.g. alkalinity) for that site. A z-score at 1 means that the respective sample show an (e.g.) alkalinity that is 1 standard deviation higher than average for the site whereas a z-score at –1 is 1 standard deviation below average . Z-scores for several rivers may be plotted in the same figure, which makes it easier to reveal general temporal trends in the data material as a whole (see Figures 2 to 4 in Paper 3).

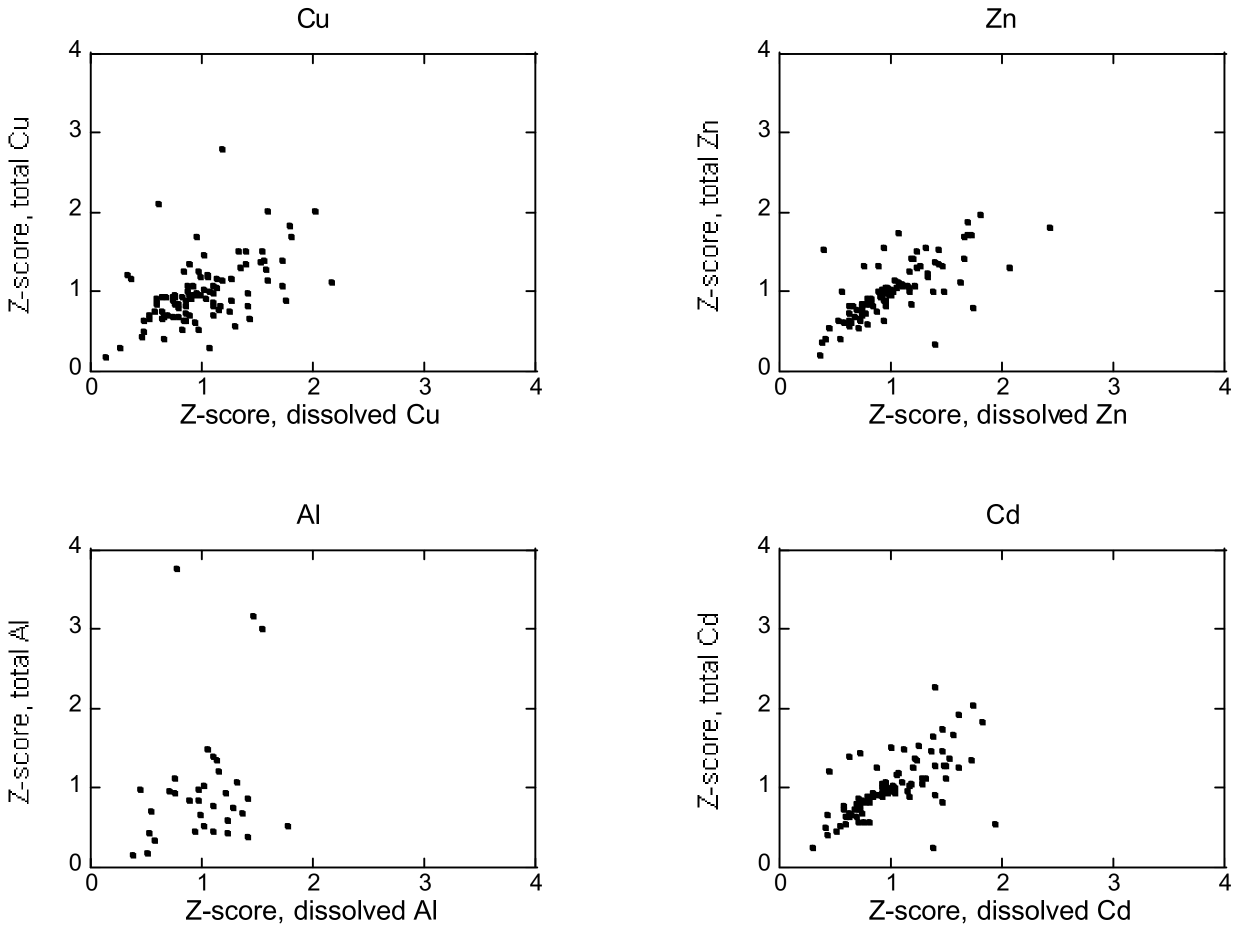

Figure 2: Normalized values (z-scores) for dissolved and total metal concentrations

As shown in Figure 2, the dissolved concentrations generally follow the totals quite well for all elements except Al. In a few cases the dissolved concentration apparently exceeds its corresponding total, e.g. on one occasion during May in Rugla (Figure A2.5). This is probably caused by a delayed dialysis equilibrium during very rapidly decreasing metal concentrations, and the phenomenon may have disturbed the calculations of dissolved fractions to some extent. At the Bjørgåsen site however, low pH causes total and dissolved concentrations to be equally high for Cu, Zn, and Cd. The almost identical path of the dissolved and the total concentrations indicate that the dialysis bags generally follow the exterior water chemistry very closely. The dissolved fractions of Zn and Cd in Hitterelva, Glåma, and Orva are also high (Figure 2, Paper 4) and indicate the same conclusion; the dissolved concentrations determined within the dialysis bags represent well the river bulk water at the sampling date (Figures A2.3, A2.6, and A2.7 in Appendix 2).

In several cases, metal concentration peaks are observed for some metals, whereas others remain low or intermediate. This is e.g. observed on October 1 in Rugla (Figure A2.5) and on July 3 in Røa (Figure A2.1), both incidents especially high in total Cu. However during a heavy rain incident in the beginning of May, Cd and Zn responded with high dissolved concentrations in Naustebekken whereas dissolved Cu in this case remained intermediate (Figure A2.2). Concentrations of Zn and Cd generally covaried very closely, but on a few occasions, e.g. in Rugla during May (Figure A2.5), dissolved Zn and Cd behaved differently. The deviations between the metals indicate that either their release mechanisms may vary, or continuous adsorption/desorption reactions at some stage in the watershed may withhold some metals longer than the others. Total concentrations of Al showed the highest temporal variations of the metals, and generally the path of the Al concentration curves deviated most from the other metals (Figures A2.1, A2.2, and A2.5).

After more than a week with dry weather, the forecast on September 29 predicted forthcoming rain. Samples were therefore collected at September 30, October 1 and October 2 to observe chemical changes on a higher temporal resolution. An intermediate rain episode came at about 16:00 and lasted to about 19:00 on October 1. Samples were collected from about 22:00 to 01.00 in Rugla, Orva, Glåma, and Røa, and for the former site this coincided well with the monitored flood maximum. New samples were then collected at all eight sites from about 12:00 the next day (October 2). The intermediate flood incident at October 1 doubled the Cu concentration in Rugla, causing the highest measured Cu concentration during 1997 in that stream (Figure A2.5). About 18 hours later it was back to the pre-flood level. The maximum Cu concentration occurred even though the precipitation that caused it was a mixture of snow and sleet in the mining area. The snow/sleet partly covered the mine tailings, which probably therefore released metals rather poorly, whereas the lowland rain probably diluted somewhat the metals that *were* released. If heavier rain episodes were monitored with similar or shorter intervals, much higher metal concentration peaks would probably have been revealed. This shows how rapid chemical changes may occur in streams, and illustrates how useful continuous logging may be in stream monitoring studies.

As discussed above, total metal concentrations are generally higher during early flood conditions for all elements. This has been attributed to flush-out from weathered materials, e.g. mine tailings, and, to some extent, particulate metals being resuspended from the stream bottom due to the higher water velocity. Several facts indicate that the latter may have contributed significantly to the Rugla Cu concentration peak discussed in the previous paragraph: First, the fact that the Cu concentrations increased despite the low water flow that was expected to seep from the snow/sleet covering the mining wastes. Secondly, the dissolved Cu concentrations decreased during the same episode (Figure A2.5). This is consistent with a scenario where no metals are released from the tailings, however particulate Cu is resuspended from the stream bottom and simultaneously diluted by lowland originating rainwater. The fact that the total concentrations of Zn and Cd did not increase as much as Cu during this episode does not necessarily contradict this conclusion: Splitting the data material in two, high and low river discharge samples, reveals that the $[\mathrm{Cu}]_{\mathrm{Total}}/[\mathrm{Zn}]_{\mathrm{Total}}$ fraction was highest during elevated river discharges in all eight rivers, on average 15 % higher. This may indicate that Cu could have a stronger tendency to resuspension that Zn and Cd. Not surprising since, as discussed above, Zn and Cd attach less readily to particles than Cu.

Metal concentrations in relation to fish stocks and guidelines

All streams in the present study are more or less influenced by previous mining activity (Table 3), and Bjørgåsen and Orva are ecologically dead due to the very high metal concentrations. According to locals, salmonid species are completely absent for several kilometers downstream the Orva inlet in Glåma, and nothing but a few whitefish (Coregonus lavaretus) had ever been caught in the studied stretch of Glåma since mining activity started in the area. Lake Djupsjøen is located some hundred meters upstream the Hitterelva sampling site, and the water chemistry and pollution levels are probably quite similar. Considerable amounts of whitefish (Coregonus lavaretus) and minor amounts of trout (Salmo trutta), grayling (Thymallus thymallus), minnow (Phoxinus aphya), and arctic char (Salvelinus alpinus) are reported in Lake Djupsjøen. However the lake is probably considerably affected by metal pollution, and some of the fish in the lake probably migrated from an unpolluted lake (Store Hittersjøen) some kilometers further upstream. Fish caught in Djupsjøen may therefore not be a part of any local fish stock (Grande *et al.*, 1996).

Table 3: Average total metal concentrations in each river (µg/L) compared to Norwegian water quality guidelines (values within "Poor" or "Very poor" water quality are shadowed)

Site	Cu2)	Zn2)	Cd2)	Al2)
Røa	3.3 ± 0.9	8.4 ± 3.6	0.0120 ± 0.0070	72 ± 62
Naustebekken	4.8 ± 2.0	90 ± 35	0.170 ± 0.076	36 ± 11
Hitterelva	35 ± 9	124 ± 17	0.23 ± 0.03	
Gaula	10.9 ± 6.4	28 ± 14	0.077 ± 0.035	
Rugla	22 ± 9	8.5 ± 2.6	0.022 ± 0.009	124 ± 147
Glåma	47 ± 38	104 ± 71	0.24 ± 0.16	
Orva	340 ± 160	850 ± 380	1.42 ± 0.61	
Bjørgåsen	3100 ± 900	6200 ± 2800	14.2 ± 4.7	
Guidelines1)				
Good	<2	<10	<0.04	<5
Less good	2 –5	10-30	0.04-0.1	5-20
Quite poor	5-15	30-60	0.1-0.2	20-50
Poor	15-50	60-110	0.2-0.5	50-100
Very poor	>50	>110	>0.5	>100

1) According to Holtan and Rosland (1992). The guidelines are mainly based on concentration factors compared to expected background levels, but to some extent on the toxicity of each element as well.

2) Average ± standard deviations.

The average metal concentrations in Glåma and Djupsjøen/Hitterelva are higher than in Rugla, a stream polluted considerably with Cu (class "Poor") but only to a minor degree with Zn and Cd. Somewhat surprisingly, no fish were however observed in Rugla during electric fishing, except trout in a minor pond 1 km downstream where it may have been able to escape into an unpolluted side stream at metal stress incidents (a waterfall separated the site and the pond from upstream migration). Naustebekken stream on the other hand was rather strongly polluted with Zn (class "Poor") and to some extent Cd but not with Cu. In Naustebekken a large population of trout existed, but the arctic char population became extinct and repopulation trials failed after the mining activity started. Possibly the Zn or Cd concentrations influenced the arctic char spawning areas in the lake, whereas the trout, spawning in running water, could do this in some unpolluted brooks running into the lake. The fact that Naustebekken, due to Zn, and Rugla, due to Cu, are both located in the class "Poor" whereas the trout populations are not influenced in the former but extinct, at least at the studied site, in the latter may therefore not necessarily lead to the conclusion that there is something wrong with the guidelines. It may as well be caused e.g. by the clean spawning areas available for the Naustebekken trout. The two last streams, Røa and Gaula, were quite clean, the latter predominantly because of substantial remediation at surrounding mining sites during the early nineties. Stream Røa however contained significant amounts of Al.

Al concentrations were only determined in three streams. This metal was rated as most critical among the metals according to the guidelines in two of these streams; Røa and Rugla (Table 3). On the basis of current Al toxicity models, it is likely however that Al will have only minor toxic effects on fish at the concentrations and pH levels concerned; at least that is the case unless the water consists of very freshly mixed neutral and acidic water bodies (Verbost *et al.*, 1995).

Lake buffering

The dissolved fraction of Cu, Zn, Cd, and Al was lower in Rugla than in Naustebekken (Paper 1). It was suggested that particle sedimentation in a lake that Naustebekken passes through caused the difference. More streams and lakes are however available in Figure 4 in Paper 4, and in this material it does not seem likely that lake and river sites should differ considerably in dissolved fractions, given similar pH and TOC conditions, Other parameters probably scatter the data material more than lake /stream origin. Lakes may however reduce toxic effects of metals running through them by diluting and smoothing metal concentration peaks. This may be viewed by the "lake buffered" metal concentrations in Naustebekken and Hitterelva (Figures A2.2 and A2.3 in Appendix 2 respectively), compared to the others (Figures A2.1 and A2.3 to A2.8 in Appendix 2).

Snowcap accumulation

Rust-coloured deposits were observed in the lower 20 cm of the snowcaps covering most of Orva during early spring (Figure P.23). The deposits probably originated from splashing droplets from the fast-flowing stream or from water sieving into the snow that sags down during mild episodes. To get some indication of whether or not the snowcap could influence the water chemistry, three samples were collected at 0, 10 and 20 cm into the snowcap from underneath (see Experimental). The results are presented in Table 4.

Table 4: Metal concentrations (µg/L) in Orva stream and in the metal deposits located in the snowcap covering the stream.

Sample	Cu	Zn	Cd	Discharge (m^3/s)
May 6				
Stream water	*147*	*583*	*0.76*	*0.26*
Snow, 0 cm 1)	2400	96	1.12	
Snow, 10 cm 1)	17500	260	2.7	
Snow, 20 cm 1)	430	9.3	0.060	
May 15				
Stream water	*720*	*1280*	*2.4*	*3.55*

1) Measured from underneath snowcap

Several factors indicated that the Cu deposits in the snowcap influenced the river water chemistry considerably during their release: The Cu concentrations were surprisingly high in the Orva snowpack (up to 100 times the river levels) whereas Zn in snow was lower than in stream water and Cd concentrations in the snow were rather close to the corresponding water concentrations (Table 4). Observations in Orva and Rugla during the May 5-8 rain episode showed that a substantial part of the winter snowcap covering a stream could be melted or mechanically broken down within a day or perhaps even only in a few hours. Additionally, the metal deposits in the snowcap were located close to the river water and were therefore probably released before the rest of the snow. Thus the metal deposits were probably mixed into the water masses within a very short time, potentially contributing to a very high metal concentration peak. During this early stage of the spring flood, the dilution effect of melting water was probably moderate as well.

To interpret the effects of Cu in the melting snowpack on the streamwater underneath, some rough assumptions were made:

1. The stream is ~5 km long, and 2/3 of it were estimated to be covered by snow.

2. From the melting in the sample bottles, the density of the snow was estimated to be 0.4 g/cm^3.

3. The 0, 10, and 20 cm snow-samples were estimated to represent the 0-5, 5-15, and 15-25 cm layers respectively in the snow, for the part of the stream covered by snow.

By calculating the corresponding water volumes and using the Cu concentrations in the snow (Table 4), it was estimated that 7.8 kg of Cu was captured in the Orva snowcap. To interpret the snowcap metal contribution to the stream water during the most critical period, one assumed that:

4. Half of the 25 cm of metal containing layer was released during six hours at May 15, the spring maximum Cu concentration.

This means that 3.9 kg of Cu were released to the water, possibly during the incident of 1997 which for some organisms located at downstream sites may have been critical for survival. Considering river discharges and metal concentrations on May 15 (Table 4), ~11.4 kg Cu were transported with the stream during six hours at May 15. If a 3.9 kg contribution of Cu from the *snowcap* were a reasonable estimation, it would have contributed with 34 % of the Cu in Orva at this incident.

It should be emphasized that the calculations are based on rather rough assumptions, and that further research with more samples is necessary to quantify accurately the influence a snowcap metal deposition may exert on water chemistry. The huge differences between Cu, Zn, and Cd concentration factors in the snow (compared to water concentrations) were rather surprising and should be further investigated.

River Glåma and discharge regulation

The stream Orva is completely dead due to the high metal concentrations in the water. It runs into the large river Glåma (called Glomma further down), which, according to locals, is almost completely dead several kilometers downstream from the Orva inlet. Thus, parameters influencing Orva water will also influence Glåma for a long distance downstream. The extra metal bursts from the snowcap in Orva (discussed above) is in this respect only one out of several negative factors which act at the same time in Glåma. Whereas the Cu concentrations in Orva increase 7-fold from the winter samplings to the spring peak sample, the corresponding Cu concentrations in Glåma 3 km downstream increase about 18-fold. At the same time, alkalinity and pH are very low as well (Figure A2.6), possibly increasing the toxic responses of the metal burst.

The extremely high metal concentration peak in Glåma is probably due to the last of the "bad luck" factors in that stream. Whereas Orva receives and loads rainwater fast, the river runoff peak in Glåma is delayed due to the long distance to Glåmas outer watershed. The presence of a large lake/dam, Aursunden (Figure 1), in its path also delays and smooths runoff peaks. When Orva water, with high metal concentrations and river discharges at early spring then enters Glåma which is still running at low winter discharge, the metal concentrations in Glåma should be expected to be very high, just as observed. To some extent the very high concentration peaks in Glåma could have been reduced somewhat. Aursunden Lake is regulated by the hydroelectric power plant Kuråsfossen Kraftstasjon. By letting out more water through during the most critical incidents, the high loads of metals from Orva could have been diluted. Unfortunately, spring flood is a period when power plants in general increase the water volume in their dams, which have been partially emptied during the electricity demanding and dry winter season. Probably due to the delay in Glåmas watershed, it seems that the river runoff in the beginning of May is naturally low (Figure 3). Thus the river discharge regulation by the power plant does not seem to have contributed to the maximum metal peak in 1997, but could have reduced it if run differently. The flood however proceeds differently each year, and it seems likely that the power plant regulation at some incidents increases the effects of the metal pollution from Orva. It is therefore suggested that considerable amounts of water should be let through at early spring rain and/or melting episodes to dilute the downstream effects of metal pollution.

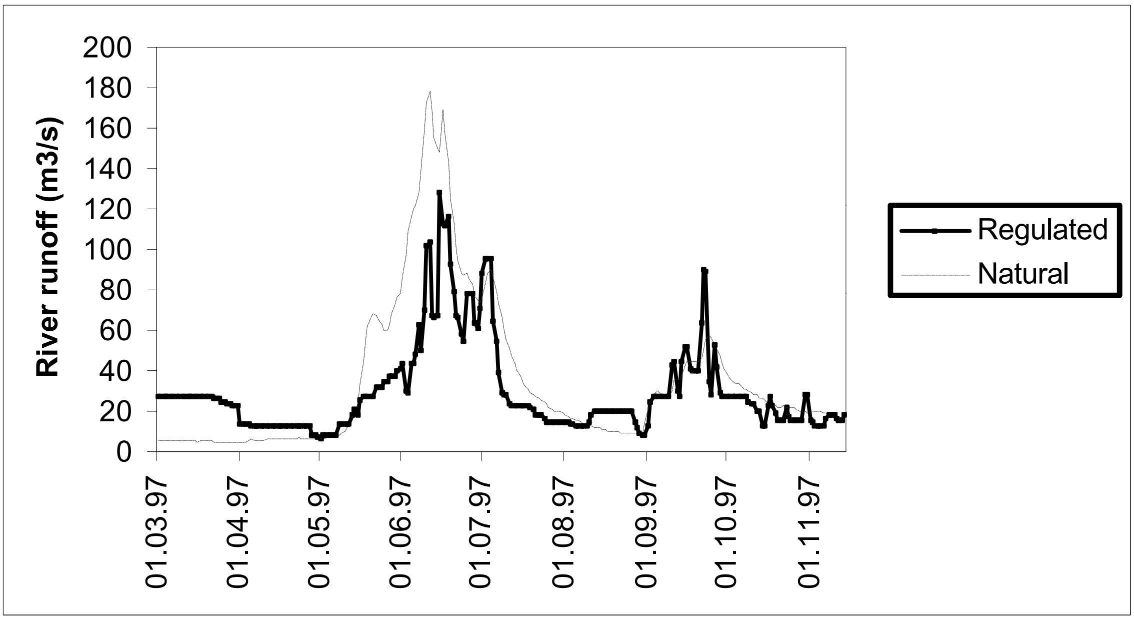

Figure 3: River runoff in Glåma, regulated (as measured) and natural (calculated; as if the stream was unregulated by the hydroelectric power plant) (Glommens og Laagens Brukseierforening, 1999).

Suggested applications of results from the present study

Following are a few examples suggesting how the present results may be used qualitatively or quantitatively in other studies.

Example 1:

In a study monitoring a moderately polluted stream, Cu concentrations around 20 µg/L, a pH of 7, and 10 mg/L TOC are found. One is concerned about what future changes in water chemistry might lead to in terms of ecological harm.

During average conditions, around 70 % of Cu in the water could be expected to exist in the dissolved state (Figure 4 in Paper 4 or Eq. 2). Many organisms are influenced by Cu concentrations of 20 µg/L, and could be sensitive to small changes in the water chemistry. Significant amounts of Cu adsorbed on particles are likely to desorb if TOC or pH decreases. One should therefore carefully control changes in the watershed that might alter the TOC or pH in the stream. Also pH, TOC, and Cu concentrations should be closely monitored at flood or other episodes where levels of these parameters may be especially unfavorable.

Example 2:

A stream receives polluted effluents from a Zn mine. The Zn concentrations are about 60 µg/L in the stream and this element is considered to be the most toxic for the water organisms. TOC is 3 mg/L and pH is about 6.5. One is concerned about decreasing pH values due to deposition of pollutants from long range atmospheric transport in the river watershed and how it will interfere with existing pollution in the area.

At low TOC values and pH around 6.5, it is likely that most Zn will already be in the dissolved state (Figure 4 in Paper 4). Further acidification is unlikely to change this. Acidification may on the other hand to some extent increase the Zn^{2+} fraction *within* the dissolved fraction, by dissociation from complexes. This may be of some concern, although the higher H^+ concentrations (as discussed above) will compete with Zn^{2+} for uptake sites on biological membranes. All in all though, it is not very likely that a minor decrease in pH will influence Zn speciation enough to harm aquatic life if they can tolerate the present metal levels.

Example 3:

A hydroelectric power plant connected to a water reservoir is located upstream an old waste fill which releases acidity and metals to the stream. Dissolved waste material is released during periods when rain flushes through the wastes. One would like to reduce the risk of negative effects from the waste material as much as possible, but fears that removing the masses may release even more in the process, and be very expensive.

One inexpensive and easy way to reduce acute toxic effects from the peaks would be, as described above for Glåma, to dilute the worst peaks by letting more water through the power plant dam. First one should consider whether rising water levels in the stream could permeate into dry layers in the waste and increase the flushout. If that seemed unlikely, one could then install instruments that continuously logged rain or water discharges, pH, and/or conductivity in the stream/waste seepage. At especially unfavorable conditions more water could be let through to dilute the incoming waste effluents.

Example 4:

Cu concentrations are on average 12 µg/L, TOC is 8 mg/L, and pH is 6.5 in a stream. During a flood episode in May, however a Cu concentration of 70 µg/L is observed, pH is 4, the TOC rises to 12 mg/L, and the alkalinity is negative. One wonders whether or not the metal level in the stream is high enough to injure aquatic life.

A substantial part (>50 %) of the Cu would probably exist in particulate/colloidal form (Figure 4 in Paper 4). Additionally, considering that on average the Cu concentration is rather low, the pH is quite neutral, and the TOC concentration is high in the stream, one could easily conclude that aquatic organisms in that stream most likely are quite unaffected by the Cu concentrations.

The metal concentrations are however unusually high during a certain stage of the flood. This coincide with a low alkalinity and a very low pH which, nevertheless an elevated TOC concentration probably elevates the dissolved fraction to ~80 % (Eq. 2 or Figure 4, Paper 4). Organisms may therefore be considered threatened by acute effects of Cu concentrations in the stream, even if the average concentrations are rather moderate.

Conclusions

The influence of pH, TOC, alkalinity, Ca concentrations, and conductivity on the concentrations and speciation of Cu, Cd, Zn, and Al have been studied in eight streams. The studied metals were predominantly bound to particles and colloids at high pH levels, but shifted to be more or less completely dissolved one or a few pH units lower. At what pH range the shift occurred clearly depended on the metal concerned and the TOC in the water. At TOC concentrations lower than 8, a 50 % dissolved fraction was estimated to occur at pH ~7.2 and ~5.8 for Cu and Al respectively, whereas for Cd and Zn a 50 % dissolved fraction seemed to occur at pH ~7.7 - slightly higher than the highest value observed in this investigation.

High TOC concentrations (> 8) seemed to accompany low fractions of dissolved metals, probably because the metals adsorbed on high molecular weight organic compounds or organic coatings on inorganic particles. A comparable dissolved fraction therefore occurred in the order of one pH unit lower in high compared to low TOC waters. Cu, Zn, and Cd occurred almost exclusively (> 90 %) in dissolved form in the two streams with average pH at 3.1 and

5.5. Six other streams with average pH from 6.9 to 7.4, showed average dissolved fractions at 54, 79, and 79 % for Cu, Zn, and Cd respectively. Most of Al, 55 % on average, was however in colloidal form in three streams in the same pH range. The dissolved and particulate fractions were 21 and 23 % respectively for this element.

In the pH neutral streams, total metal concentrations were generally high during flood conditions, and the pH was decreased. The low pH may have tended to increase the dissolved and free ionic fraction of the metals, although not necessarily their toxicity. As observed in Paper 3 however, the dissolved fraction decreased significantly at higher river discharges for Zn, Cd, and Al. Probably the higher concentration of particles in water during flood counteracted the effects of the lower pH. Alkalinity and Ca concentration, which may reduce metal toxicity, were also low during flood conditions. Some correlations that were not statistically significant may have been obscured by the fact that the maxima and minima of the different parameters did not coincide in time. Generally, but with exceptions in single streams, the total metal concentration spring peaks came first at rising flood episodes, followed by low pH, Ca concentration, and alkalinity two weeks later. Thus, at the spring maximum of metal concentrations; alkalinity, Ca^{2+} concentrations, and pH (for organisms unfavored by low pH) had not yet reached their most unfavorable condition for protecting metal stressed organisms. The flood maximum occurred two weeks after the pH, alkalinity and Ca minimum. After this point pH increased, metal concentrations decreased, and Ca concentration and alkalinity remained low.

The snowcap that covered the stream Orva accumulated and contained huge amounts of Cu, but only minor amounts of Zn and Cd. Calculations indicated that this snowcap may have markedly increased the metal concentration in the stream as it melted down during the critical period of rising flood.

It is suggested that the river runoff regulation of the river Glåma by a hydroelectric power plant could be run differently in order to level out metal peaks downstream the point where the highly metal polluted stream Orva runs into Glåma.

Gill concentrations of Cu MT in Rugla and Cd/Zn MT in Naustebekken clearly increased during run-off episodes, indicating the significance of MT in detoxifying sudden bursts of metal concentrations. Generally the Cu MT and Cd/Zn MT concentrations in gills and kidneys were high enough to account for all or almost all Cu and Cd, demonstrating the capacity of MT to regulate the toxicity of these elements. Only a minor fraction of Zn present in these organs was however bound to MT. For Zn this indicated that other detoxifying mechanisms could be more important than MT.

Further research

Considerable efforts have been made to predict metal toxic stress and accumulation from pollution in aquatic organisms during the past decades. Since the uptake varies between metal species, metal speciation techniques have been especially focused upon. Unfortunately instrumental speciation of metals in natural waters is time consuming, expensive, and best suited to separate metals into broad classes. Equilibrium models on the other hand, used to calculate the concentration of individual metal species, generally suffer from lacking data on ligand/particle concentrations/compositions or uncertain equilibrium constants, especially for organic compounds.

In the ideal case, monitoring studies of metal concentration, speciation, and toxicity should, at low cost and effort, predict rather precisely the concentrations of metal species that are available in the studied water, what toxic stress they exert, and to what extent they accumulate in water organisms. Preferably the studies should monitor metal concentrations and toxicity consecutively during temporal variations in the streams. Unfortunately, with currently available techniques, this would be extremely expensive and a huge amount of field and laboratory work would be necessary. Presently, metal pollution monitoring studies in streams and lakes are thus generally carried out by collecting occasional samples with certain intervals, e.g. once a month, and then determine total trace metal concentrations and a few other important water chemistry parameters such as pH, hardness, alkalinity, and TOC. Averages and peaks of each of the parameters are then compared to standardized water quality guidelines, sometimes also considering combinatory effects of e.g. total Cu and Ca. It is likely however that both guidelines and monitoring studies may be improved to account for the effects of metal pollution in a more precise manner. Some possible approaches to future modeling projects are suggested in Figure 4.

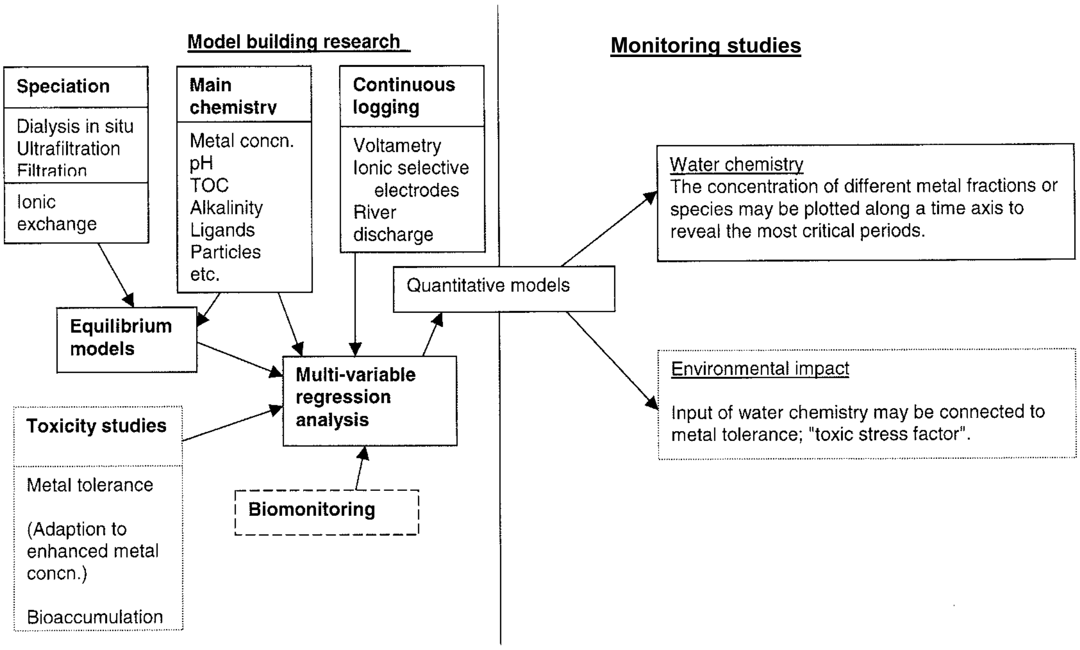

Figure 4: Possible paths for further advances in speciation and toxicity studies.

The figure is divided in two. On the left are extensive data collection projects with a large number of parameters collected. Such studies should describe as precisely as possible metal speciation, and possibly toxicity, given a wide range of water chemical/physical/biological conditions. Employing multi-variate regression analysis, these data may yield quantitative models predicting metal speciation and toxicity, given a set of more easily attainable data. Achieving this requires knowledge about which parameters significantly influence metal speciation and toxicity. For example in Paper 3, clear connections between metal speciation and combinations of pH and TOC are shown, and a simple model derived from the same data is presented in Equation 2 above. Further research could include similar data collections but from even wider ranges of chemical conditions. Using multi-variable regression analysis, fairly good quantitative models predicting metal speciation based on TOC and pH may be achieved. Including other parameters which influence metal speciation as well, such as particle content, ligand concentrations, and others, would probably also increase the precision of such models.

On the right side in Figure 4 are the monitoring studies where easily attainable data are collected and applied in the models to predict metal speciation and toxicity. Future models should include standardized procedures and sets of variables for the investigation of streams and lakes. The output of the models may also suggest which of the metals or combinations of these and other parameters that influence most significantly aquatic life, and possibly even predict what organisms or groups of organisms (e.g. benthos, fish, insects) that are most threatened.

As described above, the uptake of metal ions in an aqueous medium by an organic surface (roots or phytoplankton) may, to a first approximation, be considered to be proportional to the concentration of the free metal ion. Since many ion-selective electrodes measure the activity of the free metal ions, and have become very sensitive (Sigg and Xue, 1994), they may therefore become increasingly useful tools for metal speciation in the future. They may even allow continuous logging of metal ion activity if the calibration can be kept stable or automated and the interferences can be controlled. Biomonitoring, possibly continuous/online, may as well become increasingly important as gene technology can provide the needed tool to create organisms that respond measurable to certain pollutants in very low concentrations.

As mentioned above, accurate metal speciation is rather difficult to achieve. Possibly fractionation such as ultrafiltration, dialysis, and ion exchange prior to equilibrium models may be useful (43). E.g., by excluding most of the humic substances in water by low molecular weight cut-off dialysis, the composition of inorganic metal species would be easily computed from the total metal and ligand concentrations.

Summing up, future advances in the metal speciation techniques and the application of them may depend on combining and coordinating as well as improving the analytical tools themselves. Additionally interpretations from general knowledge about water chemistry and toxicity may be used to create statistically based models, which may describe the pollution status in waters more accurately than at present. Hopefully future technology will improve in a way that makes metal waste recirculation and minimizing more beneficial and natural resource exploitation more efficient. Advanced tools to calculate precisely just how far one can go before nature ecosystems become injured may then hopefully not be necessary.

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Pictures

Figure P.1: The mine Olavsgruva; tailings originating from these shafts are the main contributors to metal pollution in Hitterelva.

Figure P.2: About 12 million kg of Cu have been extracted from Olavsgruva, and it covers an area of 0.13 km^2, 30-50 m underground the mountain surface.

Figure P.3: Mine residuals from mine Fjellsjøgrua; Zn from this little heap places the water quality in lake Lille Fjellsjøen and the stream Naustebekken under the classification "poor" according to a Norwegian classification system (Holtan and Rosland 1992).

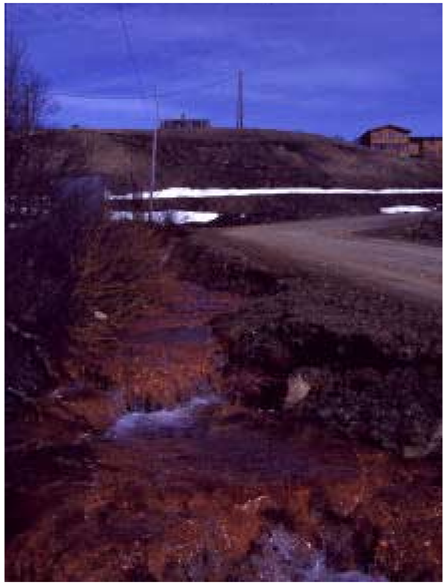

Figure P.4: Water running out from the Bjørgåsen mining area; the stream bed and even the small bushes above it are completely beige-coloured by ochre precipitate ("yellow-boy").

Figure P.5: Mine tailings from the mine Muggruva. On melting of the snow covering these heaps, weathered metal sulfides which are accumulated during the winter are flushed out to the stream Rugla.

Figure P.6: The author in front of mine tailings at Muggruva.

Figure P.7: About ten years ago mine tailings from the mines Arvedalen and Kongensgruva were levelled out over an area covering ~0.12 km^2 to reduce access of water and oxygene. This was done to reduce weathering and metal transport to the streams Orva and Glåma, but so far reduced metal concentrations have not been observed.

Figure P.8: On the other side of the small valley lies the mining area “Christanus Sextus”, which Johan Falkberget wrote about in his internationally recognised triology with the same name. The status of the mining area as a cultural treasure has so far been considered more important than environmental impacts from the heaps, and huge amounts of metals are continuously released to lake Orvsjøen and river Orva.

Figure P.9: Before environmental protection became an issue, roads were occasionally built on residuals from mining waste. These small rocks are coloured reddish-brown by iron seeping from such a road. In the background is shown a phenomenon frequently covering polluted sites around Røros; black moss.

Figure P.11: Residuals from the smelter in Røros town (there are three people on top of this heap).

Figure P.10: This little stream runs from Muggruva residuals, and contains 1.3 mg Cu/L. The green coating on stones in the stream bed possibly contains Cu precipitates.

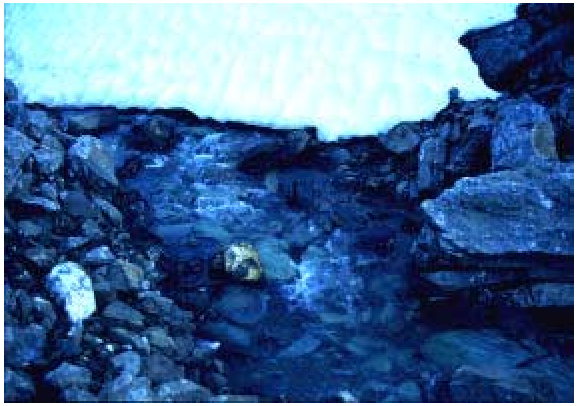

Figure P.12: Several hours of ice crushing at low river discharge in stream Røa were supposed to secure easy withdrawal of the dialysis equipment when the spring flood started. Here however is the result of one out of many subsequent surprises; the rope connecting the dialysis bags to land froze 20 cm into massive bottom ice. New hours, this time without sweating...

Figure P.13: The Naustebekken sampling site, located at the outlet of lake Lille Fjellsjøen. The iron pipe holds the pressure sensor for river discharge measurement. The mine is located on the hill behind the tree.

Figure P.14: Lake Lille Fjellsjøen from the hill shown on Figure P.13. The trout does not seem to take much notice of the high concentrations of Zn and Cd introduced along the marshy tract on the right side of the lake; locals claim that the outlet of this mine polluted brook is one of the best fishing places in the lake. In the background is Orvsjøen, almost completely dead by ~180 µg Cu/L. Aquatic organisms are however much more sensitive to most Cu pollution than man; one would have to drink more than 10 L of Orvsjøen water to achieve the Cu load of a vitamin pill.

Figure P.15: The outlet of Lille Fjellsjøen at early spring. Three to four kilometres of skiing were required to reach the site; unfortunately the weather was not always as nice as shown here.

Figure P.16: A stone in stream Hitterelva sheltered the dialysis bags.

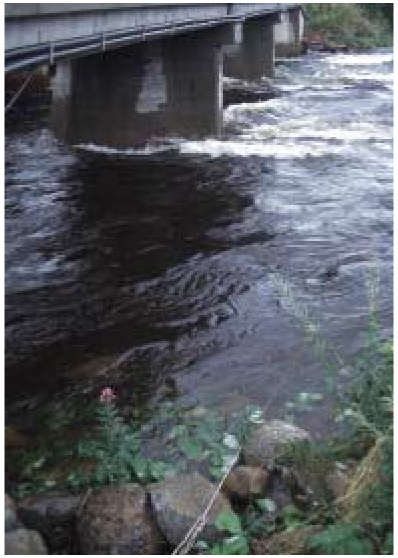

Figure P.17: The river discharge varied extremely in Gaula, and to place the dialysis bags at the right spot in this river could be a challenge. Here the dialysis equipment in the end of the rope got rough treatment by the spring flood. At some incidents one, two, or all dialysis bags were punctured and made useless due to flood incidents.

Figure P.19: At early spring flood the ice started to break up. It is not easy to secure dialysis bags and ropes against 50 m^2 ice flakes in a wild spring river.

Figure P.18: The same site as P.17 a few weeks later. Securing the equipment by placing it close to the riverbank caused better shelter at rising floods, but if the discharge suddenly was largely reduced, as here, the dialysis bags would equilibrate with fresh spring air instead of the river water.

Figure P.20: Rugla during flood ebb. Most of the snow had melted at this stage; the river discharge data logger (upper left corner) was placed at knee height two months earlier. It might have been be a better idea to set out such equipment during summer or autumn. It was very difficult to locate suitable sites below the snow-cap.

Figure P.21: At this incident the equipment was stuck on the river bottom in Glåma and had to be fetched "manually".

Figure P.23: The lower 20 cm of the snow-cap covering river Orva were completely red and extremely up-concentrated on Cu compared to the river water.

Figure P.22: Orva at intermediate flood. The river bed was completely reddish-brown by rust deposits. The water was very green and non-transparent (not visible on the picture)

Figure P.24: The little brook from the Bjørgåsen mines. The sediment deposits on the banks witness that the discharge was considerably higher when the equipment was placed out. The dialysis bags inside this bottle were not useable.

Figure P.25: The dialysis bags were transported in ~8-L bottles filled with deionized water. This is the Glåma River.

Figure P.26: The dialysis bags have been equilibrated with the river water for a week and are being pulled in for sampling and replacement.

Figure P.27: The (left) plastic bottle that protected the dialysis bag in the river was tied to a stone-filled potato bag connected to land with a rope.

Figure P.28: Drilled holes on the top and bottom of the protecting bottle ensured that water could flow through. Thin wires were tied to one end of the bags, and a knot was made on this wire ~2 cm over the bag. After having collected the old dialysis bags, the corresponding knot on the new bags was placed in one of the small slits made with a knife on top of the bottle threads. Screwing the bottle top on then sealed the wires nicely and kept the bags hanging vertically within the bottle.

Figure P.29: Raw power brings the dialysis equipment in for a new week of equilibration.

Figure P.30: Traditional 0.45 µm pore size filtration.

Paper 1

Paper I is not included due to copyright.

Paper 2

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Metal accumulation and metallothionein in two populations of brown trout, *Salmo trutta*, exposed to different natural water environments during a run-off episode

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Abstract

Cd, Cu and Zn concentrations were measured in ambient water as well as in gills, liver and kidney tissues of two natural populations of brown trout (*Salmo trutta*) during a run-off episode in two rivers with different metal compositions due to mining pollution. Metallothionein (MT) was also measured in these tissues. The two rivers, Rugla (Cu contaminated) and Naustebekken (Cd and Zn contaminated), are located in two neighboring drainage basins separated by the topographic divide near the city of Røros in the County of Sør-Trøndelag, Norway. In Rugla, the Cu concentration increased from 15 μg/l at the low water level to 41 μg/l during the run-off episode. In Naustebekken, corresponding values for Cd were 90–170 ng/l and those for Zn were 49–91 μg/l. Gill concentrations of Cu and Cd/Zn MT in both populations of native trout clearly reflect the presence of these metals in the rivers during the run-off, in accordance with the hypothesis of protection caused by MT induction. When Rugla trout were transferred to Naustebekken and vice versa, both the amounts of MT itself and the Cu contents reflected the concentration of this metal in the new environment, indicating that MT induction also protects against acutely increased metal levels. The measured levels of MT in both native and transferred trout can account for all the Cd present in the tissues, but not for all of the Cu and Zn. The capacity of MT to regulate Cd and Cu in the trout populations in their natural habitat therefore seems clearly present. Our data also indicate that the MT I and II isoforms may bind metals selectively.

Keywords: Brown trout; Cadmium; Copper; Zinc; Metallothionein; Run-off episode

1. Introduction

High levels of trace elements in freshwater environments may occur as a result of natural weathering of minerals in sediments and bedrocks or as

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a result of anthropogenic activities such as mining. Rugla and Naustebekken are two subalpine rivers belonging to two different catchments in the County of Sør-Trøndelag, Central Norway; the distance between them is less than 10 km. Rugla is part of the drainage basin Gaula running northward to the Trondheimsfjorden, while Naustebekken is part of the drainage basin Glomma running southward to the Oslofjorden (Fig. 1). Along Rugla, several Cu mines are located, the most important being Muggruva. This Cu-sulfide ore was mined between 1770 and 1919, but oxidation of mine tailings still contributes heavily to the elevated river Cu load. A Zn-blende ore (Fjellsjøgruva), mined during a test period between 1953 and 1956, is the important source for the elevated levels of Cd and Zn in Naustebekken. In spite of the nearby location and therefore being affected by the same weather conditions, the metal compositions of water from Rugla and Naustebekken rivers are quite different.

Brown trout (*Salmo trutta*) is one of the most common fish species in Norwegian streams and lakes, and the only species present in the rivers studied in this article. There has been no attempt to stock these rivers with non-native populations of brown trout. The investigated area gives a good opportunity to study responses and possible adaptations of local fish populations to distinct metal environments.

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 (Spry and Wiener, 1991). Fish inhabiting such environments have to adapt to these conditions and must tolerate sudden bursts of elevated metal concentrations. In fish, the most vulnerable organ to acute exposures is thought to be the gills (McDonald and Wood, 1993). 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 (Handy and Eddy, 1990). Liver and kidney are vulnerable organs during prolonged metal exposures, both from waterborne and dietary sources (Spry and Wiener, 1991). 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 (McDonald and Wood, 1993). A short pre-exposure to sublethal concentrations of these metals can confer protection against subsequent exposures in freshwater fish (McCarter and Roch, 1983; Bradley et al., 1985; Klaverkamp and Duncan, 1987). This may be accomplished by a mobilization of the biosynthetic apparatus so that more metallothionein (MT) can be produced in response to the second challenge (Roesijadi, 1996). Metallothioneins are considered to be involved in the regulation of essential metals such as Zn and Cu, but they also bind Cd and Hg (Cherian and Chan, 1993). The spill-over hypothesis states that toxic action of metals will take place only after the metal binding capacity of the metal binding proteins in the cells is fully exploited (Hamilton and Mehrle, 1986). Induction of MTs in fish after acute exposures to high concentrations of waterborne metals has been thoroughly documented (Roesijadi, 1992). Metallothionein induction has been shown in gills, liver and kidney, both after waterborne and dietary exposures (Spry and Wiener, 1991).

Populations of brown trout, which have sustained generations in environments with relatively high concentrations of metals, are expected to evolve toxicological resistance to both essential and nonessential metals due to natural selection. If MTs are involved in toxicological resistance, trout of such populations should induce synthesis of such proteins according to the metal environment they inhabit. However, few data exist to support this hypothesis.

In this article, it has been shown that run-off episodes with increased metal levels in the water in the two rivers caused accumulation of Cd, Cu and Zn in the gills, liver and kidney of native trout. Since the two rivers have a high Cu content, and salmonid species in general accumulate considerable amounts of this metal, both Cd/Zn and Cu MT in the tissues were determined by Cd-saturation assays. Isoforms of MT in gill tissue, developed during the episode of increased

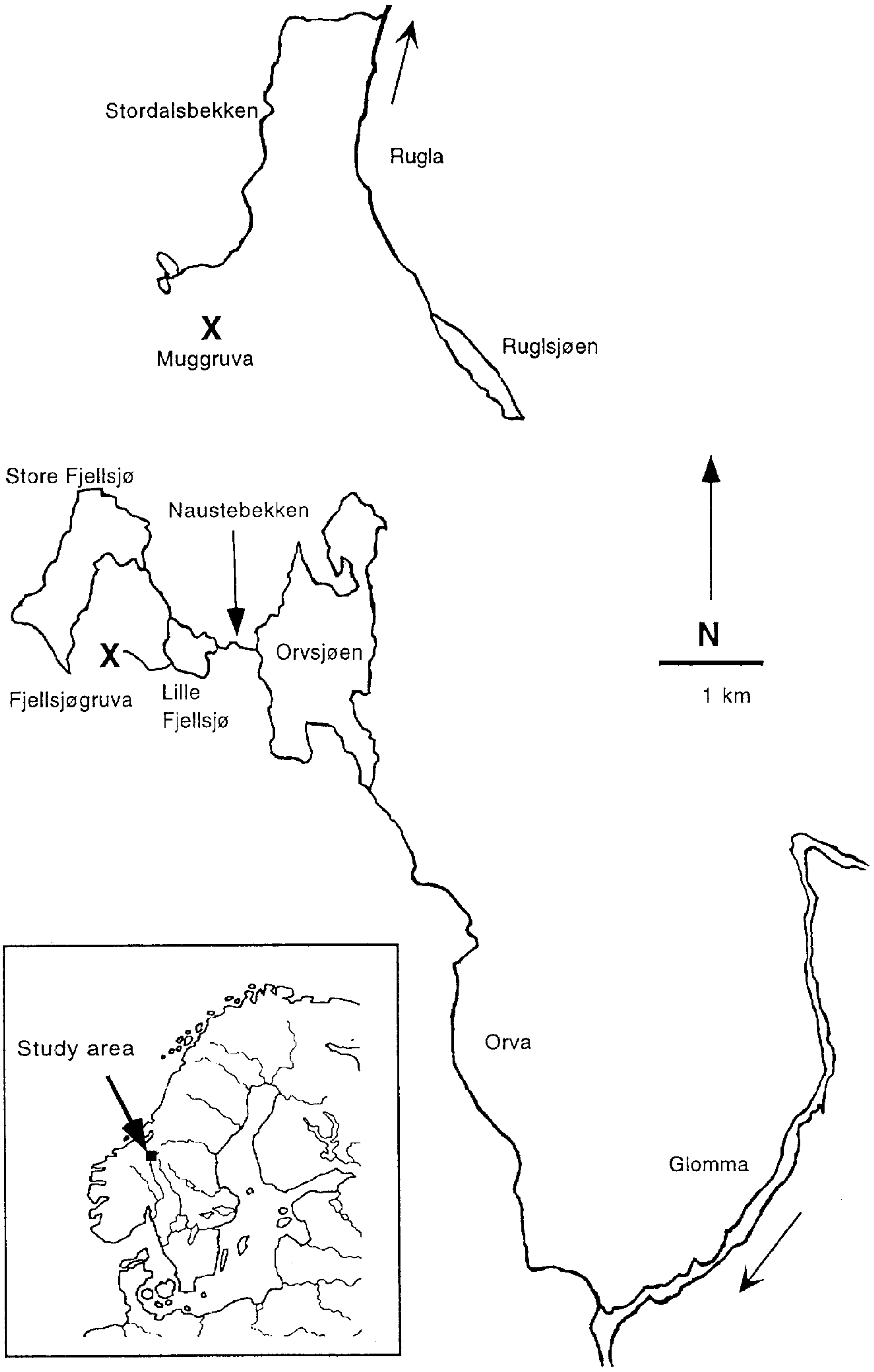

Fig. 1. Map showing the locations in Norway of the Rugla and Naustebekken rivers.

metal exposure, were also isolated, and bound metal determined. Such studies were also performed with trout transferred and kept in cages in the other river during the run-off episode. In this way, responses in both populations to the different metal environments in the two rivers could be studied.

2. Methods

2.1. Field experiments

The field experiments were performed in September and October 1997. Brown trout were captured by electric fishing and in traps. Trout were both kept in flow-through container systems in their native river and transferred to the other river. All trout were kept for at least 5 days, mostly 7–8 days, in the transferred location until sampling. The trout were not fed during this period, although some food items may have slipped into the flow-through water system. The containers contained approximately 80 l water, and the flow through the system was never lower than 10 l/min, ensuring sufficient oxygen in the containers. The water temperature during the experimental period ranged from 5.9 to 9.8°C. The containers were covered by nets and tarpaulins, to exclude the possibility of rain altering the water chemistry in the tanks. The trout were killed by a blow to the head, immediately frozen on dry ice, and transported to the laboratory within 3–5 h. They were stored at −80°C before further processing. Due to the low density of trout in these rivers, we were not able to capture as many as planned. For this reason, we had to combine trout of different age classes within each group of trout. They were divided into four groups, as described later in Table 2.

2.2. Water chemistry

Water samples for metal determination were taken before and during the run-off episode. The polyethylene bottles used for sampling were routinely washed in a washing machine, soaked in 7 M HCl, rinsed in water, soaked in deionized water and finally filled with 0.1 M HNO_3. For water sampling, the bottles were refilled with river water three times before the sample was collected. Concentrated HNO_3 was then added to 0.1 M concentration. These samples were analyzed for metals by flame (Ca, Zn) or graphite furnace (Cd, Cu) atomic absorption spectrophotometer (Perkin Elmer 5100 AAS). The pH measurements were carried out by a Radiometer PHM 80 portable pH meter, alkalinity by a Hach Digital Titrator Model 16900, and conductivity by a Radiometer CDM 80 Conductivity Meter. The measurements were performed at low water level at the end of August and compared with corresponding measurements prior to and during the run-off episode at the beginning of October that year (Fig. 2).

2.3. Metal quantification

After thawing, gills, kidney and liver were dissected out, washed in ice-cold distilled water to remove blood remnants and kept on ice. The filaments were cut off from the gill arcs before further processing. For direct metal quantification, the wet weights of tissue samples were determined. After lyophilization for 24 h, the dry weights were obtained. Tissue samples were digested in 65% HNO_3 (Suprapur; Merck), boiled and finally diluted to 0.1 M HNO_3 before metal analysis. Metal concentrations in tissue samples were determined with background correction on a Perkin Elmer 2100 AAS, equipped with graphite furnace (Model HGA-700) and autosampler (Model AS-70). Once for every tenth sample, resloping was performed (with two internal standards), and values within 10% from the long-term average of the standard solutions were accepted. Quality assurance for metal analysis was achieved by the use of standard metal solutions of Cu and Zn (Spectrosol; BDH Laboratory Supplies, Poole, UK), and Cd (Spectroscan; Teknolab A/S, Drøbak, Norway), as well as with standard reference material (Bovine liver SRM 1755b; National Institute of Standards and Technology, Gaithersburg, MD, USA).

2.4. MT quantification

After thawing, gills, liver and kidney samples were transferred to ice-cold deoxygenated 1:4 w/v 5 mM Tris–HCl (pH 8.5) and homogenized with a Glas-Col Homogeniter (Potter Elvehjem) (Suzuki and Sato, 1995). Then, 5 mM 2-mercaptoethanol was added to avoid oxidation of MT. Homogenates were centrifuged at 12 000 × *g* for 10 min, and supernatant aliquots kept at −80°C in cryo tubes before further use. The Cd-chelex assay was used to quantify Cd/Zn MT (Bartsch et al., 1990). High molecular weight proteins in the supernatants were denatured by treatment with acetonitrile, thereby avoiding heat treatment, and excessive amounts of added Cd bound to Chelex-100. After centrifugation, remaining ^{109}Cd in the supernatant solution was measured. The concentrations of total MT (Cd/Zn MT plus Cu MT) were determined by the thiomolybdate assay (Klein et al., 1990). In this case, Cu was removed from MT by ammonium tetrathiomolybdate; thereafter, excessive tetrathiomolybdate and its complexes were removed with DEAE-Sephacel. The apothionein was then saturated with Cd, and excessive Cd removed by Chelex-100. After centrifugation, the ^{109}Cd bound to MT in the supernatant solution was then measured. The concentrations of MT could be determined by assuming its molecular weight to be 7000 and a molar ratio of 7 gram-atoms of Cd per mole of protein. The assumption that total MT minus Cd/Zn MT equals Cu MT is considered valid, since other metals able to induce and bind to MT in the trout were only present in the rivers in very low amounts, as measured by inductively coupled plasma mass spectrometry and neutron activation analysis (unpublished results). To test the linearity of the Cd-saturation assays, dilutions of the commercial MT from Sigma (MT rabbit liver, lot 56H9500) were used. MT samples isolated from Naustebekken trout liver were also used, and linearity was found down to approximately 1 μg MT/g fresh weight of tissue for the Cd-chelex assay and to 10 μg MT/g fresh weight for the thiomolybdate assay. At Cd/Zn MT concentrations above 150 μg MT/g fresh weight, the Cd-chelex assay underestimated calculated values.

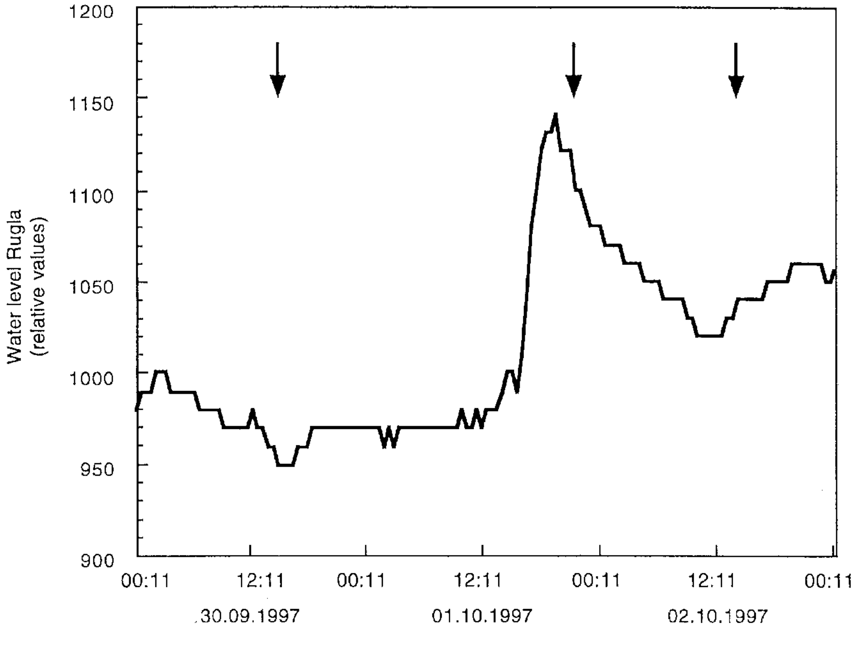

Fig. 2. Relative water levels as measured by a pressure sensor every 30 min before and during the run-off episode in the Rugla river. The abscissa shows date and time of day. The arrows indicate when the water samples for limnochemical measurements (given in Table 1) were taken. The collection of water samples under low water conditions on 28 August 1997 is not indicated.

This underestimation was found to be 9% for the rabbit liver sample of 500 μg MT, calculated as the deviation from the regression line for eight samples between 1 and 500 μg MT (each measured three times). The linearity of the thiomolybdate assay was found satisfactory throughout the measured value range. The errors of reproducibility for the Cd-chelex and the thiomolybdate assay were less than 4 and 10%, respectively.

2.5. MT isoform separation

For isoform separation, homogenates were heat-denatured for 2 min at 80°C and centrifuged at 12 000 × *g* for 10 min. Supernatant aliquots were stored at −25°C before chromatographic separation. The supernatants were applied to a Sephadex G-75 column (1.5×35.0 cm^3) and eluted with 5 mM Tris–HCl buffer (pH 8.5) at a flow rate of 42 ml/h. Absorption at 254 nm was simultaneously read with a Pharmacia LKB 2138 Uvicord S UV-monitor. The MT-containing fractions were pooled and applied to a DEAE-Sepharose fast flow column (1.5×15 cm^3). Fractions containing the MT isoforms were eluted with a linear gradient of deoxygenated 5–500 mM Tris–HCl (pH 8.5) at a flow rate of 90 ml/h. Metal concentrations in the fractions were determined by atomic absorption spectrometry (AAS) as already described. Most samples were aspired directly without dilution, otherwise they were diluted in 0.1 M HNO_3. This technique for isoform separation is in accordance to that previously used in our laboratory for preparation of MT from rat liver after Cd and Zn injections to be used for standard purposes, as described by Andersen and Daae (1988). Positive identification of Cd and Zn MT I and II isoforms was then performed by the use of both polyclonal antibodies and autoradiography. These rat MT forms have been run in parallel experiments for comparison and identification of trout MT I and II isoforms.

2.6. Statistical analysis

Statistical significance of differences between the two trout populations was tested with the Mann–Whitney U-test. This nonparametric test was used because of the low number of data points for each group. Since we had to combine trout of somewhat different age classes in some groups, a Gaussian distribution of the data could not be assumed. A *P* value lower than 0.05 was considered to be significant.

3. Results

After the dry summer, the total concentrations of Cd, Cu and Zn in Rugla and Naustebekken rivers were generally low compared with the levels observed during the fall precipitation period (Table 1). In the area, rainfall started about 12:00 h on 1 October 1997, and reached a peak between 15.00 and 16.00 h the same day, leading to a run-off maximum at approximately 19.00 h in the Rugla river (Fig. 2). In Naustebekken river, the run-off peak occurred a few hours later, because of the delaying effect of the lake Lille Fjellsjø, located between Naustebekken and the mine tailings at Fjellsjøgruva (Fig. 1). Due to this rain episode, the concentrations of Cd, Cu and Zn increased in both rivers, most dramatically in Rugla, where the concentration of Cu more than doubled. In Rugla, the conductivity, alkalinity and the total concentration of Ca decreased slightly during the run-off episode, while these parameters did not change in Naustebekken.

The trout captured in Naustebekken were generally larger than those from Rugla. The sizes of the investigated four groups of trout in terms of body mass were as follows (Table 2): Rugla trout in Rugla water (RR), 39 ± 20 g; Rugla trout transferred to Naustebekken water (RN), 48 ± 41 g; Naustebekken trout in Naustebekken water (NN), 137 ± 61 g; and Naustebekken trout transferred to Rugla water (NR), 36 ± 49 g. Table 2 also shows the total concentrations of Cd, Cu and Zn in gills, liver and kidney in the trout groups. Generally, the highest metal concentrations were found for the population inhabiting the river with the highest corresponding metal concentration. In the kidney, the highest Cu concentration was found in trout from Naustebekken, which has the lowest Cu concentration in the water.

Table 1
Limnochemical data and total metal concentrations of water from Rugla and Naustebekken rivers[a]

River	Water level	Date (1997)	pH	Conductivity (µS/cm)	Alkalinity (µEq/l)	Ca (mg/l)	Cd (ng/l)	Cu (µg/l)	Zn (µg/l)
Rugla	Low	28 August	7.3	56.7	390.0	6.8	19.0	14.8	5.5
	Medium	30 September	7.2	42.2	270.0	5.0	24.0	21.4	8.8
	Run-off	1 October					25.0	41.2	8.8
	Run-off	2 October	7.2	38.7	240.0	4.6	21.0	24.3	9.3
Naustebekken	Low	28 August	7.2	20.9	120.0	2.2	90.0	3.3	48.6
	Medium	30 September	7.2	19.6	120.0	2.2	170.0	4.4	91.0
	Run-off	2 October				2.4	150.0	3.6	80.0
	Run-off	2 October	7.0	20.5	120.0	2.2	170.0	4.4	91.1

[a] Water samples were collected at low water level in August, and before and under a run-off episode in October 1997.

Table 2
Tissue concentrations of Cu, Zn and Cd (μg/g tissue fresh weight) in four groups of brown trout (*Salmo trutta*) from Rugla and Naustebekken rivers ($n = 6$)[a]

Tissue	Metal	RR	RN	NN	NR
Gills	Cd	0.55 (0.48/0.64)	0.59 (0.54/0.60)	2.34 (1.94/3.59)	3.45 (3.15/3.74)
	Cu	1.91 (1.72/2.04)	1.68 (1.50/1.97)	1.51 (1.18/1.68)	2.63 (2.17/3.15)
	Zn	152.97 (139.20/181.98)	146.45 (142.81/150.99)	473.73 (380.25/503.43)	450.91 (405.19/503.77)
Liver	Cd	0.71 (0.69/1.00)	0.74 (0.65/0.87)	6.95 (6.05/7.79)	7.71 (7.08/8.17)
	Cu	200.08 (199.00/309.76)	305.59 (213.12/335.25)	86.99 (59.67/146.47)	149.34 (120.17/192.89)
	Zn	33.33 (30.30/34.40)	27.60 (26.61/30.29)	54.01 (39.50/98.79)	85.36 (67.44/113.11)
Kidney	Cd	1.17 (1.03/1.19)	1.35 (1.15/1.61)	15.45 (11.44/17.41)	18.32 (16.72/21.32)
	Cu	2.13 (2.08/2.30)	2.48 (2.29/2.66)	4.62 (3.82/5.11)	8.10 (7.50/8.50)
	Zn	67.20 (55.66/82.08)	63.58 (53.18/72.26)	195.76 (133.62/273.55)	296.50 (274.06/337.71)

[a] Values are 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.

In all tissues, native Rugla trout (RR) contained significantly less Cd/Zn MT than native Naustebekken trout (NN) ($P < 0.001$) (Fig. 3). Native Rugla trout contained significantly more total MT (Cu + Cd/Zn MT) in gills than native Naustebekken trout ($P < 0.05$). Thus, in the gills of native trout from both populations, the kind of metal bound to MT reflects the concentrations of metals in the rivers. In liver, however, Naustebekken trout contained significantly more total MT than Rugla trout ($P < 0.001$), while the difference found for kidney was not significant. Also included in Fig. 3 are gill and liver MT measurements of trout from the two rivers captured at low water levels in 1996 and 1997 (previously unpublished). Compared with these measurements, the concentrations of both Cd/Zn MT and total MT in gills and liver of Naustebekken trout (NN) were elevated two to four times during the run-off episode. A 12-fold increase in total MT concentration in gills of Rugla trout (RR) was found after the run-off episode, while only a two-fold increase was seen in liver. Therefore, induction of MT seems to play an important role in trout metal homeostasis in contaminated rivers, since the levels of these proteins obviously fluctuate throughout the year.

The transfer experiments did not lead to significantly altered Cd and Zn concentrations in gills, liver or kidney of either population (Table 2). In Rugla trout moved to Naustebekken water (RN), no significant differences could be seen in Cu concentrations in gills, liver or kidney either. On the other hand, when Naustebekken trout were moved to Rugla water (group NR), with nine times more Cu, a significant increase in Cu concentration in the gills was found ($P < 0.01$). In the liver, no significant Cu increase could be seen, while there was a significant increase in kidney ($P < 0.01$).

In Rugla trout (RN), no significant differences in either Cd/Zn MT or total MT concentrations were found after the transfer to the Cd/Zn-rich Naustebekken water (Fig. 3). On the other hand, the concentrations of Cd/Zn MT decreased significantly in all tissues of Naustebekken trout (NR) moved to the Cu-rich Rugla water ($P < 0.05$). An increase in total MT concentrations was found in all tissues, but significant only in gill tissue ($P < 0.001$). The levels of Cu MT in gills, liver and kidney of Naustebekken trout moved to Rugla water approached those levels found in native Rugla trout (RR).

Two separate metal binding proteins were found in gills of native Rugla and Naustebekken trout after the ion exchange experiments (Fig. 4A,B), called MT isoforms I and II. This identification of trout MT isoforms was based on metal content and on comparison with rat liver Cd and Zn MT I and II isoforms run in parallel experiments (Fig. 5 and Table 3). Measurements with AAS revealed high levels of Cu both in the so-called a-form and in MT I in native Rugla trout. In spite of excessive Cu presence, the identification of the MT isoforms was still possible because of selective affinity to radioactive ^{109}Cd (data not shown) and to elevated levels of Cd in the corresponding peaks (Fig. 4). When Rugla trout were moved to the Cd/Zn-rich Naustebekken water (Fig. 4C), more Cd was bound selectively to MT II. No clear corresponding patterns could be found for Cu and Zn.

In native Naustebekken trout (Fig. 4B), MT II in gills contained more Cd than MT I, and the amount of Cd in the MT II form was found to be much higher than for the corresponding MT II form of native Rugla trout. The amount of Cu in the MT II form was also very high compared with that found in Rugla trout. Relative large amounts of Cu were associated with the a-form and towards the end of the column, but a broad peak was also found between the MT I and II peaks (Fig. 4B). Elevated levels of Zn were found in MT I but especially in MT II. Moved to the Cu-rich Rugla water, Naustebekken trout developed a somewhat different metal binding pattern in its isoforms (Fig. 4D). The amount of Cd decreased in both MT I and MT II forms. A marked reduction in the level of Cu bound to MT II was also found, concomitant with a general reduction of metals found in the eluted fractions. A large increase in Zn binding to MT II occurred. This element also appeared in a new peak called Zn I, which eluted in front of MT I. The origin and composition of this peak were not investigated further.

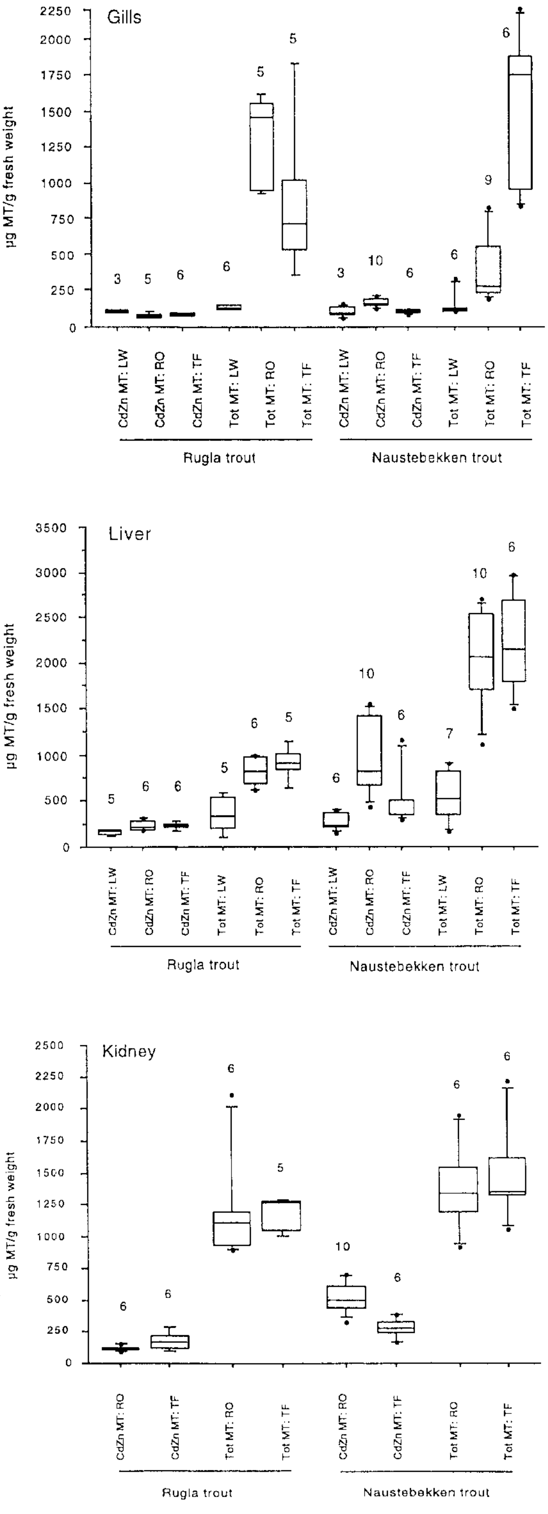

Fig. 3.

Fig. 3. Site-specific differences in metallothionein concentrations (µg MT/g fresh weight) in gills, liver and kidney of groups of brown trout (*Salmo trutta*) from Rugla and Naustebekken rivers. Cd/Zn MT was determined by the Cd-chelex assay, while total MT was determined by the thiomolybdate assay. The Cu MT concentrations equal the concentrations of total MT minus the Cd/Zn MT concentrations. Values are given as medians, quartiles and range (n = numbers in box-plot). LW, Low water; RO, run-off; TF, transferred. Trout captured at low water were not quantified for kidney MT.

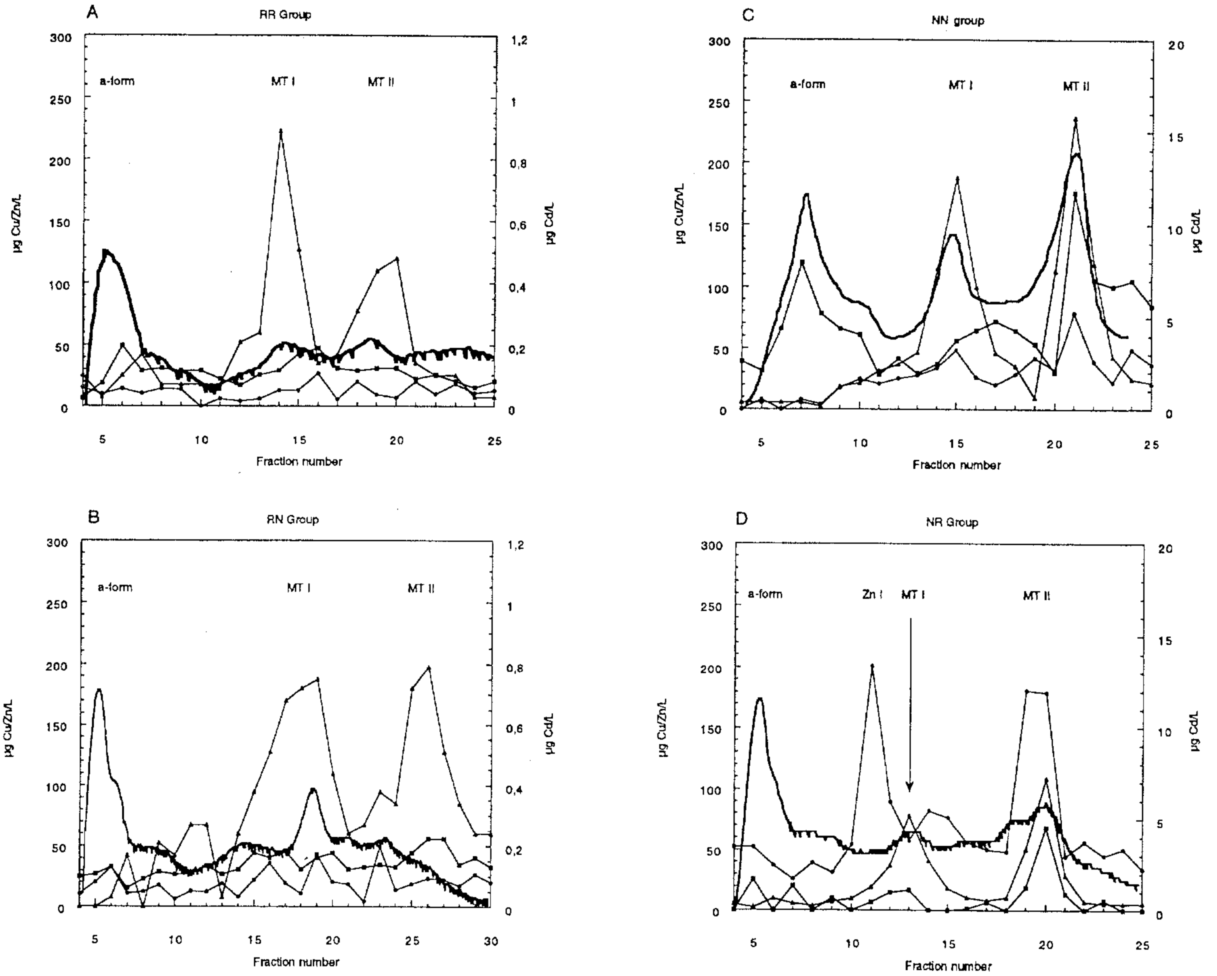

Fig. 4. Sepharose fast flow anion exchange chromatography of pooled Sephadex G-75 separated gill supernatant samples of brown trout (*Salmo trutta*) from Rugla and Naustebekken rivers. (A) Native Rugla trout, (B) transferred Rugla trout, (C) native Naustebekken trout, and (D) transferred Naustebekken trout. Relative absorbance at 254 nm (whole line, axis not shown) and metal concentrations (µg/l) are shown in eluted fractions: Cd (▲), Cu (■), Zn (●). Note the different Y2 axis in the figures.

4. Discussion

4.1. Water chemistry

During the run-off episode, the concentration of Cu in the Rugla river was more than twice as high as the corresponding values at the low water level. For shorter periods, even higher concentrations may occur, since peaks in metal concentrations may extend for hours only. In Rugla, a drop in conductivity, alkalinity and total Ca concentration could be seen during the episode, while pH remained constant. Changes in these parameters could not be seen in Naustebekken, probably due to the dilution effect of the lake Lille Fjellsjøen (Fig. 1). Metal contamination in streams often falls sharply with distance from the source (Caritat et al., 1996). Therefore, the trout in Rugla might be affected by the metals to various degrees according to their location in the river, depending on migrations and local behavior. Metal pollution from mine tailings in Naustebekken first enters Lille Fjellsjøen, making up a more defined habitat for that location. But even here, migration from

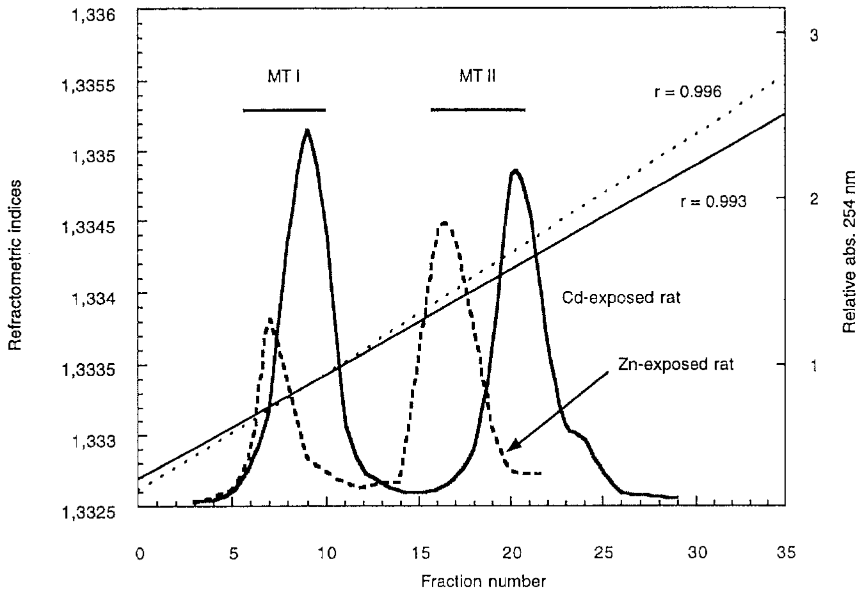

Fig. 5. Sepharose fast flow anion exchange chromatography of pooled Sephadex G-75 separated liver supernatant samples from Cd- and Zn-injected rats (*Rattus norvegicus*) (Wistar, Hannover). Absorbance at 254 nm from Cd-injected rat (whole line) and Zn-injected rat (dashed line) are shown in eluted fractions. Regression lines show the buffer gradient measured by refractometry: dotted line, Zn-injected rat; solid line, Cd-injected rat.

nonpolluted upstream areas is possible. Some of the variation in metal concentrations and MT content found within each group of trout might therefore be explained by these variables. The monitored run-off episode was neither the first nor the largest in the time period. Five other heavy rainfalls led to water levels in the Rugla river that were higher. Therefore, the measured concentrations of metals and MT in trout tissue were also influenced by these episodes. Free metal ions are believed to be the most toxic species in freshwater environments (Mason and Jenkins, 1995). The dissolved fractions of the metals studied (as fractionated in situ by dialysis bags with a molecular weight cut-off of 10–20 kDa or obstructing particles smaller than 2.5–5.0 nm in diameter) were generally higher in Naustebekken than in Rugla (unpublished data). Additionally, in Naustebekken, the Ca concentration and, concordantly, alkalinity and conductivity were lower than in Rugla (Table 1). Naustebekken water may therefore be more toxic during acute exposures, since the metals seem to be more bioavailable in this river.

4.2. Metal concentrations in tissue

Our data show that the levels of Cd and Zn in gills, liver and kidney of brown trout reflect the concentrations in the water where they live. This was expected as metals are taken up continuously both via waterborne and dietary pathways. In the gills and liver, the Cu accumulation also follows

Table 3
Refractometric indices corresponding to gradient elution of four groups of trout (*Salmo trutta*) and Cd- and Zn-injected rat (*Rattus norvegicus*) (Wistar, Hannover) metallothionein I and II isoforms during anion exchange chromatography

Group	a-Form	Zn I	MT I	MT II
RR	1.3332		1.3347	1.3355
RN	1.3330		1.3344	1.3351
NN	1.3332		1.3343	1.3352
NR	1.3332	1.3338	1.3342	1.3350
Cd-injected rat			1.3335	1.3343
Zn-injected rat			1.3333	1.3340

this pattern. Unexpectedly, the kidney of the Rugla trout contains less Cu than Naustebekken trout. Copper may, however, be eliminated through hepatic processes such as direct clearance through lysosomes and bile excretion (Nieminen and Lemasters, 1996). It has recently been documented that hepatic elimination of Cu is stimulated in Cu-acclimated rainbow trout *Oncorhynchus mykiss* relative to nonacclimated trout (Grosell et al., 1998).

Naustebekken trout transferred to the Cu-rich Rugla water received significantly elevated Cu concentrations in gills and kidney. In the liver, the increase was not found to be significant at the $P < 0.05$ level. When Rugla trout were transferred to the Cd/Zn-rich Naustebekken water, neither an increase nor decrease in metal content could be detected. In the liver, this may partly be explained by Cu being tightly bound to MT and not allowing Cd and Zn to replace this metal, thereby preventing net accumulation. The observed differences between metal accumulation of the two populations after transfer must, however, be explained otherwise. Anyway, these results indicate that Naustebekken trout may accumulate metals more effectively than Rugla trout.

The use of trout of different size within each group may camouflage differences in metal concentrations in tissues, as suggested by the high standard deviations found within each group. In both populations of native trout, a small positive correlation was found between age and Cd content in liver and kidney, but not in the gills (data not shown). With the exception of the kidney of Rugla trout, a negative correlation between age and Cu concentrations was found in all examined tissues of the two populations, while for Zn it was not possible to detect any clear age-dependent pattern. Aged fish seem to accumulate more Cd in liver and kidney than juvenile fish, while the clearance rates for Cu seem to increase with age. Trout of variable age cannot be the main explanation for the observed differences in metal accumulation after the transfer, as the mean weights of the transferred trout groups were equal.

4.3. MT content

The concentrations of MT measured by the Cd-chelex and thiomolybdate assays after the run-off episode are relatively high compared with earlier measured levels in salmonid fishes (Hamilton et al., 1987; Olsson et al., 1989). In Cd-rich environments in Austria, Dallinger et al. (1997) reported Cd/Zn MT concentrations up to 500 μg/g fresh weight in liver of arctic char *Salvelinus alpinus*, by using a modified Cd-chelex assay similar to the method used here. With differential pulse polarography method, however, Roch et al., (1986) measured total MT concentrations up to 2000 μg/g fresh weight in wild rainbow trout from a severely polluted river in Canada. This is more in line with the levels found in the present work. It should, however, be kept in mind that our methods for measuring quantitative MT levels do not involve heat treatment.

In native Naustebekken trout, the concentrations of Cd/Zn MT in gills were threefold higher than in native Rugla trout, while they were about fourfold higher in liver and kidney. Thus, the data nicely reflected the elevated levels of Cd and Zn in Naustebekken. Whereas the concentrations of total MT (including Cu MT) in gills were about fourfold higher in Rugla trout, the liver concentrations were less than one-half of that in Naustebekken trout. Therefore, in gills, but not in liver, the total MT levels in native Rugla trout reflected the elevated levels of Cu in this river.

In gill and liver tissue, a marked increase in total MT concentrations was found in both populations, when total MT levels were compared in native fish from the same river at low water levels and after the run-off episode. After transfer to the other river, Cd/Zn MT and Cu MT concentrations in the gills changed in both populations towards levels suggested by the metal content in the new environment. Our data suggest that the turnover of MT in gills is fast, 7 days of exposure to elevated environmental levels of waterborne metals may lead to a fourfold increase (Fig. 3). The most pronounced change was the marked increase of Cu MT in Naustebekken trout gills after transfer to Rugla water. Therefore, Cu seems to be a potent trigger of MT induction in gills of brown trout on an acute scale. This is in contrast to earlier findings that Cu is a poor inducer of MT in fish gills (McDonald and Wood, 1993; Grosell et al., 1997). Also, in liver and kidney, the concentrations of Cu MT increased after an acute exposure to a Cu-rich environment, but this was not followed by an increase in total MT, rather a redistribution of metals occurred (Fig. 3). The trout might not have been transferred long enough for the total MT in liver and kidney to reflect the water chemistry. In any case, these results clearly indicate that MT in gills plays a part in a first defense against waterborne metals. Because of the substantial levels of MT present, it is reasonable to believe that the trout in these rivers really faces metal concentrations that may be trying or even toxic.

4.4. Gill MTs and metal binding

After gel permeation and anion exchange separation of gill tissue supernatants from Rugla and Naustebekken trout, Cd was found in two distinct peaks. MT I and MT II, in accordance to the general belief that tissues of at least some salmonid fishes contain two main MT isoforms as is generally seen also for other vertebrates (Bonham et al., 1987; Olsson and Hogstrand, 1987; Olsson et al., 1989). The trout and rat MT isoforms eluted at somewhat different ionic strengths of the Tris buffer gradient, as shown from the refractometric indices corresponding to their elution (Table 3), which may indicate protein differences between the species. It also appears from the rat data that the isoform elution in our separating system depends on whether Zn or Cd is bound to the protein. Metallothionein isoforms from liver of Zn injected rats eluted at a slightly lower ionic strength compared with those from Cd injected rats. In accordance, the MT II form in Naustebekken trout transferred to Rugla water, thereby gaining Zn by redistribution as shown in Fig. 4D, eluted at a lower ionic strength (Table 3 and Fig. 5). Also, the other MT isoforms from gill tissue in both populations eluted at lower ionic strength after transfer. This does not seem explainable from the available data, but may well indicate metal replacements.

A third peak found in both Rugla and Naustebekken trout gills, most probably corresponding to the so called a-form (Wilhelmsen et al., 1998), eluted in front of MT I. In gills of rainbow trout, Olsson and Hogstrand (1987) also found a peak that probably corresponded to the a-form in our study, but their form did not contain metals. Our a-form was found in all anion exchange separations. In native Naustebekken trout, this form contained only Cu. When these trout were transferred to Rugla water, most of the Cu was found in MT II. The a-form contained almost no Cu in this transferred group of trout. Due to the high proportions of Cu MT in Naustebekken trout after being moved to Rugla water, it was unexpected to find that the MT isoforms in these trout contained less Cu than the MT isoforms in native Naustebekken trout. The data may therefore suggest that the presently applied anion exchange chromatographic method is not adequate for the purification of Cu-containing MT isoforms, but other explanations may also be possible.

Our results (Fig. 4) show that a high proportion of Cd is bound to MT in gills of trout living in Cd-polluted water. This is also suggested by the metal MT stoichiometry evaluations presented later in this article. When Naustebekken trout were transferred to the Cu-rich Rugla water, Zn was not only redistributed to MT II, but also to a peak called Zn I (Fig. 4D), which eluted in front of MT I. It is possible that this peak represents a metal binding protein other than MT. This may be in agreement with the hypothesis that displaced Zn is the basis for MT induction at the gene expression level (Roesijadi, 1996), since the total MT concentration increased markedly in Naustebekken trout transferred to Rugla. Anyway these results suggest that also other proteins than MT are involved in handling of Zn in fish gills.

4.5. Environmental responses

Acclimatization to elevated levels of trace elements may be achieved either by altered uptake or elimination rates, or by sequestering and thus immobilizing metal ions in metal binding proteins in the cells. Protection against metal toxicity by induction of MTs related to the metal chemistry in waters has been suggested to be an important strategy by aquatic animals (Klerks and Weis, 1987; Mulvey and Diamond, 1991; Roesijadi, 1992). In gills of Rugla trout, the concentrations of Cu MT increased markedly after the run-off episode. Correspondingly Naustebekken trout Cd/Zn MT also increased compared with the low water levels (Fig. 3). Gill concentrations of Cu and Cd/Zn MT in both populations of native trout therefore clearly reflect the total concentrations of these metals in the rivers during the run-off, in accordance with the hypothesis of protection caused by MT induction. In liver, the elevated levels of Cu MT in native Naustebekken trout after the run-off episode may be due both to dietary uptake and to the higher concentrations of dissolved Cu in this river (data not shown). It has been suggested that metal-tolerant fish are less permeable to Cd and Zn, but more permeable to Cu, either as a result of enhanced mucus production or as a result of changed metal-binding properties of the gill surfaces (McDonald and Wood, 1993). Redistribution of Cu from gills to liver and kidney may explain why Naustebekken trout had higher levels of Cu MT in these organs after an increased waterborne exposure to this metal.

When Rugla trout were transferred to Naustebekken and vice versa, the metals bound to gill MTs strongly reflected the Cu concentration in the new environments (Fig. 3). The amount of gill MT itself also reflected the new environment. Induction of MT therefore also seems to be a protection mechanism against acutely increased metal levels. These data, however, do not seem to be in accordance with those of Grosell et al. (1997, 1998), who stated that the formation of gill MT in rainbow trout could hardly be among the primary mechanisms responsible for increased Cu tolerance. Our data in fact suggest that the formation of gill Cu MT could well be an important tolerance mechanism, since acute Cu exposure led to increased gill Cu MT concentrations being higher for trout originally exhibiting the lowest Cu concentration (Fig. 3). The data also suggest that tolerance to Cu may be achieved by mechanisms such as altered uptake and elimination rates, as supported by our accumulation data which showed that Cu was concentrated to a lesser degree in tissue of trout from the Cu-rich Rugla river.

The potential metal binding capacity of MT present in the tissues can be calculated, assuming a MT molecular weight of 7000 and a metal to MT binding proportion of 7:1. The results show that in gills of all four groups of trout (native and transferred), the measured MT concentrations can account for all of the Cd and Cu, but not for Zn in gill tissue. Cd/Zn MT can account for only a small fraction of Zn present in the gills, less than 3%, indicating that MT may have only a small impact on the overall Zn regulation in this tissue. Most of the Zn in gills could be found in the high molecular weight fractions after gel permeation chromatography with Sephadex G-75 (data not shown). Zinc originally bound in the gill surface layer might have been redistributed and bound to these proteins after homogenization and chromatographic separation. This metal is extensively present in functional proteins. In liver of Rugla trout, both native (RR) and transferred, the Cd/Zn MT can account for all of the Cd, but for less than 50% of the Zn; while in liver of Naustebekken trout Cd/Zn MT can account for all of the Cd in both groups and for over 86% of the Zn in native trout (NN), but account for less than 40% of the Zn in the transferred group of trout. The results also show that Cu MT can account for 79% of the Cu in NN trout, but for less than 20% in the RR trout, indicating that liver MT is less important in trout exposed to high environmental Cu concentrations. It has been suggested that one mole of MT may bind 12 monovalent instead of seven moles of divalent Cu (Winge 1991). The reported percentiles will then represent an underestimation of the potential Cu binding capacity of MT. The corresponding metal–MT stoichiometry for the kidney shows that MT can account for all of the Cd and Cu in the four groups of trout, while it can account for less than 20% of the Zn, suggesting that this metal is mainly bound to functional proteins and not to MT in this tissue. The results reported here strongly add up to the evidence that MT both have the potential and play an important role in metal regulation.

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Paper 3

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Paper 4

INFLUENCE OF pH AND TOC CONCENTRATION ON Cu, Zn, Cd, AND Al SPECIATION IN RIVERS

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Abstract - Dissolved (<~4 nm, dialysis *in situ*), colloidal (<0.45 µm filtered, minus dissolved) and particulate (total, minus <0.45 µm filtered) concentrations of Cu, Zn, Cd, (Al) in eight (three) mining polluted rivers were determined by atomic absorption spectrometry (flame and graphite furnace). The metal size distribution in the rivers was compared to pH, Ca concentration, alkalinity, conductivity, and total organic carbon (TOC). Data plots based on the present and other studies also yielded information about the interrelations between TOC, pH and metal adsorption in rivers and lakes. More than 90% of Cu, Zn, and Cd occurred in dissolved form in two rivers with average pH at 3.1 and 5.1, whereas 54, 79, and 79% of Cu, Zn, and Cd respectively occurred in dissolved form in six pH neutral rivers. In three pH neutral rivers, on average 55% of Al was bound to colloids, whereas the dissolved and particulate fractions were 21 and 23% respectively. Our data combined with data from similar studies support equilibrium models which suggest that the percent fraction of metals adsorbed on particles rises steeply from almost zero to almost 100 % within a narrow and element-specific pH range. Changes in TOC concentration seem capable of shifting the pH to % metal absorption curves in the order of one pH unit.

Keywords: dialysis *in situ,* metal, Cu, Zn, Cd, Al, speciation, pH, alkalinity, river, aquatic

INTRODUCTION

Local topography, hydrology, biology, and geology in catchment areas, as well as local precipitation levels and climate determine the wide range of water chemistry conditions observed in lakes and streams. In addition, anthropogenic activity may add considerable amounts of polluting compounds, which will influence and be influenced by the existing aquatic systems. Mining activity is one out of many anthropogenic contributors to water pollution. When metal sulfides in mining shafts, production waste, or low quality residuals are exposed to humidity and air, the metal sulfides may be oxidized, and dissolved metals and acidity will subsequently be released to downstream water systems (Stumm and Morgan, 1981; Evangelou and Zhang, 1995). Near such sources the metal concentrations may be several orders of magnitude higher and pH may drop by several units compared to natural levels (Lemly, 1994; Robb and Robinson, 1995; Ledin and Pedersen, 1996).

Metals such as Al, As, Cd, Cr, Cu, Fe, Hg, Pb, Tl, and Zn are frequently released in large quantities during or after mining activity and may lead to major destruction of aquatic ecosystems (Davies, 1983; Williams *et al.*, 1991; Salomons, 1995; Masnado *et al.*, 1995). According to previously published work on metal concentrations in the streams (Traaen *et al.*, 1988; Røe, 1991; Arnesen and Iversen, 1994; Gundersen *et al.*, 2001), and toxicity and water quality criteria (Holtan and Rosland, 1992; United States Environmental Protection Agency, 1998), a number of metals were expected to cause toxic responses to fish and other organisms in the local rivers; in particular Cu, Zn, Cd, and Al. Biological responses in two of the rivers have been investigated simultaneously with the present work (Olsvik *et al.*, 2000, 2001).

The toxicity of different organic and inorganic metal species to aquatic ecosystems has been reviewed by Luoma (1983). Among Cu species, Cu^{2+} and possibly $Cu(OH)^+$ were considered to be most readily absorbed in organisms, whereas $Cu(CO_3)(aq)$ and $Cu(OH)_2(aq)$ appeared far less bioavailable. Several studies cited by Luoma also revealed that organic Cu and Cd complexes were bioavailable, but to a lesser extent than the most toxic inorganic species. The theory that the free metal ions accounts for most of the metal toxicity (Free-ion activity model, FIAM), has been widely used in toxicity considerations (e.g. Sunda and Hanson, 1976; Vercauteren and Blust, 1996; Brown and Markich, 2000), although some studies and reviews have criticized or found exceptions to this generalization (Pagenkopf, 1983; Campbell, 1995; Campbell *et al.*, 2000). It should be emphasized that there are huge differences in metal tolerance and uptake between biological species (e.g., Williams *et al.*, 1991; Masnado *et al.*, 1995).

The pH level is probably the single chemical parameter influencing metal speciation most significantly. Adsorption on different particles and colloids may change from almost none at low pH to almost a 100 % a few pH units higher (Schindler *et al.*, 1976; Mouvet and Bourg, 1983; Allard, Håkanson and Karlson, 1986; Stumm and Morgan, 1996). With respect to the inorganic dissolved fraction of the metals, the H^+ ion will compete with the metals for ligands such as OH^-, Cl^-, CO_3^{2-}, HCO_3^-, HS^-, S^{2-}, sulfates and phosphates. Metal complexation will again influence on adsorption abilities and bioavailability of the metals.

Due to the complexity of organic matter in natural waters, its effect on metal speciation is difficult to predict. In general humic substances tend to enhance metal cation sorption on particles at low pH and reduce metal cation sorption at high pH, but many exceptions to this rule are reported (Zuyi *et al.*, 2000). Organic compounds may be found in considerable amounts both in the dissolved, the colloidal and the particulate fraction (Tanizaki *et al.*, 1985; Patel *et al.*, 1999; Thorsen, 2000), and to which of these fractions the metals will be attached is not easy to predict. Shafer *et al.* (1999) found a clear positive relation between the concentration of dissolved organic carbon (DOC) and the dissolved fraction of Zn, indicating that DOC may attach to metals and keep them in the dissolved fraction. Allard *et al*. (1986) also found only a few percent of Cd and Zn (1 and 3 % respectively) in the organic fraction extracted from suspended particulate matter (SPM) samples, indicating that these metals may not readily attach to high molecular weight organic materials. On the other hand roughly one third of Al and Cu were organically bound. As discussed below however, the organic content of water may probably, depending on pH, influence significantly the speciation of all metals investigated. Other chemical parameters will probably also influence metal attachment to organic material; it is e.g. well established that during mixing of fresh and salty waters, the partition of metal species between solution and suspended particles is controlled by two counteracting, non-biological processes: a. Removal from the dissolved fraction by precipitation/coprecipitation mechanisms or flocculation of humic and fulvic acid – metal complexes (Sholkovitz, 1976; Hoyle *et al.*, 1984), and b. Gain to the dissolved fraction by desorption mechanisms (Van der Weijden *et al.*, 1977; Li *et al.*, 1984). It is possible that the ionic strength variation one may find between rivers and temporally within rivers may cause a similar influence on the binding of metals to organic compounds in fresh waters.

Alkalinity too will influence the speciation of metals in water by the formation of metal - carbonate or bicarbonate species. Major metal cations in water, especially Ca, are believed to compete with trace metal ions for uptake sites on cell membranes (Pagenkopf, 1983; Campbell and Stokes, 1985). Shafer *et al.* (1999) reported that elevated filterable fractions (< 0.4 μm) of Al, Cd, and Zn in non-calcareous rivers were probably caused by higher SPM and metal concentrations at such sites. The examples above illustrate that many water chemistry parameters may influence trace metal speciation. Observed correlations between metal speciation and studied water chemistry parameters may thus have been more or less influenced by confonders; i.e. other parameters directly effecting on metal chemistry and covarying with the studied parameters.

In the present work the dissolved, colloidal, and particulate fractions of Cu, Zn, and Cd were studied in eight streams. The same fractions of Al were also studied in three of these streams. The sites were chosen to cover a wide range of pH levels. Metals considered most likely to reach toxic levels towards aquatic life and the chemical parameters considered to influence their toxicity most significantly (pH, Ca, alkalinity, conductivity, TOC) were studied. Particulate (>0.45 μm), colloidal (<0.45 μm, >~4 nm) and dissolved concentrations (<~4 nm) were determined, the latter fractionated by dialysis *in situ* (Beneš and Steinnes, 1974).

The bedrock of the present study area is dominated by low to medium degree metamorphic shales intruded by a great number of sulfidic ores (Goldschmidt, 1920). Mining activity in this area was among the most substantial ones in Norway one to two centuries ago, when Norway was an important mining nation. All mining activity in this area has now ceased, and different forms of remediation have reduced the most severe metal pollution to the rivers. The main criterion used for selecting the sampling sites was to cover a wide range of metal pollution levels, mostly at or near levels where the metal concentrations could be expected to be lethal to fish. In addition the selected streams differed in discharge, altitude and main water chemical characteristics.

The purposes of this study were: 1) to reveal how the organic content and pH together influence the dissolved/particulate metal concentration ratio in rivers and lakes. 2) To predict the % fraction of the metals one may expect to find as dissolved, colloidal and particulate in natural waters, and view metal speciation differences between sites with different pH, TOC, alkalinity, and Ca concentrations.

EXPERIMENTAL

Eight mining polluted rivers in the Røros area in Norway were examined prior to and during the spring flood period (April to July) and during the autumn (August to October) of 1997. Dialyzable (dissolved), filterable and total concentrations of the elements Cu, Zn, Cd and Al were determined, for the latter element only in three of the rivers. Five sampling campaigns included the filtration procedure (< 0.45 μm) needed for calculating the colloidal fraction. Figure 2 is thus based on those five sampling campaigns, whereas all other figures and statistics are based on dialysed and total concentrations, which mostly included 13 or more sampling campaigns (except the Naustebekken and Orva streams where only 9 and 11 campaigns respectively included dialysed samples).

A 60 ml Millipore syringe, thoroughly rinsed with river water, was used to push 40 ml (rinsing), then 60 ml (sampling) river water through a Millipore 0.45 μm filter, for collecting a sample including colloidal + dissolved fractions. Dissolved species were separated by dialysis *in situ* (Beneš and Steinnes, 1974). At the river sites three to six dialysis bags were hung in the water in a thin acid-washed polyester string from the bottleneck of a 2-L wide-neck polyethylene bottle. At the top and the bottom of this bottle several 6-mm holes were drilled to ensure that a suitable water flow passed the dialysis membranes. The bottles were anchored to about 20-kg weight stone-filled bags connected to land with a 6-mm polyester rope. Two (autumn) to three (spring) dialysis bags were collected and transferred to smaller sampling bottles about once a week at the most interesting episodes. Sampling sites were chosen where the ropes and bottles could be sheltered from floating ice flakes and high water speed. Narrow dialysis tubes (4.2 cm flat diameter) were chosen for rapid equilibration (Carl Roth GmbH, 36/32, art. 0655.1, molecular weight cut-off 10000 to 20000 D/ 2.5 to 5.0 nm). Prior to use they were soaked and stirred in 0.01 M Na_4EDTA solution adjusted to pH 8 with NH_3 (Eide *et al.*, 1980) and rinsed 14 times with thorough shakings in-between. The ~40 cm long membrane tubes were then double-knotted in one end, filled with deionized water and emptied once, filled again with deionized water and sealed like a sausage with a double knot in the other end. Acid washing procedures (Beneš and Steinnes, 1974; Eide *et al.*, 1980), were avoided due to the descriptions from the manufacturer. Prior to use the low density polyethylene sampling bottles (50 ml for dialysis samples, 250 ml for total samples) were washed in a laboratory washing machine, soaked in 7 M HCl, rinsed with deionized water, and soaked in 7 M HNO_3 before they were thoroughly rinsed with deionized water again, filled with 0.1 M HNO_3 and stored in air-tight plastic bags. All other equipment used in contact with the samples was soaked in detergent solution, acetone, 7 M HCl and 7 M HNO_3. In-between and after these washing steps this equipment was rinsed with deionized water. In the end it was dried and stored in polyethylene bottles.

Samples for the determination of total metal concentrations were unfiltered, but like the dialysis samples acidified to 0.1 M with suprapure HNO_3 after collection. The term "total concentration" is used for simplicity, although metals strongly bound to particle surfaces or within lattices may not be included due to particle sedimentation in the sample bottles. Alkalinity, pH, and TOC were determined in unacidified unfiltered samples. A Radiometer PHM 80 portable pH meter was used for pH measurements and alkalinity endpoint detection. For alkalinity measurements (Norges standardiseringsforbund, 1981), a Hach digital burette (model 16900) was used. Conductivity was measured by a CDM 80 conductivity meter from Radiometer.

All Zn and Ca determinations as well as Cu determinations in Orva and Bjørgåsen streams were executed by flame AAS, whereas graphite furnace AAS was used for the rest of the metal determinations. Three replicates were analyzed for all samples and standards. Where necessary to achieve a desireable absorbance, two to six injections were dried on the tube platform prior to atomization in the graphite furnace. Calibrations were run after every ninth sample, and between these calibrations two internal standards were analyzed. Internal standards within ±10 % (Cd, Zn and Al) and

±5 % (Cu) of a long-time average were accepted; otherwise all nine samples run after the foregoing calibration sequence were reanalyzed.

The quantification limit is defined as 10σ, where σ is the standard deviation from several measurements of the analytical blank solution. Quantification limits were 0.32, 2.2, 0.023 and 11.6 µg/L for Cu, Zn, Cd, and Al respectively. At metal concentrations lower than quantification limits, all samples from a given site were reanalyzed with GFAAS. The detection limit is defined as $2t_{0.95}\sigma$, where $t_{0.95}$ is the 95 % significance t factor used in t-tests. Detection limits were 0.17, 0.70, 0.011, and 4.5 µg/L for Cu, Zn, Cd, and Al respectively. All measurements for Cu and Zn were higher than quantification limits. In Røa stream the Cd concentrations were generally lower than the quantification limits and they were therefore all excluded from the material. A few samples in the material showed values lower than detection limits for Cd (1.5 % of samples) and quantification limits for Cd and Al (12 and 8 % of samples respectively). To avoid overestimation of river site averages, none of these samples were excluded from the material when the averages were calculated. Low concentration samples were rerun several times and all parallels were averaged. The samples were randomized prior to analysis.

Recovery measurements were used to assess the accuracy of the analyses. Analysis runs were accepted when 90 - 110 % of the metal spikes were recovered in all of four randomly selected samples from the site analyzed, otherwise the series concerned was rerun.

Two (autumn) to three (spring) dialysis bags were collected at each sampling campaign. For Cu, Zn, and Cd no relative standard errors (RSE) higher than 30% were observed. For Cu and Cd a few (5.4 and 1.5% of the sampling campaigns respectively) showed RSE values higher than 20%. The agreement between the dialysis replicates was generally acceptable, but the precision for Al should preferably have been better; 17% of the sampling campaigns showed RSE values higher than 30% (but lower than 50%) for this element. Any lacking river discharge and dissolved concentration data in the subsequent figures are due to failure in pressure sensors or dialysis bags.

RESULTS AND DISCUSSION

In figure 2 the average dissolved, colloidal and particulate fractions of Cu, Zn, Cd, and Al for the eight studied rivers are presented. The rivers are ranged according to average pH values (figure 3a). A very steady pH at about 3.1 was observed at the Bjørgåsen site throughout the year, and almost all Cu, Zn, and Cd at this site were found in the dissolved fraction. In Orva pH averaged 5.5, and still more than 90 % of Cu, Zn, and Cd were dissolved. In the six other streams average pH ranged between 6.9 and 7.2 (Figure 3). In these streams all three fractions generally occurred in significant amounts for Cu, Zn, and Cd, although the dissolved fraction still was the highest. The average dissolved fractions for the six pH neutral rivers were 54, 79, and 79 for Cu, Zn, and Cd respectively. Since most solid phases of natural waters contain components of Si, Al and Fe oxides or hydroxides, the lower dissolved fraction of Cu compared to Cd may be caused by the relatively strong adsorption ability of this element to such surfaces (Stumm and Morgan, 1981). Al was only studied in three streams, all within the neutral pH range. The average colloidal fraction of Al was 55 %, whereas the average dissolved and particulate fractions were 21 and 23 % respectively. The high colloidal fraction of Al is in accordance with results from Kimball *et al.* (1995) and Pettersson *et al.* (1992).

The colloidal fractions of Cu and Zn were especially high in Røa (figure 2). This may be associated with the presence of excessive peatlands in the catchment area, causing the elevated TOC concentrations observed in this stream (figure 3b), although TOC may as well be dominated by dissolved species (Tanizaki *et al.*, 1985). Most of the metal pollutants passing the Glåma sampling site originated from Orva. Simple calculations however revealed that the metal concentrations observed were several times higher than what the water discharges observed in the two rivers and Orva metal concentrations would cause due to conservative mixing. Probably the Orva water did not mix entirely with the slowly flowing Glåma, but followed its right side along the three kilometers downstream to the Glåma sampling site. Consequently the equilibration may have been somewhat delayed, probably causing the highest dissolved fraction observed for Cu, Zn, and Cd among the pH neutral sites. The particulate fraction of metals on the other hand was also very high in Glåma, leaving almost nothing to the colloidal fraction. This is in accord with the high load of particles visually observed all through the year at this site.

In figure 4, dissolved fractions from the present study are compared with corresponding literature values. It is evident that pH as well as TOC is important in controlling the metal speciation. Generally the dissolved fraction is high at low pH and at low TOC values for all elements. Borg and Andersson (1984) found statistical evidence for this trend for Al. For Cu they reported that TOC correlated significantly in the same way, but that the correlation with pH was less obvious. For Cd they found the opposite trend as that of Cu. Their results therefore indicated that the dissolved Cu fraction was controlled mostly by organic matter content whereas Cd was more controlled pH. By combining experimental data from the present work with those from six other investigations (Borg and Andersson, 1984; Tanizaki *et al.*, 1985, 1992a-b; Arnesen, Esbensen and Grande, 1988; Røe, 1991), the general fractionation pattern of the metals over an extended range of TOC and pH values may be revealed (figure 4). From these results it appears that both pH and TOC influence or covary significantly with all metals. For Cu, Zn, and Cd dissolved fractions approaching 100 % are reported in the whole pH range from 3-7.5. Less than 80 % dissolved fractions of Cu and Cd are generally reported only in cases where pH is higher than 6.5 or TOC is higher than 6 mg/L. In the pH neutral range, dissolved metal fractions from 10 to 100 % are observed for Cd, and clearly the lower dissolved fractions occur at the high TOC sites. Probably Zn will act in a similar way as Cd, although very few high TOC data are found in the literature to support this assumption.

An interesting interpretation of the results arises when they are compared to calculated curves for pH dependence of binding of metals on solid surfaces Mouvet and Bourg, 1983; Allard *et al.*, 1986; Stumm and Morgan, 1996). Fitting such a curve from the former to available experimental Cd data (figure 4d) gives a good fit at pH values <7.5 for low TOC samples, whereas there is a lack of experimental data at higher pH values. Circumstantial evidence indicating how the experimental Cd data extend through higher pH values may be found through the Cu data. The silica adsorption curve for Cu (also from Schindler *et al.*, 1976) shows that Cu adsorption may be expected to occur at lower pH values than for Cd. The experimental low TOC data appear to support this assumption, as the dissolved fractions are much smaller for Cu than for Cd around pH neutrality. Assuming Cu and Cd fractionation acting principallly similar, but within a different pH range, the slope of the low TOC Cu data may indicate the behaviour of the dissolved fraction of Cd at higher pH values; that is; a rather steeply declining slope.

When moving the Cd-silica adsorption curve 1-1.5 pH units to the left (figure 4), it fits well the high TOC samples. This indicates that the presence of organic compounds influences metal speciation by moving the pH-to-metal adsorption curve to the left in the order of one pH unit or more compared to the case of low TOC content. Considering the steepness of the theoretical curve, which appears to be very similar to that of the experimental high TOC curve, this expresses a difference between almost all Cd dissolved (at low TOC content) to almost all Cd adsorbed (at high TOC content) within the neutral pH range. Neutral pH represents the most common condition in rivers supporting fish and this observation is therefore important from a toxicity and water use viewpoint. The pH and TOC influence on the Cd adsorption pattern observed in the present study corresponds to that of pH and fulvic acid (FA) concentrations on Cd - alumina adsorption studied in vitro by Xu (1991). Above a breakpoint at pH ~7.3, however FA reduced Cd adsorption under the conditions studied in that investigation. Similar breakpoints may also exist in pH-TOC-Cd-adsorption natural systems (figure 4d), but sufficient literature data at high pH were not available to confirm such a trend.

For the dissolved fraction of Cu (figure 4a), the Cu-silica adsorption curve (Schindler *et al.*, 1976)) appears to occur more than one pH unit below the low TOC experimental data. This simply indicates that the Cu adsorption properties with respect to the colloidal and particulate fraction in natural waters deviate considerably from those of amorphous silica. This may not be very surprising considering the complexity of colloids and particles in natural waters and the fact that adsorption properties may differ considerably between particle species (see "X" and "+" in figure 4). Moreover the solute speciation may be more important than the composition of SPM for the metal adsorption capabilities in natural water (Allard *et al.*, 1986). For the high TOC sites, the Cu adsorption-to-pH plot appears far more scattered compared to what was evident in the corresponding Cd plot (figure 4 a and d). The trend is still similar as for Cd, high TOC values clearly appear to move the adsorption curve towards lower pH. The scattering may be associated with substantial differences between the investigations concerned in other chemical parameters that influence metal speciation, such as temperature dependent equillibria, alkalinity, hardness, and the presence of numerous other ligands and competing cations. Moreover total Cu concentrations in the whole data set ranges from a few μg/L to ~10 mg/L. The FA influence on the Cu speciation may depend somewhat on the Cu concentrations within this range (Cabaniss and Shuman, 1988). This could explain some of the scattering observed in figure 4a, and similar effects may possibly also explain some of the scattering observed for the other elements.

Zn and Cd are known to have quite similar chemical properties. Since very few data for dissolved Zn fractions at high TOC were found in the literature, interpretations of the TOC influence on Zn adsorption in figure 4c will not be very reliable. The low TOC value points are though very similar to the corresponding Cd values, and it is not unlikely that similar trends would be observed for these two elements also in high TOC waters if more data were available for Zn.

Most Al data in figure 4b are from Borg and Andersson (1984). Al chemistry deviates considerably from that of the other metals discussed, but it is still interesting to observe a corresponding shift to the left in the pH to dissolved fraction curve for the higher compared to the lower TOC samples. The shift from the domination of colloidal/particulate to the domination of dissolved species seems to occur within a somewhat wider pH range for Al than for the other elements (figure 4). Figure 4 shows that Cd and Zn most readily occur in the dissolved fraction, followed by Cu and then Al.

Summing up the discussion in the last paragraphs, evidence is collected for the following three statements: 1) The studied metals are bound almost exclusively to particles and colloids at a high pH, shifting to occur almost completely dissolved within one or a few pH units lower. 2) Around what pH this shift occurs will depend on the metal concerned, and, interestingly, 3) the TOC in the water. High TOC concentrations (> 8) seem to accompany low fractions of dissolved metals, probably because the metals adsorb on high molecular weight organic compounds or organic coatings on inorganic particles. Considerable metal adsorption thus occurs around one pH unit lower in high TOC waters than in low TOC waters.

Computer simulations by Lumsdon and Evans (1995) suggest that Al precipitates as $Al(OH)_3$ (gibbsite) between pH 5.5 and 8 at concentrations higher than ~10-25 μg/L. The total concentrations from which the dissolved fractions in figure 4b are calculated are between 36 and 330 μg/L and thus above the theoretically soluble concentration. However, referring to figure 4b it is not easy to predict in what cases Al precipitate, since Lumsdon and Evans (op. cit.) calculate that organic ligands may rise the solubility of Al in water by several orders of magnitude. Al particles or Al coatings on particles may both have been produced by precipitation and could have contributed to the observed lower dissolved fractions at higher pH levels. Since appropriate stability constants of humic and fulvic material with respect to metal binding have not been determined in the investigated water systems, the amount of metals occurring as organic is not directly predictable. It is therefore difficult to interpret whether or not inorganic Al precipitations actually have occurred in cases where the solubility product is exceeded according to the total or dissolved metal concentrations and inorganic ligand concentrations.

From solubility constants and diagrams (Stumm and Morgan, 1996) and observed alkalinities, pH and dissolved metal concentrations, no precipitation with hydroxo-, carbonate-, or bicarbonate- species are likely for Cu, Zn or Cd at any site. Coprecipitation with Fe or Al species however may be possible when these metals precipitate (Scheidegger *et al.*, 1997). Ongoing precipitation will remove dissolved metal species from the water column and may thus lead to a decreased dissolved fraction of that metal. Continuous sedimentation and resuspension of metal-rich particles from the riverbed may also contribute to higher concentrations and fractions of particulate metals, on expense of the percent dissolved metal fraction.

The most obvious explanation for the shift in the adsorption curves may be related to the fact that metals adsorb to organic colloids and particles. Higher TOC levels mean larger adsorption surfaces and thus more metals may be adsorbed to organic material. The fraction of organic material occurring in dissolved form may on the other hand be surprisingly high in some cases. In two pH neutral rivers near Tokyo, Tanizaki *et al.* (1985) found TOC concentrations at 1.79 and 4.06 mg/L, the dissolved fractions of which were 67 and 61 % respectivly. Also results from Patel *et al.* (1999) and Thorsen (2000) confirm that a substantial part of the TOC in rivers may be found in the dissolved fraction. If low molecular weight material has the same affinity to metals as the high molecular weight material, a higher fraction of metals might as well be expected in the dissolved fraction in high TOC aquatic environments. There may be numerous reasons why this does not appear to be the case in the present work. Possibly TOC attached to inorganic colloids and particles as coatings may have very effective adsorbing properties. Laboratory experiments have confirmed that fulvic acid readily adsorbs to colloidal $Fe(OH)_3(s)$, $FeOOH(s)$, and $Fe_2O_3(s)$ (Ledin *et al.*, 1993). It is also possible that positively charged metal ions and negatively charged dissolved organic matter may coagulate to form colloids and particles or that metals attach easier to high, compared to low, molecular weight organic material. Results from Pettersson *et al.* (1992) indicate that the stability of Me-humic/fulvic acids (< 1.2 μm) clearly increase with increasing pH for Cu, but not significantly for Cd. The mechanisms controlling the relation between dissolved fractions, pH and TOC are therefore not necessarily the same for these two elements.

Alkalinity, conductivity and Ca concentration figures (figures 3c-e) were very similar for the six pH neutral sites. Probably the $CaCO_3$ system controlled all three parameters within this pH range. In the acidic streams, alkalinity was obviously very low or even negative since H_2CO_3 and CO_2 completely dominate the carbonate system below pH ~6. Ca concentrations were somewhat elevated in the acidic streams, whereas conductivity was extremely high and probably almost completely governed by pH. Ca, alkalinity and possibly conductivity are likely to influence dissolved fractions of the metals, Ca and conductivity because cations in general may compete with the investigated metal ions for adsorption sites, alkalinity since HCO_3^- and CO_3^{2-} may compete with particle adsorption sites for metal cations. Alkalinity, Ca concentration and conductivity differ somewhat between the investigated rivers, and do not appear to be very closely connected to pH (figure 3). Possibly due to the variations in these parameters, as well as variations in other water chemistry parameters which influence metal speciation, the figures showing the correlations between pH and dissolved metal fractions were quite scattered, especially for Cu in high TOC samples (figure 4).

It may be objected that the data plotted in figure 4 are produced by somewhat deviating methods, and that the conclusions for this reason may be subject to some degree of systematic error. E.g. most high TOC samples are from one single investigation. The low TOC data within that investigation however still supports the general trends suggested above, and no clear differences in dissolved fractions between the investigations seem evident at comparable pH and TOC levels. Furthermore the pore sizes quoted in the different studies are within a very narrow range (10000-20000 D or about 2.5-5 nm) compared to the wide range of species sizes one would expect to find in natural waters, and systematic errors large enough to influence the conclusions drawn therefore seem to be quite unlikely. Lake and river waters seem to have similar dissolved fractions of metals at comparable pH and TOC values (see low TOC data in figure 4). It is thus not likely that the TOC induced shift in the pH – dissolved metal fraction curve should arise from the fact that all high TOC samples are lake water samples.

CONCLUSIONS

At the Bjørgåsen and Orva sampling sites, average pH levels were 3.1 and 5.5 respectively. More than 90% of Cu, Zn, and Cd occurred in dissolved form in these rivers. Average pH in the six other rivers studied was in the range 6.9 to 7.4, and for this reason the dissolved fractions were lower with an average of 54, 79, and 79 % for Cu, Zn and Cd respectively. Al was only determined in samples from three pH neutral rivers. As an average, 55% of Al was colloidally bound in these rivers, whereas the dissolved and particulate fractions were 21 and 23% respectively.

Our data combined with data from similar studies support equilibrium models suggesting that the percent fraction of metals adsorbed on particles rises steeply from almost zero to nearly 100 % within a narrow and element specific pH range. Changes in TOC concentration seem to cause a distinct shift in the pH-to-% metal absorption curves. A high organic carbon content in fact seems to allow a similar adsorption to occur about one pH unit lower than in low TOC waters. Scattering of points however suggests that parameters other than pH and TOC may also influence significantly the metal speciation.

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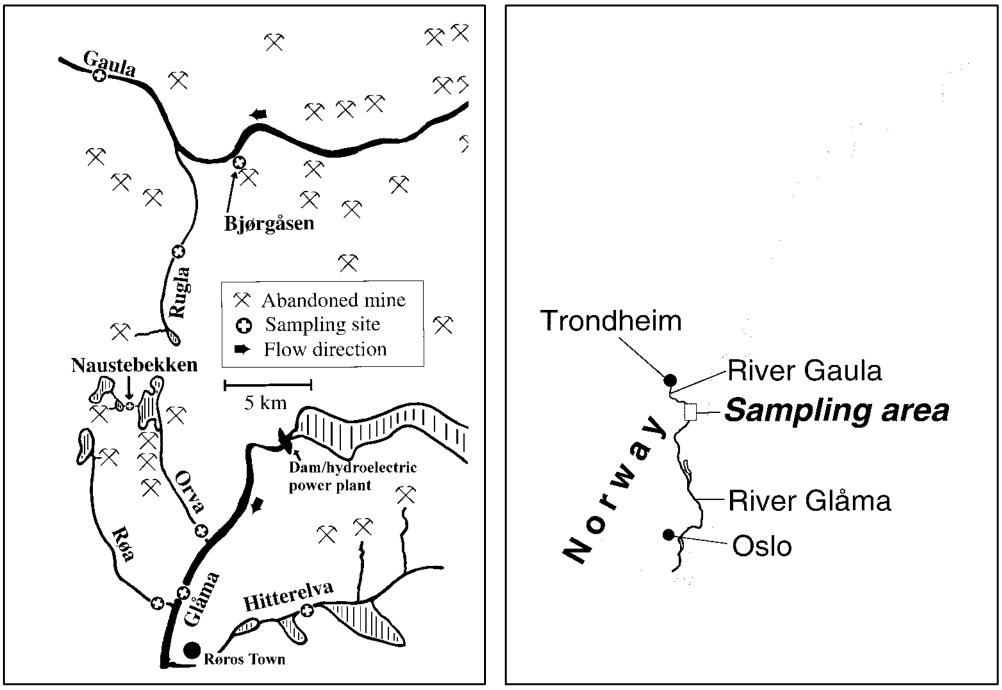

Figure 1: Map of the study area.

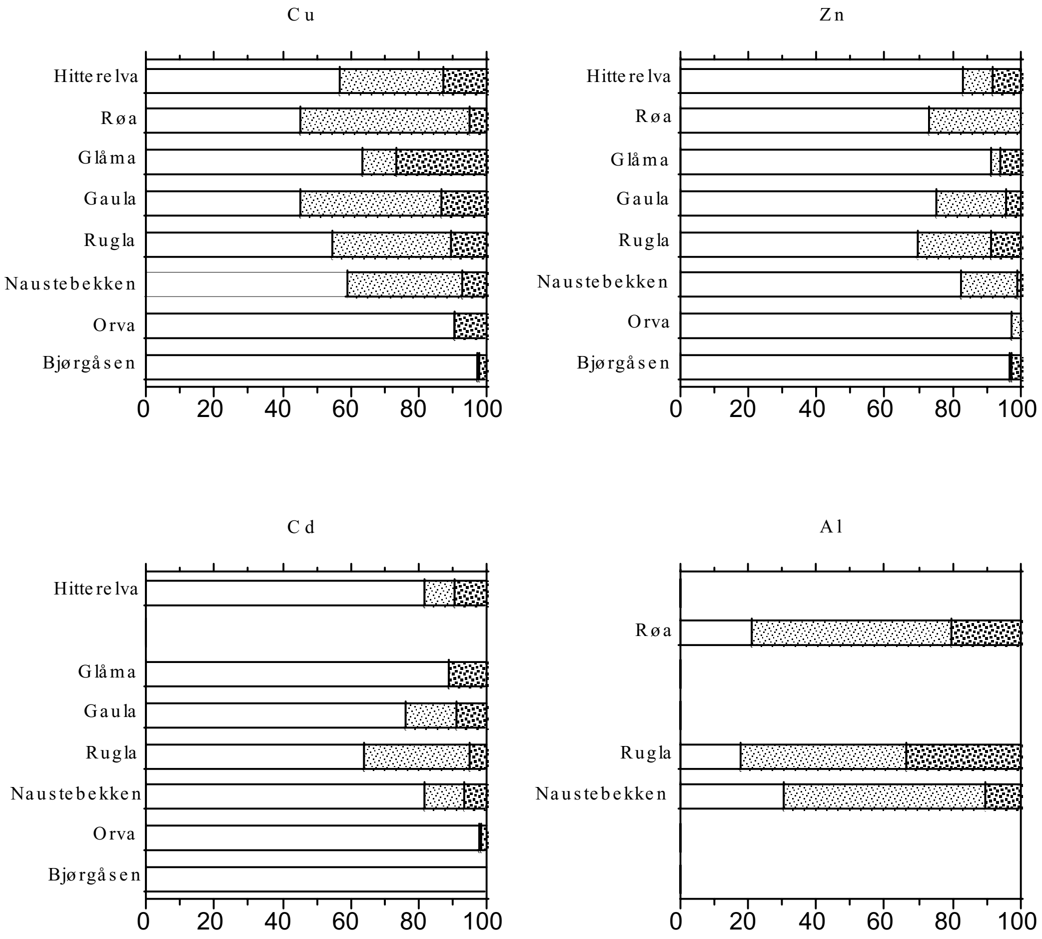

Figure 2: Percent fraction of the metals occurring as ☐ dissolved, ▨ colloidal and ▩ particulate in the rivers. The rivers are ranged according to average pH, highest pH on the top.

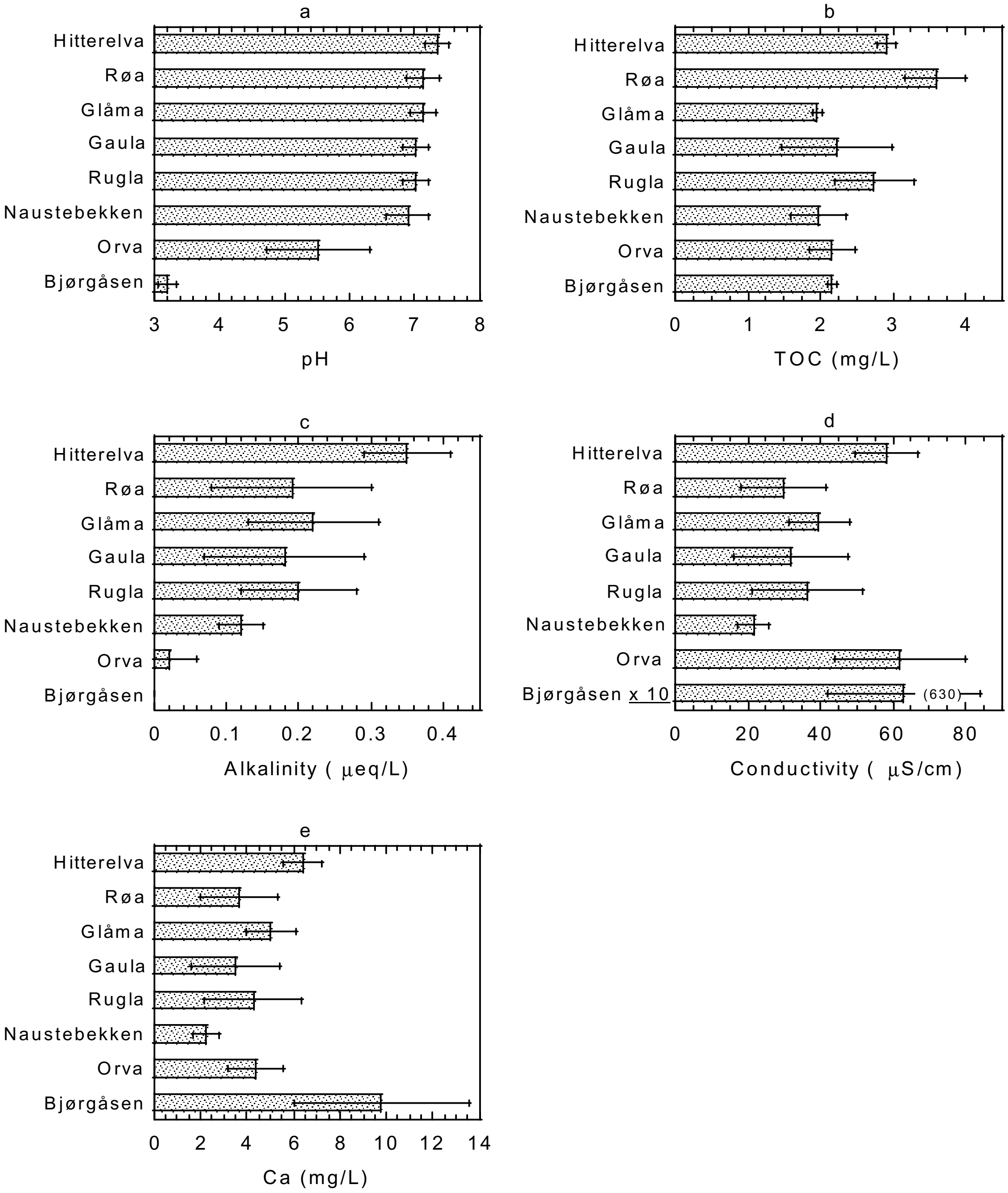

Figure 3: Average pH (a), TOC (b), alkalinity (c), conductivity (d), and Ca concentration (e) in each of the rivers. Data from all sampling campaigns (~15) are included in the averages.

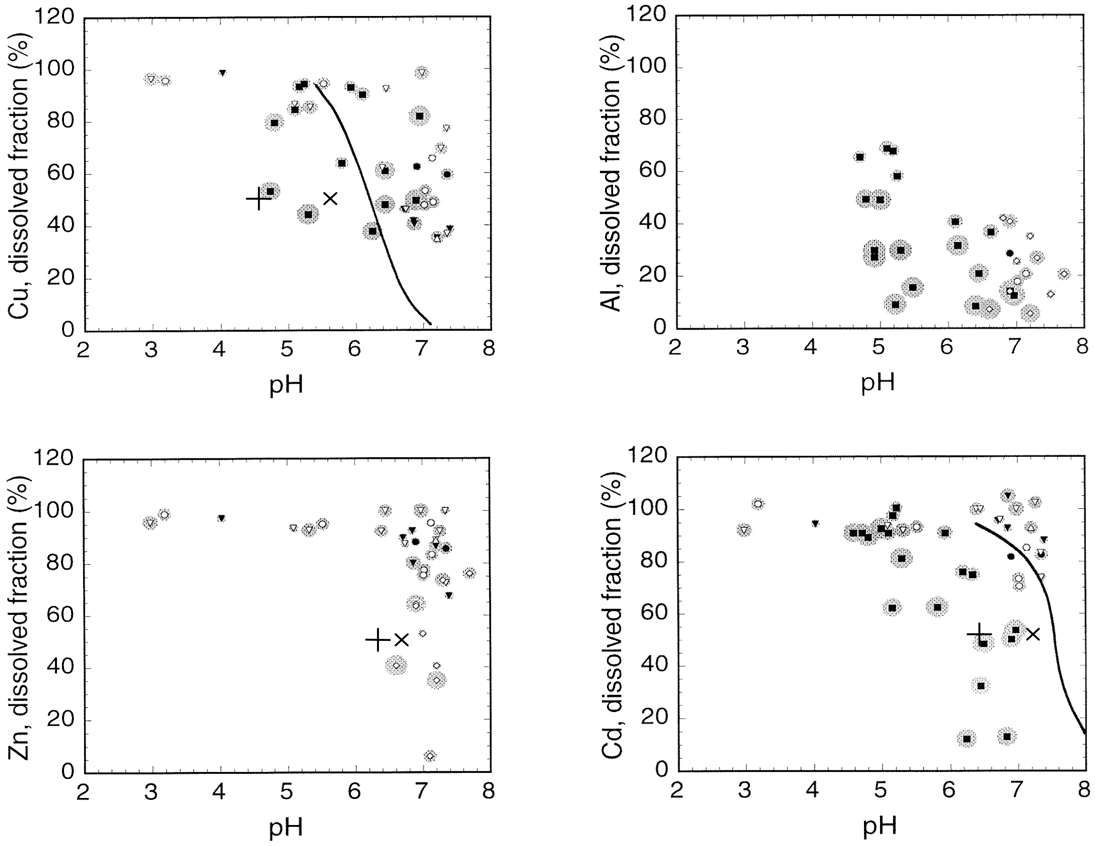

Figure 4: Dissolved fractions (% of total) of Cu (a), Al (b), Zn (c), and Cd (d) at different pH values observed in rivers (white inner points) and lakes (black inner points). Size of the outer, grey part of each point represents TOC concentration (mg/l); Largest: >8 mg/L, smallest: <2 mg/L , rest in 2 mg/L intervals. Shape of inner points represents different investigations: ○/● present work; □/■Borg and Andersson (1984); ▼/▽ Arnesen et al. (1988); △ Røe (1991); ◇ Tanizaki et al. (1985, 1992 a&b). The curves in 4a and 4d show the adsorption of Cu and Cd respectively on silica during a pH titration (Schindler et al., 1976). Similar curves may have been drawn through "X" representing (50 %) adsoption on amorphous $Fe_2O_3 \cdot H_20$ (Allard, et al., 1986) and through "+" representing (50 %) adsorption on river bottom sediments (Mouvet and Bourg, 1983); the curves were omitted for viewability of the figures.

Appendixes

Appendix 1: Analytical results for all samples and parameters

Røa

Table A1.1: Water chemistry data

Dato	Alkalinity (meq/L)	Ca (mg/L)	Conductivity (µS/cm)	pH	TOC (mg/L)	Water temp. (°C)	River discharge (m3/s)	Precipitation (mm/d) Røros	Air temp. Røros
13.04.97	0.376	6.4	50.0	7.3			0.33	4.1	1.3
21.04.97	0.414	7.1	32.6	7.4		-2	0.50	0	-1.9
06.05.97	0.308	5.9	50.7	7.1		-2	0.73	1.5	0.6
08.05.97	0.270		48.0	6.9			1.36	30	4.1
15.05.97	0.132	2.8	25.3	7.0			9.81	0	6.1
22.05.97	0.126	2.7	24.4	6.7		0	3.36	0.1	1.3
03.06.97	0.091	1.9	17.9	6.9			11.18	0	5.7
12.06.97	0.090	1.9	17.2	7.0		8	11.28	0	14.9
19.06.97	0.098	2.0	18.1	7.1		6	7.56	0	9.4
26.06.97	0.107	2.2	19.2	7.1	3.16	6	5.95	5	6.8
03.07.97	0.102	2.2	18.4	7.0		14	5.42	6.1	14.8
28.08.97	0.278	5.1	41.0	7.6			0.33	0.7	16.3
10.09.97	0.121	3.2	24.9	7.0		6	6.60	20.3	5
18.09.97	0.165	3.6	28.7	7.5		3	1.63	0	3.2
30.09.97	0.193	3.9	30.8	7.5	3.59	5	1.40	1.3	5
01.10.97		3.9					1.38	0.2	1.8
02.10.97	0.179	3.9	31.1	7.2	4.01	2	1.63	6.8	2.7

[1] 24 h average

Table A1.2: Total, filtrable (< 0.45 µm) and dialysable (< 2.5-~5 nm) metal concentrations (µg/L)

Date	Total concentrations				Filtrable concentrations				Dialysable concentrations			
	Cu	Zn	Cd	Al	Cu	Zn	Cd	Al	Cu	Zn	Cd	Al
13.04.97	2.21	9.00	0.003	15.1								
21.04.97	2.39	9.30	0.005	14.1					1.10	11.15		6.3
06.05.97	3.39	15.10	0.014	50.1					2.28	16.57		15.6
08.05.97	2.61	14.40	0.015	47.1								
15.05.97	4.20	14.60	0.029	272.4					0.51	7.27	0.020	18.6
22.05.97	3.45	8.40	0.020	103.8					1.41	6.93		14.6
03.06.97	2.46	7.40	0.019	128.4					1.76	6.37	0.016	13.4
12.06.97	2.33	5.40	0.009	65.4					1.19	4.20		13.9
19.06.97	2.85	4.50	0.008	60.9					2.27	4.80	0.006	6.9
26.06.97	2.59	5.40	0.007	83.2					0.91	3.63	0.010	5.7
03.07.97	3.70	5.30	0.007	72.0	3.03	5.50	0.020	57.0	2.77	6.33	0.027	12.3
28.08.97	1.83	2.55	0.002	8.7	1.84	2.70		8.3				
10.09.97	4.37	8.40	0.015	119.2	3.75	8.40		65.3	1.57	3.80	0.006	14.0
18.09.97	4.34	6.90	0.014	45.5	4.80	7.50		47.1	1.35	5.05		12.8
30.09.97	4.18	8.40	0.009	59.0	4.26	8.10		44.7	1.68	6.00		17.2
01.10.97	4.34	8.10	0.018	47.8								
02.10.97	4.50	9.50	0.010	38.9	4.14	9.30		51.9	2.18	7.05		14.0

Naustebekken

Table A1.3: Water chemistry data

Dato	Alkalinity (meq/L)	Ca (mg/L)	Conductivity (µS/cm)	pH	TOC (mg/L)	Water temp. (ºC)	Water level $^{1)}$	Precipitation (mm/d) Røros	Air temp. Røros
05.05.97	0.172	3.1	27.3	6.6		-3	108.33	0.1	-2.5
08.05.97	0.192	3.5	31.3	6.9			109.79	30	4.1
15.05.97	0.142	2.7	25.7	6.6			144.79	0	6.1
22.05.97	0.136	2.4	24.7	6.4		1.5	188.13	0.1	1.3
03.06.97	0.072	1.6	17.9	6.4			263.71	0	5.7
11.06.97	0.108	2.1	22.1	6.7		3	288.29	0	10.5
18.06.97	0.081	1.6	17.2	6.8		6	236.00	3.6	9
25.06.97	0.080	1.7	16.8	7.3	1.53	7	203.13	0.2	7.6
02.07.97	0.086	1.6	17.2	7.1		12	188.54	0.5	16.4
28.08.97	0.121	2.2	20.9	7.2				0.7	16.3
10.09.97	0.118	2.3	20.2	7.1		6		20.3	5
18.09.97	0.111	2.2	20.0	7.3		4		0	3.2
30.09.97	0.115	2.2	19.6	7.2	2.20	3		1.3	5
02.10.97	0.120	2.2	20.5	7	2.19	3		6.8	2.7

$^{1)}$ Measured water debts at a point in the river. Values are not calibrated to get correct discharges.

Table A1.4: Total, filtrable (< 0.45 µm) and dialysable (< 2.5-~5 nm) metal concentrations (µg/L)

Date	Total concentrations				Filtrable concentrations				Dialysable concentrations			
	Cu	Zn	Cd	Al	Cu	Zn	Cd	Al	Cu	Zn	Cd	Al
05.05.97	1.12	39.90	0.060	13.0								
08.05.97	1.44	48.40	0.065	16.7								
15.05.97	3.89	63.10	0.121	38.2					2.68	63.23	0.103	11.3
22.05.97	4.90	87.00	0.170	50.0					3.59	114.33	0.200	14.2
03.06.97	6.80	131.00	0.249	50.0					5.28	100.80	0.146	12.6
11.06.97	6.78	155.00	0.301	37.6								
18.06.97	6.54	121.00	0.236	47.9								
25.06.97	7.40	123.00	0.255	41.5					4.51	98.00	0.179	8.3
02.07.97	6.86	113.00	0.242	39.6	6.44	115.50	0.244	40.1	5.80	100.83	0.197	14.6
28.08.97	3.34	48.60	0.090	26.7	3.00	48.50	0.089	17.5				
10.09.97	4.17	72.00	0.129	35.5	3.64	69.50	0.112	29.3	1.99	50.75	0.084	7.9
18.09.97	4.60	71.40	0.122	31.5	3.80	70.90	0.109	29.9	2.18	52.95	0.097	9.8
30.09.97	4.38	91.00	0.166	34.2	4.17	89.70	0.154	33.5	2.50	77.15	0.134	13.5
02.10.97	4.40	91.10	0.169	34.8	4.60	88.70	0.154	24.9	1.98	79.70	0.168	8.4

Hittervassdraget

Table A1.5: Water chemistry data

Dato	Alkalinity (meq/L)	Ca (mg/L)	Conductivity (µS/cm)	pH	TOC (mg/L)	Water temp. (ºC)	River discharge 1) (m^3/s)	Precipitation (mm/d) Røros	Air temp. Røros
13.04.97	0.422	7.5	68.2	7.1		-1.5	0.61	4.1	1.3
21.04.97	0.412	7.5	41.9	7.3		-2	0.56	0	-1.9
07.05.97	0.456	7.8	73.3	7.2			2.15	10.5	2.9
08.05.97	0.422	7.9	74.9	7.3			3.83	30	4.1
14.05.97	0.364	6.8	65.3	7.2		-2	20.79	0	7
21.05.97		5.3	58.8	7.2		1	12.69	0.1	1.2
04.06.97	0.280	5.6	50.8	7			17.88	0	8.9
12.06.97	0.285	5.6	54.7	7.4		10	8.42	0	14.9
19.06.97	0.305	5.8	54.1	7.5		7	9.11	0	9.4
26.06.97	0.303	5.7	55.1	7.5	2.76	8	5.93	5	6.8
03.07.97	0.305	5.9	56.2	7.4		15	5.77	6.1	14.8
28.08.97		6.2					0.71	0.7	16.3
10.09.97	0.322	6.1	54.9	7.5		8	7.18	20.3	5
18.09.97	0.319	6.2	56.6	7.6		6	5.55	0	3.2
30.09.97		6.2	55.2	7.6	2.99	5	2.61	1.3	5
02.10.97	0.327	6.3	57.2	7.5	2.97	5	2.83	6.8	2.7

1) 24 h average

Table A1.6: Total, filtrable (< 0.45 µm) and dialysable (< 2.5-~5 nm) metal concentrations (µg/L)

Date	Total concentrations				Filtrable concentrations				Dialysable concentrations			
	Cu	Zn	Cd	Al	Cu	Zn	Cd	Al	Cu	Zn	Cd	Al
13.04.97	29.48	135.00	0.224									
21.04.97	27.50	133.00	0.231						13.60	117.67	0.195	
07.05.97	27.81	132.00	0.256						24.54	112.00	0.186	
08.05.97	28.87	134.00	0.234									
14.05.97	30.09	105.00	0.220						12.63	82.43	0.164	
21.05.97	61.50	170.00	0.327						38.80	148.67	0.300	
04.06.97	41.53	127.00	0.236						34.03	107.33	0.203	
12.06.97	42.84	130.00	0.253						21.04	123.67	0.215	
19.06.97	38.52	119.00	0.222						23.48	104.33	0.189	
26.06.97	33.40	111.00	0.211						16.05	102.17	0.180	
03.07.97	35.72	122.00	0.232		33.28	111.00	0.243		20.38	96.77	0.184	
28.08.97	24.33	92.70	0.188		21.30	82.80	0.152					
10.09.97	29.40	110.00	0.217		24.00	99.20	0.175		24.84	102.15	0.176	
18.09.97	33.94	118.00	0.222		27.22	108.00	0.190		15.94	95.45	0.178	
30.09.97	34.23	123.00	0.228		30.50	110.00	0.206		16.08	97.25	0.192	
02.10.97	33.40	123.00	0.226		30.53	118.00	0.205		17.48	101.50	0.190	

Gaula

Table A1.7: Water chemistry data

Dato	Alkalinity (meq/L)	Ca (mg/L)	Conductivity (µS/cm)	pH	TOC (mg/L)	Water temp. (ºC)	River discharge$^{1)}$ (m^3/s)	Precipitation (mm/d) Røros	Air temp. Røros
12.04.97	0.390	7.2	60.9	6.7		-2	1.66	0.7	-2
21.04.97	0.390	7.4	55.4	7.1		-3	1.50	0	-1.9
07.05.97	0.298	5.8	56.7	7.1			8.55	10.5	2.9
08.05.97	0.182	3.9	43.1	7.1			20.68	30	4.1
14.05.97	0.150	3.4	34.9	7		0	35.17	0	7
21.05.97	0.164	3.4	34.3	7.1		2	19.94	0.1	1.2
04.06.97	0.099	2.1	21.2	6.8			43.66	0	8.9
11.06.97	0.069	1.5	16.8	6.8		2	55.02	0	10.5
18.06.97	0.077	1.6	15.9	6.8		3	38.95	3.6	9
25.06.97	0.077	1.6	14.8	7	1.48	3	36.41	0.2	7.6
02.07.97	0.066	1.3	13.0	7		10	48.36	0.5	16.4
28.08.97	0.228	4.1	34.8	7.5		15	1.15	0.7	16.3
10.09.97	0.103	2.2	17.4	6.9		5	54.17	20.3	5
18.09.97	0.139	2.8	23.7	7.2		3	12.30	0	3.2
30.09.97	0.214	4.1	33.6	7.3	2.18	5	6.21	1.3	5
02.10.97	0.194	3.9	33.7	7.1	3.00	4	8.11	6.8	2.7

Table A1.8: Total, filtrable (< 0.45 µm) and dialysable (< 2.5-~5 nm) metal concentrations (µg/L)

Date	Total concentrations				Filtrable concentrations				Dialysable concentrations			
	Cu	Zn	Cd	Al	Cu	Zn	Cd	Al	Cu	Zn	Cd	Al
12.04.97	8.20	34.10	0.082									
21.04.97	8.52	35.00	0.092						5.16	41.27	0.082	
07.05.97	19.21	46.30	0.127						8.36	34.23	0.075	
08.05.97	25.96	48.00	0.154									
14.05.97	15.33	38.60	0.098						4.76	23.63	0.063	
21.05.97	9.10	22.80	0.067						5.16	23.50	0.071	
04.06.97	6.93	15.00	0.047						6.65	12.30	0.031	
11.06.97	22.13	41.20	0.103						2.87	7.90	0.032	
18.06.97	5.80	10.90	0.040						6.06	10.70	0.030	
25.06.97	5.12	10.80	0.036						2.22	8.13	0.021	
02.07.97	5.40	9.90	0.033		3.45	7.10	0.022		4.57	7.43	0.026	
28.08.97	4.64	10.60	0.039		4.30	11.90	0.038					
10.09.97	10.04	16.60	0.054		8.50	14.40	0.042		4.60	12.70	0.029	
18.09.97	6.98	24.30	0.064		6.34	24.80	0.060		2.47	16.50	0.042	
30.09.97	10.06	41.30	0.093		9.18	40.20	0.093		4.18	28.60	0.076	
02.10.97	11.24	38.60	0.098		10.34	38.30	0.095		4.04	33.20	0.088	

Rugla

Table A1.9: Water chemistry data

Dato	Alkalinity (meq/L)	Ca (mg/L)	Conductivity (μS/cm)	pH	TOC (mg/L)	Water temp. (°C)	River discharge[1)]	Precipitation (mm/d) Røros	Air temp. Røros
13.04.97		8.5	77.8					4.1	1.3
21.04.97	0.306	8.5	43.3	7.1		-3		0	-1.9
07.05.97	0.244	4.8	46.7	6.7			1659.00	10.5	2.9
08.05.97	0.180	3.6	37.5	6.7			1467.00	30	4.1
14.05.97	0.146	3.0	31.1	6.9		-2	840.00	0	7
21.05.97	0.182	3.7	33.6	7		2	688.00	0.1	1.2
03.06.97	0.122	2.5	23.2	6.9			850.00	0	5.7
11.06.97	0.110	1.9	22.5	6.9			759.00	0	10.5
18.06.97	0.113	2.4	22.0	6.9		7	708.00	3.6	9
25.06.97	0.132	2.5	22.7	7.1	2.23	7	648.00	0.2	7.6
02.07.97	0.127	2.3	21.8	7.2		12	708.00	0.5	16.4
28.08.97	0.386	6.8	56.7	7.3		14	346.00	0.7	16.3
10.09.97	0.142	3.2	25.5	6.9		6	708.00	20.3	5
18.09.97	0.242	4.7	38.7	7.2		4	527.00	0	3.2
30.09.97	0.272	5.0	42.2	7.2	2.69	3	476.00	1.3	5
01.10.97							587.00	0.2	1.8
02.10.97	0.241	4.6	38.7	7.2	3.31	4	567.00	6.8	2.7

[1)] Measured water debts at a point in the river. Values are not calibrated to get correct discharges.

Table A1.10: Total, filtrable (< 0.45 μm) and dialysable (< 2.5-~5 nm) metal concentrations (μg/L)

Date	Total concentrations				Filtrable concentrations				Dialysable concentrations			
	Cu	Zn	Cd	Al	Cu	Zn	Cd	Al	Cu	Zn	Cd	Al
13.04.97	6.11	6.6	0.012	17.8								
21.04.97	6.85	6.5	0.014	19.9					2.90	5.4	0.011	4.4
07.05.97	16.1	11.4	0.028	376.5					7.02	4.63	0.014	18.7
08.05.97	26.59	14.1	0.040	391.8								
14.05.97	28.2	13.5	0.041	470.2					4.01	5.77	0.029	9.2
21.05.97	15.61	7	0.018	65.7					9.11	10.8	0.024	21.4
03.06.97	21.56	7.1	0.020	107.4					13.8	5.93	0.013	17.0
11.06.97	20.96	5.7	0.017	48.3						5.27	0.015	7.5
18.06.97	23.8	6.3	0.015	55.5					12.1	3.9	0.010	11.2
25.06.97	25.75	7.1	0.015	54.0					10.1	4.6	0.010	6.2
02.07.97	30.88	7.4	0.019	83.5	28.65	6.45	0.017	24.0	17.2	4.53	0.012	11.9
28.08.97	14.81	5.5	0.019	33.9	12.685	4.6	0.018					
10.09.97	36.42	11.5	0.033	122.5	33.19	10	0.029	96.6	15.3	5.5	0.012	11.6
18.09.97	21.9	8	0.019	48.8	14.125	6.1	0.018	50.3	9.87	4.9	0.012	17.1
30.09.97	21.41	8.8	0.024	40.7	21.5	8.9	0.023	33.1	19.3	9.2	0.021	6.9
01.10.97	41.2	8.775	0.025						10.415	8.6	0.024	6.4
02.10.97	24.3	9.3	0.021	52.9	22.99	9.7	0.022	27.4	11.8	7.1	0.018	14.8

Glåma

Table A1.11: Water chemistry data

Dato	Alkalinity (meq/L)	Ca (mg/L)	Conductivity (µS/cm)	pH	TOC (mg/L)	Water temp. (ºC)	River discharge1) (m^3/s)	Precipitation (mm/d) Røros	Air temp. Røros
13.04.97	0.342	6.2	44.5	7.1		0	12.02	4.1	1.3
21.04.97	0.322	6.1	27.0	7.2		-3	12.02	0	-1.9
06.05.97		6.9	51.5	7.2			7.65	1.5	0.6
08.05.97	0.332	7.2	61.5	7.1			7.65	30	4.1
15.05.97	0.171	4.5	40.8	6.7			19.44	0	6.1
22.05.97	0.272	5.8	46.0	7		1.8	25.41	0.1	1.3
03.06.97	0.141	3.8	36.5	6.8			28.38	0	5.7
12.06.97	0.258	5.3	42.1	7.1		8	100.36	0	14.9
19.06.97	0.227	4.6	36.9	7.3		6	112.50	0	9.4
26.06.97	0.190	4.0	34.2	7.2	1.90	7	75.74	5	6.8
03.07.97	0.182	3.6	30.6	7.2		14	92.21	6.1	14.8
28.08.97	0.216	4.5	36.4	7.5		15	11.13	0.7	16.3
10.09.97	0.188	4.7	37.3	7		6	28.38	20.3	5
18.09.97	0.209	4.2	35.6	7.4		6	38.56	0	3.2
30.09.97	0.216	4.5	35.7	7.2	1.94	7	26.87	1.3	5
01.10.97		4.6					26.87	0.2	1.8
02.10.97	0.000	4.7	38.5	7.1	2.02	5	26.87	6.8	2.7

1) 24 h average

Table A1.12: Total, filtrable (< 0.45 µm) and dialysable (< 2.5-~5 nm) metal concentrations (µg/L)

Date	Total concentrations				Filtrable concentrations				Dialysable concentrations			
	Cu	Zn	Cd	Al	Cu	Zn	Cd	Al	Cu	Zn	Cd	Al
13.04.97	6.74	19.40	0.039									
21.04.97	7.99	21.10	0.054						3.57	33.03	0.053	
06.05.97	18.70	59.60	0.130						17.62	71.70	0.147	
08.05.97	125.88	271.00	0.664									
15.05.97	132.40	201.60	0.464						31.68	165.00	0.318	
22.05.97	52.12	137.70	0.291						58.30	130.33	0.272	
03.06.97	94.96	192.20	0.440						54.40	154.33	0.295	
12.06.97	39.70	73.80	0.200						20.94	56.57	0.130	
19.06.97	29.41	63.80	0.181						22.81	66.07	0.126	
26.06.97	43.48	78.20	0.188						18.00	62.17	0.141	
03.07.97	25.05	54.70	0.119		18.30	56.65	0.113		21.83	40.27	0.100	
28.08.97	8.74	12.60	0.061		7.46	33.00	0.053					
10.09.97	63.35	135.50	0.313		56.62	150.00	0.275		23.94	115.50	0.222	
18.09.97	28.56	75.00	0.132		23.19	76.40	0.144		24.99	67.35	0.139	
30.09.97	33.45	103.00	0.201		17.80	71.00	0.127		23.93	105.50	0.213	
01.10.97	51.31	135.30	0.272									
02.10.97	42.67	126.60	0.257		26.37	112.00	0.202		27.69	121.50	0.234	

Orva

Table A1.13: Water chemistry data

Dato	Alkalinity (meq/L)	Ca (mg/L)	Conduc-tivity (µS/cm)	pH	TOC (mg/L)	Water temp. (°C)	River discharge[1] (m^3/s)	Preci-pitation (mm/d) Røros	Air temp. Røros
06.05.97	0.078	6.1	70.1	6.6			0.26	1.5	0.6
08.05.97	0.118	5.0	53.6	6.6			0.49	30	4.1
15.05.97		4.0	80.6	3.4			3.55	0	6.1
22.05.97	0.038	4.3	56.3	5.9		1.2	1.21	0.1	1.3
03.06.97	0.013	3.0	43.2	5.8			4.04	0	5.7
12.06.97	0.018	3.0	40.3	6		10	4.08	0	14.9
19.06.97	0.012	3.1	42.7	6		8	2.74	0	9.4
26.06.97	0.012	3.4	49.6	5.6	2.36	8	2.15	5	6.8
03.07.97	0.012	3.2	42.7	5.8		17	1.96	6.1	14.8
28.08.97	0.004	6.9	92.7	5.1		15	0.12	0.7	16.3
10.09.97	-0.012	4.0	66.1	4.9		6	2.39	20.3	5
18.09.97	0.001	4.3	65.2	5.4		4	0.59	0	3.2
30.09.97	0.005	4.6	71.9	5.1	1.80	7	0.51	1.3	5
01.10.97		5.5					0.50	0.2	1.8
02.10.97	-0.002	5.1	91.8	5	2.32	3	0.59	6.8	2.7

[1] 24 h average

Table A1.14: Total, filtrable (< 0.45 µm) and dialysable (< ~2.5-5 nm) metal concentrations (µg/L)

Date	Total concentrations				Filtrable concentrations				Dialysable concentrations			
	Cu	Zn	Cd	Al	Cu	Zn	Cd	Al	Cu	Zn	Cd	Al
06.05.97	147	583	0.76									
08.05.97	110	332	0.45									
15.05.97	715	1280	2.42						512.00	1028	1.912	
22.05.97	241	670	1.19						252.00	652	1.119	
03.06.97	265	560	1.02						401.00	525		
12.06.97	222	509	0.94						155.00	449	0.813	
19.06.97	260	580	1.08						273.67	578	1.00	
26.06.97	296	644	1.15						244.33	576	0.997	
03.07.97	242	552	0.98		185.00	555	1.022		212.33	557	1.030	
28.08.97	291	1040	1.42		293.00	990	1.471					
10.09.97	424	905	1.74		387.00	935	1.661		339.50	852	1.476	
18.09.97	342	890	1.55		308.00	940	1.651		314.00	943	1.649	
30.09.97	411	1095	1.890		378.00	1090	1.748		410.50	1145	1.933	
01.10.97	597	1655	2.521									
02.10.97	482	1410	2.137		451.00	1400	2.094		449.50	1295	2.040	

Bjørgåsen

Table A1.15: Water chemistry data

Dato	Alkalinity (meq/L)	Ca (mg/L)	Conductivity (µS/cm)	pH	TOC (mg/L)	Water temp. (°C)	River discharge 1) (m^3/s)	Precipitation (mm/d) Røros	Air temp. Røros
12.04.97		13.8	836.9	3.1		-3	2.22	0.7	-2
21.04.97		12.9	659.1	3.1		-3	2.01	0	-1.9
07.05.97		8.3	600.2	3.2			11.45	10.5	2.9
08.05.97		8.2	746.6	3.1			27.71	30	4.1
14.05.97		6.9	591.6	3.3		-2	47.12	0	7
21.05.97		7.8	552.2	3.3		0	26.72	0.1	1.2
04.06.97		3.1	273.2	3.5			58.51	0	8.9
11.06.97		5.7	430.8	3.4		15	73.73	0	10.5
18.06.97		8.3	540.9	3.2			52.19	3.6	9
25.06.97		10.2	612.9	3.1	2.11	10	48.79	0.2	7.6
02.07.97		9.6	618.2	3.1		17	64.80	0.5	16.4
28.08.97		19.6	1266.8	2.9		17	1.53	0.7	16.3
10.09.97		8.9	522.1	3.3		7	72.59	20.3	5
18.09.97		11.2	610.5	3.2		5	16.48	0	3.2
30.09.97		11.9	634.0	3.1		5	8.32	1.3	5
02.10.97		10.2	591.5	3.2	2.20	5	10.87	6.8	2.7

1) 24 h average

Table A1.16: Total, filtrable (< 0.45 µm) and dialysable (< 2.5-~5 nm) metal concentrations (µg/L)

Date	Total concentrations				Filtrable concentrations				Dialysable concentrations			
	Cu	Zn	Cd	Al	Cu	Zn	Cd	Al	Cu	Zn	Cd	Al
12.04.97	4216	9904	21.908									
21.04.97	4246	9168	21.448						4159	9130	20.733	
07.05.97	3858	6256	14.144						4102	7140	17.446	
08.05.97	4160	6768	15.884									
14.05.97	3183	4944	11.728						3171	4941.33	12.105	
21.05.97	3269	5248	12.900						3046	5125.33	12.904	
04.06.97	1171	2096	5.248						1225	2218.67	5.683	
11.06.97	1955	3312	8.164						1386			
18.06.97	2461	4464	11.428						2294	4288	10.440	
25.06.97	2884	5440	13.276						2710	4954.67	12.230	
02.07.97	2673	4880	11.656		2595	4648	12.140		2478	4424	11.561	
28.08.97	4491	14096	22.704		4649	13632	25.744					
10.09.97	2278	5056	12.012		2224	5040	11.916		2285	5344	13.690	
18.09.97	2677	5872	14.796		2584	5880	14.752		2507	5740	13.948	
30.09.97	2964	6720	16.100		2982	6480	15.917		3047	6312	16.028	
02.10.97	2683	5424	13.096		2572	5120	13.312		2563	5164	13.461	

Table A1.17: Diel river discharges for the rivers

Date	*Røa* 1) *(m^3/s)*	*Naustebekken* 2)	*Hitterelva* 3) *(m^3/s)*	*Gaula* 3) *(m^3/s)*	*Rugla* 2)	*Glåma* 3) *(m^3/s)*	*Orva* 3) *(m^3/s)*	*Bjørgåsen* 1) *(L/s)*
01.03.97	0.33		0.26	0.54		25	0.12	0.72
02.03.97	0.33		0.26	0.60		25	0.12	0.80
03.03.97	0.34		0.26	0.66		25	0.12	0.88
04.03.97	0.28		0.26	0.57		25	0.10	0.76
05.03.97	0.34		0.26	0.62		25	0.12	0.84
06.03.97	0.36		0.26	0.70		25	0.13	0.94
07.03.97	0.31		0.3	0.67		25	0.11	0.90
08.03.97	0.29		0.29	0.64		25	0.11	0.86
09.03.97	0.29		0.25	0.59		25	0.10	0.80
10.03.97	0.28		0.25	0.61		25	0.10	0.82
11.03.97	0.28		0.26	0.68		25	0.10	0.92
12.03.97	0.27		0.25	0.60		25	0.10	0.81
13.03.97	0.27		0.29	0.82		25	0.09	1.10
14.03.97	0.26		0.24	0.75		25	0.09	1.00
15.03.97	0.26		0.24	0.72		25	0.09	0.97
16.03.97	0.25		0.34	0.66		25	0.09	0.89
17.03.97	0.25		0.32	0.66		25	0.09	0.89
18.03.97	0.24		0.29	0.61		25	0.09	0.82
19.03.97	0.24		0.29	0.65		25	0.08	0.88
20.03.97	0.23		0.35	0.59		25	0.08	0.79
21.03.97	0.22		0.42	0.55		25	0.08	0.74
22.03.97	0.22		0.36	0.55		25	0.08	0.74
23.03.97	0.21		0.34	0.54		25	0.08	0.73
24.03.97	0.21		0.33	0.53		25	0.08	0.71
25.03.97	0.20		0.27	0.52		25	0.07	0.70
26.03.97	0.20		0.29	0.52		23	0.07	0.69
27.03.97	0.20		0.32	0.51		23	0.07	0.69
28.03.97	0.21		0.32	0.51		23	0.07	0.69
29.03.97	0.21		0.35	0.51		22	0.07	0.68
30.03.97	0.21		0.39	0.53		21	0.07	0.71
31.03.97	0.22		0.44	1.60		21	0.08	2.14
01.04.97	0.23		0.5	4.70		21	0.08	6.30
02.04.97	0.24		0.51	4.82		12	0.09	6.46
03.04.97	0.26		0.52	2.94		12	0.09	3.94
04.04.97	0.26		0.73	2.01		12	0.10	2.70
05.04.97	0.27		0.78	1.78		12	0.10	2.38
06.04.97	0.29		0.56	1.78		12	0.11	2.38
07.04.97	0.30		0.49	1.75		12	0.11	2.35
08.04.97	0.33		0.47	1.74		12	0.12	2.33
09.04.97	0.35		0.46	1.67		12	0.13	2.24
10.04.97	0.39		0.47	1.77		12	0.14	2.37
11.04.97	0.43		0.49	1.92		13	0.16	2.57
12.04.97	0.40		0.54	1.66		12	0.14	2.22
13.04.97	0.33		0.61	1.74		12	0.12	2.33
14.04.97	0.40		0.55	2.36		12	0.14	3.16
15.04.97	0.43		0.5	2.00		12	0.16	2.69
16.04.97	0.40		0.54	1.84		12	0.14	2.47

1) Discharge roughly calculated from that of nearby streams by the Norwegian Water Resources and Energy Administration (NVE). 2) Water levels (not necessarily proportional to discharge) measured by pressure sensors (some dates missing). 3) Measured by NVE.

Table A1.17: Diel river discharges for the rivers (continued).

Date	*Røa* (*m^3/s*)	*Nauste-bekken* 1)	*Hitter-elva* (*m^3/s*)	*Gaula* (*m^3/s*)	*Rugla* 1)	*Glåma* (*m^3/s*)	*Orva* (*m^3/s*)	*Bjørg-åsen* (*L/s*)
17.04.97	0.42		0.59	2.11		12	0.15	2.83
18.04.97	0.50		0.56	2.09		12	0.18	2.81
19.04.97	0.53		0.58	1.81		12	0.19	2.42
20.04.97	0.51		0.56	1.64		12	0.18	2.20
21.04.97	0.50		0.56	1.50		12	0.18	2.01
22.04.97	0.43		0.63	1.37		12	0.16	1.84
23.04.97	0.37		0.56	1.29		12	0.13	1.72
24.04.97	0.41		0.49	1.34		12	0.15	1.80
25.04.97	0.43		0.53	1.26	406	12	0.16	1.68
26.04.97	0.43		0.49	1.17	376	12	0.16	1.57
27.04.97	0.43		0.43	1.15	376	12	0.15	1.55
28.04.97	0.41		0.42	1.20	376	12	0.15	1.61
29.04.97	0.38		0.4	1.49	396	7	0.14	2.00
30.04.97	0.38		0.39	2.22	416	7	0.14	2.98
01.05.97	0.35		0.6	4.83	537	6	0.13	6.47
02.05.97	0.43		0.76	8.61	1386	6	0.16	11.54
03.05.97	0.43		1.07	8.32	759	8	0.16	11.15
04.05.97	0.52		1.24	6.08	628	8	0.19	8.15
05.05.97	0.52	108	1.36	5.14	547	8	0.19	6.89
06.05.97	0.73	103	1.51	4.64	507	8	0.26	6.22
07.05.97	0.98	98	2.15	8.55	1659	8	0.36	11.45
08.05.97	1.36	110	3.83	20.68	1467	8	0.49	27.71
09.05.97	1.65	118	5.69	17.84	931	13	0.60	23.91
10.05.97	2.06	141	6.91	15.49	719	13	0.75	20.76
11.05.97	2.50	124	8.67	18.53	769	13	0.90	24.83
12.05.97	3.42	130	11.54	21.42	769	13	1.24	28.70
13.05.97	4.34	129	15.34	27.81	820	13	1.57	37.26
14.05.97	6.22	146	20.79	35.17	840	18	2.25	47.12
15.05.97	9.81	145	25	38.78	860	19	3.55	51.96
16.05.97	10.83	170	27.2	37.75	840	21	3.92	50.58
17.05.97	6.43	187	26.7	35.90	850	26	2.33	48.11
18.05.97	6.19	202	25.86	33.06	789	25	2.24	44.30
19.05.97	4.94	201	20.82	26.28	729	25	1.79	35.21
20.05.97	4.19	198	16.27	22.22	688	25	1.52	29.78
21.05.97	3.66	195	12.69	19.94	688	25	1.32	26.72
22.05.97	3.36	188	11.19	18.01	648	25	1.21	24.14
23.05.97	3.07	173	9.78	15.01	658	32	1.11	20.12
24.05.97	2.98	161	8.78	14.81	658	32	1.08	19.84
25.05.97	3.08	170	8.22	15.42	708	32	1.11	20.66
26.05.97	3.92	184	8.93	21.18	789	33	1.42	28.39
27.05.97	5.77	180	12.61	30.05	840	33	2.09	40.27
28.05.97	6.78	193	15.05	32.24	799	36	2.45	43.20
29.05.97	6.15	196	16.35	30.02	759	36	2.22	40.23
30.05.97	5.93	184	14.79	28.37	799	36	2.14	38.01
31.05.97	7.18	189	15.27	35.38	860	39	2.60	47.40
01.06.97	9.60	213	18.2	43.06	860	40	3.47	57.70
02.06.97	11.54	245	21.53	48.41	850	41	4.17	64.87
03.06.97	11.18	264	20.46	48.15	850	28	4.04	64.53

1) Discharge roughly calculated from that of nearby streams by the Norwegian Water Resources and Energy Administration (NVE). 2) Water levels (not necessarily proportional to discharge) measured by pressure sensors (some dates missing). 3) Measured by NVE.

Table A1.17: Diel river discharges for the rivers (continued).

Date	*Røa* (*m^3/s*)	*Nauste-bekken* [1)	*Hitter-elva* (*m^3/s*)	*Gaula* (*m^3/s*)	*Rugla* [1)	*Glåma* (*m^3/s*)	*Orva* (*m^3/s*)	*Bjørg-åsen* (*L/s*)
04.06.97	9.92	261	17.88	43.66	809	28	3.59	58.51
05.06.97	10.64	261	16.21	43.08	850	42	3.85	57.73
06.06.97	11.58	263	15.21	47.96	840	42	4.19	64.26
07.06.97	13.11	265	14.97	53.89	830	46	4.74	72.21
08.06.97	17.13	299	15.27	73.46	931	60	6.20	98.43
09.06.97	21.15	322	14.93	86.81	809	28	7.65	116.32
10.06.97	16.57	304	15.53	72.93	809	79	5.99	97.73
11.06.97	11.99	288	11.52	55.02	759	102	4.34	73.73
12.06.97	11.28	272	8.42	51.58	809	100	4.08	69.11
13.06.97	11.69	270	7.49	58.84	830	64	4.23	78.84
14.06.97	11.36	272	7.77	59.99	799	64	4.11	80.38
15.06.97	13.76	270	8.55	75.83	820	65	4.98	101.61
16.06.97	11.93	250	10.66	56.70	729	127	4.32	75.97
17.06.97	9.49	240	9.97	44.28	749	92	3.43	59.33
18.06.97	8.66	236	9.39	38.95	708	114	3.13	52.19
19.06.97	7.56	229	9.11	34.68	678	113	2.74	46.47
20.06.97	6.73	221	8.55	31.20	678	86	2.43	41.81
21.06.97	6.07	213	8.01	34.67	698	76	2.20	46.45
22.06.97	5.70	214	7.49	38.29	708	64	2.06	51.31
23.06.97	7.18	221	7.23	44.64	789	64	2.60	59.82
24.06.97	7.57	215	6.73	55.52	708	53	2.74	74.40
25.06.97	5.80	203	6.25	36.41	648	52	2.10	48.79
26.06.97	5.95	190	5.93	31.83	688	76	2.15	42.65
27.06.97	5.48	183	5.77	30.24	668	76	1.98	40.52
28.06.97	4.57	181	5.54	31.28	678	76	1.65	41.91
29.06.97	5.13	190	5.32	38.51	678	59	1.86	51.60
30.06.97	5.18	185	5.04	35.60	708	59	1.87	47.70
01.07.97	6.34	185	6.49	51.56	759	70	2.29	69.09
02.07.97	5.70	189	6.01	48.36	708	92	2.06	64.80
03.07.97	5.42	173	5.77	40.48	648	92	1.96	54.25
04.07.97	4.50	154	5.32	26.97	638	92	1.63	36.14
05.07.97	4.00	146	5.1	22.18	597	92	1.45	29.72
06.07.97	3.18	133	4.67	16.24	557	58	1.15	21.77
07.07.97	3.02	122	4.25	14.96	597	47	1.09	20.05
08.07.97	2.84	120	3.86	13.97	547	33	1.03	18.72
09.07.97	2.39	117	3.66	10.77	527	27	0.87	14.43
10.07.97	2.10	113	3.29	10.67	517	27	0.76	14.30
11.07.97	1.76	108	2.94	10.42	486	27	0.64	13.97
12.07.97	1.64	100	2.77	9.38	476	21	0.59	12.57
13.07.97	1.54		2.44	8.81	476	21	0.56	11.80
14.07.97	1.45		2.32	8.18	466	21	0.53	10.97
15.07.97	1.37		2.21	7.85	456	21	0.49	10.52
16.07.97	1.29		2.1	6.84	446	21	0.47	9.16
17.07.97	1.22		1.99	6.18	426	21	0.44	8.28
18.07.97	1.12		1.99	5.69	436	21	0.41	7.62
19.07.97	1.08		1.89	5.94	436	21	0.39	7.96
20.07.97	1.01		1.78	5.73	416	20	0.36	7.67
21.07.97	0.91		1.65	5.14	436	20	0.33	6.89

[1) Discharge roughly calculated from that of nearby streams by the Norwegian Water Resources and Energy Administration (NVE). [2) Water levels (not necessarily proportional to discharge) measured by pressure sensors (some dates missing). [3) Measured by NVE.

Table A1.17: Diel river discharges for the rivers (continued).

Date	*Røa* (*m^3/s*)	*Nauste-bekken* 1)	*Hitter-elva* (*m^3/s*)	*Gaula* (*m^3/s*)	*Rugla* 1)	*Glåma* (*m^3/s*)	*Orva* (*m^3/s*)	*Bjørg-åsen* (*L/s*)
22.07.97	0.87		2.37	7.58	426	17	0.32	10.16
23.07.97	0.95		2.03	7.17	416	17	0.34	9.61
24.07.97	0.78		1.56	4.83	396	17	0.28	6.47
25.07.97	0.73		1.51	4.10	386	14	0.26	5.50
26.07.97	0.73		1.49	3.87	386	13	0.26	5.18
27.07.97	0.78		1.32	5.80	406	13	0.28	7.78
28.07.97	0.73		1.12	7.49	406	13	0.26	10.04
29.07.97	0.72		1.05	7.60	406	13	0.26	10.18
30.07.97	0.61		1.13	5.84	386	13	0.22	7.83
31.07.97	0.54		1.06	4.23	376	13	0.20	5.67
01.08.97	0.49		1.06	3.52	356	13	0.18	4.71
02.08.97	0.47		1.02	3.39	366	13	0.17	4.54
03.08.97	0.51		0.96	3.27	346	13	0.19	4.38
04.08.97	0.45		0.91	2.70	346	12	0.16	3.62
05.08.97	0.43		0.86	2.23	336	12	0.16	3.00
06.08.97	0.42		0.82	1.96	316	12	0.15	2.63
07.08.97	0.39		0.77	1.84	316	12	0.14	2.46
08.08.97	0.34		0.73	1.68	326	12	0.12	2.25
09.08.97	0.35		0.68	1.63	316	12	0.13	2.18
10.08.97	0.35		0.64	1.80	316	12	0.13	2.42
11.08.97	0.33		0.6	1.37	296	14	0.12	1.83
12.08.97	0.36		0.57	1.08	326	19	0.13	1.45
13.08.97	0.35		0.53	1.13	326	19	0.13	1.51
14.08.97	0.33		0.46	0.98	316	19	0.12	1.31
15.08.97	0.30		0.4	0.75	306	19	0.11	1.01
16.08.97	0.29		0.42	0.64	306	19	0.11	0.86
17.08.97	0.29		0.4	0.58	296	19	0.11	0.78
18.08.97	0.29		0.44	0.54	296	19	0.11	0.72
19.08.97	0.29		0.37	0.59	306	19	0.11	0.79
20.08.97	0.29		0.41	0.59	316	19	0.11	0.79
21.08.97	0.27		0.44	0.54	306	19	0.10	0.73
22.08.97	0.28		0.39	0.53	336	19	0.10	0.71
23.08.97	0.28		0.38	0.75	326	19	0.10	1.00
24.08.97	0.32		0.49	0.74	346	19	0.12	0.99
25.08.97	0.30		0.66	0.98	336	19	0.11	1.31
26.08.97	0.55		0.89	1.64	376	19	0.20	2.20
27.08.97	0.35		0.79	1.65	356	19	0.12	2.22
28.08.97	0.33		0.71	1.15	346	11	0.12	1.53
29.08.97	0.31		0.83	1.09	346	11	0.11	1.46
30.08.97	1.57		1.45	5.16	850	7	0.57	6.91
31.08.97	4.35		6.22	43.85	648	9	1.57	58.76
01.09.97	1.54		8.03	14.68	547	8	0.56	19.67
02.09.97	1.34		7.92	9.14	618	12	0.48	12.25
03.09.97	1.38		10.52	13.34	507	27	0.50	17.88
04.09.97	1.17		9.06	7.69	466	26	0.42	10.31
05.09.97	1.06		7.22	5.59	446	26	0.38	7.49
06.09.97	1.01		5.92	4.17	436	26	0.37	5.58
07.09.97	1.28		5.19	4.18	456	26	0.46	5.60

1) Discharge roughly calculated from that of nearby streams by the Norwegian Water Resources and Energy Administration (NVE). 2) Water levels (not necessarily proportional to discharge) measured by pressure sensors (some dates missing). 3) Measured by NVE.

Table A1.17: Diel river discharges for the rivers (continued).

Date	*Røa* (m^3/s)	*Nauste-bekken* 1)	*Hitter-elva* (m^3/s)	*Gaula* (m^3/s)	*Rugla* 1)	*Glåma* (m^3/s)	*Orva* (m^3/s)	*Bjørg-åsen* (L/s)
08.09.97	1.27		3.77	5.14	496	26	0.46	6.89
09.09.97	3.23		3.83	18.58	830	26	1.17	24.89
10.09.97	6.60		7.18	54.17	708	28	2.39	72.59
11.09.97	2.70		7.69	21.82	648	42	0.98	29.24
12.09.97	2.46		7.26	13.81	577	42	0.89	18.50
13.09.97	2.32		4.53	10.27	678	27	0.84	13.77
14.09.97	2.99		5.78	36.66	648	28	1.08	49.12
15.09.97	2.08		9.12	24.51	618	51	0.75	32.84
16.09.97	1.67		8.06	13.65	557	50	0.60	18.28
17.09.97	1.85		5.88	13.70	547	50	0.67	18.36
18.09.97	1.63		5.55	12.30	527	39	0.59	16.48
19.09.97	1.95		4.58	14.21	607	39	0.70	19.04
20.09.97	1.95		2.68	18.87	729	39	0.71	25.29
21.09.97	2.25		2.22	44.16	698	39	0.81	59.17
22.09.97	2.58		2.47	47.09	729	72	0.93	63.11
23.09.97	2.67		2.77	42.95	668	94	0.97	57.55
24.09.97	2.45		3.86	25.91	618	83	0.89	34.72
25.09.97	2.01		4.67	14.68	567	27	0.73	19.67
26.09.97	1.76		4.3	10.13	527	27	0.64	13.58
27.09.97	1.62		3.78	7.89	507	58	0.59	10.57
28.09.97	1.48		3.38	6.39	486	39	0.54	8.57
29.09.97	1.40		2.56	5.53	486	27	0.51	7.41
30.09.97	1.40		2.61	6.21	476	27	0.51	8.32
01.10.97	1.38		2.69	5.79	587	27	0.50	7.76
02.10.97	1.63		2.83	8.11	567	27	0.59	10.87
03.10.97	1.62		3.13	8.96	587	27	0.59	12.01
04.10.97	1.65		3.51	11.62	577	27	0.60	15.57
05.10.97	1.58		3.5	9.10	517	27	0.57	12.19
06.10.97	1.34		3.29	6.97	507	27	0.48	9.34
07.10.97	1.36		3.11	6.95	507	27	0.49	9.32
08.10.97	1.37		2.94	8.19	678	27	0.50	10.98
09.10.97	1.60		2.77	13.42	577	27	0.58	17.98
10.10.97	1.45		2.77	9.21	537	23	0.52	12.34
11.10.97	1.34		2.61	7.20	496	23	0.48	9.64
12.10.97	1.39		2.34	6.27	507	23	0.50	8.40
13.10.97	1.45		3.01	6.97	466	18	0.53	9.34
14.10.97	1.32		3.31	6.35	466	19	0.48	8.51

1) Discharge roughly calculated from that of nearby streams by the Norwegian Water Resources and Energy Administration (NVE). 2) Water levels (not necessarily proportional to discharge) measured by pressure sensors (some dates missing). 3) Measured by NVE.

Table A1.18: Precipitation (three columns in the middle) and temperatures in the area

Date	Aursunden1) mm/d (685m)	Røros 1) mm/d (628m)	Fjellsjøen2) mm/d (820m)	Røros 1) T (°C)
01.03.97	0.6		-	-1
02.03.97			-	2
03.03.97		0.4	-	-2
04.03.97	6.6	4.7	-	-2
05.03.97	0.6	0.5	-	1
06.03.97	0.9	0.3	-	-1
07.03.97			-	1
08.03.97	0.6	0.5	-	0
09.03.97			-	3
10.03.97			-	2
11.03.97			-	-4
12.03.97			-	0
13.03.97			-	-4
14.03.97	0.6	0.5	-	-5
15.03.97	0.9	0.1	-	-8
16.03.97	0.6	1	-	-7
17.03.97	2.7	0.7	-	-11
18.03.97		0.1	-	-17
19.03.97			-	-14
20.03.97			-	-16
21.03.97			-	-12
22.03.97	0.9	0.5	-	-9
23.03.97			-	-14
24.03.97			-	-9
25.03.97			-	-8
26.03.97	0.6	0.6	-	-2
27.03.97	0.9	3.9	-	1
28.03.97	3	0.6	-	-2
29.03.97	2.1	1.8	-	-3
30.03.97	2.4	0.5	-	1
31.03.97	10.2	4.8	-	4
01.04.97	1.5	1	-	2
02.04.97	1.8	3.3	-	-2
03.04.97	5.4	1.4	-	-4
04.04.97	6.6	8.2	-	-4
05.04.97	4.2	2.5	-	-7
06.04.97	0.9	1	-	-7
07.04.97	1.5	1.4	-	-9
08.04.97		0.1	-	-5
09.04.97	2.1	1.3	-	1
10.04.97	0.9	0.7	-	1
11.04.97	6.3	9	-	-3
12.04.97	1.5	0.7	-	-2
13.04.97	6	4.1	-	1
14.04.97	2.1	1.5	-	-5
15.04.97	3	3.6	-	-2
16.04.97	1.5	0.2	-	3
17.04.97		0.2	-	-1
18.04.97	0.6	0.5	-	-4
19.04.97	1.5	1	-	-4
20.04.97	0.9	0.6	-	-1
21.04.97	1.5		-	-2
22.04.97	4.2	6.6	-	-5
23.04.97	0.6	0.2	-	-6
24.04.97			-	-7
25.04.97	0.9	0.5	-	-3
26.04.97	1.5	0.6	-	-2
27.04.97			-	-3
28.04.97			-	1
29.04.97			-	4
30.04.97			-	2
01.05.97	5.1	3.9	-	5
02.05.97	1.5	2	-	2
03.05.97		0.1	-	-2
04.05.97	2.1	0.5	-	-2
05.05.97		0.1	-	-3
06.05.97		1.5	-	1
07.05.97	8.1	10.5	-	3
08.05.97	18.3	30	-	4
09.05.97	0		-	1
10.05.97	0.6	5.7	-	3
11.05.97	5.4	10	-	4
12.05.97		0.2	-	6
13.05.97	0.6	0.5	-	6
14.05.97			-	7
15.05.97			-	6
16.05.97			-	5
17.05.97			-	7
18.05.97			-	2
19.05.97			-	2
20.05.97			-	1
21.05.97		0.1	-	1
22.05.97		0.1	-	1
23.05.97		0.3	-	3
24.05.97			-	3
25.05.97			-	5
26.05.97			-	7
27.05.97	0.6	1.5	-	4
28.05.97	0.9	0.9	-	4
29.05.97	4.8	5	-	3
30.05.97	0.9	1.4	-	6
31.05.97			-	9

- : Not determined

1) : From Norwegian Meteorological Institute

2) : From own logger

Table A1.18: Precipitation (three columns in the middle) and temperatures in the area (continued)

Date	Aursunden mm/d (685m)	Røros mm/d (628m)	Fjellsjøen mm/d (820m)	T (°C) Røros
01.06.97			-	8
02.06.97			-	8
03.06.97			-	6
04.06.97			-	9
05.06.97			-	11
06.06.97			-	13
07.06.97			-	15
08.06.97			-	18
09.06.97			-	14
10.06.97		0.5	-	9
11.06.97			-	11
12.06.97			-	15
13.06.97			-	16
14.06.97		0.5	-	13
15.06.97	6	8.2	-	7
16.06.97	9.3	5.1	-	5
17.06.97			-	5
18.06.97	2.4	3.6	-	9
19.06.97	0.6		-	9
20.06.97		1.2	-	11
21.06.97			-	13
22.06.97		0.8	-	11
23.06.97	5.7	4.5	-	11
24.06.97	8.1	2.5	-	8
25.06.97	0.9	0.2	-	8
26.06.97	1.2	5	-	7
27.06.97	6.5	8.3	-	10
28.06.97			-	14
29.06.97	4.5	3	-	13
30.06.97	1.2	0.4	-	15
01.07.97	14.7	24	-	18
02.07.97	0.6	0.5	-	16
03.07.97	3.9	6.1	-	15
04.07.97	0.6	0.2	-	15
05.07.97			-	14
06.07.97			-	14
07.07.97	0.9	0.2	-	15
08.07.97	1.5	2.8	-	8
09.07.97			-	8
10.07.97			-	13
11.07.97			-	16
12.07.97			-	15
13.07.97			-	17
14.07.97			-	16
15.07.97			-	15
16.07.97		0.6	-	15
17.07.97			-	15
18.07.97			-	16
19.07.97	7.2	0.1	-	13
20.07.97	0.9	4.3	-	16
21.07.97			-	15
22.07.97	6.6	2.3	-	15
23.07.97	0.9		-	14
24.07.97			-	15
25.07.97			-	16
26.07.97			-	14
27.07.97	0.3	1.9	-	13
28.07.97	2.4	4.7	-	11
29.07.97	1.5	2.5	-	11
30.07.97	0.6		-	14
31.07.97			-	13
01.08.97			-	14
02.08.97			-	14
03.08.97	1.4	5.5	-	13
04.08.97		0.1	-	14
05.08.97			-	13
06.08.97			-	17
07.08.97			-	17
08.08.97			-	17
09.08.97			-	18
10.08.97	0.9	0.6	-	12
11.08.97			-	10
12.08.97			-	13
13.08.97	6.3	0.4	-	14
14.08.97			-	11
15.08.97			-	13
16.08.97			-	14
17.08.97			-	16
18.08.97			-	15
19.08.97		0.1	-	16
20.08.97		0.9	-	15
21.08.97			-	15
22.08.97			-	15
23.08.97	6.9	4.6	-	11
24.08.97	8.7	10	-	10
25.08.97	0.6	2.8	-	12
26.08.97	12.6	8	-	16
27.08.97		0.1	-	13
28.08.97		0.7	-	16
29.08.97			-	17
30.08.97		14.5	-	15
31.08.97	53.4	47.6	-	13

- : Not determined

1) : From Norwegian Meteorological Institute

2) : From own logger

Table A1.18: Precipitation (three columns in the middle) and temperatures in the area (continued)

Date	Aursunden mm/d (685m)	Røros mm/d (628m)	Fjell-sjøen mm/d (820m)	T (°C) Røros
01.09.97	3.9	4.9	-	13
02.09.97			-	15
03.09.97	4.8	5.5	-	13
04.09.97			-	13
05.09.97	0.9	0.7	-	14
06.09.97			-	12
07.09.97	6	4.7	-	10
08.09.97	2.7	2.1	-	8
09.09.97	8.1	4.8	-	5
10.09.97	27.9	20.3	-	5
11.09.97	0.6	0.5	-	7
12.09.97	0.6	1.6	-	7
13.09.97	0.9	0.8	-	5
14.09.97	16.2	11.3	-	6
15.09.97	1.5	1	-	4
16.09.97		0.1	-	8
17.09.97	3.9	2.5	-	5
18.09.97	0.6		-	3
19.09.97	5.1	3.6	1.20	2
20.09.97	0.5	0.5	1.03	4
21.09.97	3.9	0.5	0.75	6
22.09.97	3.3	2	1.20	6
23.09.97	4.8	1.1	0.65	4
24.09.97	1.5	0.7	0.15	9
25.09.97				10
26.09.97				7
27.09.97				3
28.09.97				4
29.09.97			0.30	5
30.09.97	1.5	1.3	0.10	5
01.10.97		0.2	0.90	2
02.10.97	6.8	6.8	0.70	3
03.10.97	0.6	0.2	0.45	3
04.10.97	4.2	6.2	0.43	3
05.10.97	0.9	0.3	0.03	2
06.10.97			0.15	0
07.10.97	1.5	2.2	0.13	4
08.10.97		1.6	1.23	4
09.10.97	6.3	5.4	0.10	5
10.10.97	0.9		0.03	4
11.10.97		0.4		2
12.10.97	1.5	0.8	0.25	2
13.10.97	2.4		0.40	2
14.10.97	3.6	1.8	0.175	0

- : Not determined

1) : From Norwegian Meteorological Institute

2) : From own logger

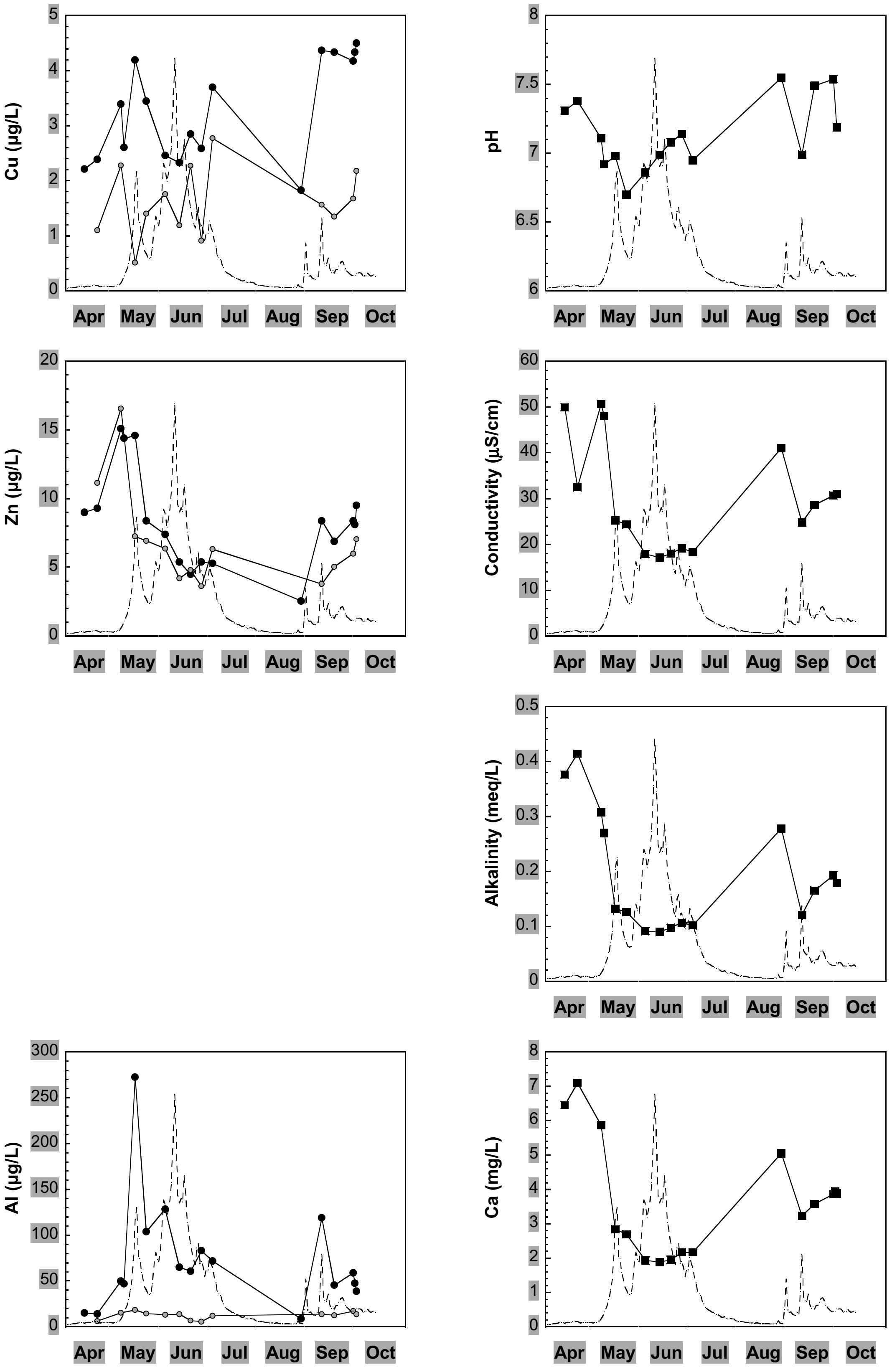

Figure A2.1: Water chemistry data and river discharge (----) in stream Røa. In left figures: Cu, Zn, and Al concn.; black points represent total concn., open points represent dissolved concn. In right figures: pH, conductivity, alkalinity and Ca concn.

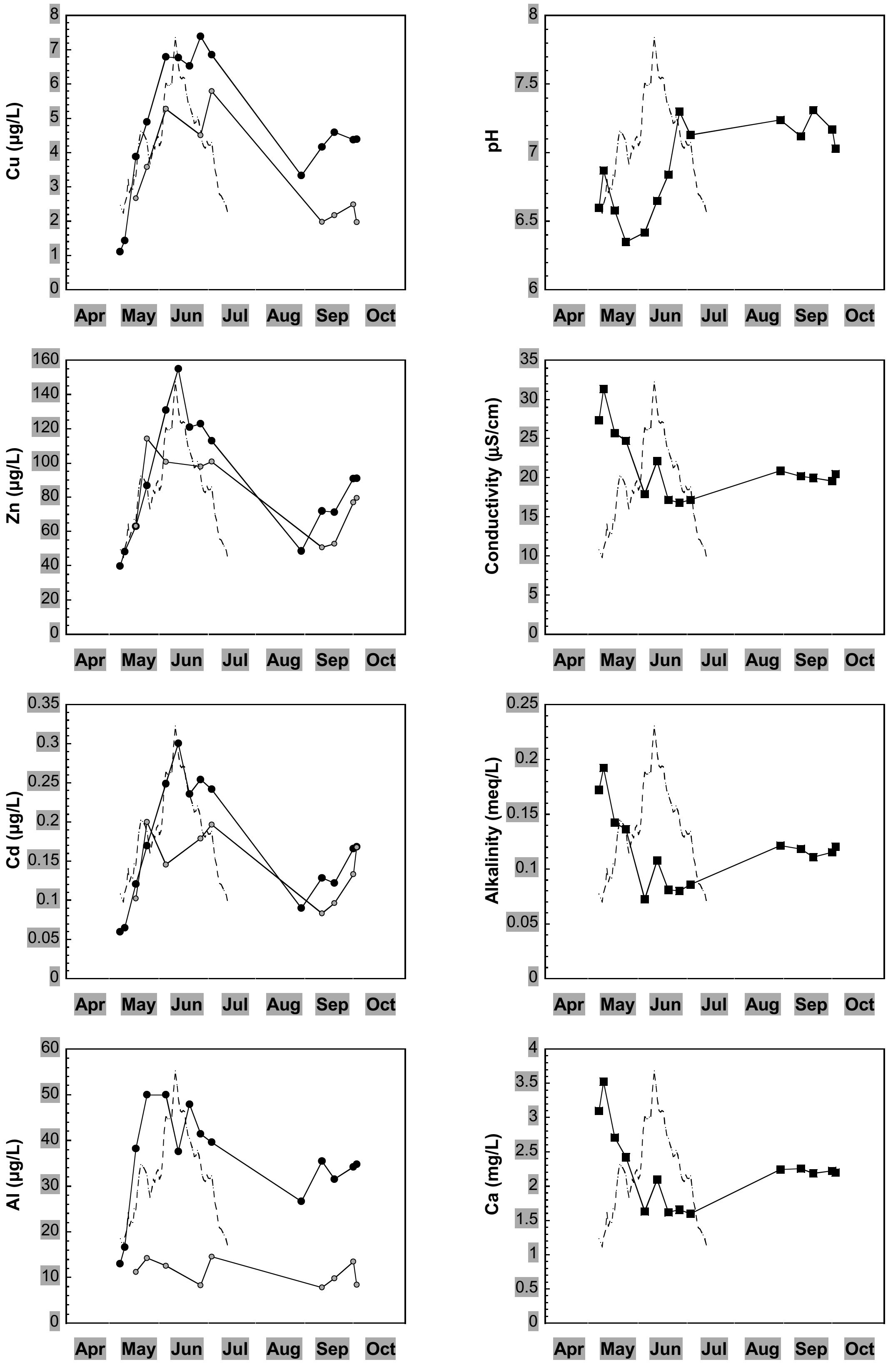

Figure A2.2: Water chemistry data and river discharge (----) in stream Naustebekken. In left figures: Cu, Zn, Cd, and Al concn.; black points represent total concn., open points represent dissolved concn. In right figures: pH, conductivity, alkalinity and Ca concn.

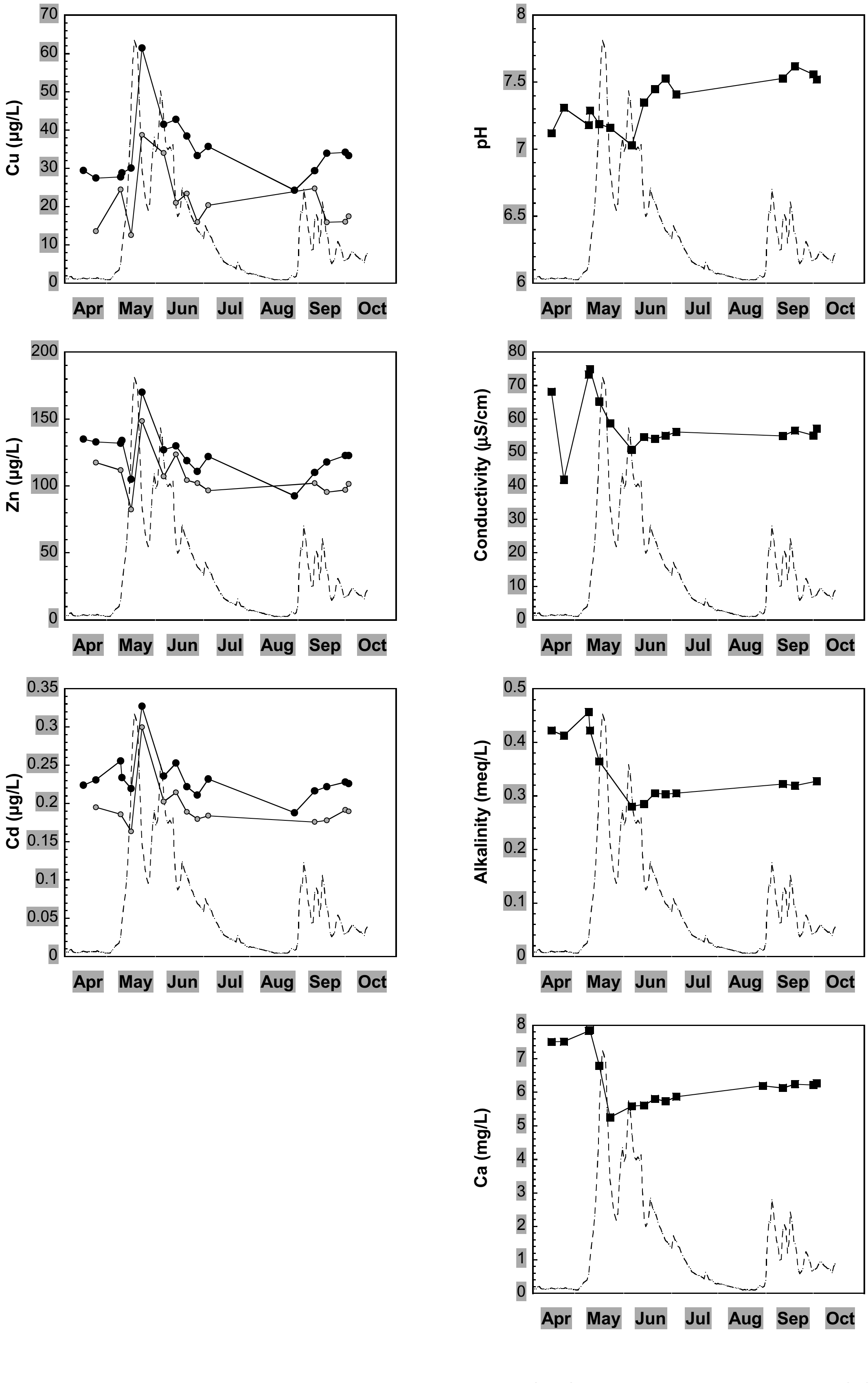

Figure A2.3: Water chemistry data and river discharge (----) in stream Hitterelva. In left figures: Cu, Zn, and Cd concn.; black points represent total concn., open points represent dissolved concn. In right figures: pH, conductivity, alkalinity and Ca concn.

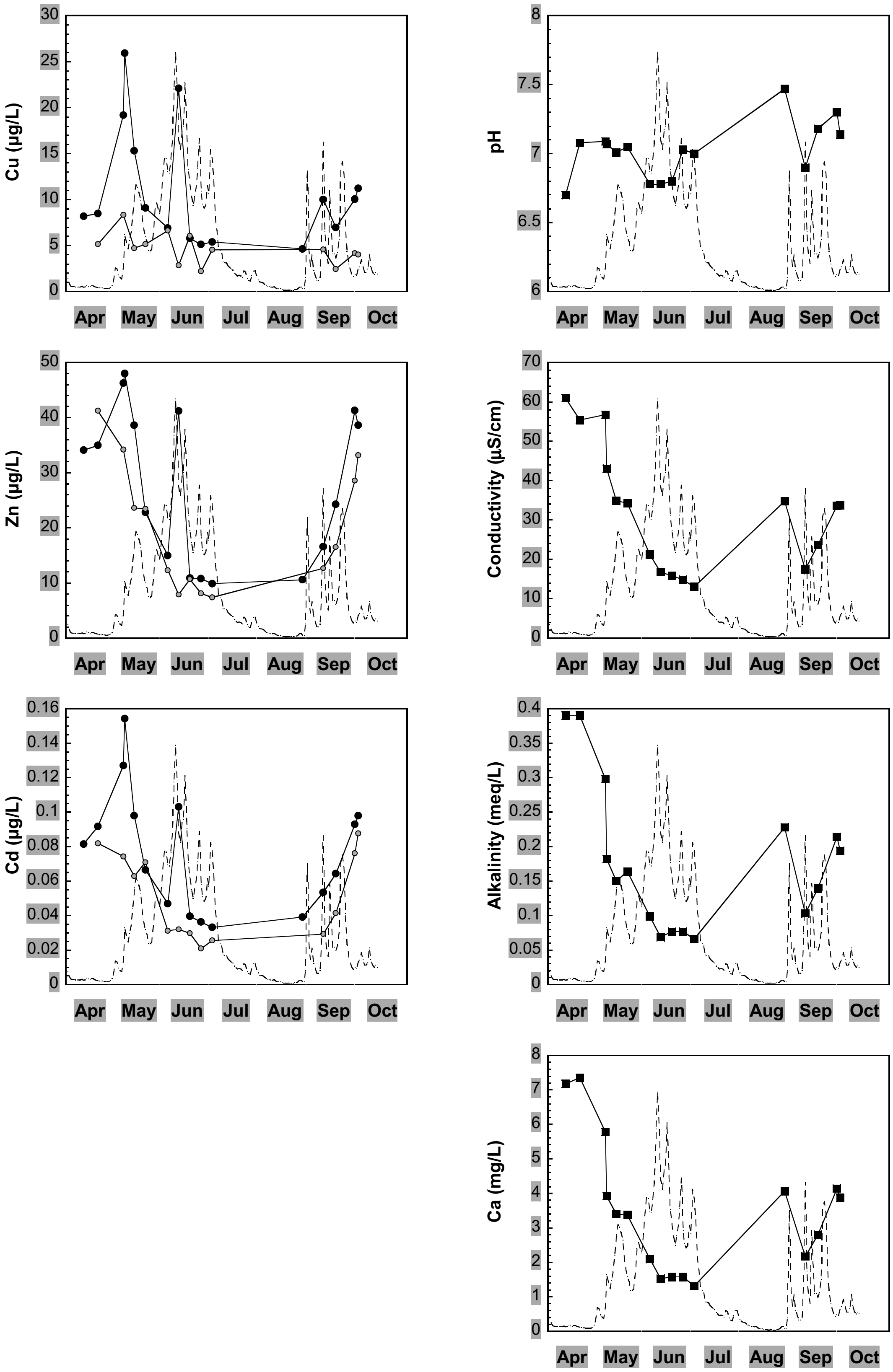

Figure A2.4: Water chemistry data and river discharge (----) in river Gaula. In left figures: Cu, Zn, and Cd concn.; black points represent total concn., open points represent dissolved concn. In right figures: pH, conductivity, alkalinity and Ca concn.

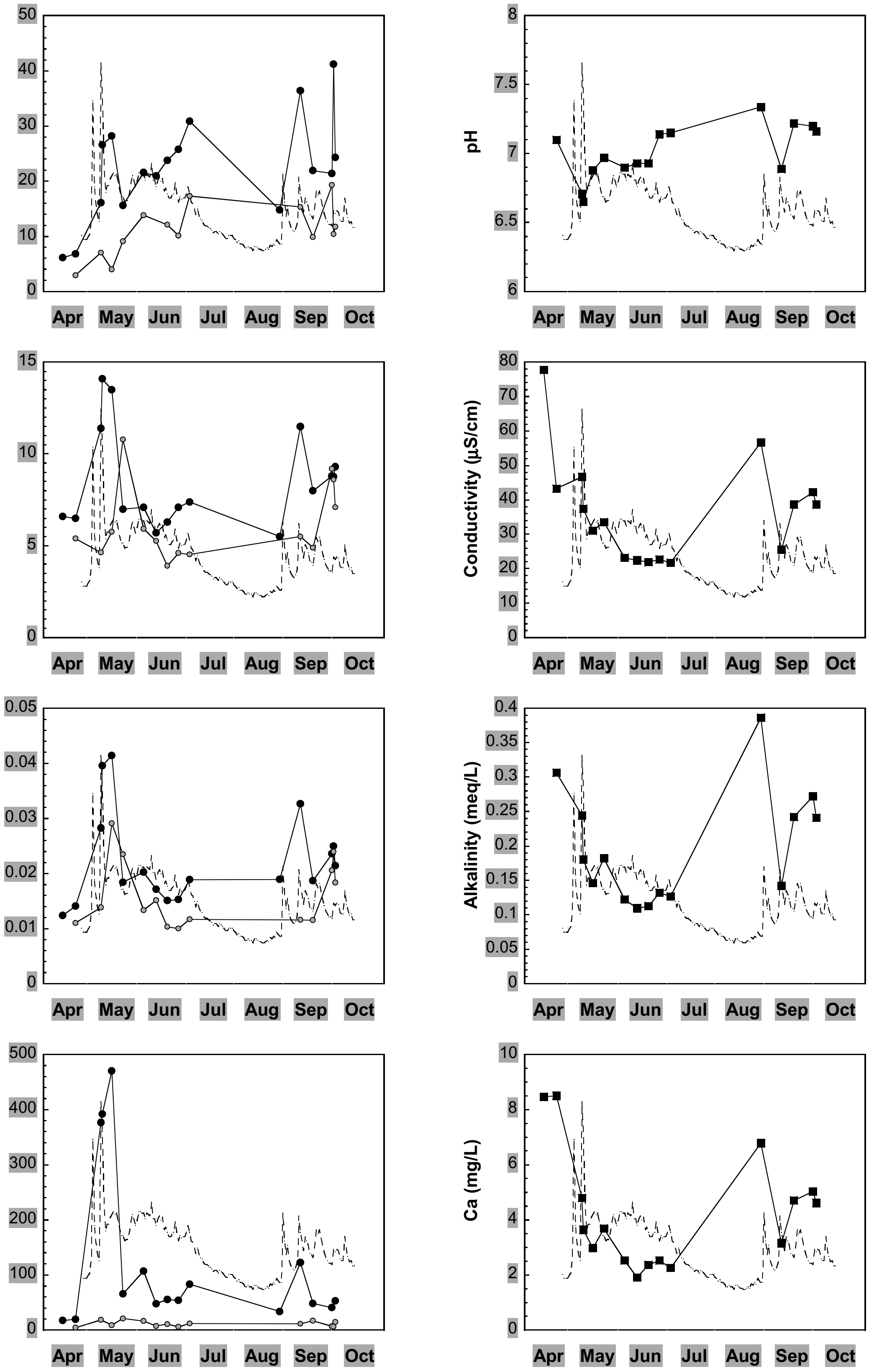

Figure A2.5: Water chemistry data and river discharge (----) in stream Rugla. In left figures: Cu, Zn, Cd, and Al concn.; black points represent total concn., open points represent dissolved concn. In right figures: pH, conductivity, alkalinity and Ca concn.

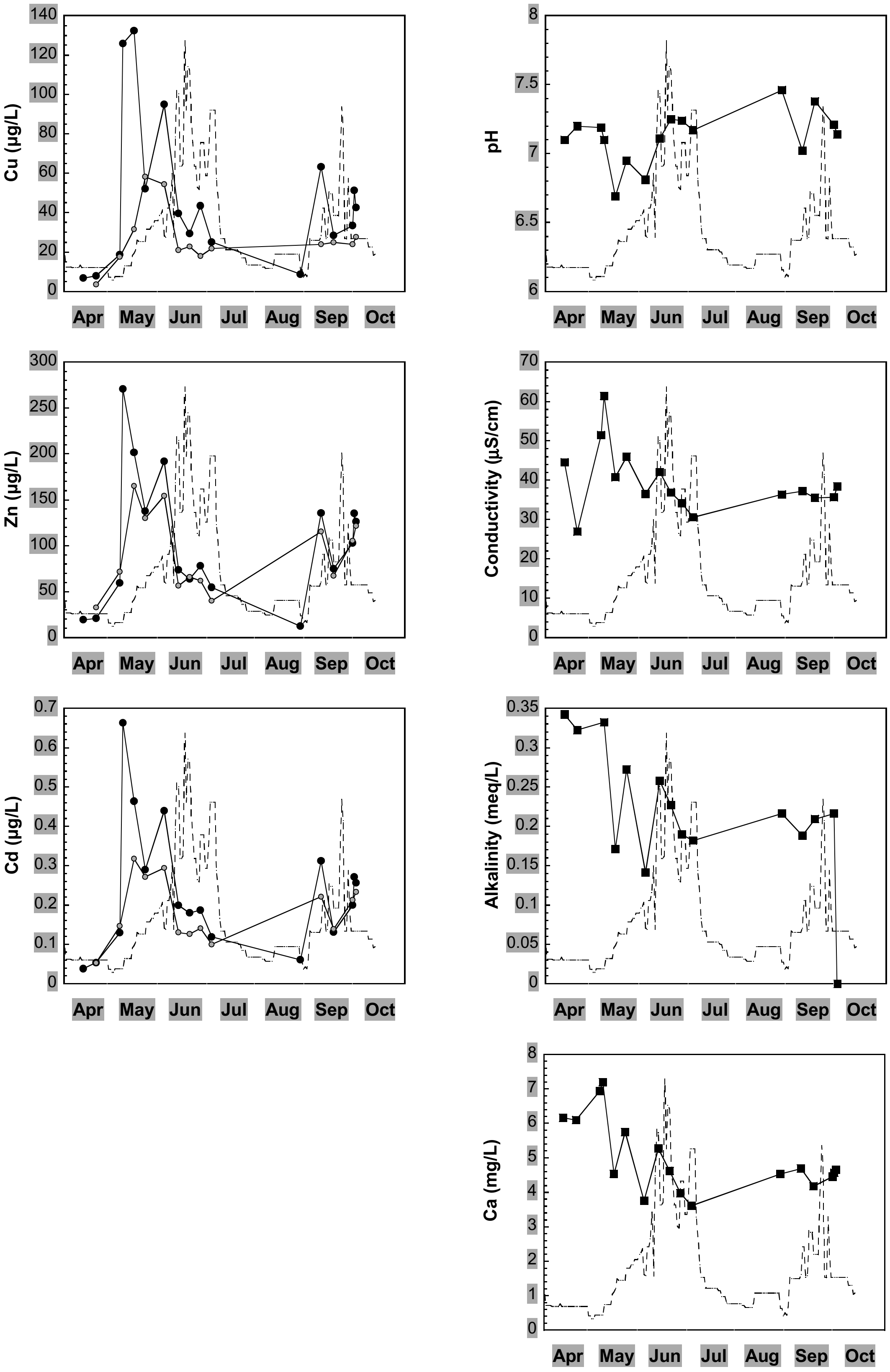

Figure A2.6: Water chemistry data and river discharge (----) in river Glåma. In left figures: Cu, Zn, and Cd concn.; black points represent total concn., open points represent dissolved concn. In right figures: pH, conductivity, alkalinity and Ca concn.

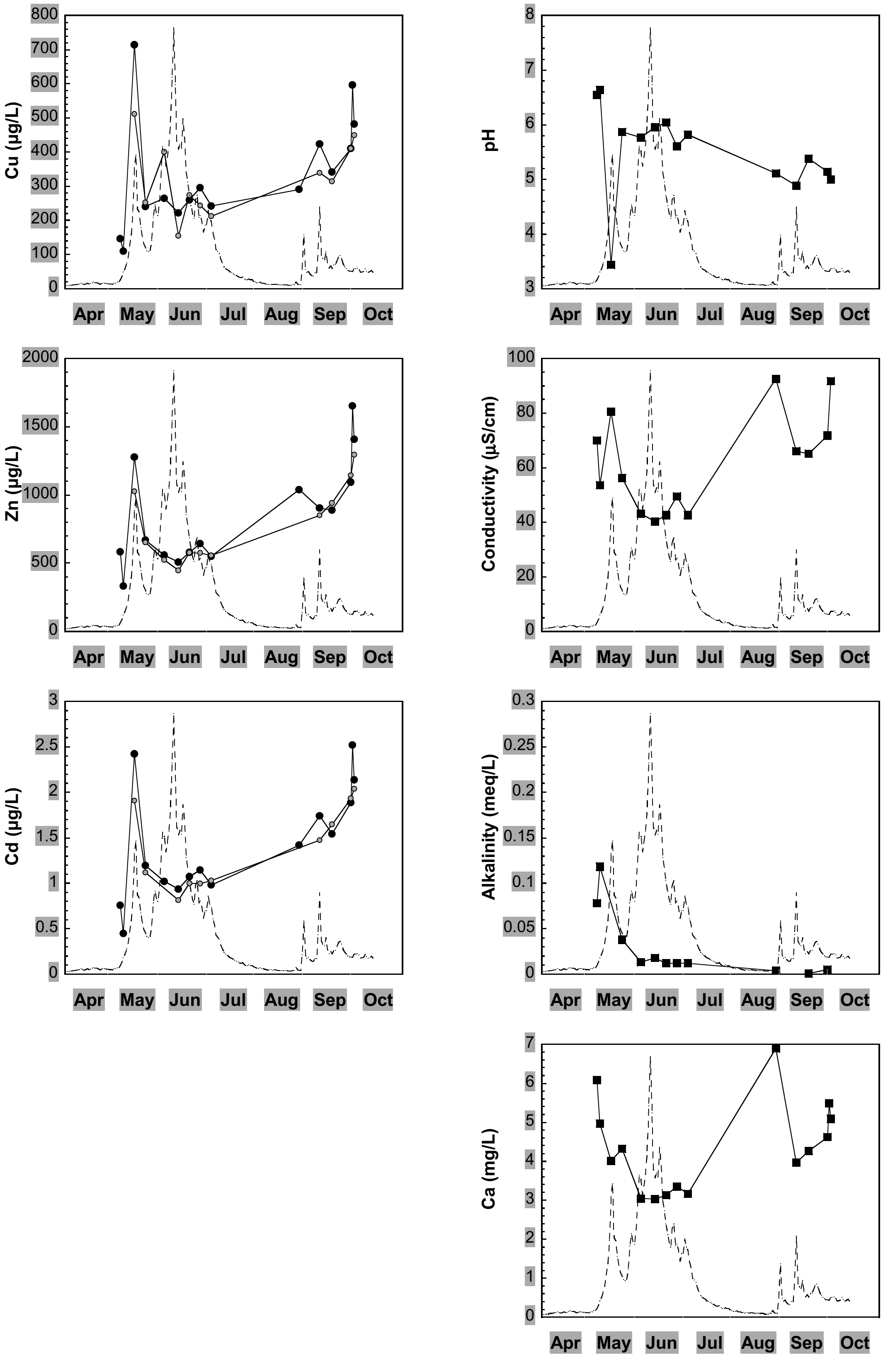

Figure A2.7: Water chemistry data and river discharge (----) in stream Orva. In left figures: Cu, Zn, and Cd concn.; black points represent total concn., open points represent dissolved concn. In right figures: pH, conductivity, alkalinity and Ca concn.

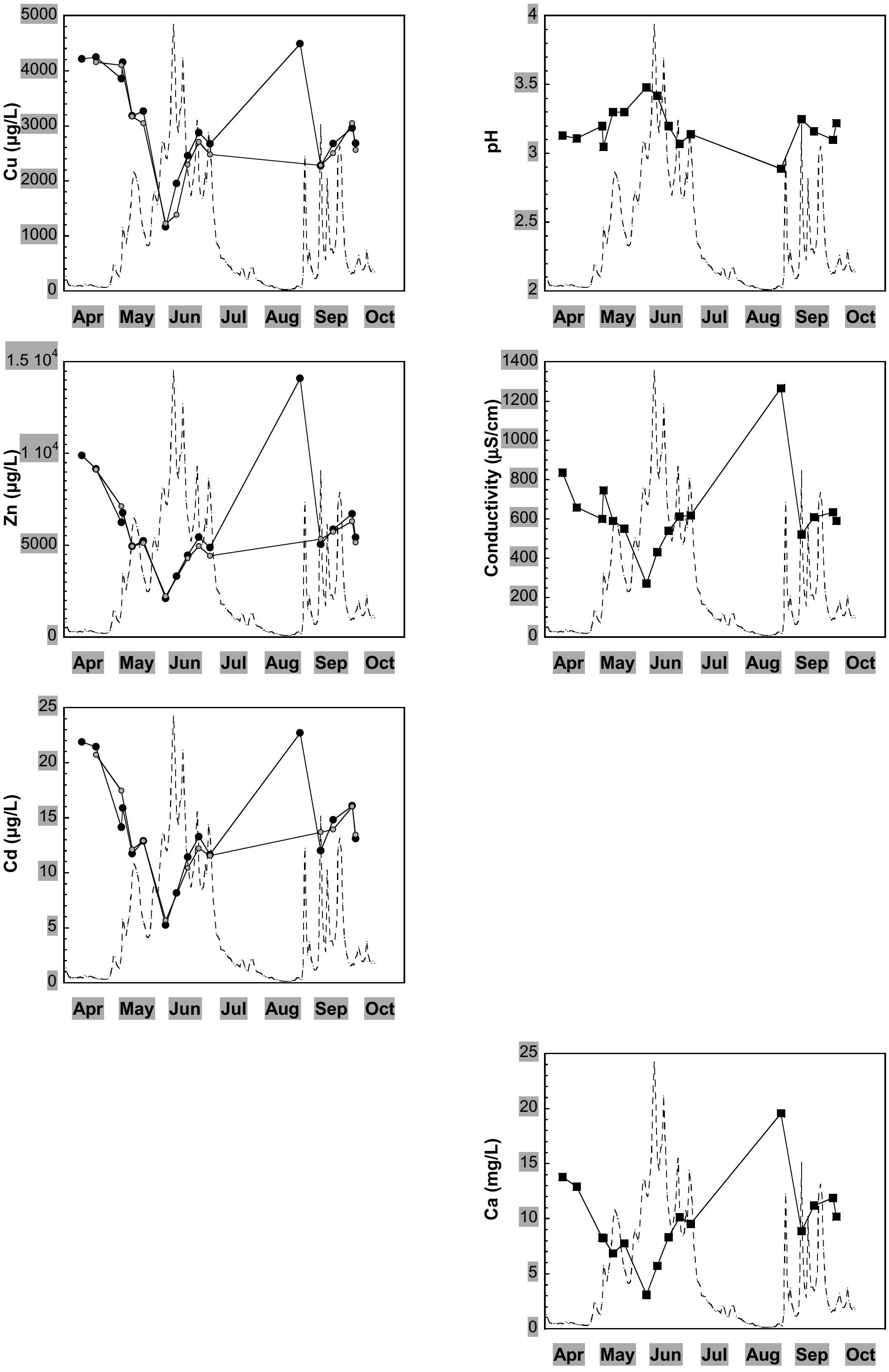

Figure A2.8: Water chemistry data and river discharge (----) at the Bjørgåsen site. In left figures: Cu, Zn, and Cd concn.; black points represent total concn., open points represent dissolved concn. In right figures: pH, conductivity, and Ca concn. (no positive alkalinity value observed).