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ORIGINAL PAPER

TOXIC METALS (Cd, Pb) IN FLATFISH, MOLLUSC *MACOMA BALTHICA*, WATER AND SEDIMENTS FROM THE SOUTHERN BALTIC SEA

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Abstract

Environmental pollution is a worldwide problem, with toxic metals being among the most noxious pollutants. Aquatic organisms can accumulate toxic elements from their environment through different pathways, including water, diet and sediments. The aim of the study was to determine the distribution of toxic metals (Cd and Pb) in tissues of three species of flatfish (*Platichthys flesus*, *Pleuronectes platessa*, *Scophthalmus maximus*), and in their environment (water, sediment and their prey mollusc *Macoma balthica*) in four regions of the southern Baltic Sea, in order to identify the most important pathways of metal uptake in these fish. The concentrations of toxic metal were measured in a graphite furnace by atomic absorption spectrometry. Toxic metal concentrations in flatfish liver were significantly higher than in the muscle tissue. The liver was the target organ for Cd and Pb accumulation. The flatfish liver, *M. balthica*, sediment, and water from the Gulf of Gdansk contained higher level of Pb than did the samples from the central Baltic Sea coast. The Pb concentrations in the liver of *P. flesus* and *P. platessa* showed positive correlations with Pb in the soft tissue of *M. balthica,* sediment and sea water. The high correlation coefficient values for Pb suggest that flatfish took up this metal through the food chain from molluscs. In turn, *M. balthica* took up Pb and Cd from sediment and water, which was confirmed by the high correlation coefficients for these metals between this mollusc and sediment and water. The positive correlations for concentrations Pb and Cd in sediment and in water indicate the next stage of the pathway along which the metals travel in the Baltic Sea environment.

Keywords: Baltic Sea, toxic metals, flatfish, *Macoma balthica*, sediment, water.

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INTRODUCTION

Marine ecosystems store metals transported by rivers and the atmosphere from local and remote industrial sources and population centers. The southern Baltic Sea, and particularly the Gulf of Gdansk, is one of the most ecologically threatened areas of this sea (HELCOM 2003). Coastal habitats of the Gulf of Gdansk are at risk from trace elements of an anthropogenic origin, as this area is polluted by the influx of contaminants from the Vistula River (Beldowski, Pempkowiak 2003, Żbikowski et al. 2006). The metal concentrations in the Ustecko-Łebskie site on the central Baltic Sea coast are low in comparison to those observed in the region of the Gulf of Gdansk. However, the central Baltic Sea coast is also contaminated by water from the Wieprz River. The Wieprz transports significant amounts of metals to the Baltic Sea, including cadmium of 0.05 to 0.49 mg dm^{-3} and lead from 0.03 to 0.30 mg dm^{-3} (Bojakowska et al. 2010). Most metals introduced into aquatic environments can be accumulated by organisms by direct contact with polluted environments or from the food chain through a series of complex physical, chemical, and biological processes. Many benthic organisms accumulate trace metals at levels reflecting those in the environment. Sediments can be a direct source of metals to sediment-dwelling or sediment-ingesting animals. Different species accumulate metals to different degrees. Benthic organisms are widely thought to be exposed to elevated heavy metal concentrations (Hendożko et al. 2010), which is why various benthic fish species are recommended for use as biomonitors of trace element pollution in marine ecosystems (Szefer at al. 2002). Benthic fish species inhabit the Baltic Sea, for example flatfish: flounder (*Platichthys flesus*), plaice (*Pleuronectes platessa*), and Baltic turbot (*Scophthalmus maximus*). These species are bottom-dwelling fish that typically prefer fine-grained to sandy sediments, where they feed mostly on benthic invertebrates, including the mollusc *Macoma balthica*. This mollusc comprises about 30% of the total weight of the food of flatfish (Florin, Lavados 2010). Beside molluscs, they feed on polychaetes and other invertebrates of the sea bed (invertebrate annelids, copepods), and small fish. The habitat and feeding preferences of flatfish render them particularly vulnerable to environmental pollution. They are also considered to be particularly sensitive to the effects of pollution and other types of habitat degradation (Skerritt 2010) and, consequently, they are valuable sentinel species, often used to monitor estuarine water quality. Additional advantages of these sedentary species are that they are common, easy to catch, and large enough for individual analyses.

The aim of the study was to determine the distribution of toxic metals (Cd, Pb) in tissues of three species of flatfish (*P. flesus*, *P. platessa*, *S. maximus*), and in their environment (water, sediment, and main prey, the mollusc *M. balthica*), in order to identify the most important pathways of metal uptake in flatfish.

MATERIALS AND METHODS

The study materials comprised the flatfish flounder (*Platichthys flesus)*, plaice (*Pleuronectes platessa)*, turbot (*Scophthalmus maximus*), the mollusc *M. balthica*, water and sediment collected in November 2013 and February 2014 at four locations in the southern Baltic Sea (Figure 1). On each date

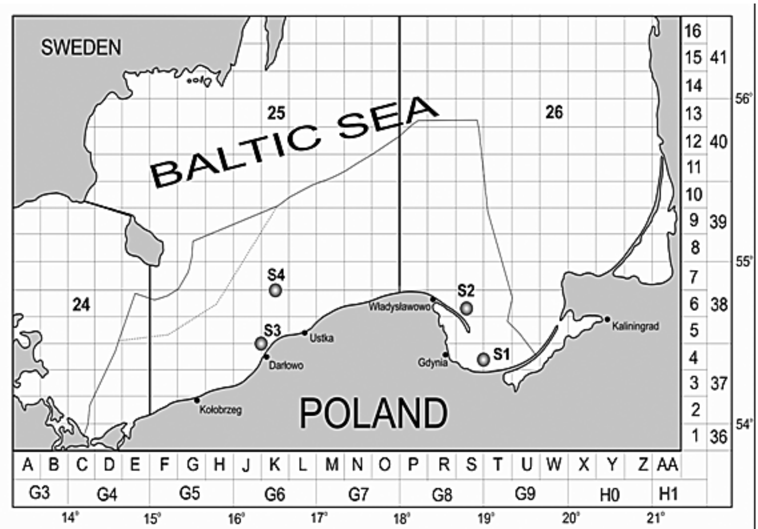

Fig. 1. Sampling sites in the southern Baltic Sea: S1 – Vistula River estuary, site in the Gulf of Gdansk, S2 – outside the Gulf of Gdansk, S3 – near the central coast of Baltic Sea, S4 – away from the central coast of Baltic Sea

and in each location, samples were obtained from three hauls. Sampling was performed in a polluted region within the Vistula River estuary: in (S1) and outside of the Gulf of Gdansk (S2); off the coast of the central Baltic Sea (S3), further away from the central Baltic Sea coast (S4). The samples collected from these areas comprised 74 flounder, 51 plaice, 50 turbot, 20 mollusc individuals (*M. balthica*), and 29 samples each of sediment and water. The fish and invertebrates were sorted according to species, sacrificed, packed into bags and frozen at -18°C. The fish were gutted before freezing. Morphometric and biological characteristics of flatfish and benthic invertebrates from the southern Baltic Sea are described in Table 1.

Sediment was collected from the four sampling sites of the Baltic Sea using polyvinyl chloride (PVC) corers. The cores were stored at 4°C until they were analyzed in the laboratory. Water samples were taken into 1 L acid-leached polyethylene bottles from the same sampling sites where flatfish and sediment were obtained. The samples were acidified with 2 ml of concentrated HNO_3 and stored at room temperature until analysis.

The samples were prepared in a clean room (US Federal Standard 209 A Class 10 000). Fish samples were prepared from single individuals. The

Table 1

Morphometric and biological characteristics of flatfish and benthic invertebrates from the southern Baltic Sea

Species	*N*	Average length (cm) *x* ± SD (min-max)	Average wet weight (g) *x* ± SD (min-max)	FCF *x* ± SD (min-max)
Platichtys flesus	74	28.5 ± 5.0 (16 - 39)	301 ± 161 (33 - 718)	1.12 ± 0.12 (0.9 - 1.2)
Pleuronectes platessa	51	29.5 ± 5.2 (19 - 41)	253 ± 117 (60 - 533)	0.88 ± 0.09 (0.8 - 1.1)
Scophthalmus maximus	50	27.4 ± 3.4 (20 - 39)	366 ± 135 (186 - 920)	1.67 ± 0.19 (1.2 - 1.9)
Macoma balthica	20	1.4 ± 0.3 (0.8 - 2.0)	0.12 ± 0.05 (0.05 - 0.20)	5.0 ± 1.6 (3.4 - 9.8)

N – number of specimens, SD – standard deviation, FCF – Fulton's body condition factor

muscle and liver were excised from the flatfish and homogenized separately. The soft tissues of *M. balthica* were separated from the shells. Batches from 1 to 2 g of fish and invertebrate tissue were weighed for analysis. The first 5-cm section of each sediment core was used in this study. The sections were dried at room temperature to constant weight, then sieved through polyethylene sieves, and only the fraction smaller than 63 mm was analyzed. About 0.5 g of sediment was weighed for analysis. The muscle and liver of flatfish, soft tissues of *M. balthica*, and sediments were mineralized with 65% nitric acid and 30% hydrogen peroxide in MARS-5 and MDS-2100 microwave ovens. The metals in the seawater samples were concentrated preliminarily by extraction to the organic phase with methyl isobutyl ketone (MIBK) using the method described by Danielson et al. (1982).

Metal concentrations were determined with atomic absorption spectrophotometry using a Perkin-Elmer 4100 atomic absorption spectrometer equipped with a graphite furnace. Metal analysis was performed in two parallel samples and the absorbance was measured twice taking the standard deviation of less than 10%. Quality assurance for each series of analyses was provided by the parallel analysis of Certified Reference Material - BCR 422 cod muscle, Standard Reference Material 1566b oyster tissue, and IAEA 433 marine sediment. Throughout the validation process, the limits of detection were set as follows: Cd – 0.1 μg L^{-1}, Pb – 1.0 μg L^{-1}, and recovery for the applied method was Cd – 105.9%, Pb – 94.5%.

The following biological parameters were recorded for the examined fish: total body length, total body weight, body condition factor. For each fish, Fulton's body condition factor (FCF) was calculated as follows:

$$FCF = W\,L^{-3} \cdot 100,$$

where: W – total weight, and L – total length of the fish.

The data were processed statistically using Statistica 8.0 for Windows (Copyright© StatSoft). Relationships between the concentrations of metals in tissues of flatfish, *M. balthica*, and that of the background environment (e.g. water and sediment) were evaluated with linear regression analysis. Analysis of differences depending on a sampling site and on a fish species were statistically analyzed by one-way ANOVA with the Tukey's test. All hypotheses were tested at a significance level of $p < 0.05$.

RESULTS

The metal concentrations in flatfish muscle tissue were significantly lower than in the liver, and often at the limits of detection of the method applied (Table 2). The liver of flounder and plaice had more Cd than the liver of turbot. This element was prevalent in the liver of flounder from the central Baltic Sea coast (S3 and S4). All of the flatfish liver samples had low levels of Pb (0.013 - 0.047 mg kg^{-1}). The metal concentrations in the sediment were the highest in region S1 (Gulf of Gdansk), and they decreased in the other regions in the following order: S2, S4 > S3 (Table 2). The concentrations of Cd and Pb in the sediment from region S1 were two to three times higher than those at sampling sites S2, S3 and S4. The metal concentrations in seawater were low in comparison to those in the sediment. Nevertheless, significant differences were noted between the studied regions (Table 2). Water from the Vistula River mouth (S1) contained the highest concentrations of Cd and Pb. In the other regions, the levels of these elements were significantly lower and decreased as follows: S2 > S3 > S4. Toxic metals occurred in the soft tissue M. *balthica* at high levels (Table 2). The levels of Cd and Pb ranged from 0.111 to 0.188 mg kg^{-1} and from 0.218 to 0.530 mg kg^{-1}, respectively. The concentrations of toxic metals in soft tissue of *M. balthica* differed between the regions, and the highest amounts of the metals were detected in individuals from region S1, while significantly lower levels were noted in the molluscs from regions S2, S3 and S4.

DISCUSSION

The gradation of concentrations of Cd in the flatfish's trophic chain was as follows: *M. balthica* > flatfish liver > sediment > flatfish muscle > water; whereas for Pb the following order was established: sediment > *M. balthica* > flatfish liver > flatfish muscle > water. The muscles of flatfish contained more Pb than Cd, while the opposite was noted in the liver. The concentrations of Cd and Pb in the liver were significantly higher than those in muscles. Similar results have been reported by other authors (Bustamante et al.

Table 2

Mean concentrations of metals in flatfish, sediment, *Macoma balthica* (mg kg^{-1} w.w.) and water (µg L^{-1}) from four regions of the southern Baltic Sea

Species	Tissue	Metal	S1	S2	S3	S4
Flounder (*Platichthys flesus*)	muscle	Cd	0.002±0.001	0.001±0.001	0.001±0.001	0.001±0.001
		Pb	0.012±0.009	0.010±0.008	0.007±0.002	0.009±0.004
	liver	Cd	$0.122\pm0.019^{a*}$	$0.072\pm0.032^{b*}$	$0.169\pm0.077^{c*}$	$0.172\pm0.044^{c*}$
		Pb	$0.038\pm0.019^{a*}$	$0.021\pm0.004^{b*}$	$0.013\pm0.006^{c*}$	$0.020\pm0.016^{bc*}$
Plaice (*Pleuronectes platessa*)	muscle	Cd	0.002±0.001	0.001±0.001	0.001±0.001	0.001±0.000
		Pb	0.013±0.002	0.011±0.006	0.010±0.003	0.011±0.004
	liver	Cd	$0.135\pm0.073^{a*}$	$0.074\pm0.015^{b*}$	$0.117\pm0.041^{a*}$	$0.082\pm0.054^{b**}$
		Pb	$0.047\pm0.020^{a*}$	$0.025\pm0.012^{b*}$	$0.014\pm0.007^{c*}$	$0.022\pm0.005^{b*}$
Turbot (*Scophthalmus maximus*)	muscle	Cd	0.002±0.001	0.001±0.000	0.001±0.001	0.001±0.000
		Pb	0.032±0.024	0.018±0.012	0.010±0.003	0.012±0.003
	liver	Cd	$0.023\pm0.001^{a**}$	$0.022\pm0.010^{a**}$	$0.062\pm0.003^{b**}$	$0.023\pm0.010^{a***}$
		Pb	$0.037\pm0.022^{a*}$	$0{,}013\pm0.011^{b**}$	$0.044\pm0.018^{a**}$	$0.022\pm0.020^{ab*}$
Sediments		Cd	0.149 ± 0.048^{a}	0.041 ± 0.025^{b}	$0.034\pm0\ 017^{b}$	0.020 ± 0.014^{c}
		Pb	4.40 ± 1.181^{a}	2.61 ± 0.365^{b}	1.70 ± 0.310^{c}	2.41 ± 0.567^{b}
Water		Cd	0.156 ± 0.078^{a}	0.08 ± 0.003^{b}	0.06 ± 0.017^{c}	0.044 ± 0.008^{d}
		Pb	0.127 ± 0.038^{a}	0.097 ± 0.022^{b}	0.053 ± 0.014^{c}	0.078 ± 0.012^{d}
Macoma balthica		Cd	0.188 ± 0.015^{a}	0.120 ± 0.028^{b}	0.111 ± 0.035^{b}	0.112 ± 0.034^{b}
		Pb	0.530 ± 0.085^{a}	0.372 ± 0.142^{b}	0.218 ± 0.080^{c}	0.289 ± 0.092^{b}

Regions: S1-Vistula River estuary in the Gulf of Gdansk, S2 – site outside of Gulf of Gdansk, S3 – coast central Baltic Sea, S4 – outside of central Baltic Sea.
Means in horizontal rows labeled with the letters *a*, *b*, *c*, *d* are significantly different respectively for Cd and Pb concentrations in regions at $p < 0.05$, after one-way ANOVA with the Tukey's test.
Means in vertical rows labeled with the asterisk *, **, *** are significantly different respectively for Cd and Pb concentrations in species at $p < 0.05$.

2003, Durrieu et al. 2005, Polak-Juszczak 2010, 2013, Staszowska et al. 2013). Al-Yousuf et al. (2000) suggest that metal accumulation in the liver (mainly of cadmium) is increased due to its tendency to form compounds with proteins (metallothioneines), whose concentrations in the liver are higher. The results indicate the flatfish's organ that accumulated the most metal was the liver, which contained as much as several-fold more Cd and Pb than muscle tissue. The levels of these metals in the liver differed depending on a species. Flounder and plaice accumulated more metals, especially Cd, in the liver than did turbot. Differences in the concentrations of metals between the species result from the type of food they ingest. The eating preferences of flatfish can be highly variable depending on the season, species, developmental stage and size of a fish (Florian Lavados 2010). Flounder and plaice feed on molluscs, polychaetes, eggs of fish and insects. In contrast, turbot's diet consists mainly of small fish.

Levels of the two toxic metals in the liver also differed depending on a region of the Baltic Sea where sampling was carried out. The levels of Cd accumulated in the liver of flounder from regions of the southern Baltic Sea were ordered as follows: S4, S3 > S1 > S2, and conversely in the liver of plaice S1, S3 > S2, S4. The concentrations of Pb in the liver of flounder and plaice were on higher levels in region S1, and significantly lower in regions S2, S4 > S3. Differences in the levels of the metals between the regions are a consequence of the pollution of the Baltic Sea in each location. Gdansk Bay is particularly contaminated by water from the Vistula River. The central Baltic Sea coastal waters are contaminated (mainly by Cd) by water from the River Wieprz. However, the influx of heavy metals is much smaller there than from the Vistula River. The sediment, water and *M. balthica* from the S1 region (Gulf of Gdansk) also contained significantly higher concentrations of Cd and Pb than did the samples from regions S2, S3, and S4. These findings coincide with those reported in earlier studies on metals in the sediment and macrozoobenthos from this area of the Baltic Sea (Pempkowiak et al. 1999, Glasby et al. 2004, Sokołowski et al. 2007, Hendożko et al. 2010). Region S1 (Gulf of Gdansk) is located at the mouth of the longest river in Poland, the Vistula River, which flows through the entire country, collecting industrial, agricultural, and municipal pollution, before it flows into the Baltic Sea (Szefer et al. 1995, Żbikowski et al. 2006, Sokołowski et al. 2007).

High concentrations of Pb in sediment, water and *M. balthica* from the Gulf of Gdansk (S1) corresponded to high concentrations of this metal in the liver of flatfish from this region (Table 3). Positive correlations in concentrations of Pb in livers of flounder and plaice, and in their diet, i.e. *M. balthica*, indicated that these fish took up metals from the environment through the food chain from the mollusk. Farag et al (2007) also suggested that the food chain pathway could be important for metal uptake in fish. Positive correlations were also detected between Pb concentration in flatfish liver and sediment and seawater (Table 3**)**. This means that bioaccumulation occurs within sediment and water, and the livers of flounder and plaice are good indicators

Table 3

Relationship between metal levels in flatfish tissues and *M. balthica*, water, sediment (correlation coefficients)

Tissue	Species	*Macoma balthica*	Water	Sediment
Muscle	*Platichthys flesus*	Cd (0.988) Pb(0.961)	Cd (0.912) Pb (0.995)	Cd (0.978) Pb (0.912)
	Pleuronectes platessa	Cd (0,948) Pb (0.935)	Cd (0.978) Pb (0.907)	Cd (0.941) Pb (0.993)
	Scophthalmus maximus	Cd (0.988) Pb (0.972)	Cd (0,912) Pb (0.907)	Cd (0.978) Pb (0.961)
Liver	*Platichthys flesus*	Pb (0.948)	Pb (0.907)	Pb (0.999)
	Pleuronectes platessa	Pb (0.963)	Pb (0.918)	Pb (0.999)
Macoma balthica			Cd (0.994) Pb (0.987)	Cd (0.987) Pb (0.969)
Water				Cd (0.974) Pb (0.919)

Levels of significance $p < 0.05$

of Pb in the Baltic Sea environment. Whereas, positive correlations between concentrations of toxic metals in *M. balthica* and in sediment and water suggest that this mollusk can be a good bioindicator of Pb and Cd in the Baltic Sea (Table 3). Positive correlations between the sediment and *M. balthica* from the southern Baltic Sea were also reported by HENDOŻKO et al. (2010).

The current results also confirmed the dependence between metal concentrations in sediment and water from the southern Baltic Sea, with high values of correlation coefficients. Positive correlations were also detected between concentrations of Cd and Pb in the muscles of flatfish and *M. balthica*, sediment and seawater (Table 3). However, very low concentrations of these metals in the muscle tissue (often at the limits of detection) do not suggest that muscles are a good indicator of environmental pollution by metals.

Other experiments on the plaice, *Pleuronectes platessa*, showed that food rather than water was the source of metals (including cadmium) for fish (BURGOS, RAINBOW 2001). In our study, only one kind of food for flatfish was analyzed (*M. baltica*). Adult individuals feed also on polychaetes, molluscs, crustaceans and small fish. Identification of the source or sources of Cd in the liver of flatfish from the Baltic Sea remains an open question for discussion and further investigations are needed, especially regarding the toxic element cadmium, in flatfish and in other fish species from the Baltic Sea.

CONCLUSIONS

The concentrations of Cd and Pb in the liver and muscle of flatfish from the southern Baltic Sea were varied and depended on a species of fish and region of sampling. The organ which accumulated most of metals was the liver of flounder and plaice. The livers of flatfish from the Gulf of Gdansk contained the most Pb, while Cd was on the highest level in the livers of fish from the central coast of the southern Baltic Sea. Inter-regional differences in the concentrations of Cd and Pb also occurred in soft tissue of *M. balthica*, in water and in sediment. Higher levels of these metals were in samples from the Gulf of Gdansk. The results of the present study indicate that the food chain is the main pathway for accumulation Pb in livers of flatfish. Flatfish take up Pb along with ingested *M. balthica* and also directly with sediment and sea water. Mollusc (*M. balthica*), in turn, takes up Pb from water and sediment. Finally, the flatfish can collect Pb directly from food and also from sediment and water, and indirectly from sediments and water via *M. baltica*. The transport pathway of metals into M. *balthica* includes the following stages: 1) directly from sediment and water to *M. balthica*, 2) indirectly from the sediments into the water, 3) from the water to *M. balthica*.

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