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Metals in the Scheldt estuary: from environmental concentrations to bioaccumulation

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Highlights

- This is the first study investigating metal distribution along the aquatic ecosystem of the Scheldt estuary
- Metal concentrations in abiotic and biotic compartments were influenced by sampling location, but not by season
- Metal concentrations in biota decreased from up- to downstream except for arsenic for which an opposite trend was found
- A potential human risk exists when fish or mussels are consumed due to high As values and in some cases due to Cd and/or Cu

Abstract

To investigate the relationship between metal concentrations in abiotic compartments and in aquatic species, sediment, suspended matter and several aquatic species (Polychaeta, Oligochaeta, four crustacean species, three mollusc species and eight fish species) were collected during three seasons at six locations along the Scheldt estuary (the Netherlands-Belgium) and analysed on their metal content (Ag, Cd, Co, Cr, Cu, Ni, Pb, Zn and the metalloid As). Sediment and biota tissue concentrations were significantly influenced by sampling location, but not by season. Measurements of Acid Volatile Sulphides (AVS) concentrations in relation to Simultaneously Extracted Metals (SEM) in the sediment suggested that not all metals in the sediment will be bound to sulphides and some metals might be bioavailable.

For all metals but zinc, highest concentrations were measured in invertebrate species; Ag and Ni in periwinkle, Cr, Co and Pb in Oligochaete worms and As, Cd and Cu in crabs and shrimp. Highest concentrations of Zn were measured in the kidney of European smelt. In fish, for most of the metals, the concentrations were highest in liver or kidney and lowest in muscle. For Zn however, highest concentrations were measured in the kidney of European smelt. For less than half of the metals significant correlations between sediment metal concentrations and bioaccumulated concentrations were found (liver/hepatopancreas or whole organism). To calculate the possible human health risk by consumption, average and maximum metal concentrations in the muscle tissues were compared to the minimum risk levels (MRLs). Concentrations of As led to the highest risk potential for all consumable species. Cadmium and Cu posed only a risk when consuming the highest contaminated shrimp and shore crabs. Consuming blue mussel could result in a risk for the metals As, Cd and Cr.

Keywords

assessment, Acid Volatile Sulphide, human health risk, metal partitioning.

Capsule:

- This is the first study that assess the metal concentrations in water, sediment, suspended matter and biota (17 taxa) along the whole Scheldt Estuary covering the whole salinity range from fresh to saline water.
- This together with the assessment of possible risk for human consumption makes this study unique.

Introduction

Anthropogenic activities such as metal industry, mining, burning of fossil fuel, have altered metal distribution and caused them to concentrate in aquatic and terrestrial ecosystems (Reck and Gordon, 2008). As they can have adverse effects on individual organisms (Wallace et al., 2000) and communities (Bervoets et al., 2005, Van Ael et al. 2014) knowledge on the distribution of metals and where they will end up in the food chain is crucial for risk assessment and for the protection of ecosystems.

Metal distribution, speciation and bioavailability is strongly influenced by the surrounding environmental conditions such as pH, salinity and organic matter content (Hatje et al. 2003). In an aquatic system, sediments often act as a sink for metals, as positively charged metal ions bind easily with negatively charged sedimentary phases such as clay particles, organic carbon, iron and manganese oxides or sulphides (Luoma and Davis, 1983; Eggleton and Thomas, 2004). Acid Volatile Sulfides (AVS) are defined as the amount of sulphides in the sediment volatilized by addition of 1 N HCl (Di Toro et al., 1990). They are produced by bacteria in anaerobic sediments through sulphate reduction and can form stable metal-sulphide precipitates. The SEM-AVS model of Di Toro et al. (1990) states that when the AVS concentration in the sediment exceeds the SEM (Simultaneously Extracted Metals) concentrations on a molar basis, all metals will be bound to the sulphides and metals are considered to be less bioavailable or toxic for aquatic organisms. However, in addition to local chemistry, metal bioaccumulation is also influenced by species-specific characteristics. The behavior and habitat preferences of a species will determine the most important uptake route of metals (De Jonge et al., 2010). Species-specific metabolic processes determine the elimination rate from the body and the ability to make them metabolically unavailable (Rainbow, 2002). These differences in exposure, pathways and biological organization in species make it hard to link environmental concentrations with tissue loads and to predict or explain bioaccumulation patterns and toxicity. Since estuaries act as the transition zone between fresh water and marine systems, the biogeochemical processes in estuaries can be important for the management of the quality of coastal waters (Van Damme et al. 2005; Schneider et al. 2016). The present study was performed in the Scheldt estuary (SE, the

Netherlands-Belgium), which receives water from densely populated and industrialized areas such as Antwerp and Brussels and which is enriched with nutrients and pollutants, including metals (Baeyens et al., 2005; Gao et al. 2013) and Persistent Organic Pollutants (POPs; Van Ael et al., 2013; Gao et al. 2013). The varying physical-chemical conditions in an estuary, make it an interesting area to study the relationships between environmental and bioaccumulated metal concentrations.

The aims of this study were (1) to evaluate the concentrations of metals in the sediment, suspended matter (SPM) and aquatic food chain in the Scheldt estuary, (2) to investigate if bioaccumulated metal concentrations can be related to concentrations in the sediment, taking into account environmental characteristics, (3) and to estimate the potential human health risk due to the consumption of metal-contaminated edible aquatic species.

To our knowledge, this is the first study to investigate metal accumulation in species from different trophic levels along the complete salinity gradient of the Scheldt estuary. Other studies focused on a part of the estuary or on one species, e.g. *Mytilus edulis* (Mubiana and Blust, 2006) and *Littorina littorea* (De Wolf et al., 2000).

Material and Methods

2.1 Field sampling

Field samples were collected during two seasons in 2010 (spring and summer) at six locations along the Scheldt estuary (Figure 1): Vlissingen, Terneuzen, Waarde, Bath (the Netherlands), Antwerp and Temse (Belgium). In summer 2011, a third time was sampled at Terneuzen, Bath and Antwerp. Sampling location 1 (Vlissingen) is situated at the mouth of the Scheldt river into to the North Sea. The sixth sampling location (Temse) is located in the upper freshwater headwaters, so the study area includes the whole salinity gradient. Fish, crabs and shrimps were sampled by means of fyke fishing (INBO, Research Institute for Nature and Forest) or trawl fishing with the vessel *Zeeleeuw* of the Flanders Marine Institute (VLIZ). Other organisms were collected manually from the shore at low tide. An overview on the collected biota samples

at the different sampling occasions is given in Table 1. The top layer (10 cm) of the surface sediment was sampled manually from the shores at low tide in the three different periods. At each location and per sampling period, three replicates were taken and pooled. A sample of surface water (500 mL) was filtered with a vacuum pump over glass fiber filters (VWR International, pore size 0.7 μm) to collect suspended particulate matter (SPM). SPM was only collected in duplicate during summer 2010.

From fish, tissues including liver, gills, kidney, intestine and muscle (caudal musculature) were collected. From crabs, gills, hepatopancreas and muscle were sampled, and from shrimps, muscle and hepatopancreas. Other species were analyzed as a whole. Oligochaeta were pooled to reach an adequate sample volume. Samples were stored at -20°C until analysis. A selection of the same samples were used to study the bioaccumulation of POPs as described in Van Ael et al. 2013.

2.2 Environmental characteristics

Water pH, O_2 content and conductivity were measured *in situ* using a WTW Multiline P4 meter during each sampling moment. Total Organic Carbon (TOC) of the sediment was determined through Loss on Ignition (LOI). Dry sediment samples were incinerated at 550°C for 4h and afterwards weight loss was determined (Heiri et al., 2001). Clay content in the sediment samples was measured with laser diffraction (Malvern Mastersizer S., Worcestershire, UK). The AVS were measured using the purge-and-trap protocol as described in De Jonge et al. 2010, by extracting them from 10 g of wet sediment with 5 mL of hydrochloric acid (HCl, 37%). The volatilized hydrogen sulphide (H_2S) was captured in a NaOH buffer. The extracted sulphide concentration was detected in the buffer with an ORION 96-16 ion-selective sulphur electrode (Ionplus, Beverly, MA, USA) (De Jonge et al., 2010). The SEM fraction was collected for metal analysis.

2.3 Metal analysis

Biota samples were oven dried at 60°C for 48 h and subsequently digested in a microwave oven in a mixture of HNO_3 (69%) and H_2O_2 (37%) (Bervoets and Blust 2003) and diluted with ultrapure water (MilliQ,

Bedford, MA, USA). For the metal concentrations in the sediment and SPM, samples were dried at 60° C for 48 h, weighed and placed into Teflon bombs. Samples were digested in a microwave oven (ETHOS 900 Microwave Labstation, Milestone, Italy) in a mixture of HNO₃ and HCl (1:3, v/v) (De Jonge et al., 2010). Afterwards, samples were diluted with ultrapure water (MilliQ) and stored until analysis.

Metal analyses (Ag, Cd, Co, Cr, Cu, Ni, Pb, Zn and metalloid As) on biota, sediment, SPM and SEM samples were performed using an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS, 7700x, Agilent Technologies, Santa Clara, CA, USA). Quality control during digestion and analysis was assured by regular analysis of blanks (including digested blanco glass fibre filters) and the use of certified reference material of the Community Bureau of Reference of the European Commission (Brussels, Belgium): CRM 609 (groundwater), CRM 505 (estuarine water), CRM 142R (light sandy soil), CRM 277R (estuarine sediment), CRM 320R (channel sediment), CRM 278R (mussel tissue) and CRM 422 (cod muscle). Recoveries were within 15% of the certified values.

2.3 Statistical analysis

Metal concentrations below the detection limit (DL) were substituted by a value of half this detection limit (Custer et al., 2000). Because of the small sampling size, differences in metal concentrations between location, season and species were investigated using the non-parametric Kruskal-Wallis with Dunn's multiple comparison test. The level of statistical significance was set at $p < 0.05$. Correlations between metal concentrations in the sediment (non-normalized and normalized for clay content and TOC) and in the tissues were studied with Spearman correlation. Statistical analyses were performed using GraphPad Prism 6.00 (GraphPad Software, Inc) and IBM SPSS Statistics 20.

3. Results and discussion

3.1 Sediment and water characteristics

Table 2 summarises the sediment and water characteristics measured at the different sampling locations. Several gradients can be observed throughout the estuary. Sediments were generally more sandy towards the sea, so the measured clay content was higher at more upwards locations. In addition highest organic carbon contents were measured at upstream locations. The presence of both clay and organic carbon favour metal binding in the sediment. Surface water electric conductivity (EC) reflected the salinity gradient in the estuary. This gradient is in accordance to the description by Meire et al. (2005). The dissolved oxygen concentration of the water increased slightly towards more downstream locations, as previously described by Van Damme et al. (2005). The highest AVS concentration was measured at the most upstream part but no clear trend towards the sea could be observed. AVS concentrations were lower than values reported by Teuchies et al. (2013) in the Scheldt who measured concentrations up to 20 $\mu\text{mol/g}$ close to our sampling site 6. However, in their study samples were taken in a tidal flat with long residence times of the overlying water and lower oxygen concentrations. Concentrations in the present study were also lower than values reported in other studies in marine (0.86 – 20.5 $\mu\text{mol/g}$, Laizhou Bay, China) (Zhuang and Gao, 2013) and freshwater environments (0.76 – 205 $\mu\text{mol/g}$, Flemish lowland rivers, Belgium) (De Jonge et al., 2010), but comparable to the values reported by Nizoli and Luiz-Silva (2012) in a Brazilian estuary.

3.2. Environmental metal concentrations

Median metal concentrations in sediment and SPM are presented in Table SII. For none of the metals the median sediment concentrations exceeded international sediment quality standards (Simpson et al. 2013; CCME, 2001; VLAREM II, 2010). At one site (location 6; Temse) however, the sediment quality standards were exceeded for As, Cd, Cr, Cu, Ni, Pb and Zn. Sediment metal concentrations were significantly

influenced by sampling location ($p < 0.04$), but not by season. For all metals the highest concentrations were measured at the most upstream location, Temse (significant for 8 out of 9 metals) (Figure 2 and Figure SI1). The most pronounced metal gradients along the estuary from up- to downstream were observed for Cd, Cu, Pb and Zn (Figure 2). For SPM metal concentrations, the same trend was observed (Figure 3 and Figure SI2) although this could not statistically be tested due to the low number of replicates ($N = 1$ to 3). Pronounced gradients were found for Ag, Cd, Pb and Zn (Figure 3). Close to Temse, the most upstream sampling location, the Scheldt receives water from the Brussels-Scheldt Canal, which might explain the higher metal concentrations. Both sediment characteristics (decreasing clay content and TOC) and mixing with unpolluted sea water can lead to a further decrease of sediment metal concentrations towards the North Sea.

Sediment concentrations in Bath were lower than the values reported by Teuchies et al. (2013) for the subtidal zone on the other side of the river. Metal concentrations in sediment at the Dutch/Belgian border (our location 4) decreased strongly between the 1970s and 1990 and weakly between 1990 and 2010 (Gao et al. 2013). Adding our measurements showed a further decrease in sediment concentrations for at least Cd, Cu, Pb and Zn.

De Boer et al. (2001) detected comparable metal concentrations in sediment from the Rotterdam harbour, but higher concentrations at the Amsterdam harbour. Copper and Cr concentrations were lower than in four Spanish estuaries. Concentrations of Pb and Zn were comparable, except for the Tinto-Odiel Estuary, which is highly polluted (Ruiz, 2001). Metal concentrations were lower than (As, Cu, Ni, Pb) or comparable to (Cd, Zn, Pb) measured by Wright and Mason (1999) in the Orwell and the Stour estuaries in England.

The median SEM concentrations are reported in Figures 4 and in Figure SI3. For Ag, SEM concentrations were below the detection limit. This is not unexpected since AgS as well as CuS are insoluble in 1-M HCl and will not be extracted by the applied AVS extraction procedure (Cooper et al. 1998; Simpson et al. 1998).

For all other metals significant differences among the sites were observed with highest concentrations upstream at site Temse. The most pronounced gradients along the estuary were observed for Cd, Pb and Zn (Figure 4). As expected SEM concentrations were always lower than total metal concentrations in the sediments.

The SEM-AVS concentrations are presented in Table SI4. All SEM-AVS concentrations had values around 0, being positive or negative depending on the variation between the replicates. Median SEM_{tot}-AVS values were positive at all locations. However, at Temse lowest concentrations were negative. These results suggest that not all metals in the sediment will be bound to sulphides and some metals will be potentially available for bioaccumulation. Several other studies reported low SEM/AVS values, indicating the absence of metal toxicity, according to the SEM/AVS concept, including Hamzeh et al. (2013) in core sediments from the Seine estuary and Oueslati et al. (2010) in sediments from the Ghar El Melh Lagoon in Tunisia. De Jonge et al. (2010) however, observed also accumulation of metals in invertebrates even at SEM-AVS < 0, especially in sediment-feeding invertebrates.

3.3. Biota

From all captured taxa, several specimens were collected to measure metals (Table 1). Two worm taxa were collected, i.e. Oligochaete and Polychaete worms without further identification. Four crustacean species were analysed on metals, i.e. two crab species, the shore crab *Carcinus maenas* and the Chinese mitten crab *Eriocheir sinensis*, the brown shrimp *Crangon crangon* and the amphipod *Gammarus pulex*. Further, one gastropod *Littorina littorea* and two bivalve species the Baltic tellin *Macoma balthica* and the blue mussel *Mytilus edulis* were analysed. Finally 8 fish species, European flounder *Platichthys flesus*, sole *Solea solea*, European smelt *Osmerus eperlanus*, shorthorn sculpin *Myoxocephalus scorpius*, eel *Anguilla anguilla*, pike perch *Stizostedion lucioperca*, pouting *Trisopterus luscus* and European seabass *Dicentrarchus labrax* were analysed on metals. Median metal concentrations in sampled biota from the whole Scheldt estuary are summarised in Table SI2 and SI3 for invertebrates and fish tissues respectively.

Highest concentrations of all measured metals were measured in invertebrates; i.e. in *L. littorea* for Ag and Ni, in the hepatopancreas of shrimp or crabs for As, Cd and Cu and in oligochaete and polychaete worms, but followed by crabs and shrimps, for Cr, Co and Pb. Only for zinc highest concentrations were measured in kidney of the European smelt.

Only few species could be captured in each of the sampled seasons and only in some cases sufficient individuals were captured to test for an effect of sampling period on metal content. This was the case for Polychaeta in Terneuzen, shrimps in Terneuzen and Waarde, shore crab in Terneuzen and Bath, mitten crab in Bath, Antwerp and Temse and sole in Bath and Antwerp. In none of the cases significant effects of season were observed.

Metal concentrations in biota at the different sites are summarized in Figure 5 and Figures SI4A-SI4T for those taxa sampled at ≥ 4 sites.

All analysed metals could be detected in all sampled species. Nonetheless, some individual samples contained low concentrations or values below the detection limit. At site 4 (Bath), most species (14) were captured, with highest median concentrations found in the hepatopancreas of shore crab (Ag, Cd), in kidney of shorthorn sculpin or muscle of shore crab (As), in oligochaete worms (Co, Ni, Pb), in Chinese mitten crab (Cu) or in *M. baltica* (Zn). However, due to the low number of replicates of captured bristle and oligochaete worms, significance could not be tested.

In fish, shrimp and crabs metals were measured in different tissues (Table SI2 and SI3). Bioaccumulation was organ-specific. In all fish species metals were measured in muscle, gill, liver, kidney and intestine and concentrations were generally lowest in muscle, with the exception of As for which concentrations in the muscle were always among the highest. The same was observed by Mhadhbi et al. 2012 who measured higher concentrations of As in muscle of sole compared to liver and kidney. For the other metals, the order of metal concentration depended on the metal and the species.

In the crabs, metals were measured in muscle, gill and hepatopancreas. Again concentrations were lowest in muscle with the exception of highest concentrations in muscle for Zn in Chinese mitten crab and for As

in shore crab. For the other metals concentrations were highest in gill (Co, Cr, Ni and Pb in both species; As in Chinese mitten crab) or in hepatopancreas (Ag and Cu in both species, Cd in shore crab). The occurrence of organ-specific metal bioaccumulation is well-known. This is in accordance with other studies on metals in fish who found in most cases lowest concentrations in muscle and depending on the exposure route, highest concentrations in liver, kidney or gills (Bervoets and Blust 2003; Dhanakumar et al. 2015; Mataba et al. 2016).

In many cases metal concentrations in the tissues were significantly influenced by sampling location, but never by season. Significant differences among locations were found in Polychaetes (Ag, As, Cd, Ni; Figure SI4A); Oligochaetes (Ag, Pb, Zn; Figure SI4B); Balitic tellin (As, Cd; Figure SI4C); brown shrimp (hepatopancreas: As, Cd, Co, Cr, Cu; muscle: Ag, As, Cd, Cr, Cu, Ni; Figures SI4D-E); shore crab (hepatopancreas: Cd, Co, Cu, Ni, Zn; gill: Ag, Cd, Zn; muscle: Cu, Pb; Figures SI4F-H); Chinese mitten crab (hepatopancreas: As, Cu; gill: As; muscle: As, Zn; Figures SI4I-K); European flounder (liver: As, Cu; kidney: Cu, Ni, Pb; gill: As, Cd, Cr, Pb; intestine: As, Cd, Cu, Ni, Pb, Zn; muscle: As, Cu; Figures SI4L-O); sole (liver: As, Cr; kidney: Cr, Cu; gill: Pb, Zn; intestine: As, Pb; muscle: Cr, Cu, Ni; Figures SI4P-T). Often a decreasing trend in biota tissue metal concentrations towards the sea could be observed although a pronounced gradient was obvious in only a few cases (Figure 5). For arsenic the opposite trend was observed with increasing accumulated concentrations towards the sea (Figure 5). This is rather unexpected since the concentrations in sediment and SPM follow a decreasing gradient towards the sea. However, Dutton and Fisher (2011) observed an increase in uptake of arsenic with increasing salinity by experimentally exposed killifish *Fundulus heteroclitus*. Moreover, Larsen and Francesconi (2003) reported higher arsenic concentrations in muscle tissue of three marine fish species from higher salinity water.

Few other studies have addressed metal concentrations in aquatic animals along the Scheldt estuary. In most cases metal concentrations in only one species were measured (e.g De Wolf et al. 2000; Mubiana et al. 2006; Wepener et al. 2008). Concentrations in *L. littorea* of As, Cd, Co, Cu, Cr, Ni, Pb and Zn collected at Terneuzen were very comparable to the concentrations in the same species measured in 2000 at the same

spot. In the present study, however, we measured median Ag concentrations that were > 10 times higher compared to 2000 (De Wolf et al. 2000). For *M. edulis* concentrations measured by Wepener et al. (2008) were comparable for Ag, As and Cd but slightly higher for Co, Cr, Ni, Pb and Zn. Kerambrun et al. 2013 collected juvenile flounders in the Scheldt at Bath in 2010 and measured a set of trace metals in gill and liver. Concentrations in the liver in the present study were very comparable for As, Ni and Pb but slightly lower for Cd (0.80 vs 3.02 µg/g dw), Co (0.45 vs 1.08 µg/g dw), Cr (0.60 vs 1.17 µg/g dw), Cu (40 vs 73.8) and Zn (120 vs 201 µg/g). These differences could be due to differences in age and/or sex of the captured fish. When comparing the mean metal concentrations in flounder liver from the whole Scheldt estuary to concentrations in flounder liver in other estuaries, concentrations for all metals in the present study were higher than in the Canche estuary in France and similar or lower compared to the Seine and Loire estuaries (Kerambrun et al. 2013). Concentrations in muscle tissue of flounder were higher than concentrations in flounders from the Vistula estuary in the Gulf of Gdansk and the Douro estuary in Portugal (0.3-1.1 µg Cu/g dw, 0.009-0.06 µg Pb/g dw; present study: 0.59-5.72 µg Cu/g dw, <DL-0.9 µg Pb/g dw) (Polak-Juszczak, 2013). Concentrations of Ag, As, Cd, Cu, Pb and Zn in sole liver, kidney and muscle in the present study were in the same range, although slightly lower, compared to concentrations measured by Mhadhbi et al. (2012) in sole from the Ria de Vigo in Spain. Compared to concentrations in muscle, gill, liver and kidney of sole from the Gironde estuary (France) concentrations of Cd, Cu and Zn in the present study were in the same order of magnitude, though again slightly lower (Durrieu et al. 2005).

Cadmium, Cu and Zn concentrations in Baltic tellin from the present study were very comparable to concentrations measured in the same species by Bordin et al. (1996) in the Western Scheldt, although sampled 20 year before the presents study.

Muscle and hepatopaneas in shore crabs from the Scottish coast contained lower metal concentrations (Cd, Cu, Pb, Zn) than the crabs in the present study. However, As concentrations were higher in the Scottish crabs (Teixeira et al., 2008). Lead concentrations in bristle worms were lower when compared to bristle worms collected at a marsh and an estuary in the southeast Gulf of California, Mexico (average 12.6 ± 4.1

$\mu\text{g Pb/g}$; present study: $5.58 \pm 4.7 \mu\text{g Pb/g}$) (Soto-Jimenez et al., 2008). The bristle worm *Nereis diversicolor* from the southwest Iberian coast (Spain, Portugal) contained lower concentrations of Cd, Pb and Zn, comparable concentrations of Cu, and higher concentrations of Ni when compared to bristle worms from the Scheldt estuary (Gomes et al., 2013). In bristle worms from the Todos os Santos Bay, in the northeast of Brazil, similar concentrations of Cu were found. Zinc concentrations were lower and Co concentrations were much higher than the bristle worms in the present study (Eca et al., 2013). From this comparison it is clear that generally spoken the concentrations of the measured metals in biota from the Scheldt estuary are similar to lower compared to other estuaries in Europe and elsewhere. When we compare concentrations in benthic fish (sole and flounder) from the Scheldt estuary with concentrations in benthic fish from uncontaminated tributaries of the Scheldt, concentrations were higher for As, Cd, Co, Pb and Zn, but in the same range or lower for Ag, Cr, Cu and Ni. Compared to contaminated tributaries concentrations were still higher for As, in the same range for Co and Pb but lower for Cd and Zn (Bervoets and Blust 2003; De Jonge et al. 2015). However, since the same species were not captured in the different aquatic systems this comparison should be interpreted with caution.

Van Ael et al. (2013) calculated the $\delta^{15}\text{N}$ values for the different species at site L2 and did find significant relationships with several accumulated POPs. For the measured metals however, no significant relationships were found with $\delta^{15}\text{N}$ indicating that accumulation did not increase with trophic level. This is in accordance to Besser et al. (2001) who did not find biomagnification for Cd and Cu and even a decrease with trophic level for Pb and Zn in a stream food web. Culioli et al. (2009) also observed a decrease in As with trophic level. The absence of biomagnification for metals in the present study is probably due to the fact that besides food, water and/or sediment are important exposure routes as observed by Van Campenhout et al. (2009).

3.4 Relation between sediment and tissue concentrations

Only two taxa (Oligochaeta and European flounder) could be captured at sufficient sites (5 or 6) in sufficient numbers (> 3 for flounder and pooled samples for Oligochaeta) to perform correlations. Less than half of the metals displayed correlations between sediment metal concentrations and bioaccumulated concentrations in fish tissues or whole oligochaete worms (Table SI5). Too few sites were sampled to construct reliable multiple regressions that take into account the effect of water and sediment characteristics.

3.5 Human health risks

In general, diet is the main exposure route to metals for humans (Castro-Gonzalez and Mendez-Armenta, 2008). Some of the species used in the present study are regularly consumed by humans; i.e. flounder, sole, shore crab, shrimp and blue mussel. To calculate the possible human health risk posed by eating these species, the median and maximum metal concentrations in the muscle or whole tissue (blue mussel) were compared to the minimum risk levels (MRLs) for chronic oral intake of As, Cd, Cr and Zn and oral intake of intermediate duration for Cu (ATSDR, 2013). Lead concentrations were compared with the guidelines formulated by the WHO (World Health Organization). However, this guideline is still under discussion (WHO, 2007). For the concentrations of Cr and As, no differentiation between the chemical forms was made. Since the toxicity of these metals depends on the chemical species, this can have consequences on the calculations. The maximum daily amount a person of 70 kg can eat without risk is calculated and presented in Table 3. The lowest edible daily amounts are found for As. When taking the median As concentrations from the present study into account, only 8.83, 10.2, 3.39, 5.32 and 1.25 gram per day can be eaten of flounder, sole, shore crab, shrimp and mussel respectively. When consuming the most contaminated organisms (highest As concentrations), only 1.57, 5.37, 1.57, 4.46 and 1.07 gram should be consumed of these species (Table 3). Since an average Belgian consumer above the age of fifteen consumes 23.9 g of fish or seafood a day (WIV, 2006), this could be a health risk with respect to As. Next to As, also

the highest concentrations of Cd and Cu in shore crab and Cd in shrimp as well as median and highest concentrations of Cd and Cr in mussel could pose a human health risk (Table 3). The shrimp and crabs with the highest Cd concentrations and the crabs with the highest Cu concentrations were all sampled at Bath, the 4th sampling location. The other metals do not pose any risk for human consumption. Several other authors addressed the human health risk of seafood consumption. Watanabe et al. (2003) reported an elevated human health risk through consumption of sea food from the lower Mississippi river, due to concentrations of As, mercury and organic compounds. Metals in ten fish species from the Salek in Slovenia posed no risk to human health (Petkovsek et al., 2012). Arsenic concentrations in 5 fish species from the Persian Gulf (Raissy and Ansari, 2014) and Cd and Pb concentrations in fish from the Mediterranean Sea (Storelli and Barone, 2013) also posed no risk to human health.

Conclusion

In conclusion, the metal concentrations detected in this study show that metals still appear to be an environmental issue in the Scheldt estuary. The consumption of contaminated sea food have potential consequences for human health. Metal bioaccumulation was species-specific and organ-specific. A direct relationship between sediment and biota concentrations was sometimes present, but often masked by other influencing factors.

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Figure 1. Sampling locations: 1, Vlissingen; 2, Terneuzen; 3, Waarde; 4, Bath (the Netherlands); 5, Antwerpen; 6, Temse (Belgium).

Figure 2. Geographical variation (median and range, Kruskal-Wallis) in sediment concentrations of Cd, Cu, Pb and Zn ($\mu\text{g/g dw}$, $n=3$; Temse $n=7$). Different letters indicate significant differences ($p<0.05$). Other metals are presented in Figure SI1

Figure 3. Geographical variation (median and range) in SPM metal concentrations ($\mu\text{g/g dw}$, $n=1-3$). Other metals are presented in Figure SI2.

Figure 4. Geographical variation (median and range) in SEM metal concentrations ($\mu\text{g/g dw}$, $n=4-8$). Other metals are presented in Figure SI3.

Figure 5. Geographical variation of accumulated metals in selected biota from the Scheldt estuary ($\mu\text{g/g dw}$). Different letters indicate significant differences ($p<0.05$). Other results on levels in biota are presented in Figures SI4A-T.

Fig 1.

Fig.2

Fig. 3

Fig. 4

Fig. 5

Table 1. Overview of the number of samples collected. For Oligochaeta the number of pooled samples is presented.

Taxon	Vlissingen		Terneuzen		Waarde		Bath			Antwerp			Temse	
	Sp	Su1	Su1	Su2	Sp	Su1	Sp	Su1	Su2	Sp	Su1	Su2	Sp	Su1
Polychaeta	1		4	14	2			2	11		1	9		1
Oligochaeta	1		1	1	2	2	1	3	2			2		
<i>Littorina littorea</i>				10										
<i>Macoma baltica</i>				2		6	2	5	9					
<i>Mytilus edulis</i>				10										
<i>Gammarus sp.</i>									5					
<i>Crangon crangon</i>	12		12	8	7	5			13					
<i>Carcinus maenas</i>	8		8	3	2	5		5	12					
<i>Eriocheir sinensis</i>					3		2	4	3	5	2	3	5	5
<i>Platichthys flesus</i>	1	4	5	6			4	11	7	6	16	7	4	1
<i>Solea solea</i>		5	7	1	2	7		9	4		7	5		
<i>Osmerus eperlanus</i>				2					7					
<i>Myoxocephalis scorpius</i>				2					6					
<i>Anguilla anguilla</i>											6			
<i>Stizostedion lucioperca</i>								3				3		
<i>Trisopterus luscus</i>				3				3						
<i>Dicentrarchus labrax</i>							3							

Sp: spring; Su1 summer 2010; Su2 summer 2011

2 Table 2. Ranges of sediment and water characteristics at all sampling locations (3<n<7).

			upstream					downstream
			Temse	Antwerpen	Bath	Waarde	Terneuzen	Vlissingen
Sediment								
TOC	%		0.70 - 9.91	1.65 - 3.43	0.49 - 3.46	0.69 - 4.39	0.66 - 0.77	0.71 - 1.76
Clay content	%		6.63 - 11.42	3.88 - 5.40	0.83 - 4.33	1.14 - 11.9	0.89 - 2.08	0.87 - 1.00
AVS	μmol/g		0.003 - 4.37	0.11 - 2.60	0.01-0.43	0.002 - 0.02	0.003 – 8.37	0.002 – 0.11
Water								
O ₂	mg/L		5.17 - 9.65	5.86 - 8.94	7.22 - 10.8	7.94 - 11.4	8.42 - 12.1	8.98 - 12.7
EC	mS/cm		0.89 - 2.38	1.85 – 3.51	10.2 - 20.5	20.3 - 27.3	31.6 - 33.9	36.8 - 41.9
pH			7.65 - 8.07	7.81 - 8.10	7.80 - 8.09	7.75 - 8.15	7.95 - 8.26	7.97 - 8.48

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Table 3. Comparison between the minimum risk levels (MRLs; chronic exposure, except for Cu: intermedian exposure) (ATSDR, 2013) and the median and maximum concentrations of As, Cd, Cr, Cu, Pb and Zn detected in flounder, sole, shore crab and shrimp muscle.

	As	Cd	Cr	Cu (intermedian)	Pb ^a	Zn
MRL (mg/kg/day)	0.0003	0.0001	0.0009	0.0100	0.0036	0.30
MRL (mg/day) for person of 70 kg	0.021	0.007	0.063	0.070	0.250	21.0
Median/maximum concentration in flounder muscle (<i>P. flesus</i>) (µg/g ww)	2.38 / 13.4	<DL / 0.03	0.22 / 0.33	0.35 / 1.19	0.02 / 0.19	9.27 / 26.2
Maximum edible amount of flounder per day (g) for a person of 70 kg	8.83 / 1.57	- / 233	285 / 190	1996 / 587	10200 / 1325	2270 / 801
Median/maximum concentration in sole muscle (<i>S. solea</i>) (µg/g ww)	2.05 / 3.91	0.01 / 0.10	0.20 / 0.33	0.26 / 0.65	0.02 / 0.13	5.29 / 7.89
Maximum edible amount of sole per day (g) for a person of 70 kg	10.2 / 5.37	730 / 70.5	320 / 193	2651 / 1075	14900 / 1965	3970 / 2660
Median/maximum concentration in shore crab muscle (<i>C. maenas</i>) (µg/g ww)	6.19 / 13.4	0.17 / 0.94	0.19 / 0.32	12.2 / 45.0	0.03 / 0.13	49.2 / 70.6
Maximum edible amount of shore crab per day (g) for a person of 70 kg	3.39 / 1.57	41.2 / 7.48	334 / 195	57.6 / 15.6	7960 / 1990	427 / 297
Median/maximum concentration in shrimp muscle (<i>C. crangon</i>) (µg/g ww)	3.95 / 4.71	0.07 / 0.30	0.33 / 0.56	7.07 / 20.7	0.10 / 0.23	16.8 / 44.0
Maximum edible amount of shrimp per day (g) for a person of 70 kg	5.32 / 4.46	101 / 23.6	189 / 113	99.0 / 33.9	2550 / 1070	1250 / 477
Median/maximum concentration in blue mussel (<i>M. edulis</i>) (µg/g ww)	16.8/19.6	3.36/5.61	1.84/2.19	7.57/8.59	1.68/3.14	95.6/145
Maximum edible amount of mussel per day (g) for a person of 70 kg	1.25/1.07	2.08/1.24	34.2/28.8	92.5/81.5	149/79.6	220/145

^a WHO, 2007; guideline under discussion

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13 **Supplementary Information**

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16
 17 Figure SI1. Geographical variation (median and range, Kruskal-Wallis) in sediment metal
 18 concentrations (µg/g dw, n=3, Temse, n=7). Different letters indicate significant
 19 differences (p<0.05).

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22 Figure SI2. Geographical variation (median and range) in SPM metal concentrations

23 (µg/g dw, n=1-3)

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27 Figure SI3. Geographical variation (median and range) in SEM metal concentrations

28 (µg/g dw, n=4-8).

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31 Figure SI4A. Boxplots of geographical variation in metal concentrations in Polychaeta
 32 (µg/g dw; n=1-18). Different letters indicate significant differences (p<0.05).

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36 Figure SI4B. Boxplots of geographical variation in metal concentrations in Oligochaeta
 37 ($\mu\text{g/g dw}$; $n=1 - 6$). Different letters indicate significant differences ($p < 0.05$).

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Figure SI4C. Boxplots of geographical variation in metal concentrations in *Macoma balthica* liver (µg/g dw; n=2-16). Different letters indicate significant differences (p<0.05).

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51 Figure SI4D. Boxplots of geographical variation in metal concentrations in shrimp
 52 hepatopancreas (µg/g dw: n=7-20). Different letters indicate significant differences
 53 (p<0.05).

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56 Figure SI4E. Boxplots of geographical variation in metal concentrations in shrimp muscle

57 ($\mu\text{g/g dw}$; n=12-20). Different letters indicate significant differences ($p < 0.05$).

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59 Figure SI4F. Boxplots of geographical variation in metal concentrations in shore crab
 60 hepatopancreas (µg/g dw; n=5-15). Different letters indicate significant differences
 61 (p<0.05).

Figure SI4G. Boxplots of geographical variation in metal concentrations in shore crab gill ($\mu\text{g/g dw}$; $n=6-17$). Different letters indicate significant differences ($p < 0.05$).

Figure SI4H. Boxplots of geographical variation in metal concentrations in shore crab muscle ($\mu\text{g/g dw}$; $n=6-17$). Different letters indicate significant differences ($p<0.05$).

Figure SI4I Boxplots of geographical variation in metal concentrations in Chinese mitten crab hepatopancreas ($\mu\text{g/g dw}$; $n=3-11$). Different letters indicate significant differences ($p<0.05$).

Figure SI4J. Boxplots of geographical variation in metal concentrations in Chinese mitten crab gill ($\mu\text{g/g dw}$; $n=3-11$). Different letters indicate significant differences ($p<0.05$).

Figure SI4K. Boxplots of geographical variation in metal concentrations in Chinese mitten crab muscle ($\mu\text{g/g dw}$; $n=3-11$). Different letters indicate significant differences ($p<0.05$).

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90 Figure SI4L. Boxplots of geographical variation in metal concentrations in European
 91 flounder liver ($\mu\text{g/g dw}$; $n=4-22$). Different letters indicate significant differences ($p<0.05$).

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94 Figure SI4M. Boxplots of geographical variation in metal concentrations in European

95 flounder kidney ($\mu\text{g/g dw}$; $n=5-26$). Different letters indicate significant differences

96 ($p < 0.05$).

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100 Figure SI4N. Boxplots of geographical variation in metal concentrations in European

101 flounder gill ($\mu\text{g/g dw}$; $n=5-27$). Different letters indicate significant differences ($p<0.05$).

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103

104 Figure SI4N. Boxplots of geographical variation in metal concentrations in European
 105 flounder intestine (µg/g dw; n=5-27). Different letters indicate significant differences
 106 (p<0.05).

Figure SI4O. Boxplots of geographical variation in metal concentrations in European flounder muscle ($\mu\text{g/g dw}$; $n=6-29$). Different letters indicate significant differences ($p<0.05$).

Figure SI4P. Boxplots of geographical variation in metal concentrations in sole liver ($\mu\text{g/g dw}$; $n=5-10$). Different letters indicate significant differences ($p<0.05$).

Figure SI4Q. Boxplots of geographical variation in metal concentrations in sole kidney (µg/g dw; n=5-12). Different letters indicate significant differences (p<0.05).

Figure SI4R. Boxplots of geographical variation in metal concentrations in sole gill (µg/g dw; n=5-10). Different letters indicate significant differences (p<0.05).

Figure SI4S. Boxplots of geographical variation in metal concentrations in sole intestine (µg/g dw; n=5-13). Different letters indicate significant differences (p<0.05).

Figure SI4T. Boxplots of geographical variation in metal concentrations in sole muscle (µg/g dw; n=5-13). Different letters indicate significant differences (p<0.05).

143 Table SI1. Median and range of metal concentrations measured in sediment and SPM ($\mu\text{g/g dw}$) from the Scheldt estuary.

Sample	Ag	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
Sediment	0.10 <DL - 0.71	7.34 5.67 - 24.6	0.14 <DL - 2.27	1.57 0.72 - 10.1	24.7 6.44 - 76.1	1.88 0.26 - 44.2	2.59 1.10 - 17.0	9.4 2.51 - 76.9	26.0 8.07 - 390
SPM	0.99 <DL - 5.07	33.8 12.4 - 55.6	1.29 <DL - 4.98	11.0 4.19 - 19.4	132 72.3 - 343	40.3 22.0 - 97.6	24.1 13.3 - 34.6	66.7 25.0 - 153	331 211 - 866

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145 <DL: below detection limit

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147

148 Table SI2. Median and range of metal concentrations measured in invertebrates (µg/g dw) from the Scheldt estuary.

Sample	Organ	Ag	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
<i>Oligochaeta</i>										
	whole	0.47 <DL - 4.23	15.2 10.2 - 63.4	0.85 0.60 – 2.10	3.56 0.89 – 6.83	11.7 4.80 - 24.6	31.9 14.3 - 121	5.53 2.14 – 9.00	12.8 3.91 - 44.3	122 60.1 - 449
<i>Polychaeta</i>										
	whole	0.64 <DL - 1.69	15.2 5.43 - 54.2	1.00 <DL - 15.8	3.56 1.04 - 7.20	0.90 <DL - 16.9	21.0 8.67 - 41.1	3.83 1.76 - 33.7	0.94 <DL - 16.7	213 70.9 - 540
<i>L. littorea</i>										
		14.2 7.25 – 22.7	20.4 13.4 – 29.0	2.38 1.80 – 4.64	1.82 0.51 – 4.65	1.53 1.02 – 1.99	139 59.8 - 253	7.06 3.78 – 17.6	1.74 1.07 – 5.82	88.1 71.9 – 117
<i>M. balthica</i>										
	whole	1.54 0.55 – 7.73	10.7 6.34 - 17.6	0.69 <DL – 1.46	1.01 <DL - 2.71	2.67 0.75 - 8.94	29.0 12.2 – 66.9	2.15 0.97 - 4.45	2.30 0.72 - 11.5	379 187 - 752
<i>M. edulis</i>										
	whole	0.07 0.05 – 0.18	16.8 11.1 – 19.6	3.36 2.02 – 5.64	1.20 0.65 – 1.43	1.84 1.46 – 2.19	7.57 5.55 – 8.59	2.57 1.70 – 3.13	1.68 1.17 – 3.14	95.6 56.4 - 145
<i>Gammarus</i>										
	whole	1.67 1.20 – 1.89	8.67 <DL – 16.3	1.45 1.26 – 1.77	<DL <DL – 0.62	1.54 0.94 – 2.96	49.9 34.9 – 62.8	1.27 0.93 – 2.74	2.10 0.80 – 3.43	84.6 76.8 - 275
<i>C. crangon</i>										
	muscle	0.40 <DL – 0.73	21.0 4.37 - 50.6	0.16 <DL – 0.99	<DL <DL – 0.31	1.52 0.58 – 1.80	28.3 10.1 – 67.0	0.23 <DL - 3.28	0.17 <DL – 1.05	72.4 52.4 - 143
	liver	2.86 <DL - 13.3	33.9 <DL - 98.2	3.75 <DL – 41.9	0.47 <DL – 5.59	1.22 <DL - 3.58	157 20.1 - 1210	0.82 <DL - 23.1	0.46 <DL - 30.2	190 91.7 - 688
<i>E. sinensis</i>										
	muscle	0.46	5.59	0.08	0.15	0.84	112	0.13	0.13	211

Sample	Organ	Ag	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
		0.12 - 0.91	2.04 - 38.1	<DL - 0.29	<DL - 1.38	0.56 – 1.57	16.5 - 249	<DL - 2.36	<DL – 0.50	95.9 – 268
	liver	1.52	3.76	0.57	0.24	0.60	464	0.70	0.13	77.9
		0.18 - 6.16	0.50 - 14.6	<DL - 4.93	<DL - 1.64	<DL – 2.07	45.5 - 1960	0.14 - 5.17	<DL - 2.85	33.1 – 229
	gill	0.96	1.13	1.13	1.49	3.59	279	2.99	4.08	91.1
		0.23 – 3.08	0.33 – 4.32	0.33 - 4.32	<DL - 11.7	0.69 - 33.5	6.22 - 478	0.21 -11.0	0.37 - 26.8	55.3 - 185
<i>C. maenas</i>										
	muscle	0.85	25.0	0.72	1.00	0.84	53.3	0.42	0.13	221
		0.23 - 1.93	9.39 - 59.5	0.12 - 4.17	0.39 - 3.82	0.36 - 1.44	13.01- 200	<DL – 7.66	<DL - 0.56	96.2 – 328
	liver	1.74	16.9	6.6	1.82	0.59	119	1.25	0.28	221
		0.42 - 7.22	8.18 - 41.6	0.63 - 35.4	<DL - 6.26	<DL - 1.72	18.8 - 1095	<DL – 9.84	<DL - 1.82	87.2 – 389
	gill	0.84	19.4	2.01	3.25	2.47	156	1.53	2.11	213
		0.36 – 1.80	11.7 - 40.3	0.44 - 7.43	0.81 - 8.21	<DL - 8.57	56.0- 342	0.49 – 8.10	0.16 - 7.60	102- 394

149 <DL: below detection limit

Sample	Organ	Ag	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
S. solea	muscle	<DL	11.0	<DL	<DL	0.76	1.08	<DL	<DL	24.0
		<DL – 0.35	3.22 – 41.9	<DL – 0.48	<DL – 0.47	0.60 – 1.56	0.44 – 3.11	<DL – 0.65	<DL – 0.61	17.0 – 37.7
	liver	2.38	6.42	<DL	<DL	0.85	133	0.30	0.43	117
		<DL – 10.3	<DL – 23.1	<DL – 1.49	<DL – 1.54	<DL – 7.68	12.1 – 400	<DL – 2.37	<DL – 9.31	69.5 – 322
	gill	<DL	0.50	<DL	<DL	1.38	4.19	0.46	1.13	112
		<DL – 0.82	<DL – 6.97	<DL – 0.53	<DL – 4.01	<DL – 5.13	0.55 – 12.1	<DL – 2.87	<DL – 3.88	72.9 - 248
	kidney	<DL	6.98	<DL	<DL	1.02	7.50	0.14	0.36	116
		<DL – 0.98	<DL – 22.1	<DL – 0.25	<DL – 1.16	<DL – 3.06	3.70 – 37.4	<DL – 1.61	<DL – 5.38	11.5 – 178
	intestine	<DL	5.37	<DL	0.14	1.03	14.7	0.60	1.25	101
		<DL – 0.60	<DL – 17.6	<DL – 0.83	<DL – 4.35	<DL – 5.50	3.68 – 65.0	<DL – 4.16	<DL – 7.44	70.1 - 189
P. flesus	muscle	<DL	10.6	<DL	<DL	0.97	1.41	<DL	0.07	34.3
		<DL - 0.51	0.50 - 64.2	<DL - 0.16	<DL - 0.31	0.42 - 1.59	0.59 - 5.72	<DL - 0.85	<DL - 0.90	18.6 - 126
	liver	0.06	7.57	0.75	0.43	0.63	37.8	0.07	0.22	139
		0.03 - 0.65	0.50 - 55.5	<DL - 2.92	<DL - 2.04	<DL – 4.85	12.4 – 133	<DL - 1.65	<DL – 1.53	84.4 - 280
	gill	<DL	2.37	0.08	0.07	1.61	2.62	0.51	1.28	169
		<DL - 0.22	<DL – 13.1	<DL - 1.10	<DL – 1.30	<DL - 5.81	<DL - 16.4	<DL - 4.97	<DL - 9.27	92.0 - 559
	kidney	<DL	6.11	0.31	0.46	0.71	6.79	0.55	0.46	198

Sample	Organ	Ag	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
		<DL - 0.58	<DL - 41.5	<DL - 1.68	<DL - 3.14	<DL - 5.32	2.98 - 20.5	<DL - 4.40	<DL - 37.5	120 - 574
	intestine	<DL	5.33	0.48	<DL	0.69	11.1	0.19	0.53	104
		<DL - 0.26	<DL - 21.7	<DL - 5.14	<DL - 18.8	<DL - 15.8	4.06 - 51.4	<DL - 12.4	<DL - 37.5	51.6 - 498
<i>O. eperlanus</i>	muscle	<DL	7.52	<DL	<DL	0.81	0.72	0.11	0.11	26.6
		<DL - 0.23	5.39 - 10.2			0.61 - 1.07	0.59 - 1.71	<DL - 0.28	<DL - 0.50	20.5 - 38.2
	liver	<DL	10.4	<DL	<DL	0.71	5.03	<DL	0.26	74.1
		<DL - 0.39	7.55 - 24.3	<DL - 0.50	<DL - 0.28	<DL - 1.44	2.90 - 7.52	<DL - 0.50	<DL - 1.18	41.5 - 127
	gill	<DL	<DL	<DL	<DL	3.22	3.26	1.19	3.72	136
			<DL - 6.01		<DL - 2.75	<DL - 9.75	1.13 - 8.62	<DL - 4.62	<DL - 13.5	117 - 196
	kidney	<DL	4.72	0.68	<DL	<DL	3.35	<DL	<DL	616
			<DL - 9.38	<DL - 1.32			<DL - 6.65			310 - 922
	intestine	<DL	<DL	<DL	<DL	<DL	9.19	<DL	<DL	96.1
			<DL - 7.75	<DL - 0.37		<DL - 0.49	2.85 - 153	<DL - 8.66	<DL - 17.0	62.7 - 332
<i>M. scorpius</i>	muscle	<DL	10.0	<DL	<DL	0.70	0.88	<DL	<DL	33.3
		<DL - 0.50	5.53 - 15.3	<DL - 0.26	<DL - 0.16	0.59 - 1.14	0.62 - 1.78	<DL - 0.24	<DL - 0.33	27.6 - 72.8
	liver	0.27	8.19	0.21	<DL	0.64	13.0	<DL	0.07	77.7
		0.07 - 1.53	4.38 - 20.5	0.10 - 0.57	<DL - 0.30	0.55 - 1.59	6.49 - 19.4	<DL - 0.16	<DL - 0.17	59.7 - 134
	gill	0.27	11.6	<DL	<DL	0.95	7.31	0.09	0.46	62.9
		<DL - 0.56	<DL - 18.3	<DL - 0.26	<DL - 0.22	0.81 - 1.35	5.64 - 12.2	<DL - 1.15	<DL - 0.97	51.3 - 82.2

Sample	Organ	Ag	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
A. anguilla	kidney	<DL	19.3	<DL	<DL	0.39	9.65	<DL	<DL	130
			13.3 – 59.2			<DL – 3.92	6.95 – 33.7		<DL – 5.04	74.7 - 817
	intestine	0.19	11.2	0.97	<DL	0.64	31.3	<DL	0.25	103
		<DL – 0.85	<DL – 16.5	<DL – 4.11	<DL – 1.24	<DL – 9.51	6.45 - 104	<DL – 1.23	<DL – 1.64	83.9 - 279
	muscle	<DL	1.53	<DL	<DL	0.69	0.78	<DL	<DL	41.9
			1.15 – 2.10	<DL – 0.19	<DL – 0.17	0.63 – 1.41	0.64 – 1.11	<DL – 0.20	<DL – 0.37	27.8 – 48.7
	liver	<DL	3.47	0.63	0.20	0.70	40.8	<DL	0.71	146
		<DL - 0.74	1.84 – 5.19	0.35 – 1.08	<DL – 0.31	0.63 – 0.89	24.2 – 84.4		0.63 – 1.28	108 – 199
	gill	<DL	<DL	0.56	0.10	1.51	5.07	0.33	0.90	137
			<DL – 1.40	<DL – 1.04	<DL – 0.25	0.85 – 1.80	3.32 – 9.72	<DL – 0.47	0.72 – 1.32	112 – 175
kidney	<DL	<DL	0.92	0.22	0.71	7.25	0.30	0.97	171	
	<DL – 1.40		<DL – 4.43	<DL – 0.66	0.55 – 0.85	4.83 – 10.4	<DL – 0.46	0.43 – 5.02	129 - 198	
intestine	<DL	1.44	<DL	<DL	0.72	15.6	<DL	0.57	158	
	<DL – 4.04	<DL – 3.86	<DL – 0.32	<DL – 0.93	0.55 – 0.84	4.23 – 45.9	<DL – 0.82	0.21 – 2.21	124 – 275	
S. lucioperca										
	muscle	<DL	6.06	<DL	<DL	0.81	1.21	0.16	0.12	26.9
			2.79 – 18.8	<DL – 0.15	<DL – 0.11	0.57 – 1.13	0.41 – 2.79	< DL – 0.28	<DL – 0.28	16.3 – 42.4
	liver	<DL	2.46	0.12	0.10	0.34	12.1	<DL	<DL	105
			<DL – 10.9	<DL – 0.41	<DL – 0.26	<DL – 0.78	5.15 – 35.8	<DL – 0.17		95.5 – 144
	gill	<DL	<DL	<DL	<DL	2.01	4.49	0.18	1.73	76.0

Sample	Organ	Ag	As	Cd	Co	Cr	Cu	Ni	Pb	Zn
			<DL – 4.98			0.71 – 3.09	2.64 – 5.84	<DL – 2.75	0.47 – 3.84	67.4 -116
	kidney	<DL	8.89 8.72 – 24.5	<DL	<DL	<DL <DL – 1.49	6.95 5.97 – 8.54	<DL	<DL <DL – 1.21	225 196 – 259
	intestine	<DL	<DL <DL – 12.5	<DL	<DL	<DL	7.64 0.05 – 16.7	<DL	<DL <DL -9.06	107 75.9 - 236
<i>T. luscus</i>										
	muscle	<DL	8.68 6.52 – 11.5	<DL <DL – 0.22	<DL	0.76 0.64 – 1.34	1.36 0.64 – 1.67	<DL <DL – 0.26	<DL <DL – 0.41	19.8 16.8 – 23.1
	liver	<DL	7.11 <DL – 10.3	<DL	<DL	0.61 <DL – 0.64	4.16 2.07 – 8.09	0.09 <DL – 2.03	<DL <DL – 1.95	35.2 18.8 – 77.9
	gill	<DL	<DL <DL – 11.3	<DL	<DL <DL – 1.00	<DL	4.66 3.96 – 6.38	<DL <DL – 2.93	<DL <DL – 4.29	107 92.2 - 138
<i>D. labrax</i>										
	Muscle	<DL	3.94 0.92 – 6.30	<DL	<DL <DL – 0.14	0.97 0.46 – 1.64	1.45 1.12 – 2.93	0.11 <DL – 0.28	0.10 <DL – 0.18	33.2 23.9 – 45.6
	Liver	0.34 <DL – 1.57	7.21 0.50 – 8.56	0.37 <DL – 0.51	0.07 <DL – 0.70	1.47 0.94 – 1.63	25.4 10.4 – 38.5	0.13 <DL – 0.38	0.23 <DL – 1.18	155 133 – 215
	gill	<DL	<DL	<DL	<DL	1.31 <DL – 2.18	1.73 <DL – 2.32	<DL	<DL <DL – 3.76	134 113 - 153

Table SI4. SEM-AVS concentrations (median, min. and max. in $\mu\text{mol/g}$) in sediments of the Scheldt estuary ($4 < n < 8$). Total: Cd+Cu+Ni+Pb+Zn

Location	As	Cd	Co	Cr	Cu	Ni	Pb
Temse	-2.25 -4.31 - -0.18	-2.30 -4.36 - -0.25	-2.26 -4.32 - -0.21	-1.75 -3.90 - 0.05	-2.10 -4.21 - 0.01	-2.08 -4.19 - -0.13	-2.00 -4.22 - 0.22
Antwerp	-0.06 -0.18 - -0.04	-0.10 -0.23 - -0.10	-0.09 -0.22 - -0.09	0.04 -0.06 - 0.09	0.06 -0.15 - 0.27	-0.05 -0.18 - -0.03	0.00 -0.17 - 0.17
Bath	0.008 -0.39 - 0.02	-0.03 -0.43 - -0.01	-0.02 -0.42 - -0.003	0.09 -0.32 - 0.27)	-0.002 -0.39 - 0.04	0.02 -0.39 - 0.09	0.00 -0.40 - 0.40
Waarde	0.01 -0.002 - 0.01	-0.002 -0.02 - -0.001	0.001 -0.01 - 0.003	0.15 0.08 - 0.19	0.002 -0.012 - 0.01	0.06 0.02 - 0.07	0.00 -0.01 - 0.01
Terneuzen	-0.03 -0.04 - 0.02	-0.06 -0.06 - -0.003	-0.05 -0.06 - 0.002	0.10 0.08 - 0.14	-0.05 -0.06 - 0.001	0.02 -0.01 - 0.05	-0.00 -0.05 - 0.05
Vlissingen	-0.002 -0.09 - 0.01	-0.02 -0.11 - -0.01	-0.02 -0.11 - 0.000	0.12 -0.02 - 0.31	-0.02 -0.10 - -0.001	0.04 -0.07 - 0.11	-0.00 -0.10 - 0.10

Table SI5. Statistics for all significant spearman correlations between sediment concentrations and tissue (liver or whole). Correlations are made with total sediment concentrations and Simultaneous Extracted Metals (SEM).

		Total Concentration		SEM	
Species	Element	r	p	r	p
<i>Oligochaeta</i>					
	Pb	0.983	0.0026	0.965	0.0294
	Zn	0.991	0.0011	0.998	0.0001
<i>P. flesus</i> Liver					
	Cr			0.974	0.0050
	Pb	0.943	0.0164	0.915	0.0294
<i>P. flesus</i> Kidney					
	Cr			0.897	0.0388
	Zn			0.888	0.0443
<i>P. flesus</i> Gill					
	Cu				0.0037
	Zn	0.986	0.0020	0.995	0.0004
<i>P. flesus</i> Muscle					
	Cr	0.892	0.0417	0.975	0.0048
	Cu	0.966	0.0075	0.963	0.0086
	Ni			0.952	0.0127
	Zn	0.885	0.0460	0.937	0.0190
<i>P. flesus</i> Intestine					
	Ni			0.975	0.0047
	Pb	0.984	0.0025	0.969	0.0006
	Zn	0.931	0.0213	0.975	0.0047

N=5