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Copper, Zinc, Arsenic, Cadmium, Mercury, and Lead in Blue Mussels (*Mytilus edulis*) in the Bergen Harbor Area, Western Norway

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Bivalves, including blue mussels (*Mytilus edulis*), have been found to be suitable biomonitor species for trace element (Goldberg 1975; Phillips 1977a; Brown and Luoma 1995; Julshamn and Grahl-Nielsen 1996). The blue mussel is capable to accumulate trace element such as cadmium and lead to a larger extent than for example fish and algae (Julshamn 1981; Julshamn and 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.

Municipal waste water system, including industrial discharges and urban run-off, heating, traffic and harbor-related pollution are the main causes of contamination of the water and sediments in Bergen harbor (Byfjorden, Western Norway) (Sekse and Kvingedahl 1992). Until 1996 the municipal wastewater from Bergen centre has been led untreated into Bergen harbor area, but from 1997 the sewage water discharge has been removed. The recipients have received approximately 116 000 person equivalents of wastewater annually until 1997. Sewage was the biggest source of copper, zinc and cadmium, while industry was the main source for mercury. Investigations have revealed that the sediments in the area are loaded with high metal levels (Skei et al. 1994). The present study was conducted to investigate changes in trace element concentration in blue mussels in the Bergen Harbor area since a previous study in 1993, and also to evaluate if the mussels can be used for human consumption according to EC's upper limits for cadmium, mercury and lead in mussels.

MATERIALS AND METHODS

Blue mussels (*Mytilus edulis*) were collected from 23 stations. Samples from the stations 1-21 were collected in March 2001 and the samples from stations 22-23 were collected in February 2002 (Figure 1). The stations 5 to 14 were the same as chosen in a previous study in 1993 (Andersen et al. 1996). 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 harbor activities. In 2001 there were seven

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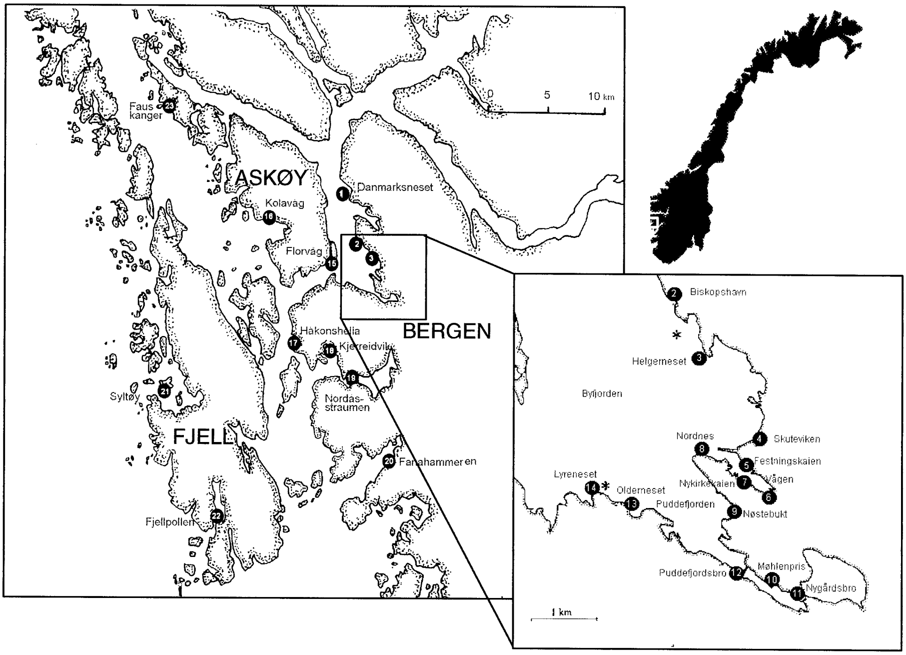

Figure 1. Map showing sampling stations (1-23) in the Bergen harbor area, Western Norway. The new sewage outlets are marked with asterisks.

mechanical and one chemical water treatment plants in use in Bergen. Stations 1-14 and 18 are located in an area where 90 % of the households were connected to municipal water treatment system. Blue mussels were collected on rocks, wooden dock piles, floating stages and harbor concrete walls, depending on the sampling site.

Blue mussels between 35-60 mm in size from each station were separated from each other and stored at -20 °C until analysis. 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 shells were cleaned from epiphytes, byssus threads were removed and the insides were rinsed in deionised water to remove sand, shell and other particles from the shell body. The mussels were left to dry on blotting paper for one hour before dissection. The length of the shells was measured and the soft tissue weight of each sample was determined. The samples were stored at -20 °C and freeze-dried for 48 hours and dry matter content was determined.
The samples were homogenized and stored 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, supra pure quality) and 0.5 ml hydrogen peroxide (30% m/V) were subsequently 1200 MEGA) for 17 minutes and the sample solutions were prepared as described

Table 1. Concentration level (Mean ± SD) of Cu, Zn, As, Cd, Hg and Pb (mg/kg dry weight) of CRM DORM 2 and TORT 2.

Metal	CRM	Mean (mg/kg)	SD	RSD (%)	Certified value (mg/kg)[a]
Cu	DORM 2	2.04	0.15	8	2.34 ± 0.16
Zn	TORT 2	189.2	6.0	3	180 ± 6
As	DORM 2	17.5	1.1	6	18 ± 1.1
Cd	DORM 2	0.048	0.01	29	0.043 ± 0.008
Hg	TORT 2	0.28	0.005	2	0.27 ± 0.06
Pb	TORT 2	0.34	0.01	2	0.354 ± 0.013

[a] 95 % confidence intervals

by Julshamn et al. (1999). The blank samples were prepared and run together with the sample solutions. Inductively coupled plasma- mass- spectrometer (ICP-MS) (Agilent 7500 C) was used to determine the concentration of copper, zinc, arsenic, cadmium, mercury and lead in the sample solutions. The ICP-MS method for those elements is accredited by the Norwegian Metrology and Accreditation Service.

Standard curves for all elements were calculated using five different concentrations for each element. The results from the determinations of copper, zinc, arsenic, cadmium, mercury and lead are presented as a mean value of three pooled samples from each station. Certified reference materials (CRM) were analysed to assess the trueness and precision for the determination of the different elements. CRMs of dogfish muscle (DORM 2) and lobster digestive gland (TORT 2) from the Institute for Environmental Chemistry, Ottawa, Canada were analysed together with the blue mussel samples. The precision was estimated as relative standard deviation (RSD %). Table 1.

For the metal concentration data from the pooled subsamples, normal distribution was assumed according to the Central Limit Theorem (Zar 1999), since the data were considered as mean values for 25 individuals. The data can hence be presented as mean values with 95% confidence intervals.

RESULTS AND DISCUSSION

The mean lengths of the mussels used for analysis from the 23 sampling sites varied between 40 and 50 mm, except shells from stations 8 (37 mm), 16 (52 mm) and 21 (59 mm). Comparisons of mean soft tissue wet weight between stations showed that they varied between 1.3 g at Station 8 and 5.4 g at Station 16. Comparing dry weight (g/100g) between the 23 sampling stations showed that there was a variation from 13.3 (Station 12) to 22 g/100g (Station 21). Some investigations have shown that accumulation of trace elements is dependent of size (Boyden 1977). The size seems to have different effect on trace element concentrations in blue mussels in polluted and unpolluted sites (Popham and D'Auria 1983). However, the metal levels of the stations 8, 16 and 21 are not conspicuous compared to the other stations (Figure 3), and hence the size differences did probably not have adverse implications in the present study.

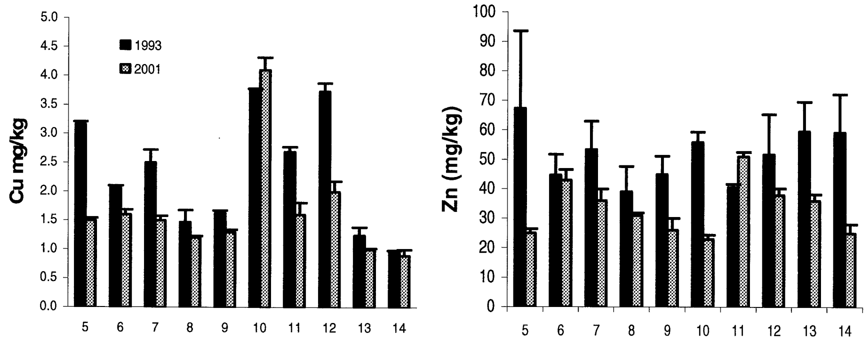

Figure 2. Concentration of Cu and Zn (mean ± SD, mg/kg w.w.) in soft part of pooled blue mussels (*Mytilus edulis*) from sampling sites in the harbor area of Bergen, 1993 and 2001. Data for 1993 are from Andersen et al. (1996). n=3 from pooled samples

Copper concentrations in blue mussels ranged from 0.8 to 4.1 mg/kg fresh weight, and the mean values are presented in Figure 2 and 3. The normal concentrations of copper in blue mussels sampled in a monitoring programme in Norway range from 0.5 to 2.3 mg/kg fresh weight (Julshamn and Duinker unpublished data). Compared with the study carried out in 1993, there is an overall decrease in copper content of blue mussels in the harbor area of Bergen (Andersen et al. 1996) (Figure 2). When human health is considered, no guidelines of copper content are given in Norway. In Spain the limit has been set to 20 mg/kg fresh weight.

The observations of some stations with copper levels above the normal range 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. 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 and Rosenberg 198; Sekse and 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.

Zinc concentrations in the blue mussels varied between 15 mg/kg and 51 mg/kg w. w. (Figure 3), while the normal concentration range of zinc in blue mussel from the monitoring program in Norway range from 8 to 23 mg/kg w. w. (Julshamn and Duinker unpublished data). Generally, zinc concentrations were lower at the present study than in 1993, except for mussels sampled at station 10 (Figure 2) (Andersen et al. 1996).

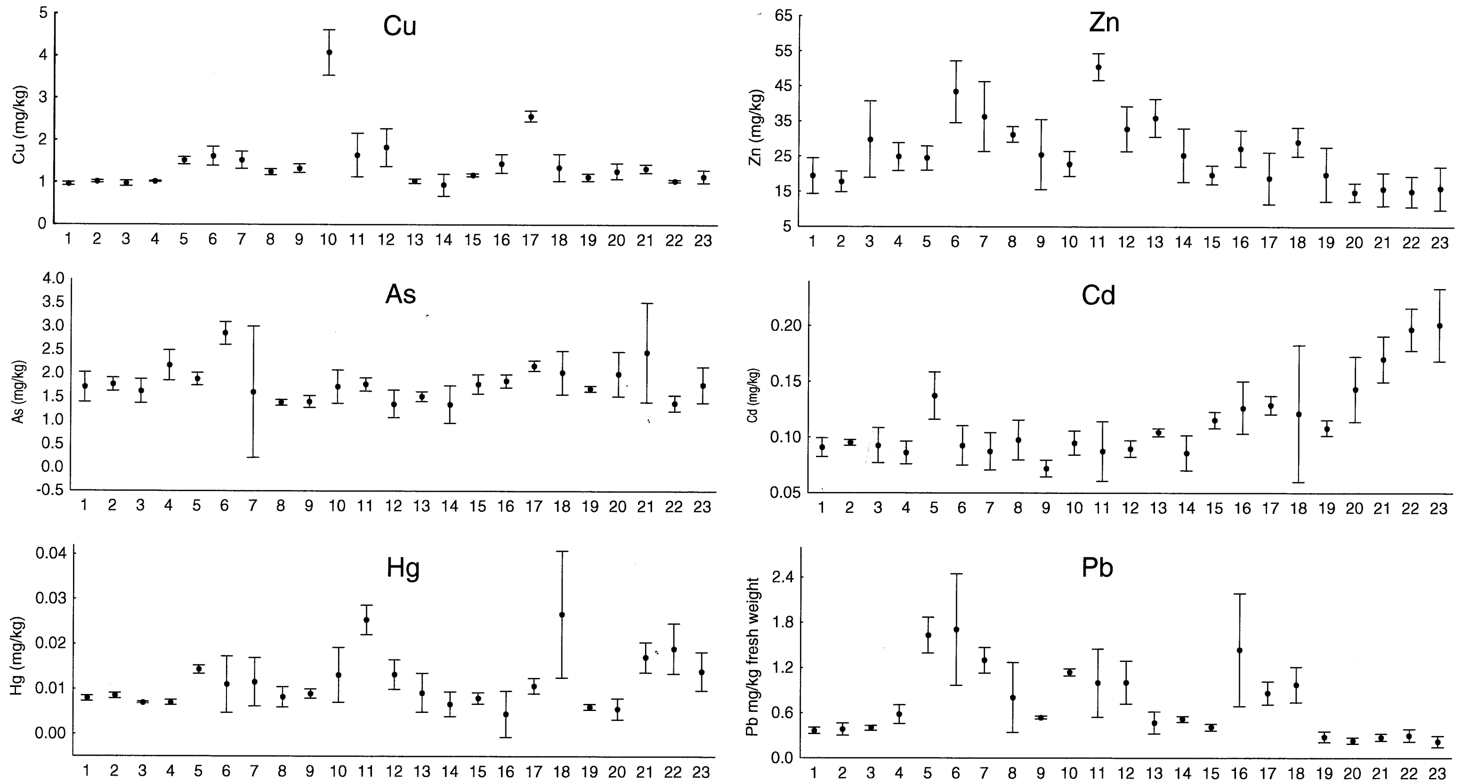

Figure 3. Trace element concentrations (mg/kg w. w.) in soft tissue of blue mussels (*Mytilus edulis*) at sampling stations in Bergen harbor area Bergen. n=3 from pooled samples. Mean with 95 % confidence intervals.

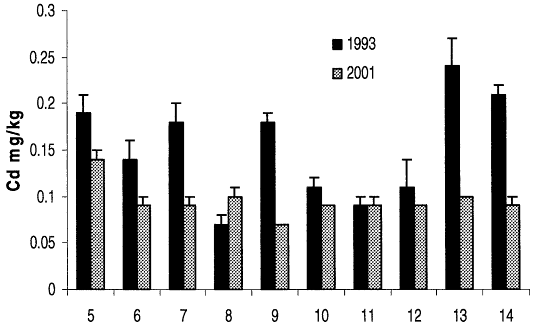

Figure 4. Concentrations of Cd (mean ± SD given as mg/kg fresh weight) in blue mussels from different sampling sites in the harbor area of Bergen sampled in 1993 and 2001. Data for 1993 are from Andersen et al. (1996). n=3 from pooled samples.

Despite the removal of the municipal wastewater discharges from the area, zinc concentrations in the Bergen centre are still high. Harbor sediments are frequently disturbed by boat traffic, and these disturbances can make zinc bioavailable to the blue mussels. This might be the main source now, when sewage water discharge has been removed from the area.

Arsenic concentrations in blue mussels in the present study varied from 1.3 mg/kg to 2.8 mg/kg w. w. (Figure 3). These values are within the normal range of arsenic in mussels obtained through the monitoring programme in Norway (2.0 mg/kg-3.2 mg/kg fresh weight) (Julshamn and Duinker unpublished data). FAO/WHO has set a maximum tolerable weekly intake of total arsenic to 15 μg/kg body weight from the human diet. 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. However, arsenic seems to be present in blue mussel in a non-toxic form (Larsen et al. 1993).

The concentrations of cadmium in the present study were found to be in the range of 0.08 to 0.20 mg/kg w.w. (Figure 3). These results are within the normal range of cadmium concentration found in blue mussels in the monitoring programme in Norway (Julshamn and Duinker unpublished data). Still, cadmium concentrations have been reduced in the harbor area of Bergen compared with the data from 1993 (Andersen et al. 1996) (Figure 4). Cadmium concentrations found in blue mussels in the area studied were clearly below the upper limit of 1.0 mg/kg fresh weight for mussels used for human consumption set by the EC (2001).

Mercury concentrations in blue mussels in the studied area ranged from quantification limit estimated to 0.01 mg/kg wet weight and up to 0.03 mg/kg

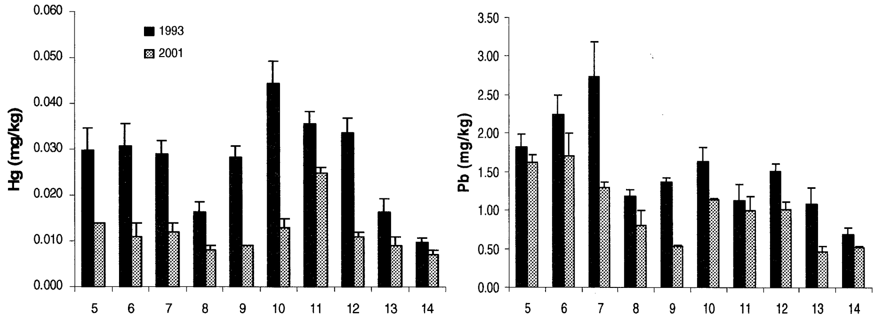

Figure 5. Concentration of Hg and Pb (mean ± SD, mg/kg w.w.) in blue mussels (*Mytilus edulis*) from sites in the harbor area of Bergen, 1993 and 2001. Data for 1993 are from Andersen et al. (1996). n=3 from pooled samples.

w.w. (Figure 3). These results are similar to those found in blue mussels in the monitoring programme in Norway (Julshamn and Duinker unpublished data).

Compared with previous studies, mercury concentration in mussels has been reduced with 50-70 % since 1993 (Figure 5) (Andersen et al. 1996). The removal of the old sewage outlets, as well as reductions in the discharges from dentist offices and laboratories may be the main reason for this (Dons and Beck, 1993).

EC (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 one tenth of this.

In the present study, lead concentrations in the blue mussels in the Bergen centre ranged from 0.4 mg/kg to 1.7 mg/kg w.w. (Figure 3). These values exceeded the normal concentration range of 0.18 to 0.57 mg/kg w.w., measured in blue mussels from the monitoring programme in Norway (Julshamn and Duinker unpublished data). Compared with the previous study (Andersen et al, 1996), lead concentrations have been reduced with 20% since 1993 (Figure 5). The EC has set an upper limit of lead concentration in mussels to 1.5 mg/kg w.w., when used for human consumption (EC 2001). At many sites in the harbor area of Bergen the lead content exceeded or was close to this limit.

Lead concentrations in blue mussels remain high at the stations in the vicinity of the main city roads (stations 5-7 and 11-12). 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 source of lead in mussels today is release from sediments that are disturbed by boat traffic.

For all the elements studied there is an overall decrease in the concentration levels in the blue mussel in the harbor area of Bergen from 1993 to 2001. The main reason for this is the removal of the municipal waste water discharge from the area from 1997. From a food safety point of view, lead concentration in blue mussels are higher than EC`s upper limit of 1.5 mg/kg w.w. in samples from two sites in the harbor area of Bergen.

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