

## Nickel, cobalt, zinc and copper levels in brown trout (*Salmo trutta*) from the river Otra, southern Norway†

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The Flåt Nickel mine at Evje in southern Norway was mined extensively from 1914 to 1945 with little regard for any potential environmental effect. Much of the ore extracted was smelted at a site adjacent to the river Otra south of Evje. Recent studies have revealed heavy metal pollution in the land surrounding the smelter and in water draining from the mine leading to concern for the aquatic ecosystem in the river Otra. Brown trout were sampled from an uncontaminated lake 9 km upstream from the smelter, from the base of the Oddebekken (a tributary draining the mine water into the Otra), from sites immediately upstream and down stream of the smelter and from a site 4 km down stream from the smelter. Fish from sites adjacent to the smelter and the base of the Oddebekken were smaller than those from the lake and down stream site. Concentrations of the metals were highest in fish sampled where the mine water entered the Otra and gradually decreased in fish further down the river. Fish from the uncontaminated lake had the lowest level of metals.

**Keywords:** Brown trout; nickel; cobalt; copper; zinc; liver; muscle; river; Norway

The Otra river valley in the Evje region of southern Norway lies on metamorphic bedrock with diorite intrusions and associated Ni mineralisation. Ni ore was worked intensively from about 1914 to 1945. The Flåt mine situated above the town of Evje was once the biggest in Europe. Much of the extracted ore, estimated at 2 000 000 tons, was processed in a smelter south of Evje.<sup>1</sup> Throughout the operation of both mine and smelter little attention was paid to the potential environmental effects of the mining and smelting of the metals.

There is now increasing concern over the possibility of toxic metals leaching from the mine tailings and from the mines themselves. Little succession has occurred in the flora at the mine site as a result of the pollution. Further studies have found elevated levels of four metals; Ni, Co, Cu and Zn in the wells used to provide local drinking water leading to suggestions that the water table has been contaminated by drainage from the mine and slag heaps.<sup>1</sup> A detailed knowledge of the mineralogy of this area suggests that Hg and As would not be expected to be present as significant pollutants. Neither metal was detected in a preliminary analysis of water samples.<sup>1</sup> Concentrations of Al were elevated but did not exceed reported lowest known biological effect concentrations.

Other work has established that the organic horizon of soil has been contaminated around the smelter and that this contamination extends for up to 1.5 km from the smelter.<sup>2,3</sup> High concentrations of all four metals were found and the soil concentrations of three of the metals Ni, Zn and Cu exceeded the threshold values recommended by ICRCL.<sup>1,4,5</sup>

Each of these metals is potentially toxic to the aquatic ecosystem in the river Otra. In particular there is concern that the metals accumulate in fish. Ni accumulates in the gills, kidney, liver, brain and white muscle of fish.<sup>6</sup> Sublethal concentrations of Ni have been reported to lead to changes in behaviour such as aggression and stress related movement.<sup>7</sup> The resultant stress may also be responsible for muscle glycogenolysis observed in some Ni poisoned fish.<sup>8</sup> Haematological parameters such as total and differential leucocyte count, erythrocyte count, haemoglobin concentration and hematocrit are also affected by Ni accumulation.<sup>7</sup> Accumulation of Co in muscle can adversely affect muscle function. A number of other systems in fish such as the lateral line system sense organs have also been reported to be affected by Co.<sup>9</sup> Zn is generally thought to be non-toxic except at very high concentrations<sup>10,11,12</sup> and trout are believed to acclimatise to high concentrations of Zn in their environment.<sup>13</sup> It has also been suggested that liver Zn concentrations may correlate with induction of metallothionein and thus act as a biomarker of exposure to heavy metals.<sup>14</sup> Exposure to Cu has been observed to interfere with lipid metabolism in the gills,<sup>15</sup> to induce apoptotic and necrotic lesions in the basal pavement cells of skin,<sup>16</sup> and apoptosis of the olfactory epithelium.<sup>17</sup> Fish sampled from a Cu contaminated lake were reported to have a high incidence of tumours.<sup>18</sup> Cu bioaccumulates in the brain and liver of fish.<sup>19</sup> Accumulation in the liver can result in liver necrosis and in the brain to neurotoxicity.<sup>20,21</sup>

The Otra is a fertile river with the diversity of habitats required to support thriving communities of trout, perch and non-migratory salmon. As all four metals polluting the Otra river valley have the potential to be toxic, there is concern for the effects of the metals on fish populations and hence the local commercial and recreational fishing community. In this study fish sampled from several sites in the river Otra have been examined for the presence of Ni, Co, Zn and Cu.

### Experimental

#### Collection of fish

Fish were collected from five sites along the river Otra over a period of one week (Fig. 1), using 20–24 mm gill nets positioned in the early afternoon and left overnight to be collected at 9 am in the morning.

The length of each fish was recorded. Fish from site B, C, D and E were immediately placed in a labelled plastic bag and frozen. In the laboratory, fish were thawed and weighed, the liver was removed and weighed separately. The operculum was removed to determined age group. Fish from site A were dissected immediately after capture. The operculum, samples of muscle tissue and the liver were removed and frozen.

#### Analysis of metals in fish tissues

Approximately 0.5 g of muscle and liver were removed from each fish, weighed and dissolved in 5 ml of concentrated HNO<sub>3</sub> in individual vials. The dissolved tissue was then analysed for

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Ni, Co, Zn and Cu. A certified reference material (DOLT-2 Dogfish liver, National Research Council of Canada) was used as a control. The recovery of metals from four samples of the certified reference material were determined: Ni (91%, 99%, 109% and 135%); Co (75%, 78%, 78% and 85%); Cu (101%, 102%, 102% and 105%); and Zn (94.5%, 97.7%, 98.3% and 100.9%). Calculated mean values  $\pm$  range were Ni ( $108.5 \pm 17.5\%$ ), Co ( $79 \pm 4\%$ ), Cu ( $102.5 \pm 1.5\%$ ) and Zn ( $97.75 \pm 3.75\%$ ).

Ni and Co concentrations were determined using a Perkin Elmer (Beaconsfield, Bucks, UK) Atomic Absorption Spectrometer 1100B and a combined Cr, Co, Cu, Mn and Ni Intensitron lamp. The analysis of the tissue samples for Cu and Zn was carried out using an Instrumentation Laboratory (Lexington, MA, USA) aa/ae Spectrophotometer 157 with individual Cu and Zn lamps and wavelength settings of 213.9 nm for Zn and 324.7 nm for Cu (Table 1). All primary standards were produced by diluting BDH laboratory standards (BDH/Merck, Poole, Dorset, UK).

#### Analysis of metals in water and sediments

Sediment samples were air dried and weighed. Moisture was driven off overnight at  $110^\circ\text{C}$  and the sample reweighed. Samples were ashed in a muffle furnace at  $450^\circ\text{C}$ , the residue was ground to a fine powder, mixed with PVA glue and pressed into a pellet under 10 tons pressure. The pellet was dried at

$110^\circ\text{C}$  overnight and stored desiccated until analysis by X-ray fluorescence spectroscopy (XRF). Water samples were analysed by ICP-AES. For both XRF and ICP-AES, replicate samples were used as internal controls. Analysis of five sets of replicate samples (three determinations for each sample) produced errors, reported as relative standard deviation (RSD), of Ni (0–9%), Co (0–10%), Cu (0–10%) and Zn (1–7%). Values determined for replicate samples analysed in commercial laboratories were in close agreement with those reported in this paper.

#### Statistical analysis

The two tailed Student's *t* test was used to test for statistical significance. Data for metal concentrations was first transformed ( $\log_{10}$ ) to a normal distribution.

#### Results and discussion

##### Population data

In April, 1996 three species of fish were caught in the nets; trout, salmon and perch. Only trout were caught in sufficient numbers for proper statistical analysis (Table 2).

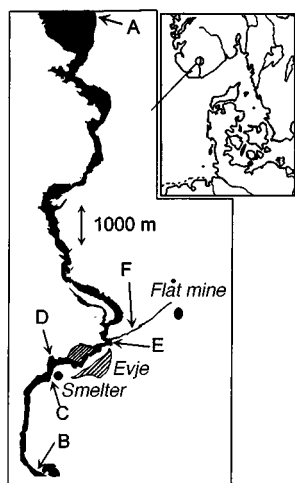
The mean and median length of fish from sites A and B were larger, and on average the fish from site B weighed more, than those from sites C, D and E. Ranking of fish by length or weight gave the same sequence: weight ranking;  $B \gg C > D > E$ ; and length ranking,  $A > B \gg C > D > E$ . The mean liver weight as a percentage of total body weight was approximately 0.7% at all sites except for site D. Livers at site D comprised approximately 0.9% of total body weight. Values could not be calculated for site A because total body weight was not determined in this group. No statistically significant differences were found between male and female fish sampled from sites B, C, D or E. In fish from site E the liver was often orange, or brown in extreme cases, as compared to the blood red colour of liver from fish sampled at site A. In large sample groups (B, C, D and E) the ratio of male to female fish approached 1:1 as would be expected. The modal average age was 2+ at sites A and C and 3+ at sites B, D and E.

The growth of trout sampled from site E, and to a lesser extent sites C and D, appeared to be adversely affected by their environment. Fish from site D had enlarged livers and fish from site E may also have suffered significant liver necrosis. It was thought that one or more of the metals polluting the Otra river valley around Evje might be responsible for these effects. Thus the liver and muscle from the fish sampled were analysed for Ni, Co, Zn and Cu.

##### Measurement of metals in fish liver and muscle

The metal concentrations measured in individual trout are summarised in Fig. 2 (liver) and Fig. 3 (muscle).

The mean concentration of Ni measured in trout was highest at site E;  $0.33 \pm 0.07 \mu\text{g g}^{-1}$  for liver and  $0.10 \pm 0.03 \mu\text{g g}^{-1}$  for muscle. The lowest concentrations of Ni were found in fish from site A;  $0.05 \pm 0.03 \mu\text{g g}^{-1}$  for liver and  $0.02 \pm 0.01 \mu\text{g g}^{-1}$  for muscle. Ni concentrations in fish from sites B, C and D were intermediate between those at site A and site E. Concentrations



**Fig. 1** Sites from which samples were collected: A, Byglandsfjord; B, Hornnes; C, South of Evje smelter; D, North of the Evje smelter; and E, Base of the Oddebekken. Water samples were collected at site F in the Oddebekken.

**Table 1** Assay performance for analysis of metals in fish

	LOD/ $\mu\text{g l}^{-1}$	RSD (%)	s/ $\mu\text{g g}^{-1}$ wet weight
Ni	0.8	8.7	$\pm 5.9$
Co	0.8	10.9	$\pm 5.9$
Zn	0.011	7.5	$\pm 5.9$
Cu	0.03	5.9	$\pm 5.7$

**Table 2** Summary of population data for trout

Site	<i>n</i>	Weight/g*	Length/cm*	Liver weight/g*	Liver (% total weight)*	Age/years	Female fish (%)
A	7	ND†	$24.7 \pm 1.0$	$0.68 \pm 0.12$	ND	2+	14.3
B	20	$119.3 \pm 6.0$	$24.0 \pm 0.6$	$0.88 \pm 0.09$	$0.73 \pm 0.06$	3+	55.0
C	24	$90.7 \pm 5.7$	$21.2 \pm 0.5$	$0.67 \pm 0.07$	$0.74 \pm 0.06$	2+	45.8
D	25	$65.5 \pm 8.2$	$19.5 \pm 0.9$	$0.58 \pm 0.10$	$0.86 \pm 0.06$	3+	52.0
E	29	$79.0 \pm 6.7$	$20.6 \pm 0.7$	$0.53 \pm 0.05$	$0.68 \pm 0.05$	3+	55.2

\* Values are means  $\pm$  confidence limit (0.95). † ND, not determined.

at sites B, C, D and E were significantly greater than those at site A ( $P \leq 0.0001$ ).

The lowest concentrations of Co were found in fish from site A, with values for liver being  $0.06 \pm 0.03 \mu\text{g g}^{-1}$  and for muscle  $0.02 \pm 0.01 \mu\text{g g}^{-1}$ . Concentrations in fish from sites B, C, D and E were similar; mean concentrations in the liver were approximately  $0.1 \mu\text{g g}^{-1}$  and in the muscle  $0.04 \mu\text{g g}^{-1}$ . Co concentrations in fish from all five sites were not statistically significantly different from each other ( $P > 0.0001$ ).

Zn concentrations at site A were lower than at all the other sites, with values for liver being  $42.8 \pm 5.2 \mu\text{g g}^{-1}$  and for muscle  $7.4 \pm 1.5 \mu\text{g g}^{-1}$ . Zn concentrations in fish from sites B, C, D and E were not statistically significantly different from each other. Mean concentrations in the liver were approximately  $60 \mu\text{g g}^{-1}$  and in the muscle  $20 \mu\text{g g}^{-1}$ . Concentrations at sites B, C, D and E were significantly greater than those at site A ( $P \leq 0.0001$ ).

Cu concentrations were lowest in fish taken from site A. Values for liver were  $29.6 \pm 12.3 \mu\text{g g}^{-1}$  and muscle  $0.52 \pm 0.28 \mu\text{g g}^{-1}$ . Highest values were found in fish taken from site E;  $117.2 \pm 33.5 \mu\text{g g}^{-1}$  in liver and  $11.5 \pm 12.2 \mu\text{g g}^{-1}$  in muscle. Values observed in the livers of fish taken from sites B, C and D were approximately  $60 \mu\text{g g}^{-1}$ ; intermediate between those from sites A and E. Cu concentrations measured in muscle of fish taken from sites B, C and D were  $7.9 \pm 1.7 \mu\text{g g}^{-1}$ ,  $5.1 \pm 0.9 \mu\text{g g}^{-1}$  and  $1.5 \pm 0.4 \mu\text{g g}^{-1}$  respectively. Concentrations in the livers of fish from sites B, C, and D, and concentrations in the muscle of fish from sites C, D, and E were significantly greater than those from site A ( $P \leq 0.0001$ ).

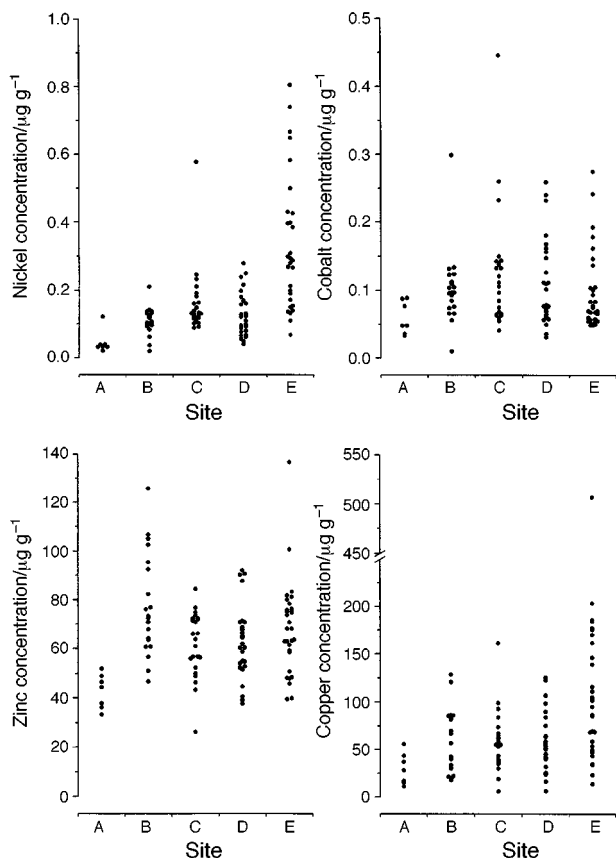
Ni, Co, Zn and Cu values determined in samples from fish taken from site A were tightly grouped at the low end of the concentration range. Similar values were also measured in some fish sampled from other sites. However, at sites B, C, D and E higher values were also measured. These observations suggest

that no fish were contaminated at site A and that not all fish from the other sites were contaminated. These results were consistent for both liver and muscle samples.

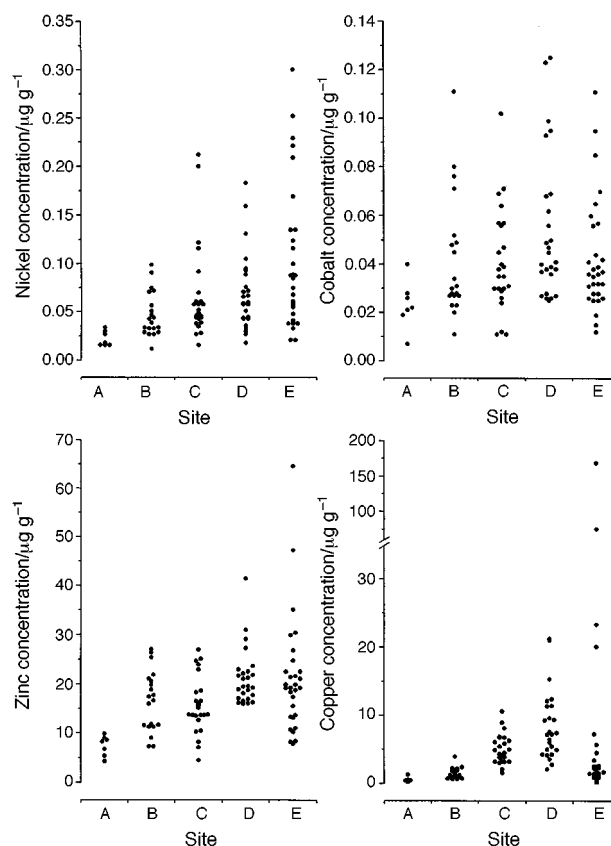
Concentrations of Zn and Cu in fish from site A were comparable to values reported elsewhere. Mean Zn concentrations for wild and hatchery-reared salmon smolts were  $16.8$  and  $11.5 \mu\text{g g}^{-1}$  wet weight, respectively. Mean Cu concentrations were  $1.9$  and  $1.7 \mu\text{g g}^{-1}$  wet weight.<sup>22</sup> We are not aware of any comparable published data on Ni and Co concentrations in fish. Fish collected at site E were found to be more highly polluted than those taken from the other sites. The effects of this pollution were also evident. Fish taken at site E were small, had relatively smaller livers and weighed less than those from other sites even though they were of comparable or greater age. No statistically significant differences were observed for fish of different ages at any site. This suggests that the difference in modal average age between fish from sites A and C (2+) and sites B, D, E (3+) (Table 2) does not affect the metal concentrations reported for each site.

### Source of contamination

Preliminary studies have been performed to determine possible sources of contamination. Ni, Co, Zn and Cu concentrations were measured in water samples taken from the Oddebekken over a period of 12 months from July, 1994 to June, 1995 (Table 3). Concentrations of each metal changed during the 12 months. The concentration of Ni ranged from  $11 \mu\text{g l}^{-1}$  in January, 1995 to  $567 \mu\text{g l}^{-1}$  in November, 1994. Co was not detected in some samples and the highest concentration detected was  $10 \mu\text{g l}^{-1}$  in September, November and December, 1994 and February, 1995. Zn concentrations ranged from  $5 \mu\text{g l}^{-1}$  in September, 1994 to  $103 \mu\text{g l}^{-1}$  in June, 1995. The concentration of Cu measured ranged from  $10 \mu\text{g l}^{-1}$  in February and June, 1995 to  $25 \mu\text{g l}^{-1}$  in September, 1994. Although the pH of the



**Fig. 2** Scatter plot of metal concentrations measured in the livers of individual trout. Sites A–E refer to locations identified in Fig. 1.



**Fig. 3** Scatter plot of metal concentrations measured in the muscle of individual trout. Sites A–E refer to locations identified in Fig. 1.

Oddebekken is low (3.9–4.7) the flux of the river Otra is so high that the measured pH of the Otra itself does not fall below pH 5. The pH of the river Otra falls along its course as humic streams drain into the river. Typical values for pH are 7 at Byglandsfjord (site A), 5.82 south of Evje (site B) and 5.77 north of Evje (site E).<sup>23</sup> The pH values of 5.7–5.8 are typical of waters draining from humic areas known to support trout. It is unlikely that changes in pH are directly responsible for the physiological effects on fish reported in this study.

Otra river bed sediment samples, collected from three sites (B, D and E) in April, 1996, were analysed for Ni, Co, Zn and Cu (Table 4). The concentrations of Ni, Co and Zn were similar at all three sites with average concentrations of 50.3  $\mu\text{g g}^{-1}$  (Ni), 14.7  $\mu\text{g g}^{-1}$  (Co) and 62.3  $\mu\text{g g}^{-1}$  (Zn). The Cu concentration ranged from 20  $\mu\text{g g}^{-1}$  at site B to 80  $\mu\text{g g}^{-1}$  at site D.

The two main sources of exposure for fish are metals in the food materials, associated non-edible particulate material and metals dissolved in the water. Although the sediment data shows that the area at the base of the Oddebekken did contain Ni, Co, Zn and Cu, the levels found were much less than those in soils surrounding the smelter.<sup>4</sup> The concentration of three metals (Ni, Co and Zn) were similar at sites B, D and E. Since the concentrations of metals found in fish from site B were considerably less than in fish from site E, it is probable that the sediment is not the main source of contamination for the fish. Superficial sediments collected from Swedish lakes contain between 100 and 500  $\mu\text{g g}^{-1}$  of Zn and between 20 and 50  $\mu\text{g g}^{-1}$  of Cu.<sup>24</sup> Sediment values from the Otra river bed are comparable with those from the Swedish survey. Concentrations of several metals have been reported for an extensive survey of Swedish watercourses.<sup>25,26</sup> The 75 percentile values for different regions ranged from 0.59–0.75  $\mu\text{g l}^{-1}$  (Ni), 6.5–16  $\mu\text{g l}^{-1}$  (Zn) and 1.1–2.7  $\mu\text{g l}^{-1}$  (Cu). In water from the Oddebekken, concentrations of Ni, Zn and Cu were greater than values reported in Swedish waters by up to three orders of magnitude for Ni and one order of magnitude for Zn and Cu.<sup>25</sup> The concentrations of Ni, Zn and Cu measured in water samples taken from the Oddebekken exceeded lowest known biological effect concentrations, reported for aquatic systems, by up to an order of magnitude.<sup>27</sup> Concentrations of metals in the river Otra were not determined in this study and only a small number of sediment samples have been analysed. In view of the effects of the aquatic environment on the fish reported here it would be of interest to determine the distribution of metals in this aquatic ecosystem.

**Table 3** Heavy metal concentrations in the river Oddebekken (in  $\mu\text{g l}^{-1}$ )

Date	pH	Ni	Co	Zn	Cu
July 1994	3.90	260	ND*	18	12
August 1994	4.18	105	7	12	11
September 1994	4.35	269	10	5	25
October 1994	4.50	299	ND	31	21
November 1994	4.35	567	10	21	21
December 1994	4.36	165	10	41	21
January 1995	4.32	11	ND	41	21
February 1995	4.50	103	10	31	10
June 1995	4.67	103	ND	103	10

\* ND, not detected.

**Table 4** Metal concentrations in the sediment of the Otra in  $\mu\text{g g}^{-1}$

Site	Moisture (%)	LOI (%)*	Ni	Co	Zn	Cu
B	0.3	3.02	48	13	69	20
D	0.4	8.87	46	15	51	80
E	2.0	14.68	57	16	67	52

\* LOI, loss on ignition.

## Conclusions

The fish from site A were found to be much less polluted than those from any of the other sites. Site E was found to be the most polluted and the fish from this area were most severely affected physiologically by their environment. Sites B, C and D were less polluted and the fish in these regions were affected less. However, concentrations of all four metals were still considerably higher than those from the control site and clearly present a serious hazard to the aquatic ecosystem.

Taken in isolation the concentrations of the metals measured in trout do not appear to constitute a risk to the health of humans consuming the fish. However, these results need to be placed in the context of metal concentrations in other local produce particularly drinking water. An assessment of the total metal exposure and the possible implications of exposure to multiple toxic metals in the diet of this population should be considered.

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