Trace metal concentrations in blue mussels *Mytilus edulis* (L.) in Byfjorden and the coastal areas of Bergen

Master Thesis in Marine Biology for the degree Candidata Scientiarium

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Sari Airas

Table of contents

1. Abstract

Blue mussels (*Mytilus edulis)* from 23 different localities were collected in Byfjorden and the coastal areas of Bergen in Western Norway in the late winter, before the spawning season. Analyses of copper, zinc, arsenic, silver, cadmium, mercury and lead were carried out with ICP-MS. Blue mussels showed elevated concentrations of copper, zinc and lead in the Bergen centre area, while arsenic, silver, cadmium and mercury concentrations were within the normal range in the area. Compared to data from 1993, there is an overall reduction in copper, zinc, cadmium, mercury and lead concentrations in blue mussels in the Bergen centre. The environmental quality in the fjords around Bergen centre can be defined as moderately polluted by copper, zinc and lead, while other coastal areas of Bergen can be defined as unpolluted. The new outlet sites for the sewage water from the Bergen centre did not indicate any elevation in trace metal concentrations in blue mussels.

Effects of size, position on the tidal zone and freezing and thawing on the trace metal concentrations in blue mussels were studied. Copper showed a negative regression slopes with length of blue mussels in polluted and unpolluted sites. Cadmium had positive regression lines with length in the polluted site but negative in the unpolluted site. Cadmium and lead concentrations were significantly higher in blue mussels growing subtidally compared with the mussels growing higher up on the tidal zone. Blue mussels dissected fresh had significantly higher arsenic, silver and cadmium concentrations than the mussels that had been frozen and thawed before dissection.

2. Introduction

2.1 Background

Marine pollution is a global environmental problem. Human activity on land, in the water and in the air contributes to contamination of sea water, sediments and organisms with harmful substances. Contaminants can be natural substances or artificially produced compounds. After discharge into the sea, contaminants can stay in the water layer in dissolved form or they can be removed from the water column to the bottom sediments in particles. Organisms can take up contaminants from the water or in particles and accumulate them in the body (Stewart, 1999). If organisms are not able to remove the substances from their body, these can be passed on to next level in food chains by biomagnification. Organisms may react differently when exposed to contaminants. Some animal groups may accumulate large amounts of contaminants without any harmful effects, while other groups might get lethal effects already in lower concentrations (Levinton, 1995; Clark, 1997). Biomonitors are organisms, which accumulate contaminants in their tissues and may be analysed to monitor the bioavailability of such contaminants in ecosystems (Viarengo & Canesi, 1991; Rainbow & Phillips, 1993).

Norwegian coast has during its history received discharges from industry, private households and transport. As a consequence, several fjord areas are seriously polluted by cadmium, lead, mercury and other metals and organic contaminants including polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (SFT, 2003). The Norwegian State Pollution Control (SFT) has developed a classification system for environmental quality of Norwegian fjords. On the basis of sediment- and water samples or concentration levels in different organisms, fjord areas can be classified to five different classes, from unpolluted (I) to extremely highly polluted (V). On this way eventual improvements or negative trends in environmental quality in the fjords can easily be followed (Molvær et al., 1997). The environmental status of several fjords have been improved recently after successful initiatives from the state-, county- and town policies, but some harbours and sites with mining and industrial activity remain problem areas (SFT, 2003). Today Norwegian State Food Control (SNT) has given limitations for sale and consumption of seafood in 28 fjord areas (SNT, 2003).

2.2. Byfjorden and the coastal areas of Bergen

Byfjorden is located in shattered fjord landscape in Western Norway (Fig. 2.1). Byfjorden is connected to coastal waters through Raunefjorden in the south and Hjeltefjorden in the west. In north it is splitted to Herdlafjorden in north-west and Salhusfjorden in north-east. Byfjorden is 200- 300 m deep and water circulation is relatively good between the fjords in the area (Linde, 1970; Helle, 1975). Bergen harbour is located in Bergen centre on the east side of Byfjorden around two shallow bays, Vågen and Puddefjorden. The harbour sediments are seriously polluted by PCB, PAH and trace metals (Anon., 2002). SNT has given a recommendation to avoid consumption and sale of seafood in Bergen area ranging from Raunefjorden in south to Herdlafjorden and Salhusfjorden in north from the Bergen centre (Fig. 2.1) (SNT, 2003).

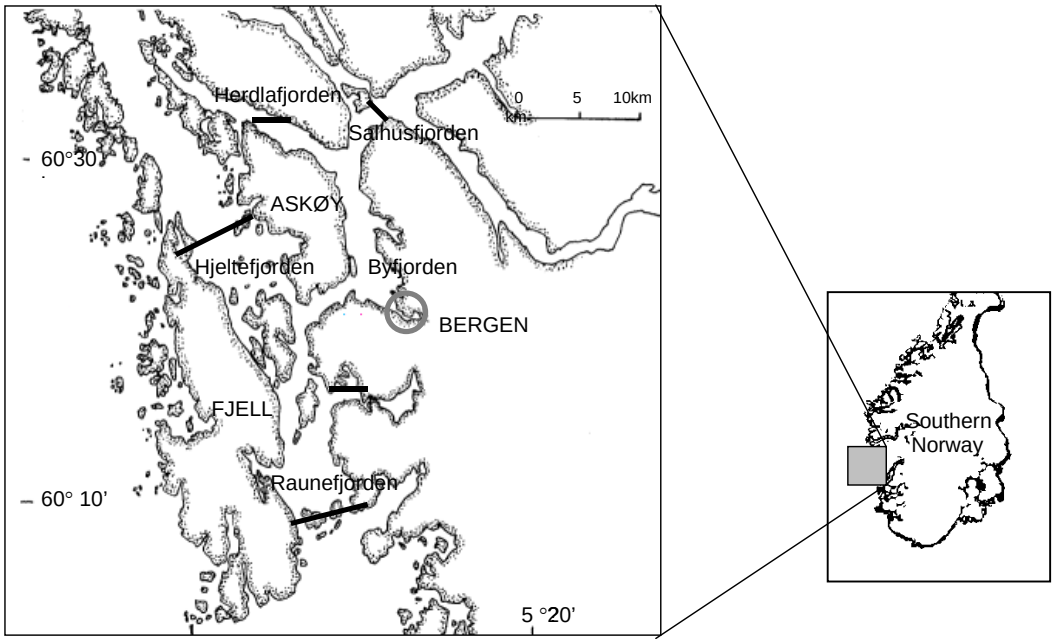

Figure 2.1. Map of Byfjorden and fjord areas around it. Bergen centre is marked with a grey circle. The area with limitations of consumption of seafood is bordered in with black lines (SNT, 2003).

Municipal waste water system, including industrial discharges and urban run-off, heating, traffic and harbour-related pollution are the main causes of contamination of the water and sediments in Byfjorden (Sekse & Kvingedahl, 1992; Anon., 2002). Until recently the municipal wastewater from Bergen centre has been leaded untreated into Byfjorden. The bays in the Bergen centre, Vågen and Puddefjorden, have received approximately 116 000 person equivalents (Pe) of wastewater annually until 1997 (Anon., 1988; Anon., 2002). One person equivalent (Pe) is an estimation of the amount of

wastewater what one person produces within a year. The input of trace metals into the municipal wastewater system was estimated to 9500 kg/year in Bergen in 1991. Sewage was the main source of copper, zinc and cadmium, while industry was the main source for mercury (Table 2.1). Urban run-off was clearly the main source of lead into the municipal waste water (Sekse & Kvingedal, 1992).

Table 2.1. Annual output (kg/year) of copper (Cu), zinc (Zn), cadmium (Cd), mercury (Hg) and lead (Pb) from different sources into municipal waste water in Bergen annually in 1991. Data from Sekse & Kvingedal (1992).

Source	Cu (kg/year)	Zn (kg/year)	Cd (kg/year)	Hg (kg/year)	Pb (kg/year)
Industry	207	750	8	38	265
Sewage	1541	2350	34	6	203
Urban run-off	529	1800	21	1	1092
Spillage from municipal waste plant	23	100	7	0	0
Totally	2300	5000	70	45	1560

However, several improvements have been made since the 1990's. Among them, two mechanical water treatment plants were built in the Bergen centre in 1997 and 1999. Now wastewater is led through a filter with 1 mm openings before it is leaded out to 40 m depth in Lyreneset and Fagernes (Fig. 2.1) (Anon., 2002). In addition, the use of leaded gasoline in vehicles has been reduced since the beginning of 1986. Also mercury output has been regulated, and since 1994 dentists and laboratories have filtered mercury out from the rest of the wastewater (Dons & Beck, 1993). On the basis of improvements mentioned above, reduction in concentrations of trace metals can be expected in the area.

2.3. Blue mussel (Mytilus edulis)

Blue mussel (*Mytilus edulis)* (Linné 1758) belongs to the family Mytilidae in phylum Mollusca (Fig. 2.2). Systematic is complicated because of the several subspecies, and the relations between these are under continues revising (Seed, 1976; 1992; Rainbow & Phillips, 1993). The species is widespread in the northern hemisphere and can be found from White Sea to Mediterranean and North Africa in Eastern Atlantic, from Canadian arctic to North Caroline in Western Atlantic and from Arctic to California and Japan in the Pacific (see Seed, 1976; 1992). Blue mussel is found on littoral and sublittoral zone, both in exposed and sheltered localities. The species tolerate wide temperature

range, as well as different salinities (Seed, 1976). It is important species both economically and ecologically, and the sales of farmed mussels reached 600 tons in Norway in 2000 (Anon., 2001).

Phylum Mollusca

Class Bivalvia

U. Class Pterioida

Orden Mytiloida

Family Mytilidae

Genus *Mytilus*

Figure 2.2. Systematic of *Mytilus edulis* (L.)

Blue mussel feeds by filtering algae, detritus and organic particles from the water with its gills. Sexual maturation takes place at the age of 1-2 years depending on the growth rate and size (Seed, 1976; Hovgaard et al., 2001). The reproductive cycle of mussels is controlled by the combination of several internal and external factors, among them nutrient reserve of individuals, food availability and temperature in the water. There might be large variations in time and form of spawning between two geographically close populations (Seed, 1976; Newell et al. 1982). In Norway the main spawning of blue mussels is in March-April (Duinker & Mortensen, 1999; Hovgaard et al., 2001). In addition, local, not synchronised spawning can appear in other times of the year (Duinker, 2002. *pers. comm.*). During spawning mussels can loose up to 70 % of their biomass (Duinker & Mortensen, 1999). Spreading of the species occurs in the larval phase, when planktonic larvae search for suitable substrate. Blue mussels favour to settle on flat shores, which receive constant wave movements, although dense populations are also found on steeper faces, dock piles and harbour walls (Seed, 1976). Adult individuals anchor themselves to substrate with byssus threads. After settlement blue mussels are able to move over substratum, but since mussels also favour growing in dense colonies, byssus threads of other individuals keep the colony together. This makes that blue mussels can not escape from unfavourable conditions or from pollution.

2.4. Trace metals

In the present study seven trace metals were analysed in blue mussels: copper (Cu), zinc (Zn), arsenic (As), silver (Ag), cadmium (Cd), mercury (Hg) and lead (Pb). All these trace metals, in addition to chromium (Cr), are included in environmental monitoring program for mussels in EU (2001), as well as in Norway (Julshamn & Duinker, 2001). Trace metals can be divided into essential elements and non essential elements. Essential elements occur naturally in all organisms. Copper and zinc are essential for many enzymatic functions. In high doses also essential elements can be poisonous and cause hazardous effects on organisms. The non essential elements do not have any positive effects on organisms and they are harmful already in low doses. They can inhibit an essential element to bind to enzyme and disturb the normal enzymatic function in the body. This group includes cadmium, mercury and lead (Aune, 1998).

Copper is used in antifouling paints on boats and fish farming equipment, electrical equipment and water pipes. Municipal wastewater, mining and processing of nickel and copperferrosulphide ($CuFeS_2$) are also notable point sources of copper into the water in Norway (Dons & Beck, 1993). In sea water copper exist both dissolved in water and bound to particulate matter (Balls, 1985). Copper is essential in respiration fro many organisms and other enzymatic functions. It is stored in liver and bone marrow in humans. In contrast, some dissolved copper salts are hazardous for many algae, bacteria and fungi, as well as fish and plankton. An overdose in humans can cause liver damage, low blood pressure, coma or even death (Dons & Beck, 1993; Blomseth & Hartmann-Pedersen, 1995). Blue mussels accumulate copper in the body although the species can regulate the uptake (Phillips, 1976a; Davenport, 1977; Davenport & Manley, 1978; Julshamn, 1981a).

Zinc was considered as a notable environmental problem in Norway in the 1990's (Dons & Beck, 1993). Its main sources are metallurgic industry, pyrite mines, galvanic industry, incineration plants and anti corrosive products, paints, plastic and rubber. Leakage to water in 1992 was estimated to 634 tons in Norway, mostly from industry and offer anodes (Dons & Beck, 1993). Many zinc compounds are soluble in water and it is accumulated in organisms. It is an essential element to all organisms, and for humans a daily intake of 9 mg zinc is needed for normal body functions (Anon., 1997). The human body can regulate uptake of zinc and overdoses can cause diarrhoea and vomiting. For

organisms in water, high doses of zinc can be acute poisonous or give chronic effects (Dons & Beck, 1993). Blue mussels can regulate zinc uptake, and is not counted as a reliable indicator species for zinc contamination (Lobel et al., 1982; Julshamn, 1981a).

Arsenic is used in impregnated wood, brass, lead accumulators and glass. Leakage to water was estimated in 1992 to 0.4 tons annually in Norway. The main sources of arsenic in southern Norway are estimated to be airborne arsenic from industrial sites and leakage from wood impregnation manufactures. Accumulation and effects of arsenic in organisms are depended on the compound. While inorganic arsenic compounds are acute poisonous for most organisms, organic compounds might be only slightly poisonous. Arsenic can cause chronic effects on embryos, damage DNA molecules or cause cancer (Dons & Beck, 1993; Berg et al., 1997). Blue mussels bioaccumulate arsenic, although fish has been found to be better indicator for the metal (Julshamn & Grahl- Nielsen, 1996). FAO/WHO has an upper limit for acceptable tolerable inorganic arsenic weekly intake of 15 μg/kg body weight in human dietary.

Silver is a trace metal which seldom is discussed when considered hazards to human health. It is used in photography in the form of silver bromide (AgBr) (Zumdahl, 1995). High silver concentrations in sediments have been reported in the vicinity of municipal sewage site and industrial sites (Sanudo-Wilhelmy & Flegal, 1992). Effects on human health are not reported in the literature, but Hill (1976) found it possible to silver ion to replace copper in chicks. Blue mussels have been analysed for silver concentrations previously (Alexander & Young, 1976; Jones et al., 2001), although Bryan & Hummerstone (1977) found deposit feeding mollusc to be better indicator for particulate silver.

International concern about effects of cadmium on organisms and environment has led to increasing focus on reduction of spillage of this element into environment. The North Sea Commissions declaration of reduction of cadmium output into water with 70 % was achieved in Norway in 1992. The main sources of cadmium are mining and processing of zinc, galvanising and paint industry and products like Ni/Cd batteries and offer anodes. The main sources into water are offer anodes used in ships and offshore industry, mining, sewage and long distance transport by air (Dons & Beck, 1993; Huse, 1999). Cadmium is found in marine waters mostly in the dissolved form (Balls, 1985). It

accumulates in fish and mammals, has long biological half life and it is acute poisonous for organisms in water and mammals. In mammals it is stored in kidneys and can cause cancer and damage kidneys (Dons & Beck, 1993). Blue mussels accumulate cadmium effectively (Phillips, 1976a; Julshamn, 1981a; Julshamn & Grahl- Nielsen, 1996). EU has sat an upper limit of 1.0 mg Cd /kg (fresh weight) in mussels used for human consumption (EU, 2001).

Mercury is an element which in some organic forms can become extremely poisonous. Sources of mercury in Norway are zinc mining, incineration plants, and products like amalgam, batteries and thermometers (Dons & Beck, 1993). Mercury is accumulated in kidneys of fish and mammals, organic mercury also in brains. Overdoses of mercury can cause damage in kidneys and central nervous system (Aune, 1998). Blue mussel takes up mercury and is a suitable indicator species for mercury contamination (De Wolf, 1975; Davies & Pirie, 1978). Upper limit of mercury in seafood is sat at 0.5 mg/kg fresh weight in EU (2001).

Lead is used in building materials and mechanical industry as well as in batteries, cables, pigments and gasoline. Use of lead in gasoline and other fossil fuels has reduced dramatically since 1990 - in Norway from 225 tons in 1990 to 4.9 tons in 1997 (Huse, 1999). Lead exists in water mostly in particulate form (Balls, 1985). It is accumulated in fish and mammals and is acute poisonous. Chronic effects can be neurotoxic, immunological or cancerous (Aune, 1998; Dons & Beck, 1993). Blue mussels take up lead from the water and food particles in similar rates and reflect environmental pollution effectively (Schulz- Baldes, 1974; Phillips, 1976a; Julshamn, 1981a). EU has set the upper limit of lead concentration in mussels to 1.5 mg/kg fresh weight, when used for human consumption.

2.5. Environmental monitoring

Environmental monitoring can include sediment or water sampling, studies of species diversity and abundance, or the use of biomonitors (Phillips, 1977a; Rainbow, 1995). The use of organisms instead of sediment- and water sampling has many advantages. Firstly, as mentioned previously, concentrations found in biomonitors tell more about a bioavailability of the pollutant in the environment. Secondly, the sampling of sediments and water might show large seasonal and temporal local variation, and time-scale monitoring might be difficult and expensive (Phillips, 1977a; Morrisey et

al., 1994). Thirdly, by using biomonitors, which have wide geographical distribution, contamination levels can be compared internationally (Mussel Watch, 1980; Rainbow & Phillips, 1993; Rainbow, 1995).

In definition, a good biomonitor species for environmental contamination tolerate and accumulate contaminants without suffering mortality and show responsiveness to changes in concentration levels. In addition, species should be abundant in the area, have long life span, sufficient sampling size and hardiness to tolerate laboratory incubation. Yet, sampling and identification of the species should be relatively easy. To reflect environmental status in a specific area, an indicator species should be sessile or have slow or limited range of movements (see Phillips, 1977a). Finally, a species should be able to accumulate pollutants similarly in different environmental conditions, and only then, according to Phillips (1977a), samples from different areas can be compared.

Bivalves, including blue mussels (*Mytilus edulis)*, have been found to be suitable biomonitor species for trace metals (Goldberg, 1975; Phillips, 1976a; 1977a; Brown & Luoma, 1995; Julshamn & Grahl-Nielsen, 1996; Riget et al., 1996). Blue mussel is capable to accumulate trace metals such as cadmium, mercury and lead to a larger extent than for example fish and algae (Julshamn, 1981a; Julshamn & Grahl- Nielsen, 1996). It has wide geographical distribution and tolerance range for different salinities and temperatures. In addition, it has sufficient size, sessile life form and is robust in laboratory conditions. It can also be transplanted to different environments (Seed, 1976; Phillips, 1977a; Cossa et al., 1980; Okumus & Stirling, 1998; Shindo & Otsuki, 1999). Despite that the species fill many of the criteria mentioned above, several biological and geochemical factors can cause large variations in contaminant levels in blue mussels. Size, sex, gut content and reproductive season of individuals and water temperature, pH, and salinity, among others, are factors which effect accumulation in mussels (Watling & Watling, 1976; Boyden, 1977; Davenport, 1977; Phillips, 1976a; 1977b; Cossa et al., 1979; 1980; Lobel & Wright, 1982; Lobel et al., 1991; Regoli & Orlando, 1994; Brown & Luoma, 1995; Shindo & Otsuki, 1999; Stecko & Bendell-Young, 2000). International monitoring programs have established some standards for sampling and sample preparation procedures to reduce sources of variation, other than the metal content itself, in contamination levels

in blue mussels. These include, among others, sampling depth and season and size of the individuals (Mussel Watch, 1980; Claisse, 1989).

2.6. Previous studies

Several environmental studies have been made in Byfjorden and coastal areas of Bergen. The most comprehensive is a report series «Byfjordundersøkelse», the co-operation program of Bergen county and the University of Bergen, in which environmental status of Byfjorden has been followed frequently with sediment and bottom fauna sampling since 1973 (Johannessen, 1974; 1981; 1982; 1983; 1984; 1985; Johannessen et al., 1991; 1992; 1993; Botnen et al.,1994; 1996; 1999; 2000; Botnen & Johannessen, 1999). Contamination levels in organisms have also been studied. Skei et al. (1994) and Knutzen et al. (1995) reported high levels of PAH, PCB, zinc, mercury and lead in blue mussels, crabs and fish, and Andersen et al. (1996) found elevated concentrations of copper, zinc, mercury and lead in blue mussels in Byfjorden (Tab. 2.2). Myhre (1998) found elevated levels of PAH, PCB and mercury in eel.

Table 2.2. Trace metal concentrations in blue mussels (mg/kg fresh weight) in Byfjorden. Data from Skei et al. (1994), Knutzen et al. (1995) and Andersen et al. (1996).

Cu	Zn	Cd	Hg	Pb	Reference
1.58- 2.98	32.7- 54.4	0.04- 0.15	0.02- 0.04	0.88- 1.76	Skei et al. (1994)
0.71- 1.32	41.6- 47.7	0.15- 0.25	0.021- 0.054	0.76- 1.38	Knutzen et al. (1995)
0.9- 3.78	32.2- 69.3	0.07- 0.29	0.01- 0.056	0.69- 2.76	Andersen et al. (1996)

2.7. The aim of the study

The aim of the present study was to examine possible changes in trace metal concentration in blue mussels since previous studies in 1993 and 1994 in Byfjorden (Andersen, 1994; Skei et al., 1994; Knutzen et al., 1995). This was done by analysing trace metal concentrations in blue mussels sampled at 23 different stations in polluted and unpolluted areas. The results were also compared with the normal background levels in the area (Julshamn & Duinker, 2001; 2002). It was also evaluated, if blue mussels in the coastal areas of Bergen are suitable for human consumption on the basis of the recommendations from EU (2001). The possible effects of the trace metal concentrations in blue mussels in the vicinity of the new sewage output sites were considered and compared with the data

from the period before discharges started (Andersen, 1994). Finally, the environmental status of Byfjorden and the coastal areas of Bergen were evaluated on the basis of the guidelines from the State Pollution Control (SFT) (Molvær et al., 1997).

In addition to the main study, effects of size, position in the tidal zone and freezing and thawing of blue mussels were studied separately with the aim of to observe sources for variation in studies of trace metal concentrations in blue mussels. The size- effect was studied by comparing five different size classes from one polluted and one unpolluted site. In the tidal- effect study trace metal concentrations in blue mussels were compared from three tidal depths. The thawing- effect study was performed to test eventual differences in trace metal concentrations between one group of blue mussels dissected fresh and another that first was frozen and thawed prior to dissection.

3. Material and Methods

3.1. Fieldwork

Blue mussels (*Mytilus edulis*) were collected from totally 23 stations. Samples from the stations 1-21 were collected between the 8th and 19th of March 2001 and the samples from stations 22-23 were collected on the 26th and 27th of February 2002 in Bergen, Askøy and Fjell (Fig. 3.1). The stations were chosen on the basis of contamination levels found previously and the aim was to cover both polluted and unpolluted sites (Andersen, 1994; Skei et al. 1994; Knutzen et al, 1995). Three reference stations, stations 21- 23, were included into the study. Municipal wastewater outlets, aqua cultural sites and traffic were avoided when locations for the reference stations were chosen. The stations 4-14 were located in the Bergen centre with heavy traffic, industry and harbour activities. Station 14 at Lyreneset and station 2 at Biskopshavn were located in the vicinity of output sites to mechanical water treatment plants. Station 16 at Kolavåg was located in a bay beside a previous municipal disposal site. Håkonshella (st.17) were located in a small craft harbour. The rest of the stations (st. 1, 18, 19, 20, 21, 22 and 23) were located in the areas with scattered settlement and with little or no industry.

In 2001 there were seven mechanical and one chemical water treatment plants in use in Bergen. Stations 1-14 and 18 were located in the area, where 90 % of the households were connected to municipal water treatment system. At stations 7 and 19, 50-75 %, and at station 20, 30-50 % of households were connected (anon., 1998). Stations 15, 16 and 23 were located in Askøy, where the municipal water treatment system consists of one chemical and seven mechanical water treatment plants and about 65 % households are connected into the communal system (Berthelsen, 2003, *pers. com*.). Stations 22 and 23 were located in Fjell, where one biochemical water treatment plant exists, which has its output at the inner Fjellspollen. About 50 % of the households are connected to the communal system. Blue mussels were collected on rocks, wooden dock piles, floating bridges and harbour concrete walls, depending on the sampling site.

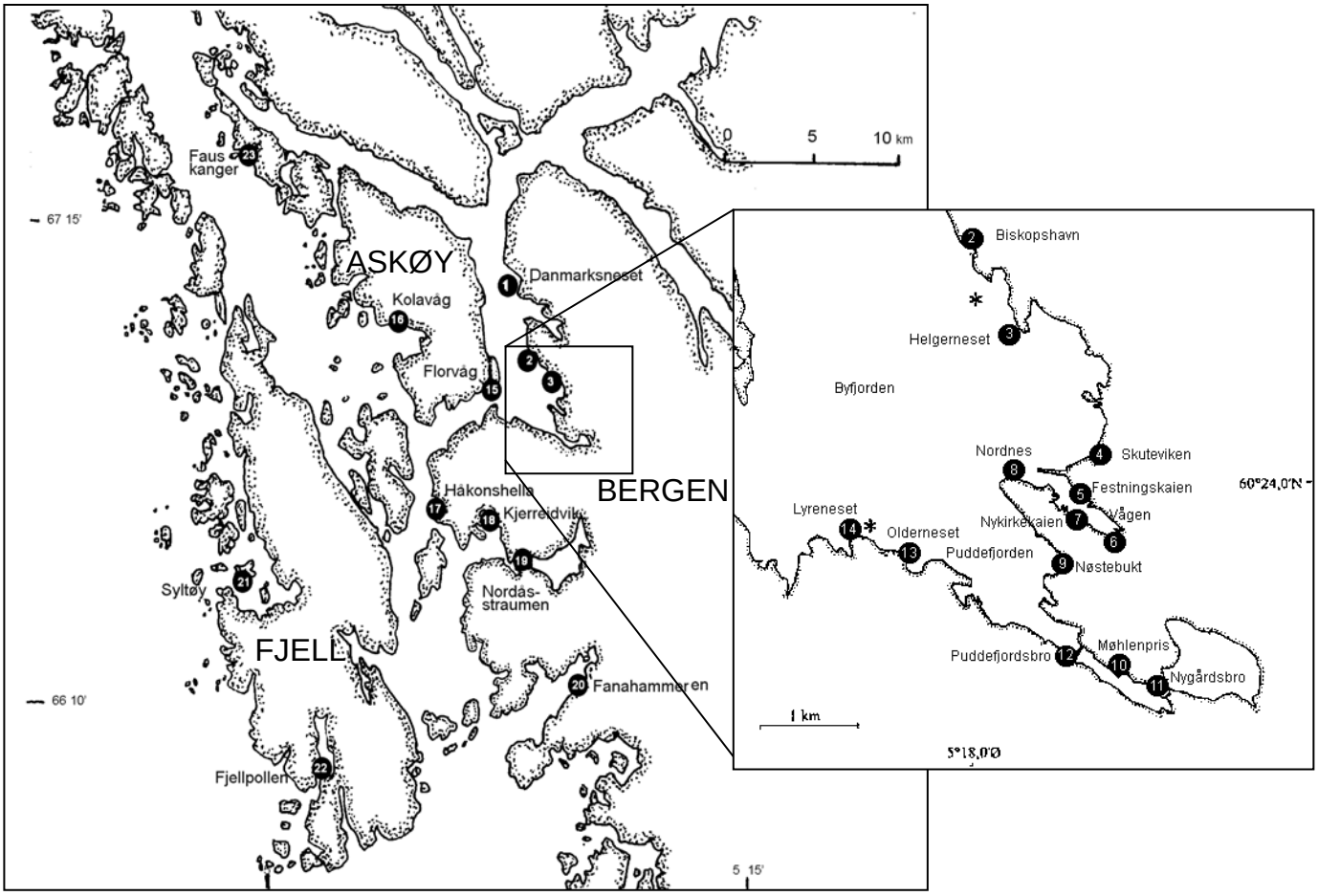

Figure 3.1. Sampling of blue mussels *Mytilus edulis* in Byfjorden and coastal areas connected to it. Stations 4-12 were located in the Bergen centre, around Puddefjorden and Vågen. The sewage outlets are marked with asteriks.

Stations:

1. Danmarksneset. Danmarksneset is located 8 km north from Bergen centre. Mussels here were collected on the rocks at a private shoreline. 1A was located 50 m south from 1B on the coastline, and the mussels had very thick shells with many epiphytes. The mussels at 1B had regular shape and thin shells.

2. Biskopshavn. Biskopshavn is located 3 km north from Bergen centre, 100 m upstream of the outflow for the new water treatment plant in Fagernes (Fig. 3.1). Annual municipal output here

presents 35 000 person equivalents annually (Pe/year). The mussels were collected on the rocks and on wooden dock pilings.

3. Helgerneset. Mussels were collected on pilings of a wooden dock in an industrial area, 2 km from Bergen centre. The water smelled of sewage and there was garbage floating in the water.

4. Skuteviken. This area has experienced recent dredging and is located next to a busy roadway. Mussels were collected on pilings of a wooden dock. Water smelled again of sewage.

5. Festningskaien. Mussels were collected on a passenger ship wharf in Bergen centre. The site has been a percipient to untreated wastewater (10 000 Pe/ year) until 1999 (Anon., 2002).

6. Vågen. The station is located by the local fish marked in Bergen centre. Area is popular small boat anchoring site. Mussels were collected from a rope hanging from the concrete harbor wall. Mussels were small.

7. Nykirkekaien. The station is located in the Bergen centre beside a ferry dock. Mussels were collected on a wooden dock. Some mussels were observed spawning at this time.

8. Nordnes. This cape point station protrudes into a busy shipping line. Mussels were small here, and collected from rocks.

9. Nøstebukt. The station is located in a ferry dock. Mussels were collected from a concrete harbor wall. The water visibility was minimal and the water smelled of sewage.

10. Møhlenpris. Station located on an industrial area, next to a cargo ship dock yards. This site has received untreated municipal wastewater of 38 000 Pe/year until 1997 (Anon., 2002). Mussels were collected on a wooden wharf.

11. Nygård. Station is located in an area with heavy traffic. Boat traffic is also frequent. The site has received untreated waste water of 5000 Pe/ year until 1997 (anon., 2002). Mussels were collected from rocks in strong stream.

12. Puddefjordsbro. The sampling site is located in an industrial area, with heavy traffic. Mussels at station 12A were collected in 70 cm depth from a concrete wall. Mussels at 12B were collected in 40 cm depth from rocks.

13. Olderneset. The station is located 2 km south-west from Bergen centre on an industrial area by a submarine bunker. Mussels were collected from a rocky shore.

14. Lyreneset. The station is located on a tidal shore with kelps and rocks. The output site for the new water treatment plant (Holen > Pe 100 000/ year) is located 30 m from the shore (Fig. 3.1) (anon., 2002).

15. Florvåg, Askøy

The sampling site is located in a small bay, close to a smaller oil harbor. Mussels were collected from a fishing gear lying at 50 cm depth.

16. Kolavåg, Askøy

The station was located on a previous communal disposal site, now covered with netting, filled with soil and the area is used for recreation. Mussels were collected from sandy shore. The mussels were large and had greenish color on shelves. Water smelled of hydrogen sulfide.

17. Håkonshella

Mussels were collected from a floating bridge in a small craft harbor.

18. Kjerreidvik. Mussels were collected from a wooden wharf.

19. Nordåsstraumen. The sampling site is located beside a bridge for heavy car traffic. Mussels were collected from a sandy shore in strong current.

20. Fanahammeren. Mussels were collected from a floating bridge in a small harbor.

21. Syltøy, Fjell. The station was located in a bay facing open Sea to the west. Large, fine shaped mussels were collected from rocks.

22. Fjellspollen, Fjell. The station was located in a small bay. Mussels were collected from rocks and a floating bridge.

23. Fauskanger, Askøy. The station was located in a shallow bay. Mussels were collected from a floating bridge.

Temperature was measured in the surface layer and it was between +4 and +6 °C during the fieldwork. Blue mussels were collected at a depth of 40-90 cm below spring low tide (LLW) using a rake or by free diving. In addition to these 23 stations, mussels for size-effect, tidal effect and thawing effect studies were collected. For the size-effect study mussels from 50 cm depth were collected at Nygård (Nyg I-V) and Fjellspollen (Fjell I-V). For the tidal effect study blue mussels were collected at Nygård from three different tidal levels at spring low tide; subtidal at 40 cm below LLW (-40 LLW), intertidal at 20 cm below LLW (-20 LLW) and 10 cm above the sea level LLW (10 LLW). Blue mussels for the thawing effect study (Fjell fresh and Fjell froz) were collected at Fjellspollen from a floating bridge.

3.2. Sample preparation

The trace metal determination was carried out following a standardised method for analysis of copper, zinc, arsenic, silver, cadmium, mercury and lead. The same procedure is used in a monitoring program for shellfish in Norway by Directorate of Fisheries in Bergen. The method is accredited according to ISO/IEC 17025 for all these metals at the laboratory of minerals and trace elements in the National Institute of Nutrition and Seafood Research, Bergen, Norway.

Blue mussels between 35-60 mm in size from each station were separated from each other by hand, put into marked plastic bags and stored at -20 °C until analysis. One sample (Fjell fresh) was dissected fresh. Blue mussels were taken out for thawing the day before sample preparation. Three pooled samples of 25 mussels each were randomly selected and prepared from every station. The mussels were marked with numbers from 1 to 25 and opened for dissection. The shells were cleaned from epiphytes, byssus threads were removed and valves were rinsed in deionised water to remove sand, shell and other particles from the shell body. The shells were left to dry on blotting paper for one hour. The length of the independent shells was measured with a caliper and the soft tissue weight of each sample was determined (Sartorius BL1500S). The soft tissues were removed carefully with a scalpel, put into a plastic box marked with a station and sample number, weighed and stored at -20 °C.

For the size-effect study, the blue mussels were sorted in five different size classes: I ≤ 3 cm, II= 3-4 cm, III= 4-5 cm, IV= 5-6 cm and V > 6 cm. For every size class three pooled samples of 25 mussels each were prepared using the same procedure as for the samples above. Blue mussels for the tidal-effect study were sorted into three groups according to the collection depth and prepared using the same method as above.

The frozen samples were freeze-dried for 48 hours. The freeze-dried samples were weighted and dry matter content was calculated as following:

$$dw\% = \frac{sdw * 100}{sww}$$

where dw%= dry weight (g/100g) in percent,
sdw= dry weight of the sample (g)
and sww= wet weight of the sample (g).

The samples were homogenized to fine powder (Retch ZM100) and stored in plastic containers at room temperature until analyses.Two parallels from each of the three pooled samples from each station were prepared according to the following procedure. Samples (0.2 g) were weighed into tetra fluorine methoxil (TFM) digestion vessels, 2.0 ml nitric acid (65% m/V) and 0.5 ml hydrogen peroxide (30% m/V) were subsequently added. The sealed containers were placed in a microwave oven (Milestone mls 12000 MEGA) for 17 minutes and the samples were heated according to the temperature program given in the Table 3.1. After complete digestion the sample solutions were cooled for 20 min to the room temperature. The sample solutions were diluted with deionised water to the total volume of 25 ml and transferred into polyethylene flasks, capped and stored at room temperature. Blank samples were processed as following: vessels were filled with digestion acids and taken through the entire procedure to monitor the average and variation of the element blank value.

Table 3.1. Digestion program used for microwave oven system

Step	Power (W)	Time (min)
1	250	1
2	0	1
3	250	5
4	400	5
5	650	5

After each digestion run the vessels were rinsed twice with filtered water (RO-water), twice with Tennards mixture (2/3 RO-water, 1/6 H_2O_2 and 1/6 HNO_3) and eight times with deionised water. Finally, the vessels were left to dry on blotting paper for 24 hours.

Inductively coupled plasma- mass- spectrophotometer (ICP-MS) (Agilent 7500 C) was used to determine the concentration of copper, zinc, arsenic, silver, cadmium, mercury and lead in the blue mussel samples. A worksheet with station and sample numbers and dilution factor was prepared for the program. Dilution factor for each sample was counted as follows:

$$\textit{Dilution factor} = \frac{\textit{25 ml}}{\textit{Dry weight (g) * 1000 (ml/g)}}$$

Standard curves for all elements were calculated using five different concentrations. Sample solutions were analysed using an accredited method for blue mussels. Blind sample number four was used to set a background for the different elements. The results from the determinations of copper, zinc, arsenic, silver, cadmium, mercury and lead are presented as a mean value of three pooled samples from each station.

3.3. Quality control

A series of 10 parallels of blanks were taken through the procedure to measure background levels of the elements. Means and standard deviation were calculated. Certified reference materials (CRM) were analysed to assess the trueness and precision of the analyses. Seven parallels of CRM dogfish muscle (DORM 2) and three parallels of CRM lobster digestive gland

(TORT 2) (Institute for Environmental Chemistry, Ottawa, Canada) were analysed together with the mussel samples. These served as reference materials for the analyses of copper, zinc, arsenic, silver, cadmium, mercury and lead. Relative standard deviation (RSD %) was used in evaluation of the precision of the methods used (Table 3.2). The precision found in the present study was compared with the values recommended by The Nordic Committee on Food Analyses (NMKL, 1996). RSD (%) was calculated as follows:

$$RSD\ (\%) = \frac{(SD)}{(X)} * 100$$

where RSD (%) = relative standard deviation in samples of CRM in percent
SD = standard deviation (mg/kg),
and X = mean (mg/kg).

Table 3.2. Recommended Relative Standard Deviation RSD (%) for precision for different analyte concentrations (NMKL, 1996).

Analyte concentration	RSD (%)
100 g/kg	2
10 g/kg	3
1 g/kg	4
100 mg/kg	5
10 mg/kg	7
1 mg/kg	11
100 μg/kg	15
10 μg/kg	21
1 μg/kg	30
0.1 μg/kg	43

3.4. Statistics

Kolmogorov- Smirnov's test for normality was used to test the normal distribution for length of the blue mussels (Zar, 1999). For the weight and the trace metal analyses the mussels were pooled in three subsamples, and the data could not be tested for normality. Normal distribution was assumed for these data according to the Central Limit Theorem (Zar, 1999) considering each pooled sample as an average. Deviations from homogeneity of variance were tested with Levene's test. The length, weight and metal data in the stations 1- 23 showed significant

heteroscedasticity and Kruskal- Wallis test followed by Newman- Keuls test for ranked numbers were used to test differences between the groups (Zar, 1999). Interactions between the trace metals and correlation of the shell length with the trace metal concentrations were tested with regression analyses (Ranta et al., 2002). Bivariate correlation analyses were used to plot variables against each other pair wise (Ranta et al., 2002). In the size-effect and the tidal-effect studies trace metal data between the groups were homogenous and the differences were tested with one- way ANOVA followed by Tukey's HSD test. In some of the metal data in the size-effect study variances were heterogeneous and Newman- Keuls test was used to test the differences between the groups, since this test has been found to be more robust to heterogeneous variances (Ranta et al., 2002). In the both studies the trace metal concentrations were tested for regression with the gradient with regression analyses and the significant correlations were plotted with bivariate correlation analyses. Length, weight and trace metal data in the thawing-effect study were tested with paired t-test for variances and differences between the groups. Level of significance in all statistical tests was 0.05. Microsoft Excel 7.0 software (Copyright © Microsoft Corporation) was used for all data tables and Statistica 5.5 (Statsoft inc., Tulsa, USA) was used for all the statistical analyses.

4. Results

4.1. Length and weight of blue mussels

Mean length and weight of blue mussels *(Mytilus edulis*) varied between 37 mm- 59 mm for length and 1.4 g -6.5 g for weight at the 23 stations studied (Fig. 4.1) (App. 1). The largest and heaviest mussels were found at Syltøy (st. 21) and Kolavåg (st. 16) with the mean lengths of 59 mm and 53 mm, and mean fresh weights of 6.0 g and 6.4 g, respectively. These mussels were significantly larger than blue mussels collected from the other stations ($p < 0.05$, Newman Keuls) (App. 2). At Syltøy some of the blue mussels collected here were larger than the normal size range (35-60 mm), but since the station represented a reference station, the mussels were included in the further analysis. Blue mussels collected at Nordnes (st. 8), Vågen (st. 6) and Håkonshella (st. 17) with the mean lengths of 39 mm, 40 mm, and 42 mm, respectively, were significantly smaller than mussels at the other stations ($p < 0.05$). These mussels were also lightest with the mean fresh weights of 1.4 g, 1.5 g and 2.4 g, correspondingly. Dry matter content varied between 13.6 g/100g (Vågen, st. 6) and 21.3 g/100g (Syltøy, st. 21) (App. 1).

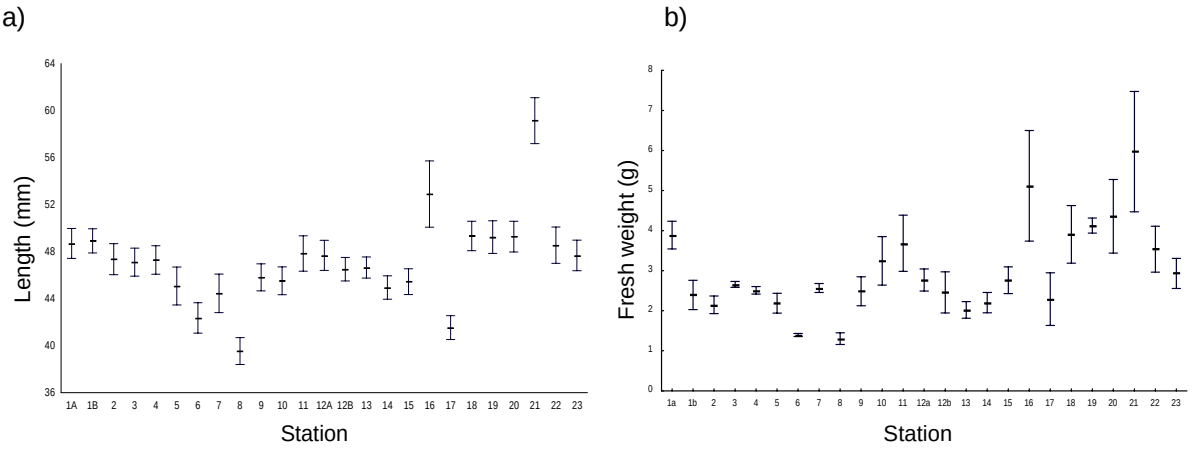

Figure 4.1.a) Mean length (mm) and b) mean fresh weight (g) of blue mussels (*Mytilus edulis)* at stations 1-23 with 95 % confidence intervals.

4.2. Quality Control

Ten blanks were analysed for background values of copper (Cu), zinc (Zn), arsenic (As), silver (Ag), cadmium (Cd), mercury (Hg) and lead (Pb) (App. 3). Zinc and arsenic had highest background values with the means 0.94 mg/kg and 0.11 mg/kg, respectively (Tab. 4.1).

Table 4.1. Analyses of background levels of Cu, Zn, As, Ag, Cd, Hg and Pb blanks (mg/kg). Mean with standard deviation (N = 10).

Metal	Cu (mg/kg)	Zn (mg/kg)	As (mg/kg)	Ag (mg/kg)	Cd (mg/kg)	Hg (mg/kg)	Pb (mg/kg)
Mean	0.03	0.94	0.11	< 0.01	< 0.01	0.01	0.02
SD	0.06	2.31	0.29	0.01	0.01	0.02	0.04

Seven parallels of Certified Reference Material (CRM) dogfish muscle (DORM 2) and three parallels of CRM lobster digestive gland (TORT 2) were analyzed for the concentrations of copper, zinc, arsenic, silver, cadmium, mercury and lead (App. 3). Copper was slightly lower in the present analysis than certified values (Tab. 4.2). Zinc and cadmium, on the other hand, were higher than the certified value. Relative standard deviation (RSD %) gained with the analyses here were within the limits for recommended relative standard deviations given by Nordic Committee on Food Analyses (NMKL, 1996).

Table 4.2. Analyses of Cu, Zn, As, Ag, Cd, Hg and Pb (mg/kg dry weight) of dogfish muscle (DORM 2) and lobster digestive gland (TORT 2). Mean and standard deviation (SD) of analyzed samples compared to certified values. Certified values are given with 95 % confidence intervals. Relative standard deviation (RSD %) of the present analysis and recommended RSD (%) (NMKL RSD %) for repeatable analysis (NMKL, 1996).

Metal	CRM	Mean (mg/kg)	SD	Certified value (mg/kg)	RSD (%)	NMKL RSD (%)
Cu	DORM 2 #	2.04	0.15	2.34 $\pm$ 0.16	8	11
Zn	TORT 2 ##	189.2	6.0	180 $\pm$ 6	3	5
As	DORM 2 #	17.5	1.1	18 $\pm$ 1.1	6	7
Ag	DORM 2 #	0.04	0.01	0.041 $\pm$ 0.013	20	21
Cd	DORM 2 #	0.048	0.01	0.043 $\pm$ 0.008	29	30
Hg	TORT 2 ##	0.28	0.005	0.27 $\pm$ 0.06	2	15
Pb	TORT 2 ##	0.34	0.01	0.354 $\pm$ 0.013	2	15

N= 7
N= 3

4.3. Trace metal concentrations in blue mussels

Copper, zinc and lead concentrations were significantly lower in blue mussels collected at the three reference stations in Syltøy, Fjellspollen and Fauskanger, compared to the blue mussels collected around the Bergen centre ($p < 0.001$, Newman Keuls) (App.4 & 5). Concentrations found in the centre were for copper 1.2- 4.2 mg/kg, zinc 23- 51 mg/kg and lead 1.0- 1.7 mg/kg fresh weight, while concentrations outside the centre were within the range of 0.22- 0.98 mg/kg for copper, 15- 36 mg/kg for zinc and 0.8- 1.4 mg/kg fresh weight for lead (Fig. 4.2). High copper concentrations were also found in

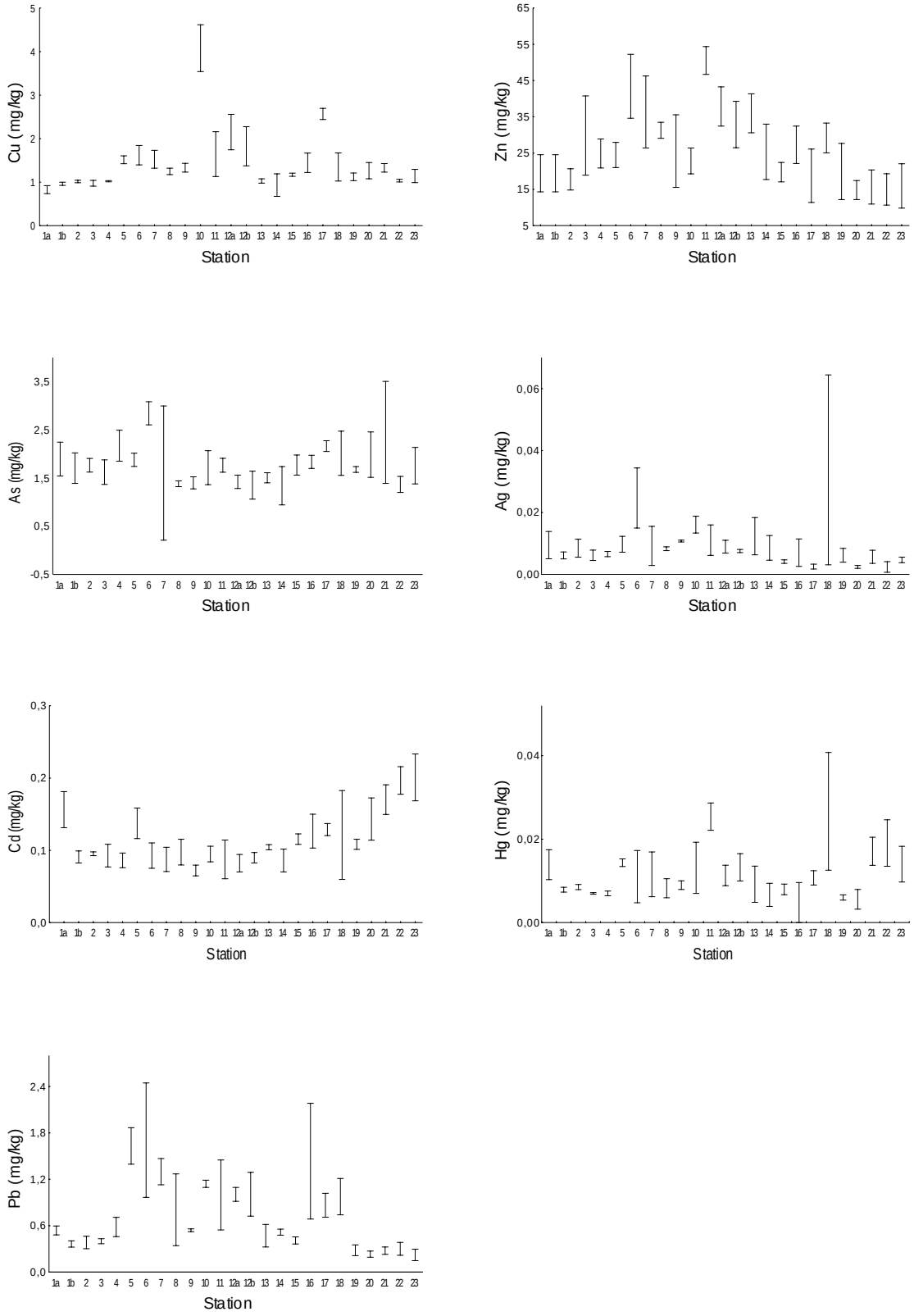

Figure 4.2. Trace metal concentrations (mg/kg fresh weight) in soft tissue of blue mussel (*Mytilus edulis)* at stations. N=3. Mean with 95 % confidence intervals.

Håkonshella (st. 17) outside the Bergen centre with the mean value of 2.6 mg/kg fresh weight. Lead was also high, 1.44 mg/kg, in blue mussels collected in Kolavåg in Askøy. In dry weight, lead concentrations exceeded 3 mg/kg at the stations 4- 18 (App. 4). Cadmium showed a tendency to increase with increasing distance from the Bergen centre. Mussels at the stations 1a, 5, 17, 19, 20, 21 and 22 had significantly higher cadmium concentrations than blue mussels in the Bergen centre ($p< 0.001$), but were in the whole area below 0.20 mg/kg fresh weight. The highest arsenic concentration were found in blue mussels collected at Vågen (st. 6), where the mean value was 20.9 mg/kg in dry weight. Otherwise, the arsenic concentrations in blue mussels were within the range of 1.3-2.8 mg/kg fresh weight, silver within the range of 0.02- 0.036 mg/kg and mercury 0.04-0.027 mg/kg fresh weight in mussels. As shown in the figure 4.2, some of the trace metal concentrations showed a large variations at some stations, like arsenic at Nykirkekaien (st. 7) and silver, cadmium and mercury at Kjerreidvik (st. 18).

4.4. Size effect study

There were no significant differences in length or weight of blue mussels between Fjellspollen and Nygård (App.6). Copper was significantly higher in the smallest blue mussels (Nyg I & Fjell I) than in blue mussels in the other size groups, although size group III at Nygård didn't indicate any differences ($p< 0.05$, Newman Keuls and Tukeys) (Tab. 4.3). Zinc, on the other hand, was significantly higher in large mussels (Nyg III-V) than in small ones (Nyg I-II) at Nygård ($p= 0.03$, Newman Keuls), while it didn't indicate any difference at Fjellspollen. Arsenic was significantly higher in the smallest mussels (Nyg I) ($p< 0.01$, Newman Keuls) and the largest mussels (Nyg V) ($p= 0.04$) compared to the other three size groups at Nygård. Cadmium was significantly higher in large mussels (Nyg IV-V) at Nygård, ($p< 0.01$, Tukeys), while at Fjellspollen cadmium was significantly higher in small mussels (Fjell I-II) than in the other size groups ($p< 0.01$, Tukeys). Lead was significantly higher in the largest blue mussels (Nyg V) compared to the other groups at Nygård ($p= 0.02$, Tukeys) while at Fjellspollen no significant differences between the size groups were observed.

Table 4.3. Trace metal concentrations (mg/kg dry weight) and dry matter content (g/100 g) in the soft tissue of mussels (*Mytilus edulis*) and standard deviation (SD) at size groups I-V at Nygård (Nyg) and Fjellspollen (Fjell). Significantly different groups marked with different letters (a,b,c,d). Size groups are defined as I≤ 3 cm, 3 cm <II≤ 4 cm, 4 cm <III≤ 5 cm, 5 cm <IV≤ 6 cm, V≥ 6 cm.

Station	Dry matter g/100 g	Cu mg/kg	SD	Zn mg/kg	SD	As mg/kg	SD	Ag mg/kg	SD	Cd mg/kg	SD	Hg mg/kg	SD	Pb mg/kg	SD
Nyg I	17.1	15.5^{a}	0.0	254^{a}	19	14.2^{a}	0.3	0.058	0.018	0.73^{a}	0.08	0.24	0.01	9.6^{a}	2.3
Nyg II	16.5	12.9^{c}	1.1	257^{ab}	10	12.6^{bc}	0.6	0.059	0.011	0.74^{ab}	0.04	0.23	0.02	10.5	1.3
Nyg III	14.7	13.0^{ab}	0.7	295^{c}	24	12.0^{bd}	0.3	0.058	0.005	0.82^{abe}	0.05	0.23	0.00	11.9	1.6
Nyg IV	14.2	12.1^{c}	1.0	298^{c}	14	11.9^{bd}	1.0	0.061	0.004	0.93^{ce}	0.06	0.25	0.01	13.2	1.1
Nyg V	14.5	11.0^{c}	0.6	290^{c}	10	13.3^{ac}	0.0	0.057	0.017	1.11^{c}	0.06	0.25	0.03	15.2^{b}	0.1
Fjell I	12.8	9.5^{a}	0.5	110	20	8.9^{a}	0.3	0.024^{a}	0.004	1.81^{a}	0.05	0.17^{a}	0.01	2.4	0.2
Fjell II	13.7	8.2	0.8	101	11	9.3^{abc}	1.0	0.016^{b}	0.004	1.60^{ab}	0.14	0.14^{b}	0.01	2.2	0.3
Fjell III	14.5	7.6^{b}	0.4	85	20	9.8^{abc}	0.1	0.013^{b}	0.002	1.31^{c}	0.04	0.14^{b}	0.01	2.0	0.2
Fjell IV	15.0	7.2^{b}	0.3	79	6	10.6^{cd}	0.3	0.013^{b}	0.001	1.29^{c}	0.08	0.15	0.01	2.2	0.3
Fjell V	15.0	6.9^{b}	0.4	91	22	11.3^{d}	0.3	0.010^{b}	0.001	1.26^{c}	0.10	0.16	0.00	2.5	0.2

There was significant positive regression between shell length of mussels and zinc and lead at Nygård (p≤ 0.01, regression analyses) (Fig. 4.4). Copper showed negative regression slopes with shell length at Nygård and Fjellspollen (p< 0.01) (Fig. 4.5). Also silver and cadmium had negative slopes with shell

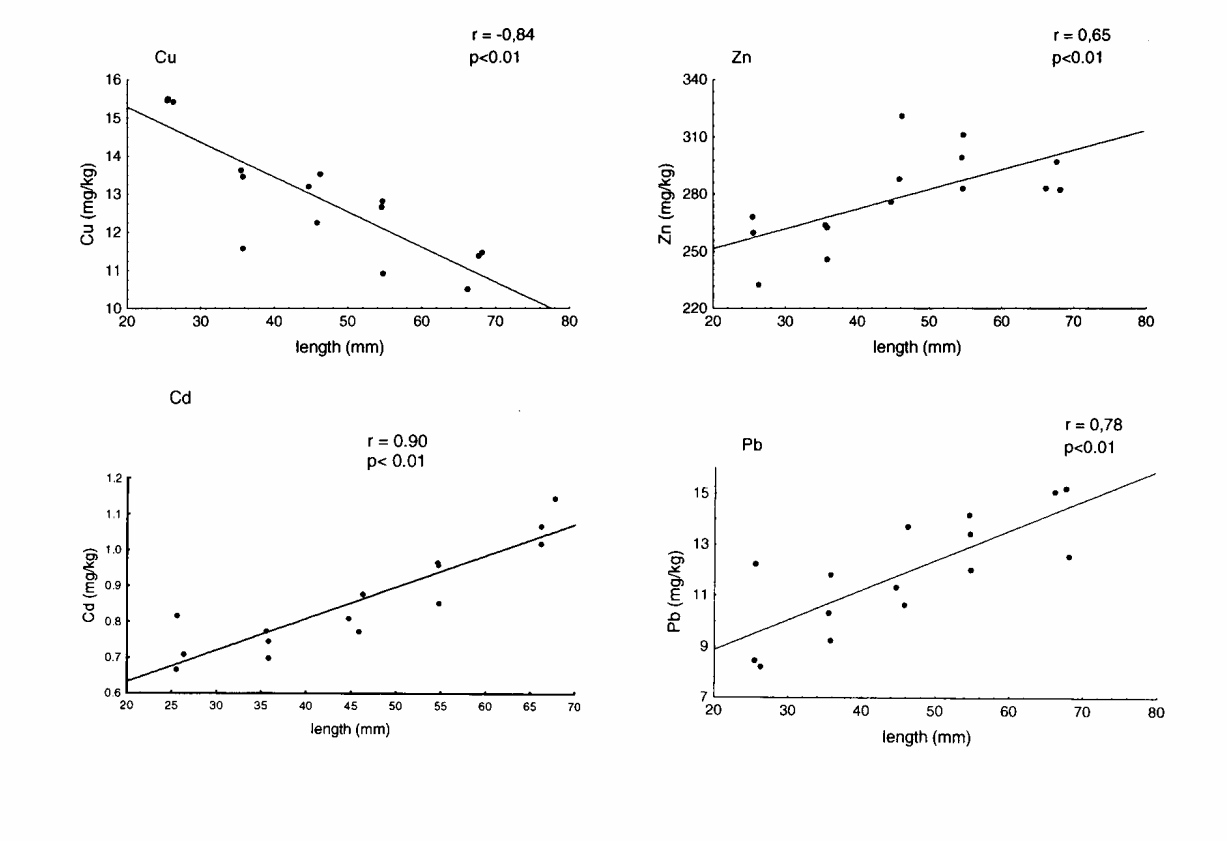

Figure 4.4. Significant bivariate correlation of Cu, Zn and Pb concentrations (mg/kg dry weight) in blue mussels (*Mytilus edulis*) with shell length (mm) in Nygård. r= correlation coefficient.

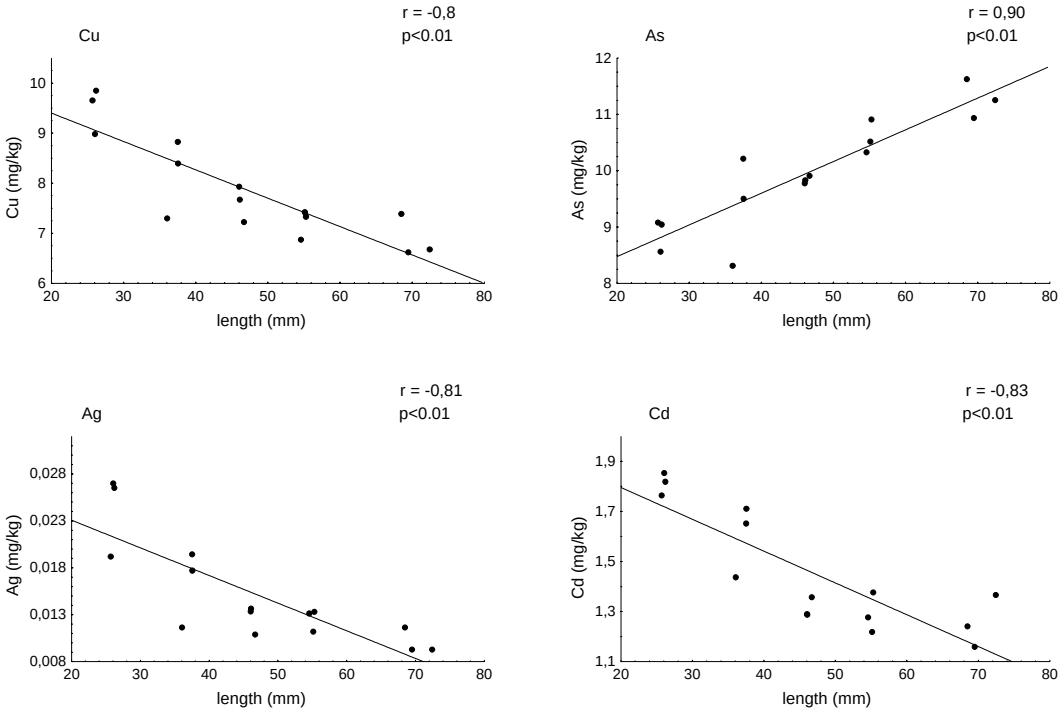

Figure 4.5. Significant bivariate correlation of Cu, As, Ag and Cd concentrations (mg/kg dry weight) in blue mussels (*Mytilus edulis*) with shell length (mm) in Fjellspollen. r = correlation coefficient.

length, but only at Fjellspollen ($p < 0.01$). At Nygård cadmium had positive regression slope with shell length ($p < 0.01$), as well as arsenic at Fjellspollen ($p < 0.01$).

Regressions between the trace metal concentrations and shell length were tested for blue mussels collected at the stations 1-23. Copper, zinc, arsenic and lead concentrations showed negative regression lines with length and were highest in small mussels ($p < 0.05$, regression analyses) (Tab. 4.4). Silver, cadmium and mercury did not indicate any regression with size (App. 12).

Table 4.4. Regression coefficients (r) for shell length and metal concentrations in mussels *M. edulis*. Significant regression marked with *.

Trace metal	r	p
Cu	-0.29	0.01*
Zn	-0.47	<0.01*
As	-0.24	0.04*
Ag	-0.19	0.11
Cd	0.14	0.22
Hg	0.08	0.49
Pb	-0.32	<0.01*

4.5. Tidal-effect study

Cadmium and lead concentrations were significantly higher in subtidal blue mussels than in mussels growing uptidally ($p < 0.05$, Tukeys) (Fig. 4.6). In contrast, copper, zinc, arsenic, silver and mercury concentrations were not significantly different between the three tidal groups (App. 9). There was significant negative regression between the sampling depth and concentrations of arsenic, cadmium, mercury and lead in mussels ($p < 0.05$, regression analyses) (Tab. 4.5). Copper showed also the same tendency having highest concentrations in mussels growing subtidally, even though the regression was not significant (App. 9).

Table 4.5. Regression coefficients (r) for trace metal concentrations (mg/kg dry weight) in mussels and sampling depth. Significant regression marked with *

Trace metal	r	B	p
Cu	-0.66	-19.7	0.05
Zn	0.25	0.1	0.52
As	-0.69	-18.0	0.04*
Ag	-0.54	-1218.0	0.14
Cd	-0.81	-166.7	<0.01*
Hg	-0.59	-839.3	< 0.01
Pb	-0.80	-9.6	< 0.001*

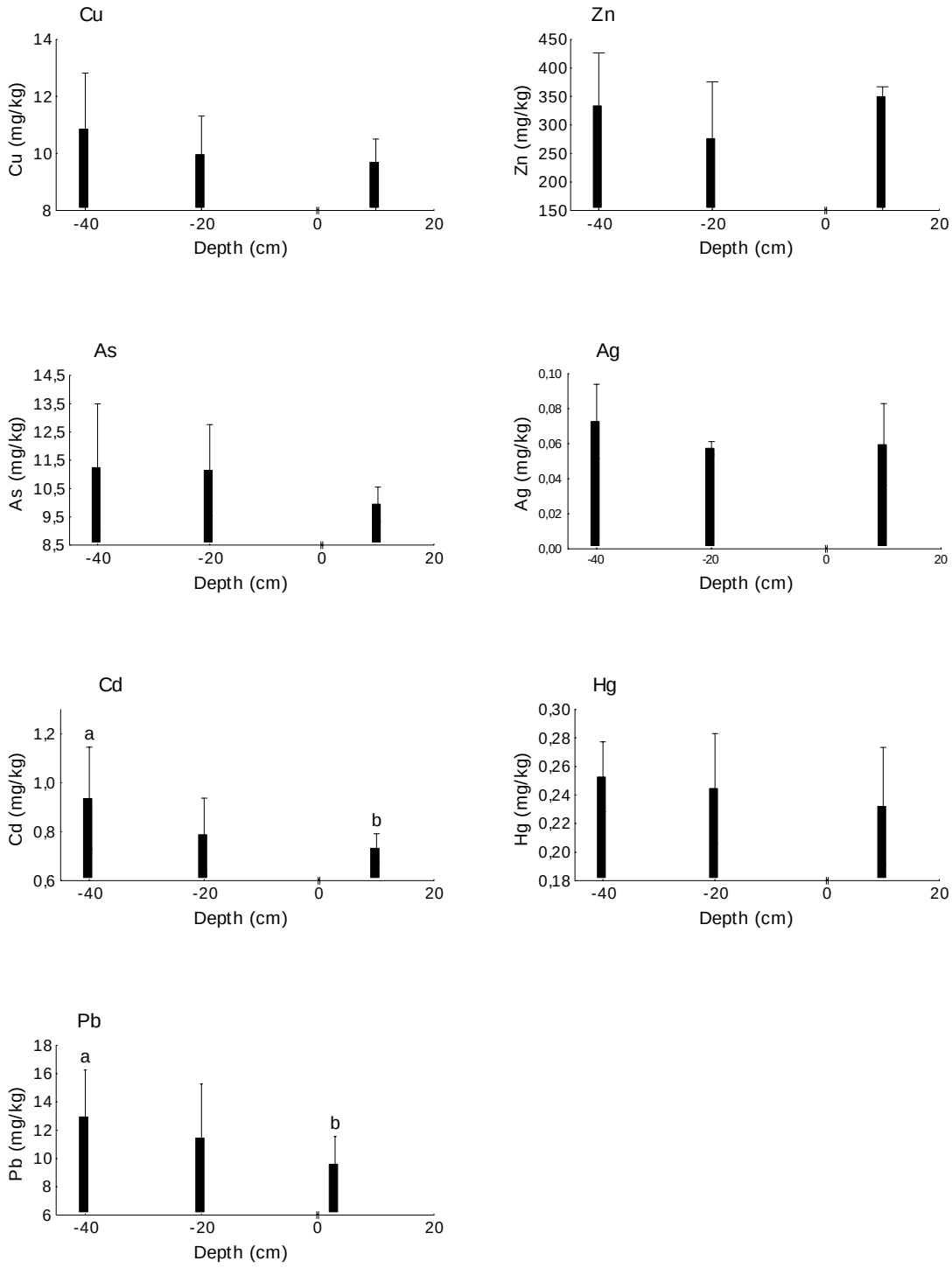

Figure 4.6. Trace metal concentrations (mg/kg dry weight) in blue mussels *(Mytilus edulis)* in three tidal groups: subtidal (-40), intertidal (-20 cm) and uptidal (10 cm). Zero in the figure illustrates the sealevel at the lowest spring tide. Mean with 95 % confidence interval. Significantly different groups marked with different letters (a,b) (One-way ANOVA)

4.6. Thawing-effect study

Arsenic, silver and cadmium concentrations were significantly higher in blue mussels which were dissected fresh (Fjell fresh) than in mussels in the control group, which first were frozen and thawed (Fjell froz) ($p < 0.05$, paired t-test) (Fig. 4.7). The opposite was found in lead concentrations; blue mussels in the control group had significantly higher lead concentrations than mussels in Fjell fresh. There were no significant differences in concentrations for copper, zinc and mercury or length in blue mussels between Fjell fresh and the control group (App.10 & 11).

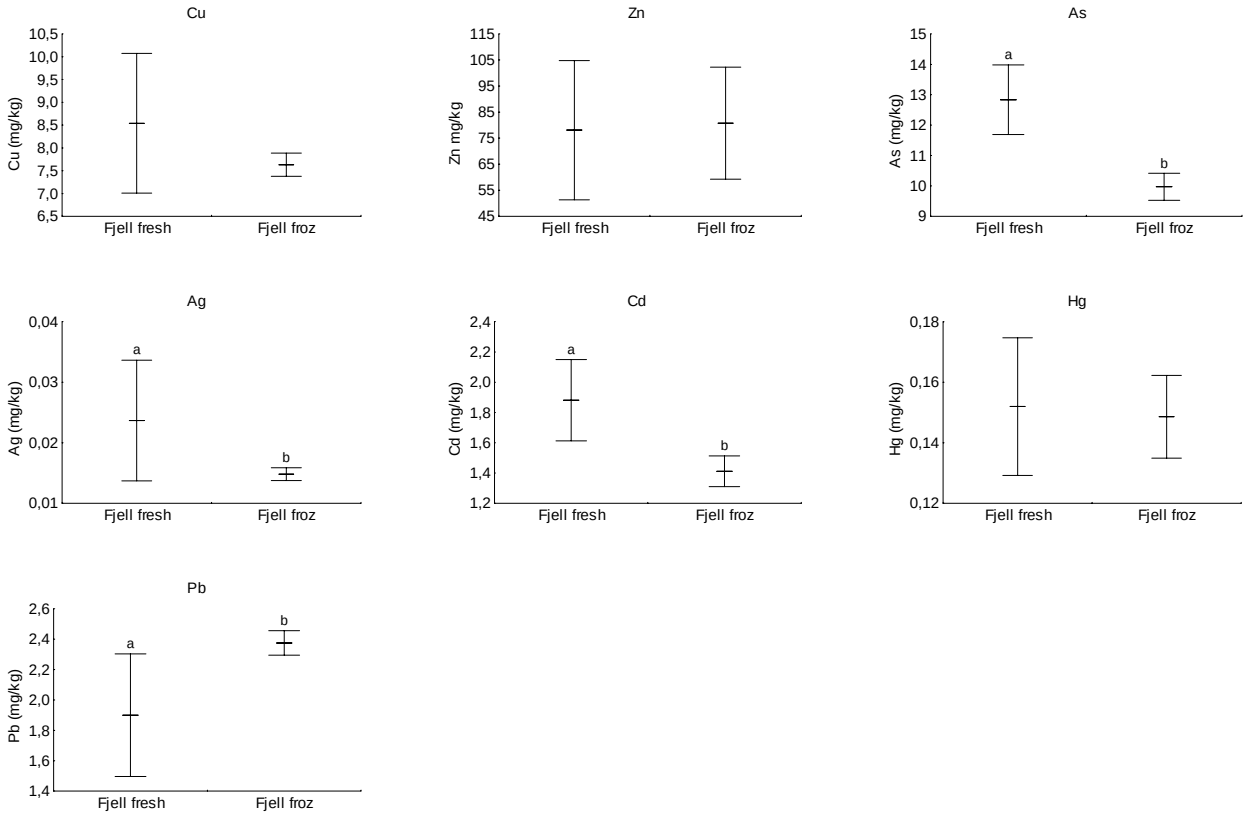

Figure 4.7. Trace metal concentrations (mg/kg dry weight) in blue mussels (*Mytilus edulis*) from Fjell fresh and Fjell froz. Mussels from Fjell fresh were dissected fresh, Fjell froz mussels were frozen before dissection. Mean with 95 % confidence interval. Significant differences marked different letter (a,b).

4.7. Metal interactions

Metal interactions at stations 1-23 were tested with bivariate correlation analyses. Copper and zinc concentrations in blue mussels showed significant positive correlation, while cadmium and lead

correlated negatively with zinc ($p< 0.05$) (Fig. 4.8). Significant positive correlation was also found between arsenic and silver, arsenic and lead, as silver and mercury and silver and lead ($p< 0.01$).

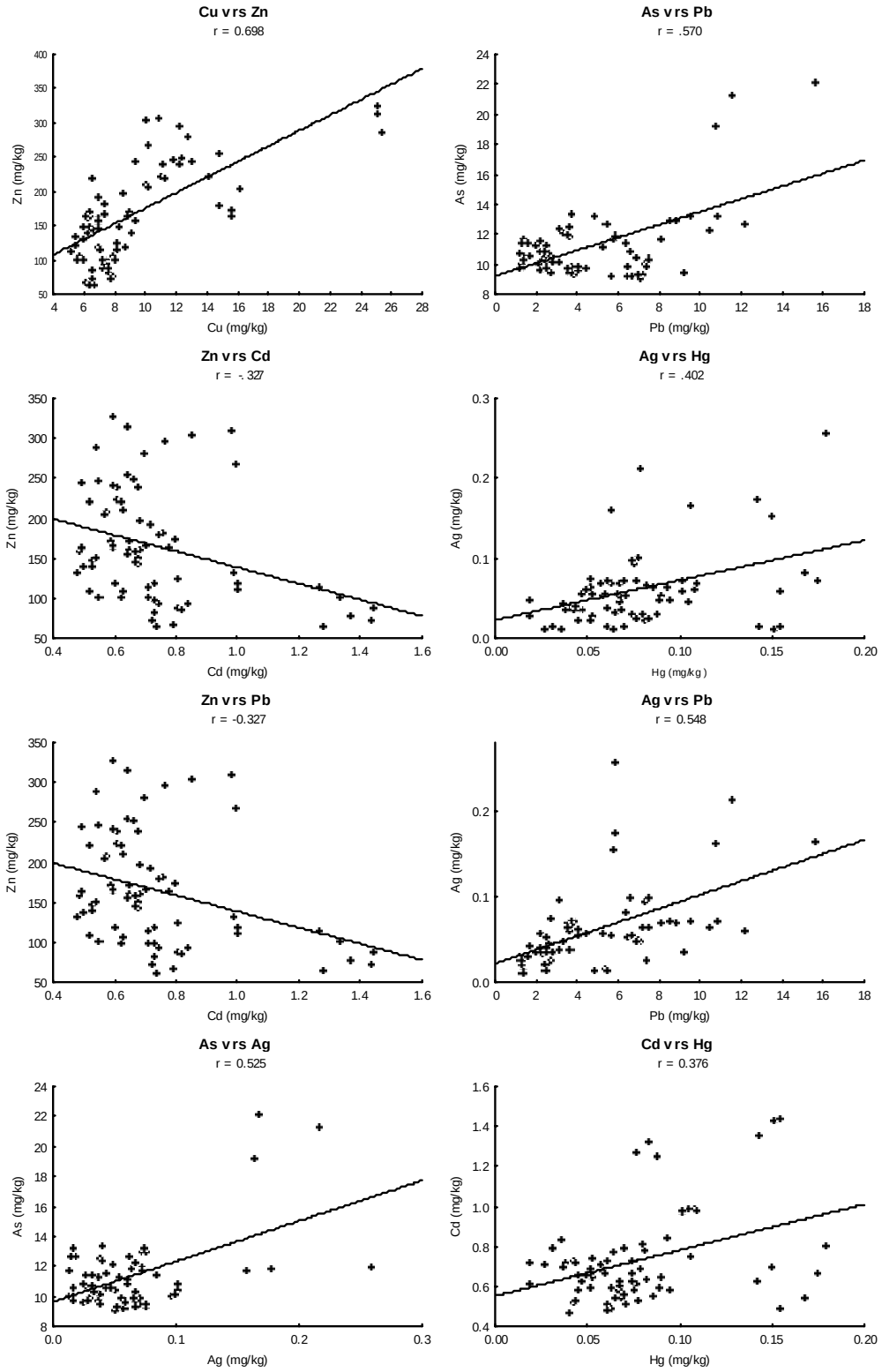

Figure 4.8. Significant correlations of trace metal concentrations (mg/kg dry weight) in blue mussels (*Mytilus edulis*) ($p< 0.05$). r = correlation coefficient.

5. Discussion

5.1. Discussion of Material and Methods

5.1.1. Blue mussels

In this study blue mussels *(Mytilus edulis*) were chosen on the basis of length of the shell (anterior-posterior axis) ranging from 35 mm to 60 mm. It is recommended, that mussels for trace metal analyses are sampled from a small size range since the metal concentrations vary with size (Boyden, 1974; Boyden, 1977; Davies & Pirie, 1978; Cossa et al., 1979; Popham & D'Auria, 1983; Regoli & Orlando, 1994), which is of special importance in comparable studies (Mussel Watch, 1980). Results from the present study are compared with a study made by Andersen (1994), who used mussels of size 35- 55 mm in length. In the present study the upper limit was increased with 5 mm, since Andersen (1994) had difficulties to find blue mussels smaller than 55 mm at some locations. The Mussel Watch programs in the Gulf of Maine and on the French coast use also blue mussels in the size range 35-60 mm (Claisse, 1989; Jones et al., 2001). In addition, blue mussels used for human consumption range often to 60 mm in size, and in the present study blue mussels were used to evaluate, if mussels are suitable for human consumption.

In the present study sexes were not distinguished before the analyses, although previous studies have reported that among certain bivalve species females and males can accumulate metals differently (Alexander & Young, 1976; Gordon et al., 1978; Watling & Watling, 1976). Lobel et al. (1989) found that females of *M. edulis* accumulated more copper, zinc, arsenic and silver than males sampled at the same site. Despite of this, most monitoring programs with mussels do not distinguish between sexes in sampling and it was assumed that in random sampling the one would catch both sexes in equal ratio in normal populations.

5.1.2. Fieldwork

In this study blue mussel sampling was done during late winter in two following years. In comparable studies it is important to do the sampling on the same time of the year, since metal concentration in mussels varies with reproductive cycle. Blue mussels loose considerable weight during spawning (Seed, 1976). Trace metal concentrations in blue mussels after spawning seem to be higher than at other periods within a year, especially in polluted waters (De Wolf, 1975; Phillips, 1976b; Cossa et al., 1979; Amiard- Triquet et al., 1986). This is due to reduced body weight after spawning while metal content still remains the same in mussels (Philips, 1976b; Boyden, 1977). The exception is arsenic, which seems to have highest concentration in mussels before spawning (Julshamn & Duinker, 2001). There was large variation in arsenic concentrations in blue mussels between the three samples collected in Nykirkekaien. The blue mussels from the station were observed spawning when sampled. The spawning might have caused the variations in arsenic concentrations at the station. Variations in metal concentrations in blue mussels in Kjerreidvik can not be explained with the spawning, since no signs of that were observed. When trace metal concentrations in mussels are calculated, the values are related to weight. Variation in weight will then be reflected to metal concentrations. Boyden (1977) suggested that instead of metal concentration, metal loads should be reported as metal content (mg/ total weight). On this way the effect of seasonal variation in weight of mussels could be avoided. On the other hand, Cossa et al. (1979) suggested that mussels used for environmental monitoring should be collected before they reach sexual maturation, since the weight varies least before the maturation. This would be time-consuming, since immature individuals are small and difficult to handle in the laboratory. The present study was compared with the study of Andersen (1994), who also sampled mature individuals in late winter, before the spawning season, in the same geographical area.

Blue mussels for the present study were collected during spring low tide at 50- 70 cm depth to avoid that mussels would have had contact with the surface layer of the water. Sampling depth can effect metal concentration in mussels due to differences in chemical properties of water layers or due to varying food availability in different water layers (Nielsen, 1974;

Phillips, 1976b; Seed, 1976). The surface microlayer contains organic particles and metals to a larger extent than deeper layers (Xhoffer et al., 1992). Mussels growing in the uptidal zone have contact with water surface more frequently than subtidal mussels and this could increase uptake of metals by mussels. In comparative studies it is recommended that sampling depth is the same in all studies (Mussel Watch, 1980). The present study was compared with the study of Andersen (1994), who also sampled below 30- 50 cm depth under spring low tide. Effect of sampling depth is further discussed in connection with the tidal-effect study later in this chapter.

Salinity affects feeding rate and metal accumulation in mussels, as well as metal solubility in water (Phillips, 1976b; 1977b; Davenport, 1977). Phillips (1976b) found that cadmium uptake was increased and lead uptake was decreased in *Mytilus edulis* near to fresh water inputs of trace metals. During spring, snow melting and heavy rains increase freshwater runoff from land. In the present study salinity was not measured, but on the basis of other studies (Botnen et al., 2000), stations locating in shallow bays seem to have lower salinity than stations facing more open water. The reference stations Fauskanger and Fjellspollen were located in the shallow bays and the fresh water input might influence on trace metal concentrations in blue mussels here. Fresh water input is also increased in the vicinity of sewage outlets, which in this study would have effect on blue mussels at Biskopshavn and Lyreneset.

5.1.3 Sample preparation and trace metal analyses

In the present study, gut content of the blue mussels was remained for the metal analyses, although intestines of mussels can contain sediment particles and phytoplankton, which may contain high concentrations of metals (Abdullah & Royle, 1974; Stephenson et al., 1978; Lobel et al., 1991). This can cause an overestimation of metal concentration in organisms. For example, Stephenson et al. (1978) found following metal loads of intestine / total body of mussels in percentages: 6 % for copper, 1 % for zinc, 10 % for silver, 11% for cadmium and 20 % for lead. Intestinal tract of fish can easily be removed before analyses, while in bivalves,

digestive gland often serves as a storage for biological metals and must be included for the analyses (Georges & Pirie, 1980; Lobel et al., 1991). Depuration for 2-3 days was found to be a sufficient method to remove non-biological metals from the bivalve intestines (Lobel et al., 1991; Brown & Luoma, 1995). On the other hand, depuration of mussels for longer periods of times might cause loss of some metals from the mussels depending on the biological half lives of metals (Mussel Watch, 1980). Second, wild blue mussels collected for human consumption are not depurated before preparation, and to monitor food safety, gut content should be included for the analyses. This is why it was decided to include gut content for the analyses in the present study.

There are two methods used to prepare mussels for the metal analyses; 1) mussels are dissected fresh and then frozen before analyses and 2) mussels are first frozen and then dissected. In the present study mussels were frozen prior to dissection. This can have some effect on metal concentration measured in further analyses. Some of the metals can be lost from the body in melting water and the results gained in the analyses might be lower than in mussels dissected fresh. On the other hand, many monitoring programs are based on sampling in a wide geographical area, while the analyses are made in one central laboratory. The transport of mussels without freezing them might be difficult, or even impossible. Freezing might be the only way to gain material in large scale monitoring programs with mussels. Freezing in this study should not give any error, when compared with the previous studies in the area, since the present study was compared with studies, where mussels were also frozen before dissection (Andersen, 1994). Since no literature was found about possible effects of freezing on metal concentrations in mussels, a separate study of the effect of thawing was made and the results are discussed later in this chapter.

Mussels were rinsed with deionized water before dissection to remove sediment particles and other debris from the shell body. It is possible that some of the particles were still remained in the soft tissues and were taken to the analyses. Particles might have contaminated samples with sediment bound metals.

Freeze drying might cause some metal losses due to volatilisation of metals or it could cause contamination by other samples (Mussel Watch, 1980). In this study certified reference material processed on the same time with mussel samples did not give any indication of this.

Samples in this study were homogenised with a powerful food processor. The blade and other parts were brushed and vacuum cleaned between samples from different stations. Washing with water was done only at the end of each day. Samples which were assumed to contain less metal were treated first. This might have caused contamination of samples and eventually could have increased variation of metal concentrations in the mussels within the stations.

Inductively coupled plasma-mass spectrophotometry was used for the metal analyses. This method has been found to give better quantitative data than other emission sources due to high stability, low background and low detection limits (Skog et al., 1988). Certified Reference Material (CRM) was used to evaluate trueness and repeatability of the analyses. An ideal CRM would have been of bivalve mollusc with similar metal concentrations as in the present study. Since no CRM material of molluscan was available, bovine liver and intestinal gland of lobster were used instead.

5.1.4 Statistics

In this study 75 individuals were analysed in three subsamples for each station. Gordon et al. (1980) stated that pooling of many individuals into a single sample would reduce variances and give a better population mean prior to the sampling of individuals, as long as the number of individuals in each pool is the same. In the present study, despite of pooling, weight and metal data showed large variations. To reduce the variation in length and metal data, a smaller size range could have been used. For example, Skei et al. (1994) and Knutzen et al. (1995) have used blue mussels in the size range 30-50 mm and Julshamn et al. (2001) in the

size range 30-40 mm. However, the present study was compared with the study of Andersen (1994), in which larger size range (35- 55 mm) was used and it was chosen to prioritise

similarity in the size ranges above the risks of the weakening of statistical methods. In the cases where variances were not homogenous, the Newman Keuls test was used, since this test seems to be more robust for Type 1 error compared to the Tukeys HSD test (Ranta et al., 2002). Metal data in mussels tend to be skewed, and many authors prefer to operate with log transformed data for statistics (Boyden, 1974; Lobel & Wright, 1982). The use of pooled samples in the present study avoids this problem, since these are considered as averages that are normally distributed according to the Central limit theorem (Zar, 1999), so log transformation was not used.

5.2. Discussion of Results

5.2.1. Trace metal concentrations in blue mussels

Lead (Pb)

In the present study, lead concentrations in blue mussels (*Mytilus edulis*) were high in the Bergen centre, ranging from 0.4 mg/kg to 1.7 mg/kg fresh weight. These values exceed the normal concentrations, 0.18- 0.57 mg/kg fresh weight, measured in blue mussels in the Hordaland county (Julshamn & Duinker, 2002). Compared with previous studies (Andersen, 1994), lead concentrations have reduced with 20% since 1994 (Fig. 5.1). EU has set upper limit of lead concentration in mussels to 1.5 mg/kg fresh weight, when used for human consumption (EU, 2001). Blue mussels from the Bergen centre exceed this limit and are not suitable for human consumption.

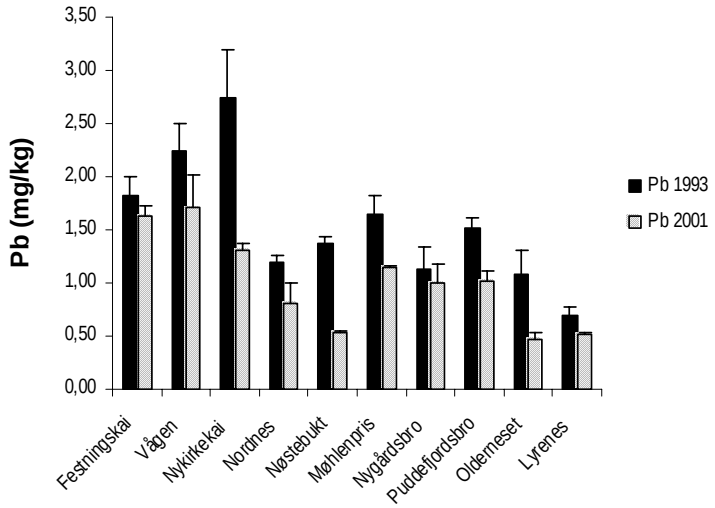

Figure 5.1. Mean ± SD (mg/kg fresh weight) of lead (Pb) in soft part of pooled blue mussels (*Mytilus edulis*) from various sampling sites in Byfjorden, Bergen, 1994 and the present study, 2001. Data from 1993 is reported by Andersen (1994).

Lead concentrations in blue mussels remain high at the stations in the vicinity of main city roads (Festningskai, Vågen, Puddefjorden and Nygårdsbro). This indicates that the urban run-off washed from the roads might still be one of the main sources of lead into the water today. Another possible explanation to high concentration of lead in mussels today is that the

sediments in the Bergen centre contain large amounts of lead, which can be withdrawn to water column, when the harbor sediments are stirred by ship traffic (Anon., 2002).
The lead values found here are similar to that of concentrations, 0.52 mg/kg-1.5 mg/kg fresh weight, found in Hardangerfjorden (Julshamn et al., 2001), and lower than found along the East coast of the UK, 3- 28.8 μg/g dry weight (Widdows et al., 1995).

According to some time-scale monitoring programs, lead has been reduced in many problem areas. For example, in the Gulf of Maine lead concentration in mussels has been reduced from 5.4 mg/kg to 1.8 mg/kg dry weight in the period of 1993- 1998 (Jones et al., 2001). Even more dramatic improvement has been recorded by Julshamn et al. (2001) in the Hardangerfjord, where lead concentration in blue mussels has been reduced from 120 mg/kg in 1983 to 1.5 mg/kg fresh weight in 1998. This can be due to improvements in water treatment systems, reductions in industrial discharges or, as in Hardangerfjord, a combination of technical improvements like covering the polluted sediments with fibre cloths and reductions in industrial discharges (Julshamn et al., 2001).

Mercury (Hg)

Distinct from lead, mercury concentrations in blue mussels in the studied area were low and ranged from quantification limit to 0.03 mg/kg fresh weight in mussels. These results are similar to that found in blue mussels in Hordaland county (Julshamn & Duinker, 2002). Compared with previous studies, mercury concentration in mussels has been reduced with 50-70 % since 1994 (Fig. 5.2) (Andersen, 1994). The most dramatic reduction in mercury concentrations were found in Møhlenpris and Nøstebukten. The removal of the old sewage outlets, as well as reductions in the discharges from dentist offices and laboratories might be the main reason for this (Dons & Beck, 1993).

Like lead, sediments in Vågen and Puddefjorden are highly polluted by mercury, containing up to 5 mg/kg mercury on the upper layer (Molvær et al., 1997; Anon., 2002). Despite of this, blue mussels seem to be unaffected in the area, which may indicate that sediment bound mercury is stable and is not bioavailable to blue mussels. Generally, harbor sediments are

unstable, and boat traffic might stir sediments periodically bringing mercury back to the water column, although there was no evidence for this in the present study. EU (2001) has set an upper limit of mercury in mussels at 0.5 mg/kg fresh weight, if used for human consumption. Blue mussels in the present study had concentrations one tenth of this, and can be considered as safe for human consumption.

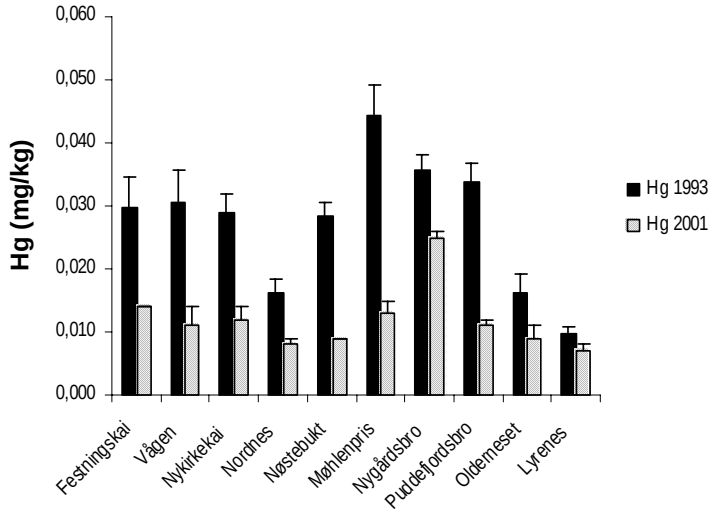

Figure 5.2. Mean ± SD (mg/kg fresh weight) of mercury in soft part of pooled blue mussels (*Mytilus edulis*) from various sampling sites in Byfjorden, Bergen, 1994 and the present study, 2001. Data from 1993 is reported by Andersen (1994).

Mercury concentrations found here are much lower than those found in Scotland, 0.05- 0.2 mg/kg fresh weight (Davies & Pirie, 1978), slightly lower than those found in the Gulf Watch program in the Gulf of Maine, 0.23 ± 0.14 mg/kg dry weight (Jones et al., 2001) and similar to those found in Irish waters in 1999, 0.01- 0.05 mg/kg fresh weight (McGovern et al., 2001).

Cadmium (Cd)

The concentrations of cadmium between 0.08 mg/kg and 0.2 mg/kg fresh weight in the present study are within the normal range found in blue mussels in the Hordaland county (Julshamn & Duinker, 2002). Cadmium concentrations have been reduced in the Bergen centre compared with the data from 1994 (Andersen, 1994) (Fig. 5.3). Reduction in Oldernes, et and Nøstebukt was over 50 %. Cadmium concentrations found in blue mussels in the whole study area were clearly below the upper limit of 1.0 mg/kg fresh weight for mussels used for human consumption set by EU (2001).

The cadmium values in the present study showed an interesting tendency to increase with increasing distance from the centre. Blue mussels sampled in Fjellspollen and Fauskanger had cadmium concentrations of 0.20 mg/kg fresh weight, while blue mussels in the Bergen centre had the concentrations ranging from 0.08 mg/kg to 0.14 mg/kg fresh weight. Similar trend was found by Andersen (1994) in the same area. The water solubility and the uptake of cadmium by blue mussels increase with decreasing salinity, and higher cadmium concentrations have been found in blue mussels growing in the brackish water (Phillips, 1976b; 1977b). Fauskanger and Fjellspollen were both located in shallow bays with limited water circulation and the salinity of the water here might have been reduced due to snow melting in the sampling period. This might explain partly the higher concentrations at these two stations. Another explanation might be that the coastal waters bring atmospheric cadmium more frequently to the stations locating outside the Byfjorden, which has more limited connection to the coastal water. The fresh water effect or elevated cadmium levels were not observed in the vicinity of the new water treatment plants at Lyreneset and Biskopshavn.

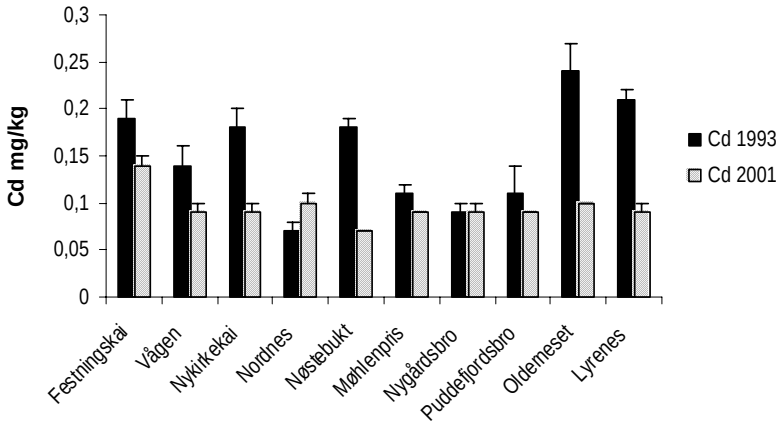

Figure 5.3. Mean ± SD (mg/kg fresh weight) of cadmium in soft parts of pooled blue mussels (*M. edulis*) from various sampling sites in Byfjorden, Bergen, 1994 and the present study, 2001. Data from 1993 is reported by Andersen (1994).

Comparing the results of the present study with other studies, higher cadmium concentrations, 1.0 mg/kg fresh weight, were found in Hardangerfjorden by Julshamn et al. (2001), and in the Gulf of Maine, >2.8 mg/kg dry weight, by Jones et al. (2001).

Arsenic (As)

Arsenic concentrations in blue mussels varied from 1.3 mg/kg to 2.8 mg/kg fresh weight. These values are within the normal range of arsenic in mussels, 1.97 mg/kg- 3.23 mg/kg fresh weight, from the Hordaland county (Julshamn & Duinker, 2002). FAO/WHO has set a maximum tolerable weekly arsenic intake to 15 μg/kg body weight in human dietary. With a mean concentration of 1.8 mg/kg found in the present study, the maximum weekly intake would be 500 g mussels for a person of 60 kg.

In this study high concentrations of arsenic were found both in the centre and in areas with scattered settlement. This indicates high background levels or diffuse arsenic sources in the whole area studied. Possible sources may be burning or leakage of arsenic from impregnated wood. Arsenic levels in mussels should be followed in the future.

Julshamn & Duinker (2001) found that arsenic concentrations in blue mussels are at its highest in the period before spawning, in March. This would indicate that arsenic could be stored in gonads and eggs of mussels, since arsenic concentrations are reduced in June, right after spawning (Julshamn & Duinker, 2002). This might be one reason why it was found high arsenic concentrations in blue mussels in the present study, and it can be assumed that the concentrations will sink in blue mussels in the summer.

Silver (Ag)

Silver concentrations in blue mussels were low, and varied from the quantification limit (0.01 mg/kg dry weight) to 0.36 mg/kg fresh weight in the main study. Similar concentrations were found in farmed mussels sampled in Hordaland in 2001 (Julshamn & Duinker, 2002). Much higher silver concentrations have been reported in the harbor areas in the Gulf of Maine (Jones et al., 2001) and in California (Alexander & Young, 1976).

Zinc (Zn)

Zinc concentrations found in blue mussels varied between 15 mg/kg and 51 mg/kg fresh weight, while the normal values in the Hordaland county lie within the range of 17 - 22 mg/kg

fresh weight (Julshamn & Duinker, 2002). The highest values were found at Vågen and inner Puddefjorden and exceeded the normal values with 30-40 %. Generally, zinc concentrations were lower at the present study than in 1994, except at Nygårdsbro, where zinc concentrations were higher at the present study (Fig. 5.4) (Andersen, 1994). EU has not set any upper limit of zinc in mussels so far.

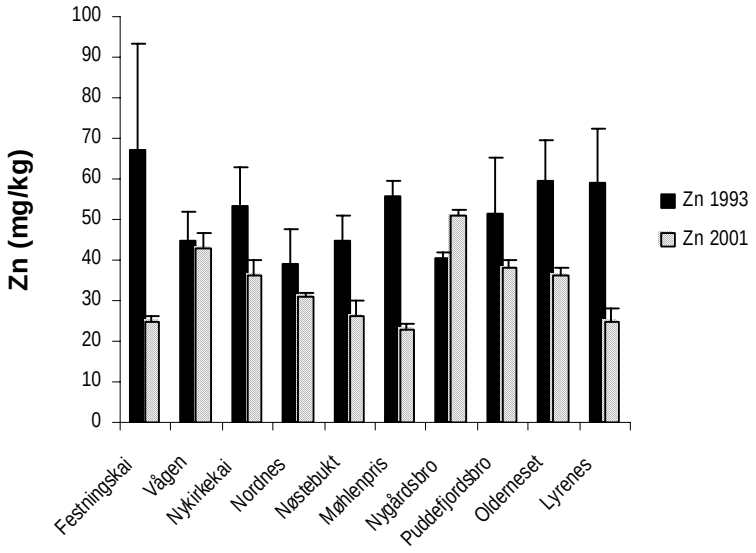

Figure 5.4. Mean ± SD (mg/kg fresh weight) of zinc in soft parts of pooled blue mussels (*Mytilus edulis*) from various sampling sites in Byfjorden, Bergen, 1994 and present study, 2001. Data from 1993 is reported by Andersen (1994).

Despite the removal of the municipal wastewater discharges from the area, zinc concentrations in the Bergen centre are still high. The sediments in Vågen and inner Puddefjorden are markedly polluted by zinc (Anon., 2002). Harbor sediments are frequently disturbed by boat traffic, and this might result that zinc is withdrawn into the water from contaminated sediments and that metal is bioavailable to the blue mussels. This might be the main source now, when sewage water outflow has been moved from the area.

Copper (Cu)

Copper concentrations in blue mussels ranged from 0.8 to 4.1 mg/kg fresh weight. Normal values of copper in blue mussels in the Hordaland county are 0.5- 2.0 mg/kg fresh weight (Julshamn & Duinker, 2002). Stations at inner Puddefjorden and Vågen, as well as at Håkonshella had the highest values ranging from 1.6 mg/kg in Vågen to 4.1 mg/kg in Møhlenpris. Compared with previous studies there is an overall decrease in copper content of blue mussels in the Bergen centre (Andersen, 1994) (Fig. 5.5). There was a slight increase of copper in mussels at Møhlenpris. The measured copper values are similar found in Gulf of Maine (Jones et al., 2001) and lower than in Oslofjorden (Green & Knutzen, 1992) and Hardangerfjorden (Julshamn & Grahl-Nielsen, 1996). When human health is considered, no guidelines of copper content are given in Norway. In Spain the limit has been set for 20 mg/kg fresh weight (Anon., 1992), and on the basis of this, mussels in the area can be considered as safe for human consumption.

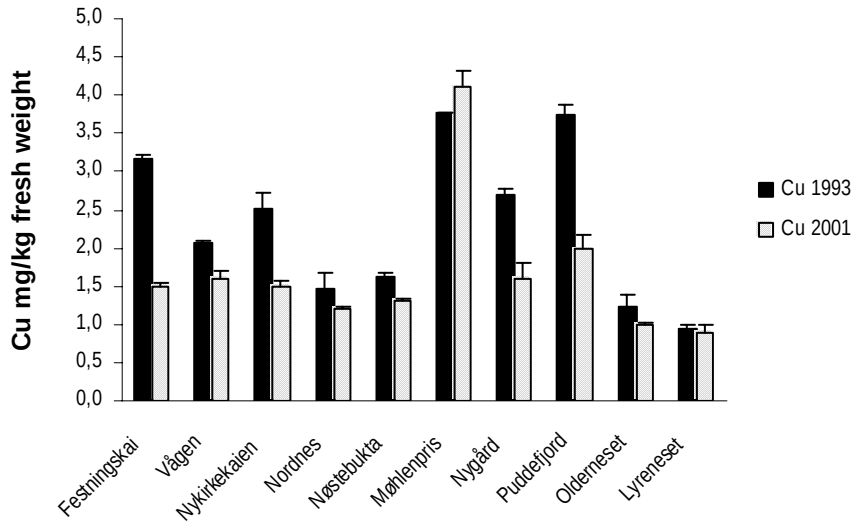

Figure 5.5. Mean ± SD (mg/kg fresh weight) of copper in soft part of pooled blue mussels (*Mytilus edulis*) from various sampling sites in Byfjorden, Bergen, 1994 and present study, 2001. Data from 1993 is reported by Andersen (1994).

The results from this study indicate that despite of the removal of the municipal wastewater discharge from the Bergen centre, copper is still bioavailable for mussels in the area. Copper is used in antifouling paints on boats. There is heavy boat traffic in the Bergen centre, and the samples in Håkonshella were taken from a small craft harbor. Young et al. (1979) reported extremely high concentrations, up to 127 mg/kg dry weight, of copper in blue mussels in the

harbor area in Southern California. There is also a large dry- dock in Puddefjorden. On a dry-dock, old paint is removed from ships by sandblasting. Spillage from sandblasting operations and leakage from removed paint particles has previously been recognised as a direct source of copper to water (Degerman & Rosenberg, 1981; Sekse & Kvingedal, 1992). Meiggs (1980) found that both mussels and sediments were remarkable contaminated by copper and zinc in the vicinity of a dry dock. Use of copper in antifouling paints on boats might be the main cause of the elevated levels of copper in blue mussels found in the present study.

5.2.2. Size- effect study

Copper concentrations were significantly higher in small mussels (< 4 cm) than in large ones (> 6 cm) at the both stations. Similar results have been reported previously for copper (Boyden, 1974; Boyden, 1977; Cossa et al., 1980; Popham & D'Auria, 1983; Riget et al., 1996). Cadmium behaved opposite, being significantly higher in large mussels. This corresponds with previous findings in unpolluted waters (Riget et al., 1996), while Boyden (1977) found no correlation with cadmium and size in mussels, and Cossa et al. (1980) reported negative correlation with size. At Fjellspollen silver was found to be significantly higher in small mussels than in large. Zinc and lead, on the other hand, were significantly higher in large mussels at the polluted site (Nygård). Riget et al. (1996) reported similar results for lead but found zinc to be independent of size. However, both negative and positive correlation between zinc, as well as lead, and size are reported (Boyden, 1974; Boyden, 1977; Cossa et al., 1980; Popham & D'Auria, 1983). De Wolf (1975) found higher concentrations of mercury in small individuals than in large ones. In the present study, like in Riget et al. (1996), mercury did not indicate any correlation with size, although in the present study, some significant differences were found between the size groups II and III compared to other groups at Fjellspollen. As a conclusion, in the present study, size seems to have different effect on trace metal concentrations in blue mussels in polluted and unpolluted sites, when it comes to zinc, lead, arsenic and silver. Popham & D'Auria (1983) noted the same in the case of zinc and lead concentrations in a contaminated and an uncontaminated area. Differences in metal accumulation in mussels can be due to environmental stress, different

seasonal growth patterns or metal uptake rates between size groups, or due to the effect of other metals in the water (Strong & Luoma, 1981; Widdows et al., 1995; Riget et al., 1996; Stewart, 1999). However, it can be concluded, like in Amiard- Triquet et al.(1986), that although

there is variation in metal concentrations between different size groups, a general picture of environmental condition in the two different areas could clearly be seen: The trace metal concentrations except cadmium, were higher in polluted site, Nygård, than in unpolluted site, Fjellspollen, in all size classes.

5.2.3.Tidal- effect study

In the present study cadmium and lead were significantly higher in the subtidal blue mussels growing in 40 cm below the spring low tide than in mussels on the upper shore, 10 cm above the spring low tide mark. In contrast, Philips (1976b) recorded highest values of zinc, cadmium and lead in the intertidal mussels, and De Wolf (1975) found highest mercury concentrations as well in intertidal mussels. Phillips (1977b) reported that zinc and cadmium concentrations in blue mussels collected at low salinity waters were higher than in mussels from high salinity waters. In the present study the sampling site, Nygård, was located in a narrow sound, where the water current is quite strong. It is possible likely, that the strong water movement at the site prevents the formation of vertical layers in the water and the mussels in all tidal zones have the similar salinity environment. Blue mussel take up cadmium in dissolved form, while lead is taken up from food particles and from the water in similar rates (Shulz- Baldes, 1974). Mussels growing uptidally will have less direct contact with water and less time to feed, than the mussels living continuously beneath the water surface. Previous studies have reported that intertidal mussels have slower growth rates than mussels growing subtidally (Seed, 1976; Griffiths, 1981). This can be due to reduced access to food during low tide or increased light exposure, which may inhibit growth (Seed, 1976). This might be one of the reasons, why cadmium and lead were significantly higher in subtidal mussels. In the present study uptidal mussels were significantly lighter than mussels at the intertidal zone. In the size- effect study discussed previously, lead was significantly higher in the large mussels

than in the small ones. The results here in the tidal effect study might reflect the size effect in lead concentration rather than the tidal effect studied.

5.2.4. Thawing effect study

In the thawing- effect study arsenic, silver and cadmium were significantly higher in the blue mussels dissected fresh than in the mussels that first were frozen. The differences were of the magnitude of 20-50 %. Lead, on the other hand, was significantly higher in blue mussels, which first were frozen. The circulation system in blue mussels is partly open. This means that blood, or hemolymph, circulates in blood veins between different organs but freely within organs. Hemolymph transports dissolved nutritive products, oxygen and trace metals from gills to the other parts of the body (Field, 1921; Hovgaard et al., 2001), although the transport system for trace metals is not fully understood (Simkiss & Mason, 1983). When mussels freeze ice crystals may damage cells and organs. When thawed, hemolymph and cell fluids may leak out from the organs and veins, and some of the trace metals can be lost from the body this way. This may be an explanation for higher concentrations of trace metals found in the blue mussels dissected fresh in the present study, although it does not explain why lead was higher in mussels which first were frozen. Mussel Watch (1980) recommended that mussels used for environmental monitoring should be dissected fresh to avoid leakage of hemolymph. In the other hand, Field (1921) stated that hemolymph can be leaked out from drained fresh mussels by a simple press. This can also happen when mussels are prepared without freezing for biomonitoring. Further investigations should be made upon the thawing-effect on trace metal concentrations in blue mussels. If significant differences are found, clear procedure should be stated further for the International Mussel Watch programs to avoid confusion between the two existing methods.

5.2.5. Metal interactions

In the present study several trace metal interactions were recorded. There was significant positive correlation between copper and zinc, while zinc and cadmium and zinc and lead correlated negatively with each other. Copper-zinc correlation has been recorded previously

by Phillips (1976b) and Riget et al. (1996), while Phillips (1976a) found no effect of other metals on uptake of cadmium or lead. It has been well reported that deficiency of an essential element like zinc can increase the binding of a toxic metal to enzyme binding sites for the essential elements (see Mussel Watch, 1980; Stewart, 1999). Cadmium is an effective competitor for zinc, and this is confirmed also in the present study with negative regression slopes between zinc and lead. In the other hand, in the study of the 23 stations along the Bergen coast, the highest cadmium concentrations were found in blue mussels collected at the reference stations, while in zinc polluted waters in the centre cadmium concentrations were low. This could indicate that cadmium was not taken up by blue mussels in the presence of high zinc or copper content or that cadmium was within normal range and did not interfere with zinc.

5.2.6. The blue mussel as a biomonitor

After a definition, a good biomonitor is an organism which can respond to different contamination levels in the environment (Phillips, 1977b). In the present study trace metal concentrations in blue mussels reflected contamination level in the environment in the case of lead, copper and zinc. The highest contamination levels of these metals were found in the Bergen centre and the lowest in the reference stations and stations furthest away from the centre. In previous studies blue mussel have been found to be a good biomonitor of lead, mercury and cadmium (Davies & Pirie, 1978; Shulz- Baldes, 1974; Julshamn & Grahl-Nielsen, 1996), while algae (*Ascophyllum nodosum*) and oyster (*Ostrea edulis)* were found to be better indicator species for copper pollution (Julshamn, 1981a; Julshamn, 1981b). Blue mussel can regulate uptake of copper in the body depending on salinity, season, temperature and presence of other metals like zinc, cadmium and lead (Phillips, 1976a; 1976b; 1977b; Davenport, 1977; Davenport & Manley, 1978; Amiard- Triquet et al., 1986). Szefer & Szefer (1991), in the other hand, reported that copper and zinc were accumulated more efficiently by blue mussels than lead. Cadmium concentrations as well, may be influenced by the presence of other metals and the salinity in the water (Phillips, 1976a; Elliott et al., 1986). Blue mussels have also been found to be able to equilibrate to different zinc concentrations when

transplanted, and that the species can regulate zinc uptake effectively, even in highly polluted waters (Boyden, 1977; Julshamn, 1981a; Lobel et al., 1982). In addition, Julshamn & Grahl-Nielsen (1996) reported that fish was better indicator in arsenic contamination than blue mussels. Despite of the findings in previous studies, blue mussel in the present study was a good indicator for lead, copper and zinc, while in the case of arsenic, silver, cadmium and mercury the concentrations in blue mussels did not indicate any pollution gradient either in relation to the Bergen centre or to the sewage water outlets. To gain more precise evaluation of the contamination levels in the area, suspension feeders could be used to monitor the trace metal availability from the sediments (Rainbow & Phillips, 1993).

5.2.7. Evaluation of the effect of the new sewage outlets.

Mussels sampled in Lyreneset and Biskopshavn beside the outlets of the new water treatment plants did not indicate any elevation in trace metal concentrations when compared to other stations of with previous data sampled before the output started (Andersen, 1994). This indicates that mechanical water treatment is a sufficient method to remove trace metals from the wastewater. Another explanation might be that the discharge of wastewater into the deep water might lead trace metals further away from the actual output site, and elevated concentration of trace metals might be found further away along the water stream. Sanudo-Wilhelmy & Flegal (1992) reported high silver concentrations in sediments in the vicinity of municipal sewage site. Similar findings were not detected here. Recent studies have reported increased organic material in the vicinity of the new sewage outlet in Lyreneset and effects on the bottom fauna (Botnen et al., 2000). Metals, like lead and copper, show affinity to organic particles (Bryan & Hummerstone, 1977; Xhoffer et al., 1992). The bottom sediments in the vicinity of the sewage discharge might accumulate these trace metals with time. Further studies of the chemical composition of sewage water should be made in order to measure the magnitude of trace metal concentrations in the outgoing sewage water. In addition, if trace metal concentrations seem to increase in sediments under the sewage outlets, improvements of the water treatment should be evaluated.

5.2.8. Environmental quality of the coastal waters of Bergen

Environmental quality of Byfjorden is generally good when compared to the criteria from the State Pollution Control (Molvær et al., 1997). Table 5.1 shows the concentration levels of

trace metals in blue mussels at five different quality classes from class I, unpolluted, to class V, highly polluted. On the basis of the present study, the area from Helgerneset to Nordåsstraum (stations 4-18) is moderately polluted by lead and belongs to the class II, while the rest of the stations are unpolluted and belong to the class I (Tab. 5.1). The whole study area is unpolluted by silver, cadmium, and mercury, while Vågen and Puddefjorden are moderately polluted by zinc and copper. Copper is also high in Håkonshella, which belongs to class II, moderately polluted. Arsenic concentration in blue mussels were very close to the boundary value of 10 mg/kg dry weight between the classes I and II, ranging from 9.2 to 12.9 mg/kg dry weight, except at Vågen, where 20.9 mg/kg dry weight was measured. The area can be defined then as slightly or moderately polluted by arsenic.

Table 5.1. Classification of environmental quality in Norwegian fjords on the basis of trace metal concentrations (mg/kg dry weight) in blue mussels after Molvær et al. (1997). Environmental quality classes are termed as: I =unpolluted or slightly polluted, II = moderately polluted, III = remarkably polluted, IV = highly polluted and V = very highly polluted.

Environmental quality class	Cu mg/kg	Zn mg/kg	As mg/kg	Ag mg/kg	Cd mg/kg	Hg mg/kg	Pb mg/kg
I	< 10	< 200	< 10	< 0.3	< 2	< 0.2	< 3
II	10 - 30	200 - 400	10 - 30	3 - 10	2 - 5	0.2 - 0.5	3 - 15
III	30 - 100	400 - 1000	30 - 100	10 - 20	5 - 20	0.5 - 1.5	15 - 40
IV	100 - 200	1000 - 2500	100 - 200	20 - 40	20 - 40	1.5 - 4	40 - 100
V	> 200	> 2500	> 200	> 40	> 40	> 4	> 100

5.2.9. Concluding remarks

The conclusions of the present study may be summarised as following:

I Blue mussels in the Bergen centre are moderately polluted by lead, zinc and cadmium.

II Reductions in copper, zinc, lead, cadmium, mercury and lead concentrations in blue mussels were observed in the Bergen centre compared to the data from 1993. The magnitude of the reduction varied between 10-50 %

III There was no increase in trace metal concentrations in blue mussels collected in the vicinity of the new sewage outlet in Lyreneset compared to the data from the area before the discharges were started.

IV The environmental quality of the coastal waters of Bergen and Byfjorden varies from the class I, non polluted, to the class II, moderately polluted.

V On the basis of recommendations from EU, blue mussels from the Bergen centre can not be used for human consumption because of the elevated concentrations of lead.

VI Size has different effects on the trace metal concentrations in blue mussels in polluted and unpolluted sites. Copper, silver and cadmium were significantly higher in small mussels than large ones in the unpolluted site, while zinc, cadmium and lead were significantly higher in large mussels in the polluted site.

VII Cadmium and lead were significantly higher in blue mussels growing subtidally compared to blue mussels on intertidal and uptidal zones.

VIII Freezing and thawing of blue mussels before the analyses of trace metals have significant effects on metal concentrations. Arsenic, silver and cadmium were significantly higher in blue mussels dissected fresh compared to blue mussels that first were frozen and thawed.

6. References

Abdullah MI, Royle LG. 1974. A study of the dissolved and particulate trace elements in the Bristol Channel. *Journal of Marine Biological Association of the United Kingdom* 54:581-597.

Alexander GV, Young DR. 1976. Trace metals in Southern Californian mussels. *Marine Pollution Bulletin* 7:7-9.

Amiard- Triquet JC, Amiard- Triquet C, Berthet B, Metayer C. 1986. Contribution to the ecotoxicological study of cadmium, lead, copper and zinc in the mussel *Mytilus edulis*. I. Field study. *Marine Biology* 90:425-431.

Andersen V. 1994. En undersøkelse av tungmetaller i blåskjell (*Mytilus edulis*, Linné 1758) i Bergen havneområde. *Hovedfagsoppgave i Marinbiologi*. Institute for Fisheries and Marine biology University of Bergen. 107 pp.

Andersen V, Maage A, Johannessen PJ. 1996. Heavy metals in blue mussels (*Mytilus edulis*) in the Bergen harbour area, Western Norway. *Bulletin of Environmental Contamination Toxicology* 57:589-596.

Anonym. 1988. *Rammeplan for avløpsdisponering i Bergen*. Hovedrapport 1988. *Bergen Kommune*, VVA-avdeling. Main plan for the municipal sewer system in Bergen 1988. 77 pp.

Anonym. 1992. A compilation of standards and guidance values for contaminants in fish, crustaceans and molluscs for the assessment of possible hazards to human health. *Monitoring Manual- Principles and Methodology of the Joint Monitoring Programme*. Oslo and Paris Commissions. Update 1992. A7.2/92-E.

Anonym. 1997. Norske nærinsgsstoffanbefalinger 1997. *Statens ernæringsråd*, Oslo. 1997. 17 pp.

Anonym 1998. *Hovedplan for avløp og vannmiljø* 1997-2007, Bergen Kommune, teknisk Utbygging. The main plan for the municipal sewer system and the water environment 1997-2007, Bergen, section for technical building. 37 pp.

Anonym. 2001. Aquaculture in Norway- 2001. *Norwegian Fish Farmers' Association* 2001. 15 pp.

Anonym. 2002. Tiltaksplan for Bergen havn. Aktivitet 1: Tilførsler/ kildeområder, Delrapport. *Fylkesmannens miljøvernavdeling, Hordaland*. 2002. 110131-01. The action plan for the Bergen Harbour, discharges and sources, Hordaland county, Department for the Environment. 57 pp.

Aune T. 1998. *Fremmedstoffer i mat: tilsetningsstoffer og miljøgifter i næringsmidler*. 1. Ed. Høyskoleforlaget, Kristiansand. Toxins in food: additional compounds and contaminants in food stuffs. 275 pp.

Balls PW. 1985. Copper, lead and cadmium in coastal waters of the western North Sea. *Marine Chemistry*. 15:363-378.

Berg V, Eriksen GS, Iversen PE. 1997. Forslag til strategier for kartlegging av miljøgifter i marine organismer i norske havner og fjorder. Strategies for monitoring of contaminants in marine organisms in Norwegian harbors and fjords. SNT-Report 1997/10. 25 pp.

Berthelsen T. 2003. Personal comments. Askøy commune, department of technical building.

Blomseth LH, Hartmann-Pedersen P. 1995. *Grunnstoffene- universets byggesteiner*. Universitetsforlaget AS Oslo. 1995. 257 pp.

Botnen HB, Hjohlman S, Johannessen PJ, Tvedten ØF. 1994. «Byfjordundersøkelsen» overvåking av fjordene rundt Bergen 1993. *IFM- Report* 1994/39. Institute for Fisheries and Marine Biology, University of Bergen. 157 pp.

Botnen HB, Tvedten ØF, Johannessen PJ, Hjohlman S. 1996. «Byfjordundersøkelsen» overvåking av fjordene rundt Bergen 1994- med oppsummering av resultater fra 1973- 1994. *IFM- Report* 1996/11. Institute for Fisheries and Marine Biology, University of Bergen. 157 pp.

Botnen HB, Hjohlman S, Johannessen PJ. 1999a. «Byfjordundersøkelsen» overvåking av fjordene rundt Bergen- marinbiologisk miljøundersøkelse av Store Lungegårdsvann, Solheimsviken, Damsgårdsundet og Puddefjorden i 1996 og 1997; samt fjæreundersøkelse på Fagernes i 1998. *IFM- Report* 1999/3. Institute for Fisheries and Marine Biology, University of Bergen. 93 pp.

Botnen HB, Johannessen PJ. 1999b. «Byfjordundersøkelsen» overvåking av fjordene rundt Bergen- marinbiologisk miljøundersøkelse av Store Lungegårdsvann, Solheimsviken, Byfjorden, Skuteviken, Puddefjorden, Vestrepollen, Vågsbøpollen, Kviturdvikspollen, Grunneosen og ved Fagernes i 1998. *IFM- Report* 1999/10. Institute for Fisheries and Marine Biology, University of Bergen. 71 pp.

Botnen HB, Hjohlman S, Johannessen PJ. 2000. Byfjordundersøkelsen» Overvåking av fjordene rundt Bergen- Miljøundersøkelse 1999. *IFM-Report* nr. 8, 2000. Institute for Fisheries and Marine Biology, University of Bergen. 101 pp.

Boyden CR. 1974. Trace element content and body size in molluscs. *Nature* 251:311-314.

Boyden CR. 1977. Effect of size upon metal content of shellfish. *Journal of Marine Biological Association of the United Kingdom*. 57:675-714.

Brown CL, Luoma SN. 1995. Use of the euryhaline bivalve *Potamorcorbula amurensis* as a biosentinel species to assess trace metal contamination in San Francisco Bay. *Marine Ecology Progress series* 124:129-142.

Bryan GW, Hummerstone LG. 1977. Indicators of heavy-metal contamination in the Looe estuary (Cornwall) with particular regard to silver and lead. *Journal of the Marine Biological Association of the United Kingdom* 57:75-92.

Claisse D. 1989. Chemical contamination of French coasts. The results of a ten years Mussel Watch. *Marine Pollution Bulletin.* 20:523-528.

Clark RB. 1997. *Marine Pollution*. 4. Ed. Clarendon press, Oxford. 161 pp.

Cossa D, Bourget E, Piuze J. 1979. Sexual maturation as a source of variation in the relationship between cadmium concentration and body weight of *Mytilus edulis* L. *Marine Pollution Bulletin* 10:174-176.

Cossa D, Bourget E, Pouliot J, Puice J, Chanut P. 1980. Geographical and seasonal variations in the relationship between trace metal content and body weight in *Mytilus edulis*. *Marine Biology* 58:7-14.

Davenport J. 1977. A study of effects of copper applied continuously and discontinuously to specimens of *Mytilus edulis* (L.) exposed to steady and fluctuation salinity levels. *Journal of Marine Biology Association of United Kingdom. 57:63-74.*

Davenport J, Manley A. 1978. The detection of heightened sea-water copper concentrations by the mussel *Mytilus edulis*. *Journal of the Marine Biological Association of the United Kingdom* 587:843-850.

Davies JM, Pirie JM. 1978. The mussel *Mytilus edulis* as a bio-assay organism for mercury in sea water. *Marine Pollution Bulletin* 9:128-132.

Degerman E, Rosenberg R. 1981. *Miljöeffekter av småbåtshamnar och småbåtar*. Statens Naturvårdsverket. Report 1981: PM 1399. Environmental effects of small craft harbors and vessels. 22 pp.

De Wolf P. 1975. Mercury content of mussels from West European Coasts. *Marine Pollution Bulletin* 6: 61-63.

Dons C, Beck. PÅ. 1993. Priority hazardous substances in Norway. *SFT-Report* 93:22. TA-nr 985/1993. 115 pp.

Duinker A, Mortensen S. 1999. Kvalitet av skjell- et kritisk punkt for en voksende eksportnæring. *Norsk Fiskeoppdrett* 19:30-32.

Duinker A. 2002. Personal comments. The National Institute of Nutrition and Seafood Research, Bergen, Norway.

Elliott NG, Swain R, Ritz DA.1986. Metal interaction during accumulation by the mussel *Mytilus edulis planulatu*s. *Marine Biology* 93:395-399.

EU. 2001. Commission Regulation (ED) No 466/2001. Setting maximum levels for certain contaminants in food stuffs. 13 pp.

Field IA. 1922. Biology and economic value of the sea mussel *Mytilus edulis. Bulletin of the US*

Bureau of Fisheries 1921-22: 127-259.

Georges SG, Pirie JS. 1980. Metabolism of zinc in the mussel, *Mytilus edulis* (L.): a combined ultrastructural and biochemical study. *Journal of Marine Biological Association of United Kingdom*. 60:575-590.

Goldberg ED. 1975. The Mussel Watch- A first step in global marine monitoring. *Marine Pollution Bulletin* 6:111.

Gordon M, Knauer GA, Martin JH. 1980. *Mytilus californianus* as a bioindicator of trace metal pollution: Variability and statistical considerations. *Marine Pollution Bulletin* 11:195-198.

Green NW, Knutzen J. 1992. Miljøundersøkelse i indre Oslofjord. Delrapport 2. Miljøgifter i organismer 1992. *NIVA- Report* 0-921315. Environmental monitoring in the inner Oslo-fjord, contaminant in organisms. 54 pp.

Griffiths RJ. 1981. Production and energy flow in relation to age and shore level in the bivalve *Choromytilus meridionalis* (Kr.) *Estuarine, Coastal and Shelf Science* 13:477-493.

Helle HB. 1975. Byfjordundersøkelsen 1973- 1974. Oseanografisk resipientundersøkelse av fjordene rundt Bergen. *Delrapport nr. 1. Geophysical Institute*, University of Bergen. 72 pp.

Hill CH. 1976. Mineral interrelationships. In: Prasad AS, Oberleas D. 1. Ed. *Trace elements in human health and disease. II. Essential and toxic elements.* Academic Press, INC, London. 281-300 pp.

Hovgaard P, Mortensen S, Strand Ø. 2001. *Skjell - biologi og dyrking*. 1. Ed. Kystnæringen Forlag & bokklubb AS, 2001. 255 pp.

Huse A. 1999. Environmentally hazardous substances in products. Data for 1997. *SFT-Report* 99/03. TA-1613/1999. 73 pp.

Johannessen PJ. 1974. Biologisk resipientundersøkelse av fjordene rundt Bergen. Byfjordundersøkelsen *1973- 1974*. *Delrapport nr. 2*. University of Bergen. 85 pp.

Johannessen PJ. 1981. «Byfjordundersøkelsen». Resipientundersøkelse av fjordene rundt Bergen. *Report nr. 1.* Tidsrommet fra oktober 1979 til og med desember 1980. University of Bergen. 180 pp.

Johannessen PJ. 1982. «Byfjordundersøkelsen». Overvåkingen av fjordene rundt Bergen 1981. *Report nr. 2*. University of Bergen. 110 pp.

Johannessen PJ. 1983. «Byfjordundersøkelsen». Overvåkingen av fjordene rundt Bergen 1982. Report nr. 3. Institute of Marine Biology. *Reportseries* 1983/3. University of Bergen. 67 pp.

Johannessen PJ. 1984. «Byfjordundersøkelsen». Overvåkingen av fjordene rundt Bergen 1983.

Report nr. 4. Institute of Marine Biology. *Reportseries* 1985/19. University of Bergen. 88 pp.

Johannessen PJ. 1985. «Byfjordundersøkelsen». Overvåkingen av fjordene rundt Bergen 1983. Institute of Marine Biology*. Reportseries* 1985/20. University of Bergen. 73 pp.

Johannessen PJ, Botnen HB, Risheim I. 1991; «Byfjordundersøkelsen». Overvåking av fjordene rundt Bergen 1990. *IFM- Report* 1991/11. Institute of Fisheries and Marine Biology. University of Bergen. 108 pp.

Johannessen PJ, Risheim I, Tvedten ØF, Botnen HB. 1992. «Byfjordundersøkelsen». Overvåking av fjordene rundt Bergen 1991. *IFM- Report* 1992/10. Institute of Fisheries and Marine Biology. University of Bergen. 108 pp.

Johannessen PJ, Hjohlman S, Tvedten ØF, Risheim I, Botnen HB. 1993. «Byfjordundersøkelsen». Overvåking av fjordene rundt Bergen 1992. *IFM- Report* 1993/18. Institute of Fisheries and Marine Biology. University of Bergen. 172 pp.

Jones SH, Chase M, Sowles J, Hennigar P, Landry N, Wells PG, Harding GCH, Krahforst C, Brun GL. 2001. Monitoring for toxic contaminants in *Mytilus edulis* from New Hampshire and The Gulf of Maine. *Journal of Shellfish Research* 20:1203-1214.

Julshamn K. 1981a. Studies on major and minor elements in molluscs in Western Norway. VII. The contents of 12 elements, including copper, zinc, cadmium and lead in common mussel (*Mytilus edulis*) and brown seaweed (*Ascophyllum nodosum*) relative to the distance from the industrial sites in Sørfjorden, inner Hardangerfjorden. *Fiskeridirektoratets Skrifter, Serie Ernæring* 1:267-287.

Julshamn K. 1981b. Studies on major and minor elements in molluscs in Western Norway. I. Geographical variations in the contents of 10 elements in oyster (*Ostrea edulis*), common mussel (*Mytilus edulis*) and brown seaweed (*Ascophyllum nodosum*) from three oyster farms. *Fiskeridirektoratets Skrifter, Serie Ernæring* 1:161-182.

Julshamn K, Grahl-Nielsen O. 1996. Distribution of trace elements from industrial discharges in the Hardangerfjord, Norway: A multivariate data-analysis of saithe, flounder, and blue mussel as sentinel organisms. *Marine Pollution Bulletin* 32:564-571.

Julshamn K, Torpe EK, Børnes C, Sæthre LJ, Maage A. 2001. Cadmium, lead, copper and zinc in blue mussels (*Mytilus edulis*) sampled in the Hardangerfjord, Norway. *Journal of Environmental Monitoring* 3:539-542.

Julshamn K, Duinker A. 2001. Overvåkningsprogram for skjell 2000. *Metaller i skjell.* Institute of Nutrition of Directorate of Fisheries, Bergen, Norway. 2001. 9 pp.

Julshamn K, Duinker A. 2002. Overvåkningsprogram for skjell 2001. Unpublished data.

Knutzen J, Skei J, Johnsen TM, Hylland K, Klungsøyr J, Schlabach M. 1995. Miljøgiftundersøkelser i Byfjorden/Bergen og tilliggende fjordområder. Fase 2. Observasjoner i 1994. Environmental monitoring in Byfjorden/Bergen and the coastal waters. *NIVA- Report* 95/3351. 163 pp.

Levinton JS. 1995. *Marine Biology- Function, Biodiversity, Ecology*. 1. Ed. Oxford University Press, New York. 420 pp.

Linde E. 1970. Hydrography of the Byfjord. Report 20. Geophysical Institute, University of Bergen. 38 pp.

Lobel PB, Wright DA. 1982. Total body zinc concentration and allometric growth ratios in *Mytilus edulis* collected from different shore levels. *Marine Biology* 66:231-236.

Lobel PB, Mogie P, Wright DA, Wu BL. 1982. Metal accumulation in four molluscs. *Marine Pollution Bulletin* 13:170-174.

Lobel PB, Belkhode SP, Jackson SE, Longerich HP. 1989. A universal method for quantifying and comparing the residual variability of element concentrations in biological tissues using 25 elements in mussel *Mytilus edulis* as a model. *Marine Biology* 102:513-518.

Lobel PB, Belkhode SP, Jackson SE, Longerich HP. 1991. Sediment in the intestinal tract: A potentially serious source of error in aquatic biological monitoring programs. *Marine Environmental Research* 31:163-174.

McGovern E, Rowe A, McHugh B, Costello J, Bloxham M, Duffy C, Nixon E. 2001. Trace metal and chlorinated hydrocarbon concentrations in shellfish from Irish waters, 1997-1999. Marine Environment and Health Services No. 2, 2001. Marine Environment and Health Services Division Dublin. Ireland.

Meiggs TO. 1980. The use of sediment analysis in forensic investigations and procedural requirements for such studies. In: Baker RA editor. *Contaminants and Sediments. 1. Fate and transport, case studies, modelling, toxicity, contaminants.* Ann Arbor Science Publishers, Inc. 1980. 558 pp.

Molvær J, Knutzen J, Magnusson J, Rygg B, Skei J, Sørensen J. 1997. Classification of environmental quality in fjords and coastal waters. A guide. SFT 97:03. Ta-1467/1997. 36 pp.

Morrisey DJ, Underwood AJ, Stark JS, Howitt L. 1994. Temporal variation in concentrations of heavy metals in marine sediments. *Estuarine, Coastal and Shelf Science* 38:271-282.

Mussel Watch, 1980. The International Mussel Watch, 1980. *Report of a workshop sponsored by the environmental studies board commission on natural resources.* National Research Council. National Academy of Sciences, Washington, D.C. 245 pp.

Myhre JP. 1998. Biomarkører i ål (*Anguilla anguilla* L.). Miljøgifteksponering i laboratorieforsøk og feltundersøkelse i fjordsystemet rundt Bergen*. Hovedfagsoppgave i marinbiologi til graden candidatum scientarium*. Institute for Fisheries and Marine Biology, University of Bergen. 91 pp.

Newell RIE, Hilbish TJ, Koehn RK, Newell CJ. 1982. Temporal variation in the reproduction cycle of *Mytilus edulis* L. (Bivalvia, Mytilidae) from localities on the east coast of the United States. *Biological Bulletin.* 162:299-310.

Nielsen SA. 1974. Vertical concentrations gradients of heavy metals in cultured mussels*. New Zealand Journal of Marine & Fresh Water Resources*. 8:631-636.

NMKL. 1996. *Validering av kjemiske analysemetoder*. NMKL-Prosedyre nr. 4 (1996). Nordic Committee on Food Analyses 1996. Validation of methods for chemical analyses. 25 pp.

Okumus I, Stirling HP. 1998. Seasonal variations in the meat weight, condition index and biochemical composition of mussels (*Mytilus edulis* L.) in suspended culture in two Scottish sea lochs. *Aquaculture.* 159:249-261.

Phillips DJH. 1976a. The common mussel *Mytilus edulis* as an indicator of pollution by zinc, cadmium, lead and copper. II. Relationship of metals in the mussel to those discharged by industry. *Marine Biology* 38: 71-80.

Phillips DJH. 1976b. The common mussel *Mytilus edulis* as an indicator of pollution by zinc, cadmium, lead and copper. I. Effects of environmental variables on uptake of metals. *Marine Biology* 38: 59-69.

Phillips DJH. 1977a. The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments- a review. *Environmental Pollution* 13:281-311.

Phillips DJH. 1977b. The common mussel *Mytilus edulis* as an indicator of trace metals in Scandinavian waters. I. Zinc and cadmium. *Marine Biology* 43:283-291.

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

Rainbow SP, Phillips DJH. 1993. Cosmopolitan biomonitors of trace metals. *Marine Pollution Bulletin* 26:593-601.

Rainbow SP. 1995. Biomonitoring of heavy metal availability in the marine environment. *Marine Pollution Bulletin* 31:183-192.

Ranta E, Rita H, Kouki J. 2002. *Biometria- Tilastotiedettä ekologeille*. 8. Ed. Helsinki University Press, Helsinki. *Biometria- Statistics for the* ecologists. 569 pp.

Regoli F, Orlando E. 1994. Seasonal Variation of trace Metal Concentrations in the Digestive Gland of the Mediterranean Mussel *Mytilus galloprovincialis*: Comparison between a Polluted and a Non-Polluted Site. *Archives of Environmental Contamination and Toxicology* 27:36-43.

Riget F, Johansen P, Asmund G. 1996. Influence of length on element concentrations in blue mussels (*Mytilus edulis*). *Marine Pollution Bulletin* 32:745-751.

Sanudo- Wilhelmy SA, Flegal AS. 1992. Anthropogenic silver in the Southern California bight- a new tracer of sewage in coastal water. *Environmental science and Technology* 26:2147-2151.

Schulz-Baldez M. 1974. Lead uptake from seawater and food, and lead loss in common mussel *Mytilus edulis*. *Marine Biology* 25:177-193.

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

Seed R. 1992. Systematics evolution and distribution of mussels belonging to the genus *Mytilus*. *American malacological bulletin.* 9:123-137.

Sekse M, Kvingedal K. 1992. Industriutslipp av miljøgifter til kommunalt nett. Hovedrapport. *Bergen Kommune. Miljøvernavdelinga, Fylkesmannen i Hordaland. Industrial discharges of environmental contaminants to the municipal water treatment system. 64 pp.*

SFT. 2003. Statens forurensingstilsyn. Miljøtilstand i norske fjorder 1984- 2000. An official homepage for Norwegian State Pollution Control (SFT). *Environmental status in Norwegian Fjords 1984- 2000.* http://www.sft.no/

Shindo K, Otsuki A. 1999. Establishment of sampling strategy for the use of blue mussels as an indicator of organotin contamination in the coastal environment. *Journal of Environmental Monitoring* 1:243-250.

Simkiss K, Mason AZ. 1983. Metal ions: Metabolic and toxic effects. In: Hochachka PW editor. *The Mollusca. 2. Environmental biochemistry and physiology*. Academic Press, INC, London. p.102-165.

Skei J, Knutzen J, Klungsøyr J. 1994. Miljøgiftundersøkelser i Bergen havneområde og Byfjorden 1993. Fase 1. Miljøgifter i spiselige organismer og bunnsedimenter. *NIVA-Report* 94/O-93017. Environmental contaminants in organisms with nutritive importance. 88 pp.

Skog DA, West DM, Halles FJ. 1988. Fundamentals of Analytical Chemistry, 5th edition, 1988. Saunders College Publishing. 554-577. 847 pp.

SNT. 2003. Statens Næringstilsyn. Kostholdsråd i norske fjorder 2002. An official homepage for Norwegian State Food Control (SNT). http://www.snt.no/nytt/kosthold/fisk_skalldyr/kyst.html. 20.02.2003.

Stecko JRP, Bendell-Young LI. 2000. Uptake of ^{109}Cd from sediments by the bivalves *Macoma balthica* and *Protothaca staminea*. *Aquatic Toxicology* 47:147-159.

Stephenson MD, Martin JH, Martin M. 1978. State Mussel Watch, 1978. Annual Report. Trace metal concentrations in the California Mussel at areas of special biological significance. Moss Landing Marine Laboratory, California.

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

Strong CD, Luoma SN. 1981. Variations in the correlation of body size with concentrations of Cu and Ag in the bivalve *Macoma Balthica*. *Canadian Journal of Fisheries and Aquatic Sciences* 38:1059-1064.

Szefer P, Szefer K. 1991. Concentration and discrimination factors for Cd, Pb, Zn and Cu in benthos of Puck Bay, Baltic Sea. *The Science of the Total Environment* 105:127-133.

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

Watling HR, Watling RJ. 1976. Trace metals in *Choromytilus meridionalis*. *Marine Pollution Bulletin* 5:91-94.

Widdows J, Donkin P, Brinsley MD, Evans SV, Salkeld PN, Franklin A, Law RJ, Waldock MJ. 1995. Scope for growth and contaminant levels in North Sea mussels *Mytilus edulis*. *Marine Ecology Progress Series* 127:131-148.

Xhoffer CL, Wouterr L, Van Grieken R. 1992. Characterisation of individual particles in the North Sea Surface Microlayer and Underlying Seawater: Comparison with Atmospheric Particles. *Environmental Science & Technology* 26:2151-2162.

Young DR, Alexander GV, McDermott- Ehrlich D. 1979. Vessel- related contamination of Southern California harbours by copper and other metals. *Marine Pollution Bulletin* 10:50-56.

Zar JH. 1999. *Biostatistical analysis*, 4. Ed. Prentice Hall, Upper Saddle River, New Jersey. 718 pp.

Zumdahl SS. 1995. *Chemical Principles*, 2. Edition. D.C. Heath and Company, Lexington, 1995. 1043 pp.