The influence of marine and lacustrine humic substances on the accumulation of cadmium by the Baltic mussel *Mytilus trossulus**

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Abstract

The accumulation of cadmium by the Baltic mussel *Mytilus trossulus* in the presence of marine and lacustrine humic substances (HS) was investigated under laboratory conditions. The tested organisms were exposed to Baltic Sea water (salinity 7.0 PSU, pH 7.85) spiked with cadmium (50 μg Cd l^{-1}) and humic substances (6.0 mg HS l^{-1}), isolated from either marine (6 sampling sites) or lacustrine (8 sampling sites) environments. Experiments were carried out at a constant seawater temperature of (10°C±1°C). The exposure time was 21 days.

On average, the humic substances, a fraction of naturally occurring organic matter, were found to stimulate cadmium accumulation in the mussels. Lacustrine humic substances stimulated cadmium uptake to a lesser extent than the marine ones. Cadmium was accumulated preferentially in the hepatopancreas, and to a smaller extent in the gills and muscles, regardless of the presence, properties and

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origin of the humic substances. However, the effect was modified by the dissimilar physical and chemical properties of the substances, which were related to their place of origin.

1. Introduction

The prevention of pollution and the protection of the natural environment, including that of the oceans, have become one of the main priorities at the end of the 20th century. Nevertheless, heavy metal concentrations in natural waters are still rising (Clarck, 1989). Heavy metals are accumulated in the tissue of living organisms, causing harmful effects at relatively low concentrations. Some metals are then moved along the trophic chain to fish and humans.

The accumulation of heavy metals by marine organisms is frequently reported on in the literature (Denton, 1981; Amiard-Triquet *et al.*, 1986; Ahsanullah and Williams, 1991). The results of these studies indicate that benthic organisms in general and mussels in particular exhibit exceptionally high heavy metal accumulation factors (Theede *et al.*, 1979; Riisgård *et al.*, 1987). Cadmium, a metal easily bioaccumulated by benthic organisms, exhibits a particularly toxic influence even at small concentrations (Ray, 1984). Therefore, its quantity in water has to be monitored. Of still greater importance is the investigation and understanding of the rules governing the migration and distribution of the metal in the ecosystem. The fact that the accumulation of heavy metals, including cadmium, by marine biota depends on a number of biotic and abiotic factors is well documented (O'Hara, 1973; Cain and Luoma, 1986; Coimbra and Carraca, 1990). Among the latter, dissolved organic matter is attracting increasing attention (Ray, 1984; Shaw, 1994; Kożuch *et al.*, 1997).

Constituting a substantial fraction of the natural organic matter dissolved in Baltic waters, humic substances (HS) are naturally occurring complexing agents (Pempkowiak, 1989; Kożuch *et al.*, 1992). It was found in both field and laboratory studies that marine HS modify the bioaccumulation of cadmium to a considerable extent, owing to the formation of complexes with the metal (Kożuch and Pempkowiak, 1992; Pempkowiak and Kożuch, 1993). Additional, detailed laboratory studies have shown that the structural features and molecular weight of marine HS influence the accumulation rate of cadmium by mussels (Pempkowiak *et al.*, 1994; Kożuch and Pempkowiak, 1996). Since the HS present in lacustrine and marine environments differ in their physical and chemical properties (Harvey *et al.*, 1983; Summers *et al.*, 1987), their role as modifiers of metal contents in biota is likely to differ. However, no attempt has yet been made to compare marine and lacustrine humic acids.

This paper presents data indicating elevated cadmium uptake by the Baltic blue mussel in the presence of marine humic substances (MHS) as compared to lacustrine substances (LHS) of this kind. The differences are due to the dissimilar physical and chemical properties of the humic substances tested and to the place of origin of the substances.

2. Materials and methods

Baltic blue mussels *Mytilus trossulus*, collected from the Gulf of Gdańsk during the period from November 1996 to March 1997 and selected according to size (25–35 mm in length), were used in the cadmium accumulation experiments.

Laboratory tests on the accumulation of cadmium from seawater by the mussels were performed in glass aquaria filled with continuously aerated Baltic Sea water (salinity 7.0 PSU, pH 7.85, temperature $10°C \pm 1°C$ containing cadmium (concentration 50 $\mu g\,Cd\,l^{-1}$) and marine (experiment I) or lacustrine (experiment II) HS (6.0 $mg\,HS\,l^{-1}$). Seawater depleted of HS (0.0 $mg\,HS\,l^{-1}$) with added metal (concentration 50 $\mu g\,Cd\,l^{-1}$) served as a control in each experiment. The time of exposure was 21 days.

The marine humic substances (MHS), used in the first experiment, were isolated from both the surface and bottom seawater and from the surface bottom sediments sampled in the Pomeranian Bay and the western Gulf of Gdańsk (southern Baltic Sea). The lacustrine humic substances (LHS), utilised in the second experiment, were isolated from the water of seven lakes located in south-western Norway (Trehorningen, Hellerudmyra, Aurevann, Maridalsvann, Birkenes, Humex, Gjerstad). HS were isolated from water according to a standard procedure based on sorption on Amberlite XAD–2 resin, followed by desorption with ammonium hydroxide. Briefly, water was filtered through Whatman GF/F filters, acidified to pH 2.0 with concentrated HCl, and passed through a glass column (3×35 cm) filled with Amberlite XAD–2 resin. HS adsorbed onto the Amberlite were eluted with 0.5 M NH_4OH. The excess ammonium hydroxide was removed in a rotary evaporator and the concentrated HS solution was stored in a refrigerator at 4°C. HS from the sediments were isolated by alkaline extraction, followed by acidification of the extract and separation into fulvic and humic fractions. The solution containing fulvic acids was passed through a column filled with Amberlite XAD–2 resin. The fulvic acids were then desorbed with 0.5 M NH_4OH, concentrated in a rotary evaporator and stored in a refrigerator at 4°C. Details of the isolation procedure are given elsewhere (Pempkowiak *et al.*, 1994).

The isolated HS were further characterised by analysis of their physical and chemical properties. These included the elemental composition

(C, H, N), UV/VIS spectra (the substances were dissolved in 0.2 mol l^{-1} $NaHCO_3$; spectra recorded on a Beckman DU–68 spectrophotometer), FTIR spectra (KBr pellets, Bruker IFS66 spectrophotometer) and ^1H NMR spectra (50 mg HS were dissolved in 1 ml 0.2 mol l^{-1} NaOD in D_2O; spectra recorded on a Unity Plus 500 MHz Varian spectrometer).

During a three-week-long exposure to cadmium a number of mussels were removed from the aquaria every seventh day and their internal organs (gills, hepatopancreas and the 'rest' of the soft tissue) analysed for cadmium content. Oven-dried soft tissue was wet-digested with 3 ml concentrated HNO_3 followed by 1 ml concentrated $HClO_4$. The acids were evaporated and the residue redissolved in 5 ml 0.1 mol l^{-1} HNO_3. The measurements were carried out in a Video 11E (Thermo Jarrell Ash) atomic absorption spectrophotometer.

3. Results and discussion

HS tested in the cadmium accumulation experiments and their places of origin are listed in Tab. 1. Tab. 2 gives the accumulation rates of cadmium by mussels, equal to the directional coefficients of the relationship $C_{Cd} = (C_{Cd})_o + bt$ between the cadmium concentration in the soft tissue of *M. trossulus* (C_{Cd}) and the time of exposure (t) expressed in $\mu g\,Cd\ g^{-1}\ day^{-1}$ (Kożuch, 1995). It follows from these data that cadmium accumulation rates range from 1.71 $\mu g\,Cd\ g^{-1}\ day^{-1}$ ('rest', marine HS7) to 38.51 $\mu g\,Cd\ g^{-1}\ day^{-1}$ (hepatopancreas, lacustrine HS7), depending on the

Table 1. Origin of marine and lacustrine humic substances, utilised in the experiments on accumulation of cadmium by the mussel *M. trossulus*

Humic substances – origin					
marine environment (Baltic Sea)				lacustrine environment (Norwegian lakes)	
Pomeranian Bay		Gdańsk Bay			
water		water		water	
surface water	(HS1)	surface water	(HS5)	Trehorningen	(HS1)
bottom water	(HS2)	bottom water	(HS6)	Hellerudmyra	(HS2)
				Aurevann	(HS3)
bottom sediments				Maridalsvann	(HS4)
fulvic acids	(HS3)	fulvic acids	(HS7)	Birkenes	(HS5)
humic acids	(HS4)	humic acids	(HS8)	Humex	(HS6)
				Gjerstad-limed	(HS7)
				Gjerstad-unlimed	(HS8)

Table 2. Accumulation rates of cadmium [μg Cd g^{-1} dry wt. day^{-1}] in the soft tissue and organs of *M. trossulus* during exposure of mussels for 21 days in seawater enriched with cadmium (50 μg Cd l^{-1}) and marine (experiment I) or lacustrine (experiment II) humic substances (6.0 mg HS l^{-1}); control – 50 μg Cd l^{-1}, 0.0 mg HS l^{-1}

Sample number	Hepatopancreas b	± SD*	Gills b	± SD*	'Rest' b	± SD*	'Total' soft tissue b	± SD*
marine humic substances (experiment I)								
control	12.00	1.17	7.26	0.80	3.14	0.31	4.57	0.32
HS1	12.70	0.67	7.75	0.80	3.39	0.42	6.12	0.47
HS2	8.06	0.71	3.57	0.94	3.47	0.47	4.40	0.44
HS3	18.96	0.54	9.88	1.13	4.90	0.57	7.23	0.38
HS4	17.77	3.78	7.91	1.22	3.99	0.70	6.89	1.17
HS5	19.19	2.94	7.81	0.59	3.36	0.75	6.75	0.45
HS6	15.10	1.77	7.28	0.65	4.33	0.43	7.10	0.34
HS7	6.10	0.71	3.60	0.38	1.71	0.25	3.10	0.30
HS8	17.20	0.60	9.10	0.89	2.40	0.23	4.27	0.31
lucastrine humic substances (experiment II)								
control	27.42	5.13	11.87	1.76	10.76	0.83	14.14	0.90
HS1	26.97	3.88	15.60	0.86	10.01	1.11	13.79	1.43
HS2	17.40	2.20	15.10	3.20	10.31	2.16	12.68	2.33
HS3	26.91	3.72	15.05	2.40	9.20	1.29	12.28	1.68
HS4	35.80	3.51	19.62	4.87	9.95	1.96	15.56	1.96
HS5	21.16	2.51	10.70	1.15	5.97	1.11	9.98	1.05
HS6	34.76	4.83	22.09	2.77	9.92	2.62	16.23	2.23
HS7	38.51	6.43	14.40	2.16	10.15	1.51	16.09	2.28
HS8	32.70	5.21	14.70	2.22	9.90	1.25	14.80	1.23

* ± SD – standard deviation of the directional coefficient b in the relationship $C_{Cd} = (C_{Cd})_o + bt$ between cadmium concentration in the soft tissue of *M. trossulus* (C_{Cd}) and the time of exposure (t).

organ in question and the origin of HS. This agrees well with the cadmium accumulation rates in the Baltic blue mussel reported earlier (Kożuch, 1995; Kożuch *et al.*, 1997).

The accumulation rates of cadmium by mussels in the presence of MHS range from 1.71 μg Cd g^{-1} day^{-1} ('rest', HS7) to 19.19 μg Cd g^{-1} day^{-1} (hepatopancreas, HS5). These rates are half as fast as those found in experiments with LHS. The latter range from 5.97 μg Cd g^{-1} day^{-1} ('rest', HS5) to 38.51 μg Cd g^{-1} day^{-1} (hepatopancreas, HS7). This might have been due to the fact that the life activity of the mussels collected in February

and subsequently used in the accumulation experiment with LHS is more intense than that of the mussels collected in November and then used in the accumulation experiment with MHS. Recently, Gosling (1992) suggested that the physiological activity of mussels is subject to seasonal variation. The comparison of cadmium accumulation rates in mussels growing in water without added HS (control) during the first and second experiment (Tab. 2) confirms this hypothesis.

The calculated metal accumulation rates by the 'total' soft tissue and various organs of *M. trossulus* in the presence of the investigated HS in relation to the controls are shown diagrammatically in Fig. 1. A strong stimulatory effect of HS on cadmium accumulation in marine organisms has already been reported for marine HS (Pempkowiak *et al.*, 1989; Kożuch and Pempkowiak, 1992; Pempkowiak *et al.*, 1994). The present data follow the previously reported pattern. However, the data in Fig. 1 and Tab. 2 go beyond these findings, indicating as they do the varying stimulatory effect of HS isolated from the two environments on cadmium uptake by mussels. In the case of MHS (experiment I), the accumulation rates of metal in the total soft tissue of organisms grown in water containing MHS exceed those in mussels grown in water devoid of these substances (control), except for MHS isolated from the bottom water of Pomeranian Bay (HS2), and fulvic (HS7) and humic (HS8) acids isolated from bottom sediments of the Gulf of Gdańsk. On average, MHS increase the accumulation rate in the hepatopancreas by a factor of 1.40 ± 0.21 ($n = 6$) as compared to the control. LHS (experiment II) stimulated cadmium accumulation by mussels to a lesser extent than the marine ones. The ratio of the average accumulation rate by the hepatopancreas in the presence of LHS to that of the control is 1.06 ± 0.27 ($n = 7$). No statistically significant differences were found in the accumulation rates of the metal by the total soft tissue of mussels in the presence of various LHS (Tab. 2), with the exception of the very high accumulation rate recorded in the case of the LHS isolated from the limed lake Gjerstad.

The differences between marine and freshwater HS could be due to their dissimilar physical and chemical properties (Kożuch, 1995; Kożuch *et al.*, 1997). Selected properties of HS tested in the two experiments were measured (Tab. 3). The observed stimulatory effect of MHS on cadmium accumulation rates in mussels seems to be correlated with the specific elemental composition of the substances. The much higher C/N index in LHS confirms the greater contribution of terrestrial organic matter in these substances as compared to the marine ones (Bojanowski *et al.*, 1981). Analyses of the ^{1}H NMR and IR spectra of the HS tested indicate that MHS display a more aliphatic character, in contrast to the

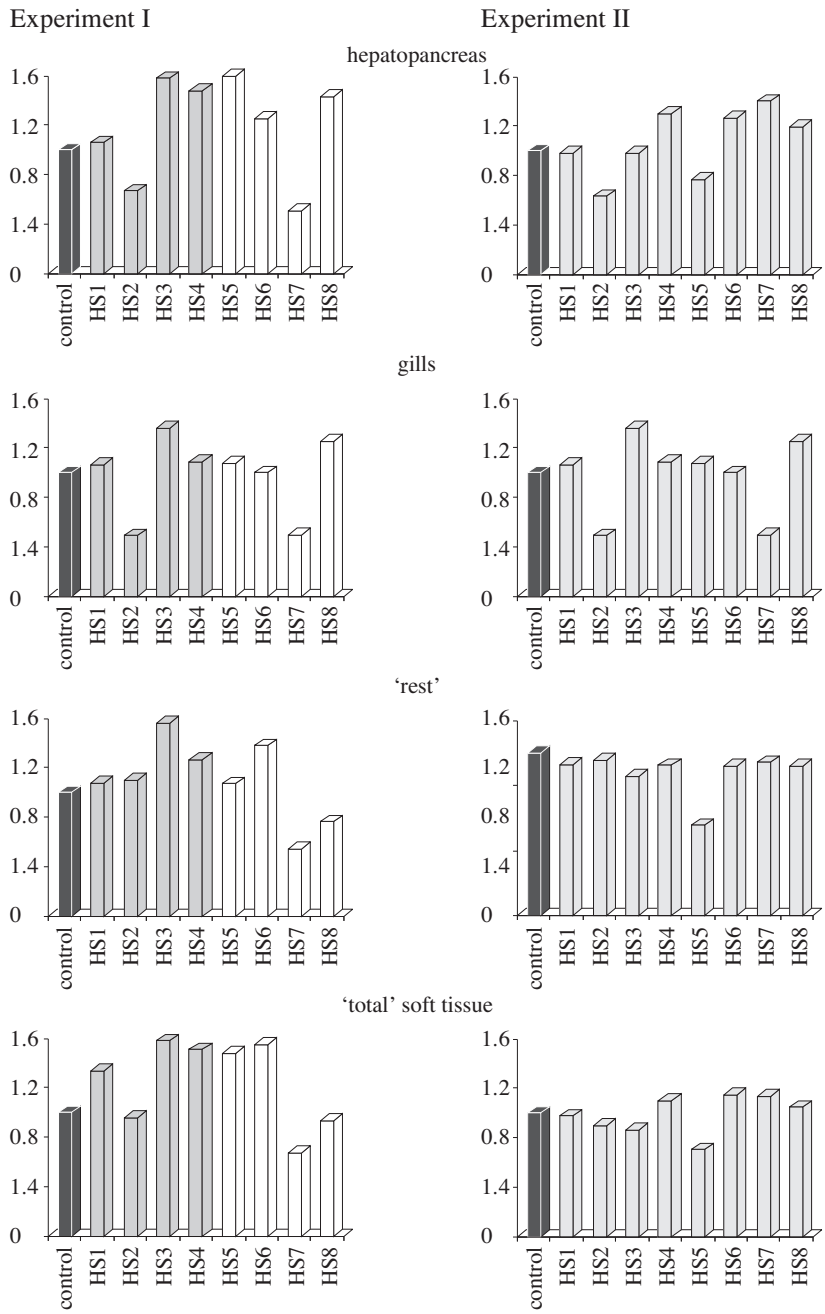

Fig. 1. Accumulation rates of cadmium [μg Cd g^{-1} dry wt. day^{-1}] in the 'total' soft tissue and organs of *M. trossulus* during exposure of mussels for 21 days in seawater enriched with cadmium (50 μg Cd l^{-1}) and marine (experiment I) or lacustrine (experiment II) humic substances (6.0 mg HS l^{-1}) in relation to the accumulation rates in control (50 μg Cd l^{-1}, 0.0 mg HS l^{-1})

Table 3. Selected physical and chemical properties of the investigated marine and lacustrine humic substances, utilised in the experiments on the accumulation of cadmium by the mussel *M. trossulus*

Sample number	Ash	Organic matter	Elementary composition of organic matter					Spectra			
			C	H	N	C/H	C/N	^{1}H NMR	IR	UV/VIS	
	[%]	[%]	[%]					H_{ar}/H_{al}	1600/1450	$E_{2/3}$	$E_{4/6}$
marine humic substances (experiment I)											
HS1	1.4	98.6	47.86	6.17	5.68	7.76	8.43	0.074	1.00	6.24	8.28
HS2	2.4	97.6	46.78	6.39	6.47	7.32	7.23	0.044	1.00	6.37	7.09
HS3	15.0	85.0	59.46	7.40	6.33	8.03	9.39	0.086	1.25	4.77	3.66
HS4	18.0	82.0	63.84	8.94	8.68	7.14	7.35	0.104	1.56	2.22	2.56
lacustrine humic substances (experiment II)											
HS1	57.5	42.5	52.2	6.0	2.2	8.70	23.73	0.129	1.166	3.92	11.52
HS2	32.9	67.1	50.3	5.3	1.0	9.49	50.30	0.054	2.621	3.32	9.80
HS3	56.0	44.0	45.8	5.9	2.3	7.76	19.91	0.104	1.525	3.30	9.41
HS4	68.4	31.6	43.9	5.3	4.7	8.28	9.34	0.072	0.337	3.70	9.53
HS5	67.8	32.2	48.0	5.6	2.6	8.57	18.46	0.098	0.517	4.75	10.89
HS6	36.7	63.3	52.7	5.4	1.0	9.76	52.70	0.074	2.291	3.50	10.15
HS7	60.6	39.4	53.2	6.0	2.0	8.87	26.60	0.071	2.065	3.14	6.87
HS8	49.6	50.4	50.8	5.6	1.5	9.07	33.87	0.069	2.497	3.56	10.49

lacustrine ones, which are more aromatic. Moreover, LHS are characterised by a higher aromatic ring content. This inference is suggested by both the ^{1}H NMR spectra (H_{ar}/H_{al} LHS $= 0.091 \pm 0.023$, $n = 6$; H_{ar}/H_{al} MHS $= 0.068 \pm 0.021$, $n = 4$) and the IR spectra (1600 cm^{-1}/1450 cm^{-1} aromatic index of LHS $= 2.03 \pm 0.57$, $n = 6$; MHS $= 1.20 \pm 0.26$, $n = 4$). The higher aromatic index of LHS and, therefore, the limited complexation of cadmium by these substances (Kożuch and Pempkowiak, in press) is correlated with their lesser stimulatory effect on the accumulation rates of the metal by *Mytilus* as compared to HS isolated from the marine environment (Kożuch and Pempkowiak, 1996; Kożuch *et al.*, 1997). This is reflected by UV/VIS measurements. The higher aliphatic index derived from the UV/VIS spectra (equal to the ratio of absorption at 465 nm to that at 665 nm – $E_{4/6}$) for MHS isolated from surface (HS5) and bottom (HS6) water of the Gulf of Gdańsk contrasts with the smaller values of the index in the sedimentary HS. This correlates well with the fact that the stimulatory effect of substances isolated from water is greater than that of such substances isolated from

the sediments on rates of cadmium accumulation by the mussels. This is in agreement with the results of earlier studies (Kożuch, 1995; Kożuch and Pempkowiak, in press).

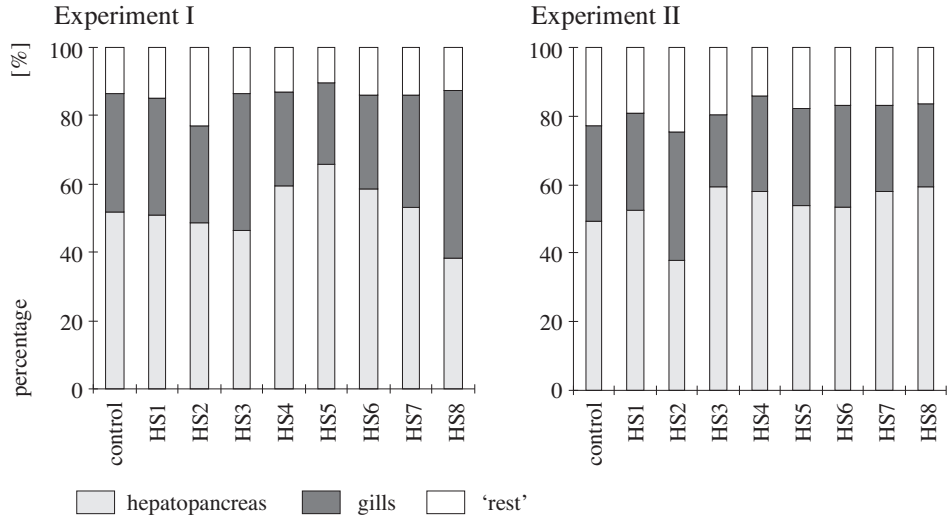

Fig. 2. Percentage distribution of cadmium in the inner organs of the soft tissue of *M. trossulus* after 21-day long exposure of mussels in seawater enriched with cadmium (50 μg Cd l^{-1}) and marine (experiment I) or lacustrine (experiment II) humic substances (6 mg HS l^{-1}); control – 50 μg Cd l^{-1}, 0.0 mg HS l^{-1} in seawater

The percentage distribution of cadmium in the inner organs of the soft tissue of *M. trossulus* after a 21-day exposure in seawater containing cadmium and marine or lacustrine HS is presented in Fig. 2. Cadmium was accumulated mainly in the hepatopancreas, less so in the gills and to a still smaller extent in the 'rest' of the soft tissue, regardless of the presence of various marine and lacustrine HS in the seawater. It had been reported earlier that cadmium is stored largely in the hepatopancreas (Kożuch and Pempkowiak, 1992), most probably in the form of complexes with metal-bonding protein-thionine (Coombs, 1979; Nolan and Duke, 1983; Bebianno and Langston, 1991). The average contribution of cadmium stored in the gills in relation to the total content of the metal is larger in mussels exposed to MHS ($\bar{x} = 0.34 \pm 0.07$, $n = 7$) than in those exposed to lacustrine ones ($\bar{x} = 0.26 \pm 0.03$, $n = 8$). This may indicate that humus-cadmium complexes are being absorbed on the gill surfaces, and that the ensuing enhanced transport of cadmium through membranes is the mechanism responsible for the elevated cadmium content in the soft tissue of mussels in the presence of MHS.

References

Ahsanullah M., Williams A. R., 1991, *Sublethal effects and bioaccumulation of cadmium, chromium, copper and zinc in the marine amphipod Allorchestes compressa*, Mar. Biol., 108, 59–65.

Amiard-Triquet C., Berthet B., Metayer C., Amiard J. C., 1986, *Contribution to the ecotoxicological study of cadmium, copper and zinc in the mussel Mytilus edulis. Experimental study*, Mar. Biol., 92, 7–13.

Bebianno M. J., Langston W. J., 1991, *Metallothionein induction in Mytilus edulis exposed to cadmium*, Mar. Biol., 108, 91–96.

Bojanowski R., Pempkowiak J., Kupryszewski G., 1981, *Humic substances in the Baltic sediments, isolation and preliminary characterisation*, Oceanologia, 7, 105–120.

Cain D. J., Luoma S. N., 1986, *Effect of seasonally changing tissue weight on trace metal concentrations in the bivalve Macoma balthica in San Francisco Bay*, Mar. Ecol. Prog. Ser., 28, 209–217.

Clarck R. B., 1989, *Marine pollution*, Clarendon Press, Oxford, 220 pp.

Coimbra J., Carraca S., 1990, *Accumulation of Fe, Zn, Cu and Cd during the different stages of the reproductive cycle in Mytilus edulis*, Comp. Bioch. Physiol., 95, 265–270.

Coombs T. L., 1979, *Cadmium in aquatic organisms*, [in:] *The chemistry, biochemistry and biology of cadmium*, M. Webb (ed.), Elsevier, Amsterdam, 93–139.

Denton G. R. W., 1981, *Influence of temperature and salinity on the uptake, distribution and depuration of mercury, cadmium and lead by the brack-lip oyster Saccostrea echinatea*, Mar. Biol., 64, 317–326.

Gosling E., 1992, *The mussel Mytilus: ecology, physiology, genetics and culture. Developments in aquaculture and fisheries sciences*, Elsevier, Amsterdam–London–New York–Tokyo, 589 pp.

Harvey G. R., Boran D. A., Chesal L. A., Tokar J., 1983, *The structure of marine fulvic and humic acids*, Mar. Chem., 12, 119–132.

Kożuch J., 1995, *The influence of selected properties of humic substances on the accumulation of cadmium by the mussel Mytilus trossulus*, Ph. D. thesis, Uniw. Gdańsk, Gdynia, (in Polish).

Kożuch J., Pempkowiak J., 1992, *The influence of humic substances on the accumulation of cadmium from seawater by the blue mussel Mytilus edulis*, [in:] *Proc. 12th Baltic Mar. Biol. Symp., Helsingor, Denmark, 1991*, E. Bjornestad, L. Hagerman and K. Jensen (eds.), Olsen and Olsen, Fredensborg, 97–100.

Kożuch J., Pempkowiak J., 1996, *Molecular weight of humic acids as a major property of the substances influencing the accumulation rate of cadmium by the blue mussel (Mytilus edulis)*, Environm. Int., 22, 585–589.

Kożuch J., Pempkowiak J., *Influence of marine humic substances with different properties on the accumulation of cadmium by the Baltic mussel Mytilus trossulus*, (in press).

Kożuch J., Pempkowiak J., Grzybowski W., 1992, *Complexing properties of humic substances towards cadmium and their influence on the accumulation of cadmium by Mytilus edulis*, Bull. Sea Fish. Inst., Gdynia, 1, 15–18.

Kożuch J., Radłowska M., Pempkowiak J., 1997, *Humic substances of different origin influence the accumulation of cadmium by the mussel Mytilus edulis trossulus to a varying degree*, [in:] *Proc. 14th Baltic Mar. Biol. Symp., Pärnu, Estonia, 1995*, E. Ojaver (ed.), Estonian Acad. Publ., Tallinn, 80–85.

Nolan C. V., Duke E. J., 1983, *Cadmium accumulation and toxicity in Mytilus edulis: involvement of metallothioneins and high-molecular weight protein*, Aquat. Toxicol., 4, 153–163.

O'Hara J., 1973, *The influence of temperature and salinity on the toxicity of cadmium to the fiddler crab Uca puligator*, Fish. Bull., 71, 149–153.

Pempkowiak J., 1989, *Origin, sources and properties of humic substances in the Baltic Sea*, Ossolineum, Wrocław, 146 pp., (in Polish).

Pempkowiak J., Bancer B., Legeżyńska E., Kuliński W., 1989, *The accumulation and uptake of cadmium by four selected Baltic species in the presence of marine humic substances*, [in:] *Proc. 21st Europ. Mar. Biol. Symp., Gdańsk, Poland, 1986*, R. Z. Klekowski, E. Styczyńska-Jurewicz and L. Falkowski (eds.), Ossolineum, Gdańsk, 599–608.

Pempkowiak J., Kożuch, J., 1993, *Field and laboratory studies on the influence of humic substances on the accumulation of cadmium by Mytilus edulis*, Stud. i Mater. Oceanol., 64, 245–251.

Pempkowiak J., Kożuch J., Southon T., 1994, *The influence of structural features of marine humic substances on the accumulation rates of cadmium by a blue mussel Mytilus edulis*, Environm. Int., 20, 391–395.

Ray S., 1984, *Bioaccumulation of cadmium in marine organisms*, Experientia, 40, 13–23.

Riisgărd H. U., Bjornestad E., Mohlenberg F., 1987, *Accumulation of cadmium in the mussel Mytilus edulis: kinetics and importance of uptake via food and seawater*, Mar. Biol., 96, 349–353.

Shaw P. J., 1994, *The effect of pH, dissolved humic substances and ionic composition on the transfer of ion and phosphate to particulate size fractions and epilimnetic lake water*, Limnol. Oceanogr., 39, 1734–1743.

Summers R. S., Cornel P. K., Roberts P. V., 1987, *Molecular size distribution and spectroscopic characterisation of humic substances*, Sci. Total Environm., 62, 27–37.

Theede H., Andersson I., Lehnberg W., 1979, *Cadmium in Mytilus edulis from German coastal waters*, Meeresforschung, 27, 147–155.