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Trace metals in the shells of blue mussels (*Mytilus edulis*) from the Poland coast of Baltic sea

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Abstract In this study, bioaccumulation of the heavy metals (Hg, Pb, Cd, Cu, Zn, Cr, Ni, Fe, Mn, V, Li, Al) in the shells of *Mytilus edulis* were investigated. Shells of *Mytilus edulis* were collected in May to September 2005 from 12 stations on the Polish coast of Baltic Sea. At each sampling sites 17–330 different size of shells were collected during 2005, respectively. Due to heavy industrial activities in the region, the bay has the polluted coastal waters of Poland. Shells were analysed by ICP AES and Coleman MAS 50 CV atomic absorption spectrometer. As expected from the similarity found between the relationships of metal content length and dry the concentrations of the different metals in the shells did not seem to depend on the shell length. No significant differences were detected in metal concentration between different shell lengths. Given their geographical distributions, as well as their abilities to show up spatial and temporal changes in metal bioavailabilities confirmed here, it is concluded that the mussel *M. edulis* a suitable candidate to be used in biomonitoring surveys of the Poland coast of Baltic.

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Introduction

Trace metals in coastal waters are derived from a variety of natural and anthropogenic sources. Urban and industrial developments along the coastal areas, rivers and estuaries contribute to the major part of the anthropogenic metal load of the sea (Ridgwig et al. 2003; Cobelo-Garcia et al. 2004). Trace metals can be bioaccumulated in aquatic organisms. Due to the concerns over this accumulation and their toxic effects to humans consuming these organisms, monitoring programmes for metals in environmental (biotic and abiotic) samples have been widely established and implemented. The bioaccumulation of metals in benthic macro invertebrates can provide an indication of the extent and magnitude of environmental contamination that is temporarily introduced via the water column and sediment. Thus, biomonitoring by employing living organisms such as mussels as sensors plays a vital role in governmental and industrial strategies to identify, assess, control, and reduce pollution problems (Krishnakumar et al. 1994, 1995).

It has been recognized that the soft tissues of marine molluscs are generally more efficient accumulators of metals than shells (Brown and Depledge 1998). Consequently, most attention has been directed to the soft tissues of the organisms while studies on the use of shells as indicators are few (Brix and Lyngby 1985; Bourgoin 1990; Foster and Chacko 1995; Prakash et al. 1996; Puente et al. 1996; Foster et al. 1997; Price and Pierce 1997; Giusti et al. 1999; Richardson et al. 2001). Nevertheless, studies on metal accumulation in shells are also useful since they can be used as a record of environmental metal levels (Chow et al. 1976; Sturesson 1976; Rhoads and Lutz 1980; Carriker et al. 1980, 1982; Koide et al. 1982; Al-Dabbas et al. 1984).

Shells have important practical advantages over the use of the soft tissues to monitoring metal contamination of the aquatic environment such as: (1) reveal less variability (Bourgoin 1990; Lingard et al. 1992), (2) integrate elemental concentrations over the life of the animal, (3) preserve the metals after death giving an idea about what the concentrations were in the past (Ferrel et al. 1973; Sturesson 1978; Carriker et al. 1980; Carell et al. 1987) and (4) offer considerable advantages with respect to both sample preservation and storage. Generally, the metal concentrations in the soft tissues show greater variability than in shells usually due to seasonal weight changes (associated with physiological conditions, reproductive state) and consequently, shells may provide a more realistic indication of the degree of contamination/pollution. Indeed, some authors when considering the metal concentrations in the soft tissues of marine molluscs, to avoid seasonal variations, have chosen to correlate them with shell weight, using metal/shell weight indices (Soto et al. 1995, 1997). Most of the studies regarding metal concentrations in the shells are on bivalves and particularly on genus *Mytilus* used as sentinel organisms in biomonitoring studies (Brix and Lyngby 1985; Bourgoin 1990; Szefer and Szefer 1990;Puente et al. 1996)

Blue mussel=common mussel (*Mytilus edulis*, Linnaeus 1758) lives in the Atlantic waters of European coast and the Mediterranean and Black and Baltic Seas. It also inhabits the coastal waters of Island, southern part of Greenland and the Atlantic and Pacific coastal waters of the North America (Zatsepin et al. 1988). Blue mussel is found on littoral and sublittoral zone, both in exposed and sheltered localities. The species tolerate wide temperature range, as well as different salinities (Seed 1976). Blue mussel feeds by filtering algae, detritus and organic particles from the water with its gills. Sexual maturation takes place at the age of 1–2 years depending on the growth rate and size (Seed 1976; Hovgaard et al. 2001). The reproductive cycle of mussels is controlled by the combination of several internal and external factors, among them nutrient reserve of individuals, food availability and temperature in the water. Blue mussels favour to settle on flat shores, which receive constant wave movements, although dense populations are also found on steeper faces, dock piles and harbour walls (Seed 1976). Adult individuals anchor themselves to substrate with byssus threads. After settlement blue mussels are able to move over substratum, but since mussels also favour growing in dense colonies, byssus threads of other individuals keep the colony together. This makes that blue mussels cannot escape from unfavourable conditions or from pollution.

The Gulf of Gdansk (Fig. 1) lies at the southern end of the Baltic Sea. In the north the Gulf is delimited by the Hel Peninsula, and its western part consists of the Bay of Puck, the innermost shallow

Fig. 1 Study area and location of sampling sites

part of which has its own pattern of water circulation. To the east of Gdansk are the three main outlets of the River Vistula, the basin of which occupies more than half of Poland with the major industrial regions and cities of Katowice, Krakow and Warsaw. It is not surprising, therefore, that the Gulf is considered metal polluted. The discharge of the Vistula is a key source of trace metals into the waters of the Gulf, and affects their subsequent distribution in water and sediment. The waters of both the Szczecin lagoon and Pomeranian bay are polluted mainly from industrial sources and also eutrophic. The Szczecin Lagoon and the Pomeranian Bay are supplied by the Oder River, the second largest river in Poland after the Vistula, with a length of 854 km and an outflow of 14.5 km^3 $year^{-1}$. This river drains the heavily industrialised heartland of Silesia and transports 90 t Cu $year^{-1}$, 792 t Zn $year^{-1}$, 15.5 t Cd $year^{-1}$ and 104 t Pb $year^{-1}$ to the Baltic (Neumann et al. 1996).

The primary objective of this field study was to assess the degree of pollution impact on mussel shells (*Mytilus edulis*) living at various sites of Polish coast of Baltic Sea.

Materials and metods

Shells of *Mytilus edulis* were collected in May to September 2005 from 12 stations on the Polish coast of Baltic Sea (Fig. 1). At each sampling site 17–330 different size of shells were collected during 2005, respectively. The specimens were sorted with respect to their sizes (Group A – <1.5 cm; Group B – 1.6–2.0 cm; Group C – 2.1–2.5 cm; Group D – 2.6–3.0 cm; Table 1).

After washing and drying, each shell was individually pulverized in an agate mortar and the resulting homogeneous fine powders stored for subsequent analysis. 1 gr of homogenized shells powder digested with 5 ml HNO_3+$HClO_4$ (3.5/1.5) for 24 h and samples were heated at 70°C. After this step into the sample solution was added 2–3 ml deionised water and for 2 h sample were heated at 98°C. The sample solutions were diluted with deionised water to the total volume of 100 ml and transferred into polyethylene flasks, capped and stored at room temperature. After dilution of solution, Hg^{2+} was reduced to Hg with $SnCl_2$ (20% solution in concentrated HCl). Readings of Hg were made at 253.7 nm using a model Coleman MAS 50 CV atomic absorption spectrometer.

For other metals, after dissection, 0.5 g shell samples were digested with a mixture of 4 ml HNO_3+$HClO_4$ (4/1). The resulting mixture was placed on a hot plate at 50°C and then 20–30°C and after that 2 ml HNO_3 were added and was placed on a hot plate at 200°C. The final residue were diluted with 15% HNO_3 (1 ml) and 4 ml H_2O. Readings of Pb and Cd were made using a model Perkin Elmer ZL 4110 (GF AAS) and other metals were made at using Jobin Yvon JY24 ICP AES.

Results and discussion

Organisms suitable for use as biomonitors provide integrated measures of the supply of trace metals available to them in an environment, accumulating the metal taken up from all sources such as from water and from food (Phillips and Rainbow 1993; Rainbow 1995a). They accumulate trace metals in proportion to the integrated ambient availabilities. It is appropriate, therefore, to refer to high accumulated metal concentrations in a biomonitor organism as indicative of high ambient availabilities to that biomonitor. Although all biomonitors must be net accumulators of the metal concerned, different species in the same habitat may show different patterns of metal contamination, according to the different routes of uptake available to them or to any differences in the physiological handling of metals taken up by different routes (Phillips and Rainbow 1993; Rainbow and Phillips 1993; Rainbow 1995a). It is for this reason that the use of a suite of biomonitors is to be recommended in any investigation of the metal contamination of an aquatic habitat (Phillips and Rainbow 1993; Rainbow 1995a).

Concentrations of 12 metals (Hg, Pb, Cd, Cu, Zn, Cr, Ni, Fe, Mn, V, Li, Al) measured in shells sampled from the Poland coast of Baltic Sea are summarized in Table 2. Levels of Hg ranged from 0.002 to 0.008 μg/g across various sites, with the highest mean level found in Międzyzdroje and Ustronie Morskie (0.008 μg/g). The highest level of Pb was recorded in the Międzyzdroje samples (0.432 μg/g), followed by Ustka (0.194 μg/g).

Relatively low concentrations of Cd were noted in Jurata (0.009 μg/g) while the highest concentrations

Table 1 Biometric data for four size classes (Group A – >1.5 cm; Group B – 1.6–2.0 cm; Group C – 2.1–2.5 cm; Group D – 2.6–3.0 cm) of mollusc *Mytilus edulis* from the southern Baltic

Sampling area	Date	Size (cm)	Number	Size group
Międzyzdroje	02.05.2005	1.5	14	VA
		2.0	48	VB
		2.5	35	VC
		3.0	30	VD
		Mean	22	V
Dziwnów	22.08.2005	Mean	18	VIII
Rewal	22.08.2005	1.5	101	IIIA
		2.0	76	IIIB
		2.5	42	IIIC
		3.0	11	IIID
		Mean	54	III
Niechorze	17.08.2005	1.5	49	IIA
		2.0	37	IIB
		2.5	42	IIC
		3.0	53	IID
		Mean	30	II
Mrzeżyno	15.08.2005	Mean	18	XII
Kołobrzeg	15.08.2005	Mean	49	X
Ustronie Morskie	22.08.2005	1.5	14	IA
		2.0	48	IB
		2.5	35	IC
		3.0	30	ID
		Mean	24	I
Unieście	09.08.2005	1.5	69	IVA
		2.0	98	IVB
		2.5	76	IVC
		3.0	46	IVD
		Mean	39	IV
Ustka	12.08.2005	Mean	17	VII
Łeba	06.09.2005	Mean	19	VI
Jurata	05.09.2005	Mean	22	XI
Sopot	06.09.2005	Mean	23	IX

were detected in Ustka (0.114 μg/g). Cu concentrations in the shell samples ranged from 2.450 μg/g in Kołobrzeg to 4.170 μg/g in Międzyzdroje. The highest mean level of Zn was found in Międzyzdroje (101.27 μg/g), followed by Niechorze (9.490 μg/g), Unieście (8.520 μg/g) and Ustronie Morskie (6.070 μg/g).

Concentrations of Cr did not show much variation between sampling sites (1.070–1.390 μg/g), with the lowest and the highest mean levels found in Rewal (1.070 μg/g) and Ustka (1.390 μg/g), respectively. The highest average concentration of Ni was observed in Dziwnów (2.240 μg/g), while there were not important differences in Ni concentrations among the remaining stations (1.460–2.240 μg/g).

Highest mean concentrations of Fe were found in Ustka (23.85 μg/g) and Łeba (22.19 μg/g). Fe levels tended to fluctuate between sampling station. The lowest mean level of Fe was recorded in Rewal (0.570 μg/g). Levels of V ranged from 0.958 to 1.364 μg/g, with the greatest mean concentration found in Międzyzdroje. The lowest mean level of Mn was recorded in Jurata (23.40 μg/g) and the highest in Dziwnów (131.2 μg/g). Shells of *M. edulis* (Table 2), from the all stations, contained the high levels of Mn which are suspected to be natural in origin.

Levels of Li in Jurata (3.484 μg/g), Rewal (1.057 μg/g) were relatively high, while levels recorded in the other stations ranged between 0.275 and 0.795 μg/g. Ustka had the lowest level of Li (0.275 μg/g). Concentrations of Al did not show much variation between sampling sites (119.0–214.0 μg/g), with the lowest and the highest mean levels found in Kołobrzeg and Łeba, respectively.

Table 2 Concentrations of metals in various stations of *Mytilus edulis* (μg/g dw)

Study area	Metal concentrations (μg/g)											
	Hg	Pb	Cd	Cu	Zn	Cr	Ni	Fe	Mn	V	Li	Al
Międzyzdroje	0.008	0.432	0.103	4.170	10.27	1.300	2.000	4.950	74.40	1.364	0.446	164.0
Dziwnów	0.005	0.010	0.098	3.070	5.660	1.360	2.240	1.070	131.2	1.284	0.375	154.0
Rewal	0.005	0.090	0.060	2.820	5.140	1.070	1.460	0.570	41.40	1.087	1.057	163.0
Niechorze	0.002	0.108	0.073	2.750	9.490	1.090	1.720	ns	35.10	0.958	0.650	158.0
Mrzeżyno	0.002	0.019	0.094	3.300	4.450	1.340	2.180	ns	60.00	1.361	0.795	145.0
Kołobrzeg	0.002	ns	0.080	2.450	3.340	1.160	1.820	20.25	62.10	1.011	0.705	119.0
Ustronie Morskie	0.008	0.160	0.110	2.910	6.070	1.150	1.510	5.520	47.10	0.979	0.642	166.0
Unieście	0.003	0.111	0.101	3.320	8.520	1.180	1.800	21.94	63.30	1.165	0.605	170.0
Ustka	0.005	0.194	0.114	3.020	5.560	1.390	2.030	23.85	65.60	1.224	0.275	163.0
Łeba	0.005	0.117	0.031	2.890	2.650	1.280	1.900	22.19	32.60	1.239	0.345	214.0
Jurata	ns	0.011	0.009	3.230	2.240	1.290	2.020	17.70	23.40	1.159	3.484	142.0
Sopot	0.005	ns	0.064	2.680	3.550	1.150	1.680	10.47	60.40	0.999	0.290	128.0

Some of these differences may well be attributable to changes in anthropogenic input of metals over even that limited period, or to changes in physicochemical factors such as salinity which affect the uptake of many trace metals (Phillips and Rainbow 1993; Rainbow 1995b, 1997).

As expected from the similarity found between the relationships of metal content-length and dry the concentrations of the different metals in the shells did not seem to depend on the shell length. No important differences between metal concentration at different shell lengths were detected (Table 3; Fig. 2).

Table 3 Concentrations of metals in various size of *Mytilus edulis* (μg/g dw)

Station		Hg	Pb	Cd	Cu	Zn	Cr	Ni	Fe	Mn	V	Li	Al
Międzyzdroje	A	0.005	0.308	0.129	4.200	7.710	1.390	nd	nd	77.10	1.163	0.506	176
	B	0.008	0.083	0.072	2.360	3.850	0.890	1.260	8.370	25.90	0.677	0.627	101
	C	0.008	0.395	0.234	6.120	5.790	1.310	1.870	0.010	55.60	1.202	0.412	158
	D	0.003	0.081	0.193	3.070	4.700	1.490	2.060	11.60	76.30	1.333	nd	155
Rewal	A	nd	0.153	0.729	3.300	6.250	1.110	1.440	1.110	34.90	0.912	0.809	163
	B	0.002	0.130	0.114	2.970	4.220	1.090	1.720	3.540	35.20	0.942	0.852	155
	C	0.005	0.043	0.043	2.640	5.010	1.030	1.440	0.310	46.80	0.909	0.714	147
	D	nd	0.068	0.068	3.020	9.280	1.140	1.670	2.390	31.60	1.073	0.540	194
Niechorze	A	nd	0.055	0.053	3.140	10.38	1.160	1.410	nd	35.20	0.984	0.934	172
	B	0.002	0.042	0.030	2.740	8.620	1.040	1.360	nd	25.40	0.883	0.764	161
	C	0.005	0.078	0.049	2.500	6.820	1.030	1.330	nd	32.20	0.871	0.638	149
	D	0.002	0.103	0.050	2.600	8.190	1.020	1.520	nd	40.60	0.918	0.695	138
Ustronie Morskie	A	0.003	0.012	0.018	2.730	4.730	1.000	1.130	2.970	51.70	0.743	1.235	161
	B	0.005	0.328	0.045	2.750	4.420	1.040	1.440	10.20	41.50	0.843	0.729	175
	C	0.005	0.157	0.099	3.000	5.150	1.050	1.340	2.700	27.70	0.905	0.822	164
	D	0.005	0.231	0.106	2.620	4.810	0.950	1.390	nd	29.40	0.922	0.550	146
Unieście	A	0.005	0.314	0.135	3.680	9.300	1.140	1.860	nd	50.90	1.087	0.741	163
	B	0.005	0.122	nd	2.880	5.360	0.900	nd	1.070	37.80	nd	0.500	127
	C	0.005	1.990	0.217	8.510	7.430	1.060	1.870	9.710	52.20	0.684	0.492	390
	D	0.005	0.384	0.089	3.010	6.010	1.050	1.800	nd	52.60	1.083	0.375	137

Group A>1.5 cm, Group B – 1.6–2.0 cm; Group C – 2.1–2.5 cm; Group D – 2.6–3.0 cm

nd Not detected

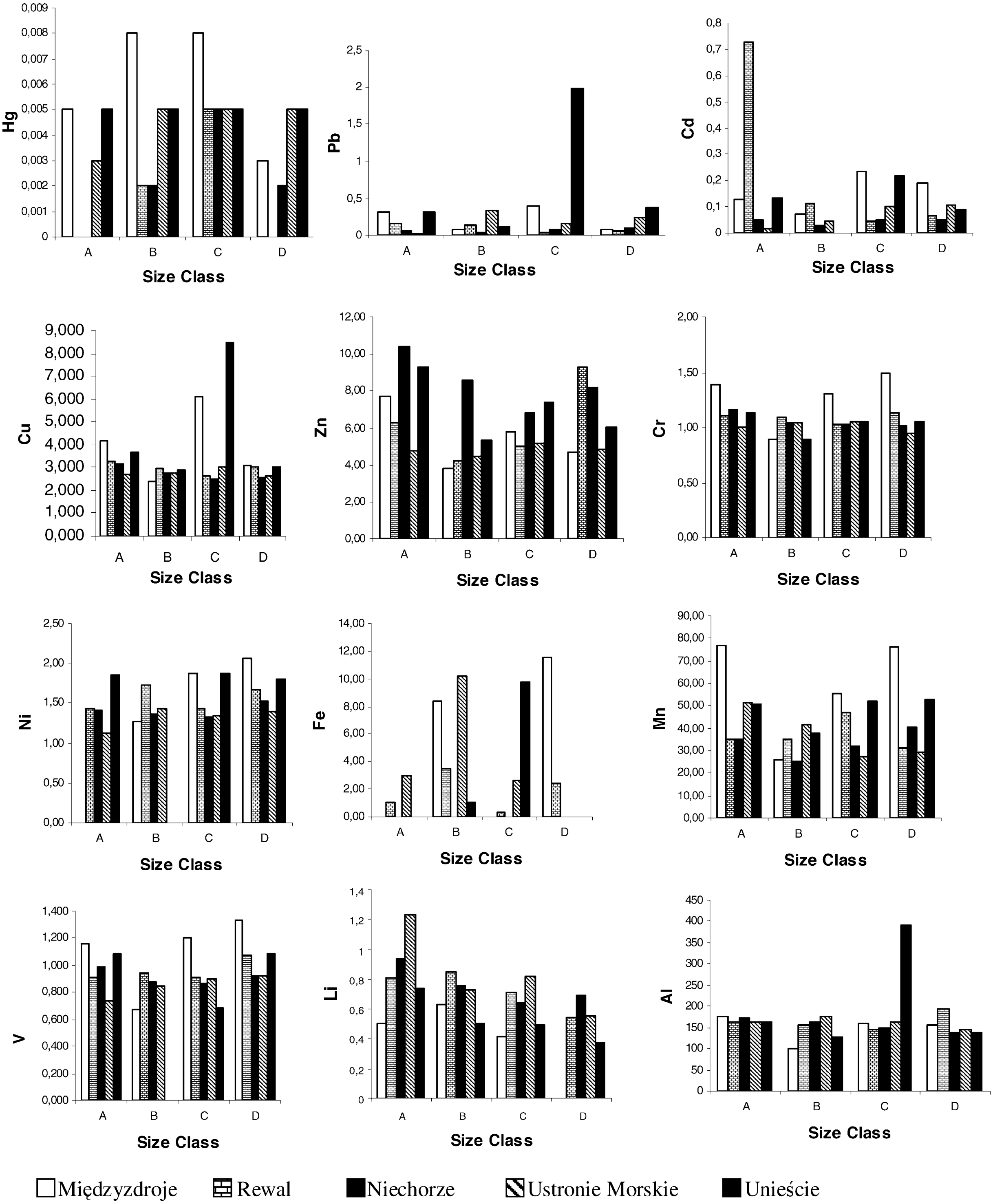

Fig. 2 Inter-regional distrubution of selected metals in the shells of successive size classes of *M. edulis*

Some investigations have shown that the accumulation of heavy metals is dependent of size (Boyden 1977; Latouche and Mix 1982). However, there is no general agreement on how the metal concentration is affected by size. Knutzen and Skei (1990) defined "high background levels" for the heavy metals in sediments and biota to indicate areas affected by heavy metal pollution.

Mussels have played a key role in the development of biomonitoring programmes for heavy metals, especially *Mytilus* as in the US Mussel Watch Program (Goldberg et al. 1978, 1983; Farrington et al. 1983; Lauenstein et al. 1990) and in NW Europe, for example Scandinavia (Phillips 1977, 1978). Individual species are widespread, for the most part as a result of people's intervention such as the cases of *M. edulis* and *M. galloprovincialis* (Seed 1992).

Several metals have been sporadically determined in shells of Baltic mollusc (Brix and Lyngby 1985; Szefer and Szefer 1985, 1990; Szefer 1986; Ikuta and Szefer 2002). According to Brix and Lyngby (1985) the concentrations of Cd, Cr, Hg and Zn in the shells of *M. edulis* from the Limfjord, Danish waters, decreased significantly with shell weight while their contents as well as those of Cu and Pb exhibited positive trends with increasing of size, similarly to the distribution patterns of all these metals in the soft tissue. Compared the results of this study in general, in our study no significant relation between metal concentrations at different shell size were detected.

The lack of spatial patterns is related to the regulation of internal metal concentrations by *M. edulis*. Amiard-Triquet et al. (1986) concluded that the use of mussels as a bioindicator of pollution seems doubtful for metals, since the concentrations of these trace elements in the organisms are largely independent of their levels in ambient seawater. Phillips (1980) also suggested that mussels should not be used as an indicator of copper in the marine environment. The effects of other metals on the net uptake of copper could not be easily eliminated or allowed for, and is extremely erratic and affected by salinity, temperature, other metals and changes in their relative concentrations.

Given their geographical distributions, as well as their abilities to show up spatial and temporal changes in metal bioavailabilities confirmed here, it is concluded that the mussel *M. edulis* is suitable candidate to be used in biomonitoring surveys of the Poland coast of Baltic.

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