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Metal Content of Marine Mussels from Western Scheldt Estuary and Nearby Protected Marine Bay, The Netherlands: Impact of Past and Present Contamination

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In 1953 the coastal areas of south-west Holland were subjected to heavy flooding due to a high tidal wave during a storm. The flood left many people dead and destroyed a vast area of agricultural land. To prevent future flooding, the Dutch government commissioned the 'DeltaWorks Project', which involved construction of a series of dams, dykes and bridges across river channels flowing into the North Sea. By the 1970s, most channels leading into the Eastern Scheldt (ES) (Fig. 1) were dammed and with completion of a storm-surge barrier across the sea-end in 1987, ES was effectively turned from an estuary into a protected marine bay. Consequently, input of chemical contaminants from land based sources into ES decreased dramatically (Nienhuis et al. 1994). However, persistence of heavy metals particularly in sediments was anticipated, though it was never clear how long this residual contamination would continue to affect the biota (De Jonge and De Jong 2002).

Adjacent to ES is Western Scheldt estuary (WS) formed from river Scheldt (Fig.1), which together with its tributaries drains one of Europe's most populated and industrialized regions of Belgium, parts of France and Holland. WS is considered one of the major sources of metal pollution for the North Sea (van Eck and De Rooij, 1993). WS exhibits both pollution and salinity gradients and recently we presented data showing temporal and spatial variations in heavy metal levels in mussels (*Mytilus edulis*) along the WS (Mubiana et al. 2005).

Though ES is comparatively less impacted by pollution as compared to WS, there are also marked contrasts in the behaviour of trace metals in the two systems in terms of physical and chemical speciation (Gerringa et al. 1996, 1998; Zwolsman et al. 1997). Therefore, the aim of the current study was to evaluate levels of several heavy metals (As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in tissues of mussels (*M. edulis*), in order to assess whether levels in ES have gone down relative to WS. Several locations were selected in ES and WS following a land-to-sea gradient, which in WS represent both environmental metal pollution and salinity gradients. In the ES, the selected sites also followed the past pollution gradient, which for most metals, still exist in sediments (Gerringa et al. 1996; Zwolsman et al. 1997). Lessons from this study regarding the persistence of

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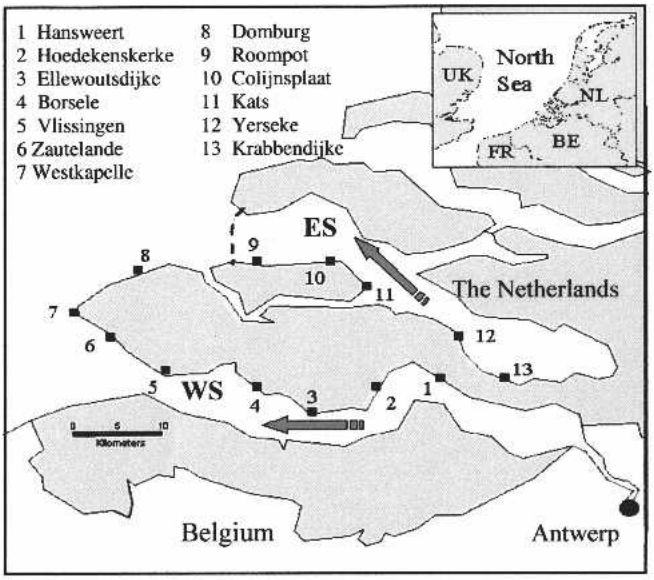

Figure 1. Map showing sampling locations and arrows indicate the direction of decreasing past (ES) and present (WS) environmental concentrations of metals.

metals in mussels from ES can be applied to other places around the world where similar management measures are being considered. The study provides a basis for setting a realistic time frame within which results can be expected following implementation of some environmental pollution remediation or reduction measures. The study also highlights the importance of some environmental factors in modifying the relationship between metal concentrations in the environment and that obtained in the biota.

MATERIALS AND METHODS

At each sampling location (Fig. 1), 30 – 35 mussels were collected during the summer of 2002. In order to avoid possible interference due to differences in either body size or tidal exposure, as was demonstrated in previous studies (Mubiana et al. *in press*), animals of the same body size (40 - 45 mm shell length) were always collected along the waterline during low tide. Salinity and *p*H were also measured. From each location, three sediment samples (top 10 cm) were collected for particulate organic carbon (POC), particle size distribution and metal analysis. Suspended particles were collected on membrane filters (0.45 µm), which were then digested in acid for metal analysis. In the study, dissolved metals were not determined since previous studies showed high short-term variability due to either season or tidal cycle (Zwolsman et al. 1996, 1997; Gerringa et al. 1996).

Metal contents of mussels were determined with ICP-MS (UltraMass 700, Varian,

Australia) after microwave tissue digestion of each individual in a mixture of 5-ml concentrated HNO_3 and 0.25-ml H_2O_2. As quality control, three reference mussel tissue samples (CRM 278R, Institute of Reference Materials and Measurements, Geel, Belgium) were included in each sample rack and as shown in Table 1, recoveries were within 7 % of certified values. To determine total metal concentrations in sediments and SPM, these samples were digested in closed bombs using an industrial microwave digestion system (Ethos 900, Milestone, USA) in a mixture of 4-ml HNO_3, 4-ml HF and 50-ml H_3BO_3. Easily extractable or reducible metals from sediments were determined in aliquot samples (three replicates) by centrifugation (30 min.) in 0.1 M hydroxylamine hydrochloride and the supernatants were analysed using ICP-MS. Easily extractable metals are often used to estimate metal bioavailability from sediments (i.e. Luoma 1989).

Table 1. Recoveries for reference mussel tissue samples (CRM 278R, Community Bureau of Reference, Geel, Belgium).

	Fe	Co	Ni	As	Cd	Cr	Cu	Mn	Pb	Zn
Measured	134	0.32	0.76	5.8	0.34	0.77	9.37	7.15	1.95	80.0
(μg/g)	(3)	(3)	(5)	(3)	(3)	(10)	(2)	(3)	(3)	(3)
Certified	No certified			6.07	0.348	0.78	9.45	7.69	2.00	83.1
(μg/g)	values			(2)	(3)	(8)	(1)	(3)	(2)	(2)
Recovery (%)				**95.4**	**98.8**	**99.0**	**99.2**	**93.0**	**97.3**	**96.2**

Values in brackets are relative standard errors (%) rounded to the nearest percent.

RESULTS AND DISCUSSION

Figure 2 shows metal concentrations in mussels from WS and ES and there was a general decreasing trend of concentrations towards the North Sea in both areas. The data was further analysed using Analysis of Covariance (ANCOVA) to evaluate differences between WS and ES (main effect) with salinity and distance (from the sea) as continuous predictor variables or covariates. Results in Table 2 showed that, except for Cd, the effect of distance was significant ($p<0.05$) while the effect of salinity was only significant for As, Cd, Co, Fe, Mn, Pb and not for Cr, Cu, Ni, Zn. After taking into account the effects of salinity and distance, ANCOVA revealed overall differences between ES and WS for Cd, Cu, Fe and Mn, of which only Cd is a non-essential metal. It is known that within a certain range, body concentrations of essential metals in many organisms are regulated in response to either environmental conditions (i.e. concentrations) or biological requirements (Phillips 1976). Therefore, differences in metal-body concentrations between ES and WS for the essential metals (i.e. Cu, Fe, Mn) may in part be due to biological regulation as well as differences in total metal pollution between ES and WS. Generally, concentrations of most metals in mussels from ES were comparable with the more polluted WS, which was contrary to expectations.

Lack of clear differences in terms of body concentrations of metals between ES and WS is not consistent with differences in the levels of metal pollution of these areas (i.e. van Eck and De Rooij 1993; Gerringa et al. 1996). However, the two

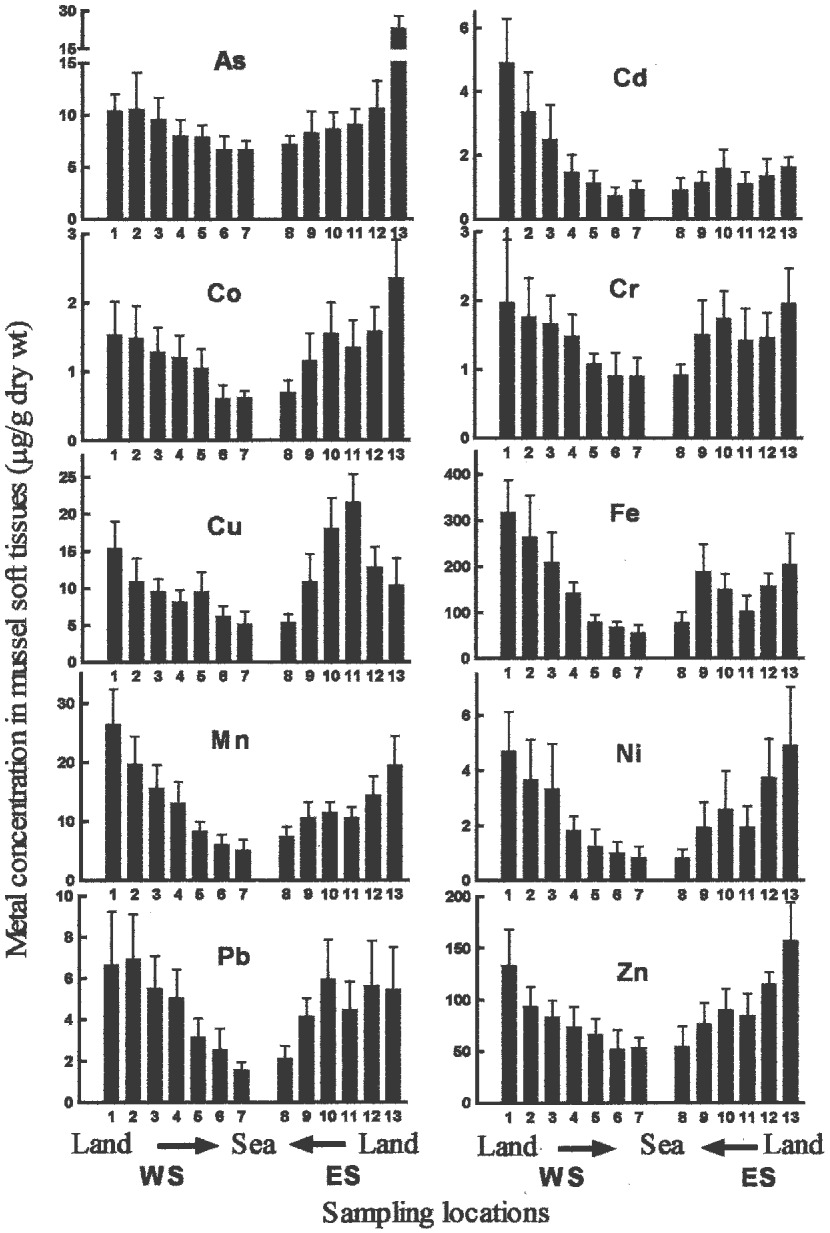

Figure 2. Metal concentrations (+SD) in mussels from ES and WS (The Netherlands). 1- Hansweert, 2- Hoedekenskerke, 3- Ellewoutsdijke, 4- Borsele, 5- Vlissingen, 6- Zautelande, 7- Weskapelle, 8- Domburg, 9- Roompot, 10- Colijnsplaat, 11- Kats, 12- Yerseke and 13- Krabbendijke.

areas have distinctively different physical, geochemical and environmental characteristics (i.e. Nienhuis et al. 1994; Gerringa et al. 1996, 1998).

In the case of WS, lower than expected metal concentrations in mussels may have been due to some water quality and pollution reduction measures taken in recent years by Belgium partly as international commitment to reduce pollution towards the North Sea (1984 North Sea Conference, Bremen Germany). Since then, decreases of 50 – 96 % in metal discharges have been reported from Belgium (Mubiana et al. 2005). Without such reductions, metal content of mussels in the estuary (WS) would be even higher than the values presented in this current study.

Table 2. ANCOVA results comparing metal concentrations in mussels from ES and WS (main effect) with salinity and distance from the sea as covariates.

Effect		MS	F	*p*		MS	F	*p*
Salinity	**As**	234.2	24.0	0.000	**Cd**	35.92	63.7	0.000
Distance		817.9	83.6	0.000		0.02	0.03	0.862
WS vs ES		18.31	1.87	0.174		3.56	6.31	0.013
Salinity	**Co**	0.847	4.88	0.029	**Cr**	0.287	1.43	0.234
Distance		9.369	54.1	0.000		2.504	12.4	0.001
WS vs ES		0.235	1.36	0.246		0.67	3.33	0.071
Salinity	**Cu**	4.62	0.21	0.647	**Fe**	75000	30.5	0.000
Distance		134.4	6.21	0.014		35400	14.4	0.000
WS vs ES		258	11.9	0.001		11700	4.74	0.032
Salinity	**M**	338	18.1	0.000	**Ni**	0.061	0.04	0.838
Distance		250	13.4	0.000		64.04	45.6	0.000
WS vs ES		198	10.6	0.002		0.501	0.36	0.550
Salinity	**Pb**	21.12	7.92	0.006	**Zn**	1711	2.63	0.108
Distance		37.92	14.2	0.000		39000	59.8	0.000
WS vs ES		13.41	3.03	0.072		388	0.62	0.442

With respect to ES, there are several factors that may have enhanced metal bioaccumulation to levels higher than would be expected based on the degree of environmental contamination. Studies have persistently shown significant concentrations of heavy metals in the sediments and since ES is almost cut-off from inland inflow, the only significant source of metal contamination in the sediments is from past pollution (i.e. Gerringa et al. 1996; Zwolsman et al. 1997; De Jong and de Jong 2002). The hypothesis is that the re-suspension of sediments-bound metals in ES coupled with some favourable environmental conditions somehow makes once inaccessible metals more bioavailable for uptake by mussels. Therefore, some of the key physiochemical factors with potential to facilitate such a process are discussed and contrasted between ES and WS.

As shown in Table 3, there were marked differences between ES and WS in terms of salinity, pH and suspended particulate matter (SPM). For example, though there is less SPM in ES, carbon content of both SPM and sediments is much higher compared to WS. Consequently, the ingestible particles in ES are of higher

nutritional value for filter feeders including bivalves. Therefore, intake of carbon-rich particles by mussels in ES may enhance assimilation of particulate metals more than in WS. Furthermore, total metal concentrations in sediments (Table 4) revealed no obvious differences between ES and WS, though the situation can be different if metal concentrations were normalized to a specific sediment component like clay content. However, in this study such calculations were not necessary. As shown in Table 4, total metal loads in sediments in the two areas were comparable, but due to differences in sediment characteristics, ES generally showed higher percentage of easily extractable metals (except for Fe and Mn). It is generally accepted that easily extractable metals are a better representation of bioavailable metals from sediments (Luoma 1989). Therefore, on this basis, ES is also likely to present higher metal bioavailability from sediments compared to WS. In addition, as shown in Table 3, the majority of particles (69-77%) in ES were small enough (<20 μm) to be filterable by mussels, thereby presenting another factor that favours higher direct uptake of particulate metals in ES.

Table 3. Range values (minimum – maximum) of some environmental parameters measured at locations in ES and WS during sampling.

	Salinity (psu)	*p*H	SPM (mg dry wt/L)	POC in dry SPM (%)	POC in dry Sediment (%)	Particles <20 μm (%)
WS	16 - 35	7.9 - 8.04	155 - 168	2.8 - 3.6	1.1 – 2.3	5.8 - 9.7
ES	33 - 34	8.1 - 8.2	30 - 48	37 - 45	21 – 24	69 - 77

Table 4. Metal concentrations (mean ±SD) in sediments and suspended particles at two sampling locations of Hansweert in WS (1) and Wemeldinge in ES (2)

Metal	Area	Total metal concentrations in SPM (μg/g)	Total metal concentrations in sediments (μg/g)	Easily extractable metals from sediments as % of total metal
Cd	**1**	2.10 (0.08)	0.44 (0.31)	1.10 (0.03)
	2	1.4 (0.12)	0.25 (0.01)	1.60 (0.01)
Co	**1**	2.53 (0.03)	0.48 (0.04)	0.60 (0.03)
	2	1.5 (0.14)	0.42 (0.07)	4.52 (0.57)
Cr	**1**	36.2 (1.0)	6.28 (0.54)	0.10 (0.07)
	2	20.1 (1.3)	5.73 (0.79)	0.37 (0.33)
Cu	**1**	30.4 (0.4)	3.63 (1.55)	0.11 (0.01)
	2	14.4 (5.2)	3.93 (0.08)	0.80 (0.71)
Fe	**1**	16333 (169)	4318 (251)	0.90 (0.01)
	2	10428 (102)	4229 (291)	0.41 (0.23)
Mn	**1**	655 (6)	86.6 (4.3)	31.0 (3.13)
	2	430 (14)	133 (4.6)	24.0 (4.81)
Ni	**1**	12.9 (0.1)	2.11 (0.09)	2.40 (1.0)
	2	4.98 (0.7)	2.78 (0.24)	2.70 (1.0)
Pb	**1**	36.6 (0.6)	5.91 (0.1)	1.10 (0.26)
	2	12.2 (0.6)	5.26 (0.3)	1.50 (0.23)
Zn	**1**	142.6 (1.3)	59.7 (2.1)	0.70 (0.03)
	2	62.7 (2.1)	41.1 (3.1)	3.60 (0.17)

Overall results as demonstrated in Tables 3 & 4, clearly indicate that prevailing environmental conditions favours higher metal bioavailability in ES compared to WS, though in terms of total metals in the environment WS appear to be more polluted with heavy metals. Furthermore, besides differences in physical speciation of metals, studies have also demonstrated differences in chemical speciation between ES and WS (Gerringa et al. 1996, 1998). According to Gerringa and co-workers (1996), chemical speciation of Cu was suggested to be the main factors influencing uptake by *Macoma balthica* in ES and WS. For most metals, chemical speciation in natural seawater is complex due to high concentrations of dissolved inorganic salts. It was shown that in ES, there were clear relationships between copper speciation, POC and DOC but such relationships in WS were not clear. It was also demonstrated that in ES, dissolved metals tended to associate more with dissolved organic ligands whereas in WS in part due to high concentration of inorganic suspended particles (Table 3), metal partition favoured inorganic complexation (Gerringa et al. 1996, 1998). Studies involving different organisms have shown that uptake is mostly depended on free metal ion in the medium. As a result, complexation with DOM in other organisms tends to decrease metal uptake and toxicity (i.e. Hudson 1998). However, according to recent studies, this phenomenon is not valid in mussels, where complexation of metals with DOM appears to enhance uptake (i.e. Roditi et al. 2000; *Mubiana et al. submitted*). The theory is that as mussels absorb DOM for nutritional requirements (Dame 1996), metals associated with DOM are somehow co-absorbed into mussel tissues. Therefore, in mussels, unlike most non-filter feeders, high DOC may result in increased metal uptake and accumulation. Therefore, higher (than expected) tissue concentration of metals observed in this study regarding mussels from ES is clearly consistent with this hypothesis. Finally, another important factor is salinity, which due to complexation, was expected to favour high accumulation of metals in the upper part of WS where salinity is low. As shown in Table 2, the salinity component in ANCOVA significantly accounted for some of the variations for As, Cd, Co, Fe, Mn and Pb though not for Cr, Cu, Ni and Zn. Salinity also influences animal physiology, though the consequences on metal accumulation in mussels are complex and still not clearly understood (Mubiana et al. *in press*). Furthermore, in most estuaries, salinity is often inversely correlated with environmental contamination due to proximity to inland sources upstream (Phillips 1976).

Overall, this study showed that in complex estuarine environments, metal uptake and accumulation in the biota not only depend on the level of environmental pollution, but can be simultaneously influenced by a battery of factors, some of which act in opposite directions or can enhance uptake to an extend that differences in environmental contamination are masked. Therefore, provided the conditions are favourable, residual contamination in sediments can have long-term impact on biota even long after implementation of some remediation measures.

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